CHARACTERIZATION OF SOYMILK PRODUCED BY CONTINUOUS FLOW HIGH PRESSURE THROTTLING PROCESS

by

LITHA SIVANANDAN

(Under the Direction of Rakesh K. Singh and Romeo T. Toledo)

ABSTRACT

Standard soymilk processing uses filtration or centrifugation step to remove coarse solids in the comminuted soy. The objectives were to produce soymilk from dehulled beans by Gaulin homogenizer or produce sterile soymilk using microfluidizer-throttling or continuous flow high pressure throttling (CFHPT) to retain retaining all essential soybean solids. The soymilk was characterized for particle size distribution, rheological and ultra-structural properties, to establish an empirical model to characterize the distribution of particle size of particles in the soymilk, and to evaluate the consumer acceptability. Whole dehulled soybeans were blanched, mixed with deionized water, and comminuted coarsely in a food-processor. An intermediate comminution step in Megatron (process M) or Fitzmill (process F) or Stonemill (process S) was followed by homogenization at selected pressures using Gaulin homogenizer or microfluidizer-throttling or CFHPT system. The combined process M and CFHPT treated samples at the highest pressure showed the smallest particle size and the highest apparent viscosity. All samples showed pseudoplastic flow behavior. Ultrastructural images elucidated particle microstructure in the soymilk and homogeneity of suspended particles. The very small fat globules at highest CFHPT pressure treatment of process M were seen entrapped in the network and was uniformly distributed. Thus combined process M and the highest CFHPT pressure was considered the best

treatment. Therefore, the high pressure throttling process will allow utilization of the whole soybean to produce excellent quality soymilk with high emulsion stability. The increase in the CFHPT flow rate significantly affected size reduction of particles of soymilk. The empirical models were established which can be used to predict the size of particles in soymilk, at different volume fractions, processed using high pressure throttling processes at various pressures and flow rates. Consumer acceptability test showed that more research is needed to make a soymilk that appeal to the taste of the American consumer before the CFHPT process can be used commercially to produce soymilk. Thus, soymilk with all the essential solids can be made available to the public and processors benefit from the high processing yields since none of the essential solids of the beans are discarded.

INDEX WORDS: high pressure throttling, soymilk, particle size distribution, rheological properties, ultrastructural properties.

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DEDICATION

To my princess, Ipsita, my sweet heart, Kaushlendra, my mother Lalitha, and my father Sivanandan.

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CHAPTER 1

INTRODUCTION

Popularity and high demand of non-thermal processes as potential methods of food preservation has been observed in recent years and high pressure treatment is one among them due to the fresh-like properties offered by the high-pressure treated products (Amornsin 1999; Rastogi and others 2007). While the scrutiny of minimally processed food continues, high pressure treatment attained much attention and foods treated with high pressure were reported to have superior color, flavor, nutrient retention, lower microbial count, vitamin content and functional properties due to its non-thermal and non-chemical application (Adapa and others 1997).

Conventional homogenization, developed by Auguste Gaulin in 1899 (French patent number 295,596), has been extensively adopted in food applications, especially in dairy industry and in other areas dealing with oil and water emulsions (Peck 2004; Zamora and others 2007). Classical homogenization used <100 MPa for standard industrial processes by forcing fluid foods through narrow gap of 100-300 μm in the homogenizer valve, with an upstream pressure of about 20-60 MPa, to stabilize emulsions against creaming and coalescence and obtain products with appropriate rheological properties (Kessler 1981).

Use of high hydrostatic pressure (HHP) as a means of preserving milk was done 100 years before [by Hite (1899) and cited by (Rastogi and others 2007)] and later it was extended to high pressure application to preserve fruits and vegetables [Hite and others (1914) as cited by (Rastogi

and others 2007)]. HHP uses high isostatic pressure (equal pressure applied with a pressurized fluid from all sides of the pressure vessel at the same time) to inactivate microbes and alter the physicochemical and functional properties of the pre-packaged food (in packages of flexible films or other material) kept in high pressure vessels (Moorman 1997; Rastogi and others 2007). Pressure may be applied directly by high pressure vessels or indirectly by pressure intensifiers attached to the vessels (Rastogi and others 2007). The increase in temperature (3 °C per 100 MPa) of the liquid component of the product due to high pressure application is a disadvantage of this processing technique (Rastogi and others 2007). The food cools down when the pressure is released (decompression). Though HHP processed foods posses superior nutrient and sensory quality compared to thermally-processed foods, some problems such as accelerated enzymatic browning in vegetables and lipid oxidation in muscle tissues are unavoidable (Rastogi and others 2007). Also, bacterial spores are highly pressure resistant (upto 1200 MPa), and a combination of moderate heat treatment (or any other pre-treatment such as ultra sound) with pressure treatment could be effective in initiation of spore germination or inhibition of germinated bacterial spores and inactivation of piezo-resistive microorganisms (Rastogi and others 2007). This process temperature can go upto 90-121 °C in combination with pressures of 500-800 MPa to inactivate spore forming bacteria such as *Clostridium botulinum* (Rastogi and others 2007). The high cost of capital investment for new equipment limits commercialization of HHP. The high labor cost and low productivity of the batch process adds to its disadvantages (Moorman 1997). Apart from the high initial cost and engineering limitation of huge pressure vessel, long processing time (30 min to 1 h) is an added concern.

Growing interest is seen in dairy industry for non-thermal process which combines efficient microorganism reduction with a maximum retention of the physicochemical properties of the

product (Lopez-Pedemonte and others 2006). Ultrahigh pressure homogenization (UHPH, also known as high pressure homogenization (HPH) or dynaimic high pressure) uses pressures above 200 MPa. UHPH machines were developed with some technological modifications in the conventional homogenizer such as incorporating high pressure pumps and intensifiers (driven by hydraulic pump), specifically designed high pressure valves made of ceramic material (ceramic seats and needles) to withstand the high pressures upto 350 MPa, modified geometry compared with classical homogenizing valves (APV-Gualin homogenizers) to generate difference in the fluid flow directions, and narrower gaps between the valve and the seats (Floury and others 2004; Thiebaud and others 2003). A second pneumatic valve after the first valve supports a pressure upto 50 MPa. The first stage valve is refrigerated by constant water circulation of water at ambient temperature in an external jacket built around it; this is to avoid the loss of homogenization performance due to temperature increase and rapid expansions or contractions of the first stage valve. The residence time of the fluids in the high pressure section is in the order of seconds.

High pressure throttling was developed as a means of continuous microbial inactivation of fluid foods at The University of Georgia, Athens (Moorman 1997). Throttling valve (or micrometering valve) is a fine restriction such as a partially closed valve or porous plug to control the flow of the fluid through a micro-metering valve. CFHPT uses high pressure (up to 310 MPa) to pressurize liquid foods and to continuously throttle the foods from that high pressure to atmospheric pressure for the purpose of inactivating microbes and modifying proteins which should change the rheological properties of the food (Moorman 1997). Thus, the name continuous flow high pressure throttling (CFHPT) aptly suits the process. As the fluid material exits the throttling valve, temperature rise occurs (which is proportional to the pressure applied),

it is held at that elevated temperature, and the product is then very quickly cooled to prevent over processing of the product which lead to a reduction in overall quality (nutrition and sensory).

Advantages of CFHPT processing include simultaneous homogenization and sterilization which can benefit physical properties, texture, and stability of the sterilized product during storage. CFHPT treatment has a wide range of applications in the manufacture of functional ingredients. An excellent result on particle size reduction and narrowing the distribution was earlier reported by Peck (2004) where the CFHPT processed blueberry-whey beverage showed no aggregation of particles during storage in addition to the significantly smaller size offered by the treatment compared with thermally processed beverage. Moorman (1997) reported increase in viscosities of CFHPT treated milk concentrations (ultrafiltered) and increase in apparent viscosities and water holding capacity of yogurt made from CFHPT treated milk. These changes in the properties of milks and yogurt were due to the modification of dairy proteins by the CFHPT process. Therefore, the author suggested that CFHPT treatment of skim milk could be utilized for improving the "mouth feel" of skim milk without added ingredients such as polysaccharides. High shear rates can increase the surface area to volume ratio, which can increase viscosity of certain fluid materials leading to improved texture, taste, and flavor characteristics (Areekul 2003).

Microfluidization is a homogenization technique developed by Cook and Lagacé (1985) in which the liquid food in a high pressure chamber is split into two streams in the interaction chamber and collides with each other at high speed at a 180 ° angle (Lemay and others 1994; Tunick and others 2002). Smaller particle size and narrower particle size distribution was exhibited by the foods processed by this process. Paquin (1999) explained that the size reduction observed was due to the cavitation which resulted from collisions of the fat globules while the

two streams were united. The hybrid process of CFHPT and microfluidization introduced a micrometering valve (which was used in CFHPT process) in place of interaction chamber in the microfluidizer (Moorman 1997; Amornsin 1999).

Soymilk is in growing demand in Western countries too due to its immense health benefits and it is one of the traditional nutritious beverages used for thousands of years in oriental countries (Sloan 2005). The beany flavor of soy in food products are still a constraint for its complete acceptance among the American consumer (Torres-Penaranda and others 1998; Wilson 1996). Standard soymilk processing uses thermal treatment of fine soy slurry from which the coarser bean particles (okara – having 27% protein, dry basis) are already removed (Kumar and others 2003; Lakshmanan and others 2006; Chan and Ma 1999; Liener 1981). Several processes were developed for soymilk processing to include the whole bean solids in the product (Hand and others 1964; Nelson and others 1976; Mustakas and Mayberry 1964) and one of the milestones in the soy to process soymilk with effective utilization of cotyledon solids was the Illinois process of producing soymilk which removed only 10-12% of water-soluble bean solids in the final product which also avoided residue formation (Rosenthal and others 2003; Nelson and others 1976; Kuntz and others 1978). CFHPT treated blueberry-whey beverage was preferred by sensory panelists over the heat treated beverage indicated the higher retention of flavors in the CFHPT processed beverage as opposed to the thermally-processed item (Peck 2004). Also, the shelf-life of the CFHPT beverage was higher as it was visible by its superior quality on day 35 as against the visible spoilage of the thermally-processed product at day 65. Though lot of works has been done in soymilk processing, studies on the effect of these processes in the properties of soymilk is lacking.

The inclusion of whole bean cotyledons solids in the soymilk is very essential for increasing the consumer demand of this healthy drink. The excellent physicochemical and sensory properties exhibited by the liquid foods (blueberry beverage, honey, milk, yogurt) processed using high pressure throttling process shows unlimited application of this process in continuous liquid food processing. Thus this study was undertaken with the following objectives:

1. To process soymilk produced from whole dehulled soybeans using pressurized homogenization and high pressure throttling process and to study its effects on particle size distribution, rheological and ultrastructural properties exhibited by the soymilk.
2. To study the effect of flow rate used in high pressure throttling process on the particle size reduction of soymilk and to establish a model to predict the particle size of soymilk processed using high pressure throttling processes.
3. To study the consumer acceptability of the whole soybean milk processed by high pressure throttling in comparison to a commercial soymilk.

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CHAPTER 2

REVIEW OF LITERATURE

This chapter deals with review of the studies done in the past in the areas related to high pressure treatment, soymilk processing and evaluation, and the properties of high pressure treated foods.

2.1 High hydrostatic pressure (HHP) processing

Use of high hydrostatic pressure (HHP) as a means of preserving milk was done 100 years before [by Hite (1899) and cited by (Rastogi and others 2007)] and later it was extended to high pressure application to preserve fruits and vegetables [Hite and others (1914) as cited by (Rastogi and others 2007)]. HHP uses high isostatic pressure (equal pressure applied with a pressurized fluid from all sides of the pressure vessel at the same time) to inactivate microbes and alter the physicochemical and functional properties of the pre-packaged food (in packages of flexible films or other material) kept in high pressure vessels (Moorman 1997; Rastogi and others 2007) Pressure may be applied directly by high pressure vessels or indirectly by pressure intensifiers attached to the vessels. The increase in temperature (3 °C per 100 MPa) of the liquid component of the product due to high pressure application is a disadvantage of this processing technique (Rastogi and others 2007). This temperature rise is still higher (8-9 °C per 100 MPa) if the food contains significant amount of fat (Rasanayagam and others 2003). Also a decrease of pH in apple juice by 0.2 units per 100 MPa increase in pressure was reported earlier (Heremans 1995). The food cools down when the pressure is released (decompression). This heat of compression

with the pressure application could be utilized effectively in combination since the temperature of the product can be raised from 70-90 °C to 105-120 °C by compressing the food to 700 MPa (Meyer 2000). If no heat is lost or gained from the walls of the pressure vessel during pressure holding, the original temperature of the food is reached (Rastogi and others 2007). Also, it is important, but difficult, to maintain the isothermal conditions during the pressure holding period to avoid the uneven temperature distribution due to heat transfer across the pressure vessel walls. Structural changes are unavoidable in the food if entrapped air is present in it (for example strawberry, lettuce) (Rastogi and others 2007). Microbial cells are destroyed by HHP, which includes changes in the morphology, the cell membrane and the wall of microorganisms, and modifies biochemical reactions and genetic mechanisms (Patterson 2005).

The Le Chatelier's principle states that phenomena (transition temperature of lipids and water, conformation of macromolecules, and other chemical reactions) which accompany the volume reduction are enhanced by the pressure and vice versa (Rastogi and others 2007). In addition to the shift in reaction equilibriums to the most compact state, an increase or decrease in reaction rate constant is also observed with pressurization. For heating one liter of water from 20 to 25 °C at 400 MPa requires 19.2 kJ only as against 20.9 kJ at atmospheric pressure (Rastogi and others 2007). Thus the covalent bonds of food constituents are less affected due to the low energy levels involved in this process (Rastogi and others 2007). The rate of inactivation of pathogens and spoilage microorganisms increases in the temperature range of 45 to 50 °C and therefore, preservation of acid foods ($pH \leq 4.6$) is the most obvious application of HHP (Rastogi and others 2007).

Though HHP processed foods posses superior nutrient and sensory quality compared to thermally-processed foods, some problems such as accelerated enzymatic browning in

vegetables and lipid oxidation in muscle tissues are unavoidable (Cheftel 1995). Also, bacterial spores are highly pressure resistant (upto 1200 MPa), and a combination of moderate heat treatment (or any other pre-treatment such as ultra sound) with pressure treatment could be effective in initiation of spore germination or inhibition of germinated bacterial spores and inactivation of piezo-resistive microorganisms (Knorr 1995; Rastogi and others 2007). This process temperature can go upto 90-121 °C in combination with pressures of 500-800 MPa to inactivate spores forming bacteria such as *Clostridium botulinum* (Rastogi and others 2007). The high cost of capital investment for new equipment limits commercialization of HHP (Mertens and Deplace 1993). The high labor cost and low productivity of the batch process adds to its disadvantages (Moorman 1997). Apart from the high initial cost and engineering limitation of huge pressure vessel, long processing time (30 min to 1 h) is an added concern. The pressure vessels with internal volume of 700 L can be operated only at 500 MPa, which also needs thick walls to accommodate the applied pressure (Mertens and Deplace 1993). Though Japan, Portugal and France have several HHP processed foods available in the market, the commercially available HHP processed food products are limited to guacamole and oysters in USA (Rastogi and others 2007).

HHP denatures proteins, inactivates enzymes, gelatinizes starches, and inactivates microorganisms, while the loss of colors, flavors, aromas, vitamins and other nutrients is minimal (Pothakamury and others 1995). Apart from this, there are no toxic substances or off-flavors produced in foods processed due to HHP application (Rastogi and others 2007). Puppo and others (2005) reported droplet size distribution (surface frequency) of high pressure processed soy protein isolate dispersions of 10 g/l protein at pH 8 (50mM Tris-HCl buffer). With the high pressure treatment of proteins, a shift in droplet populations towards smaller size (<1

μm) were observed for these emulsions. This decrease in particle size was about 25-30% in this treatment. Also, the extent of high pressure treatment was effective in reducing the particle size distribution curve from 10 μm to 3-5 μm for the other treatment emulsion at pH 3 (Glycine-HCl buffer 50mM). Lakshmanan and others (2006) reported that the peptide profiles were not affected though aggregates were observed in the soluble protein fraction in thermal- and pressure-treated samples of soymilk. They also reported increased emulsion stability and decreased hydrophobicity for high pressure processed soymilk. Increase in solubility and surface hydrophobicity of soy protein isolate emulsion at pH 3 was reported by Puppo and others (2004). Further in another study, a better potential of protein adsorption was suggested due to the displayed decrease in bridging flocculation (Puppo and others 2005). This bridging was described as the adsorption of proteins in two different droplets during emulsion formation and resulting reduction in bridging flocculation was due to increased adsorption of proteins in oil-water interface with increased pressure application.

Puppo and others (2005) showed high pressure processing treatment of soy protein isolate, β-7S and A-11S polypeptides displayed increased ability to anchor at the oil-water interface. When proteins gets absorbed at the oil/water interface (soymilk) and help the droplet against creaming, flocculation, and coalescence, the emulsion stability increases (Lakshmanan and others 2006). Dickinson and Pawlowsky (1996) reported that a HHP treatment of globular protein, bovine serum albumin (BSA), in the range of 400-700 MPa, substantially changed the rheological behavior of flocculated emulsions of BSA and the anionic polysaccharide dextran sulfate in ways which were qualitatively different from heat treatment. A study on model oil-in-water emulsions made from sodium caseinate and β-lactoglobulin subjected to HHP of 450 MPa reported by Dumay and others (1996) observed no change in oil droplet size of both emulsions while the

pressure sensitive β-lactoglobulin emulsions exhibited an increase in viscosity. So the changes in viscosity were assumed to have no relation to the changes in oil droplet size but rather to changes in emulsifying protein, and HHP processing may be used to modify functional properties in novel ways. HHP processing (300 MPa) of calcium caseinate suspensions showed colloidal integrity of the calcium caseinate aggregates was disrupted and it resulted in the decrease in turbidity (Lee and others 1996). Increased resistance to HHP-induced changes was exhibited by colloidal systems of lower pH, especially at higher calcium concentrations.

High pressure treatment improves protein gelation properties and has been successfully used to producing foods (Hoover 1993) for guacamole and oyster. The protein denaturation which occurs with high pressure treatment is a result of hydrogen, hydrophobic and ionic bonds breakage rather than covalent bond disruption, which results in off-flavors (Rastogi and others 2007). Pressurization studies (392-980 MPa) conducted on egg white and yolk resulted in firm gels with natural taste and these gels with no loss of vitamins and amino acids were softer, more elastic, and more digestible than heat-treated gels (Hoover 1993). Only structures of large molecules such as proteins, enzymes, polysaccharides, and nucleic acid are altered by high pressure treatment, while the small molecules such as amino acids, vitamins, and flavor compounds remain unaffected. Puppo and others (2004) reported that the high pressure treatment induced physicochemical changes of soybean proteins at pH 8 with increase in surface hydrophobicity, a partial unfolding of 7S and 11S fractions, and an aggregation of protein (with 11S fraction). The physicochemical changes in soymilk due to pressurization and theory of protein aggregation were reinstated by Puppo and others (2005). They could explain that the combined effect of increase in surface hydrophobicity, partial denaturation, and more disordered structure were giving a better opportunity for adherence at the oil-water interface. This gave a

clearer picture to the size reduction and the increase in the percentage of adsorbed proteins as pressure treatment levels increased. The emulsifying activity index of 7S and 11S fractions of soybean proteins at pH 7.5 was increased with high pressure treatment of 200-600 MPa (Molina and others 2001).

2.2 High pressure homogenization (HPH)

Conventional homogenization, developed by Auguste Gaulin in 1899 (French patent number 295,596), has been extensively adopted in food applications, especially in dairy industry and other areas of dealing with oil and water emulsions (Peck 2004; Zamora and others 2007). Classical homogenization used <100 MPa for standard industrial processes by forcing fluid foods through narrow gap of 100-300 μm in the homogenizer valve, with an upstream pressure of about 20-60 MPa (Kessler 1981), to stabilize emulsions against creaming and coalescence and obtain products with appropriate rheological properties. Walstra (1983) explained that under turbulent conditions that occur during homogenization, more dense materials are displaced by convection movements and then the adsorption of aggregates are predominated.

Growing interest is seen in dairy industry for non-thermal process which combines efficient microorganism reduction with a maximum retention of the physicochemical properties of the product (Lopez-Pedemonte and others 2006). Ultrahigh pressure homogenization (UHPH, also known as high pressure homogenization (HPH) or dynaimic high pressure) uses pressures above 200 MPa. UHPH machines (Stansted Fluid Power Ltd., Essex, UK) were developed with some technological modifications in the conventional homogenizer such as incorporating high pressure pumps and intensifiers (driven by hydraulic pump), specifically designed high pressure valves made of ceramic material (ceramic seats and needles) to withstand the high pressures upto 350

MPa, modified geometry compared with classical homogenizing valves (APV-Gualin homogenizers) to generate difference in the fluid flow directions, and narrower gaps between the valve and the seats (Thiebaud and others 2003; Floury and others 2004b). A second pneumatic valve after the first valve supports a pressure upto 50 MPa. The first stage valve is refrigerated by constant water circulation of water at ambient temperature in an external jacket built around it; this is to avoid the loss of homogenization performance due to temperature increase and rapid expansions or contractions of the first stage valve (Lopez-Pedemonte and others 2006). The residence time of the fluids in the high pressure section is in the order of seconds in most cases (Thiebaud and others 2003).

The fluid is forced through a small gap and then it is exposed to ultra rapid pressure depression without temperature change which helps in nucleation and growth of gas filled bubbles (or cavities) within the body of the liquid (Guerzoni and others 1999). The collapse of such cavities could transmit several localized forces to surfaces or particles, including the microbial cell. The fluid velocity very rapidly increases (even above sonic velocity) when depressurization occurs, which generate an elongational flow, very intense velocity gradients, and probably stretching effects. Floury and others (2004b) calculated a maximum fluid velocity of 200 m/s (assuming that the total pressure drop was split equally between the fluid kinetic energy and frictional forces), which was in agreement with the Poiseulle's equation, for a valve gap of ~2 μm in a Stansted nm-GEN 7575 single stage homogenizer operating at 340 MPa. With the same type of homogenizer, the temperature of liquid passing through the high pressure valve increased in proportion to the pressure applied by ~15 °C per 100 MPa (Thiebaud and others 2003).

Numerical simulations of pressure field at the gap outlet predicted quite large areas of low pressure, turbulence, cavitation, and fluid re-circulation (Floury and others 2004a). Turbulence is said to be the predominant mechanism in the high pressure homogenizer (Walstra 1983) even though laminar shear and cavitation may also play an important role. Turbulence leads to the break up of the dispersed phase into small droplets. Coalescence occurs when the relative motion between the drops results in their collision. The shelf life, as well as the texture of the emulsion, greatly depends on the drop size distribution, which can be adjusted by controlling the rate of drop breakage and coalescence during emulsion formation. According to Walstra (1975), mean drop size was found to vary as $D_{(4,3)} \propto P_h^{-0.6}$ (where $D_{(4,3)}$ is the particle size diameter and P_h is the homogenizing pressure). This result was in agreement with drop breakage in a locally isotropic turbulent flow field (Floury and others 2000). The average fat drop size was smaller at higher homogenizing pressures (from 7 to 40 MPa). Even though the relation $D_{(4,3)} \propto P_h^{-0.6}$ was obtained with low dispersed phase fractions, the value of the exponent decreased at higher emulsion fat contents, leading to larger drop sizes, possibly due to the opposing effect of drop coalescence (Mohan and Narsimhan 1997). Bacterial inactivation in high pressure homogenization processes is achieved by pressure, exposure to hydrodynamic cavitations, impingement against static surfaces, high turbulence and fluid shear, and temperature rise which occurs due to heat dissipation of kinetic energy in the high pressure valve (Lopez-Pedemonte and others 2006). The residence time of the food at this high temperature can be reduced to less than 1 s if the heat exchanger is placed immediately after the valve (Thiebaud and others 2003).

In the homogenizer, severe processing conditions such as high pressure, shear stress and temperature can lead to a deterioration of proteins stabilizing properties (Floury and others 2000). However, while it is already established that protein structure is susceptible to

modification by high-pressure processing, it is not known to what extent the new technology, such as dynamic high-pressure homogenization, allows systematic modification of the rheology and texture of protein-stabilized emulsions. Applications of ultrahigh pressure homogenization (pressures more than 200 MPa) are mainly found in pharmaceutical and biotechnology sectors (Floury and others 2000). Other applications include formulation of fine food emulsions, disruption of dense cell microbial cultures and subsequent recovery of intracellular metabolites, inactivation of bacteriophages, and modification of functional properties of hydrocolloids (Floury and others 2002a; Floury and others 2002b; Moroni and others 2002; Thiebaud and others 2003). The increase of the pressure level in homogenizers permits to reduce the mean droplet size of the emulsions produced and thereby improve the shelf life of the products by reducing creaming rate, deflocculating of clusters of primary fat globules, and dispersing agglomerates uniformly (Floury and others 2000). The energy threshold necessary to break these clusters apart may not be reached by lower pressure homogenization. High-pressure homogenization is also expected to increase the surface activity of the emulsifying molecules; it may improve the efficiency of the product (coating ability or penetration action for example). Roesch and Corredig (2003) reported that after heating oil-in-water emulsions containing commercial fraction of soy protein concentrate and soybean oil at 82 °C for 2 min before homogenization at 80 MPa displayed a gel-like behavior, stability to creaming and no separation during quiescent storage for 20 days at 4 ºC. Roesch and Corredig (2003) reported increasing viscosity with increase in soy protein concentrate (SPC) in 10% and 6% oil-in-water emulsions at 0 and 10 days of storage. The emulsions exhibited shear-thinning behavior and hysteresis and the author attributed the shear-thinning behavior to the presence of aggregated protein-protein droplets due to phase separations at high concentrations of SPC.

Zamora and others (2007) reported that the main peaks of ultra-high pressure homogenized milks were between 0.1 and 0.3 μm as against the 3.8 μm for the raw milk. This size reduction was increased with increase in pressure applied from 100 to 300 MPa, but at 330 MPa an increase in size [$D_{(v,0.9)}$, $D_{(4,3)}$] was observed. Above a critical pressure application (~200 MPa), the increased susceptibility of fat globule coalescence was observed in previous studies (Floury and others 2000; 2004b). The possible reasons behind this increase in average size of particles and widened particle size distribution of the second stage ultra-high pressure homogenized milk compared with single stage were attributed to the higher heterogeneity occurred due to partial agglomeration of very small, insufficiently coated globules that collide within the second valve (Zamora and others 2007).

Zamora and others (2007) reported that small fat globules (< 0.5 μm), as revealed by nile red fluorescence in confocal micrographs of homogenized- milk, were entrapped in (or had become a part of) the proteinaceous network and a fewer larger globules (~1.5 to 2 μm) were retained in the serum. The smaller fat globules disrupted the continuity of strong protein network thus obstructing the availability of casein to the network as they were tied to the newly formed fat globules. The small fat globules also acted as weak centers in the network, and thereby a decrease in gel strength, thick and lumpy strands, and a concomitant coarser texture was observed. These weaker gels were formed more for the homogenized, homogenized-pasteurized milks. Single stage ultra-high pressure homogenization above 200 MPa resulted in the smallest particle size and narrowest distribution in that study. In this treatment, fat globules did not disrupt the proteinaceous structure; rather they acted as casein micelles in the structure and strengthened the rennet gel firmness. The study also revealed lower levels of nile red and FITC fluorescence when the pressure treatment was increased to 300 MPa. This was explained as due

to more than 50% of their particles in the micrograph were beyond the resolution threshold (0.23 μm/pixel). In the same study, at 330 MPa, the broader size distribution of 2-stage ultra-high pressure homogenized milk was observed and this phenomenon was also revealed in confocal micrographs. The reason for the increase was attributed to the aggregation of well-defined small fat globules within dense proteinaceous structures.

Roesch and Corredig (2003) attributed the presence of large particles in the image taken with integrated light scattering to partly due to large soy fiber structures. From microscopic observations, those fiber particles had size between 50 and 100 μm and this size range did not seem to be affected by either heat treatment or by high-pressure homogenization. The microstructural observations of the different components of commercial soy protein concentrate (SPC) in oil-in-water emulsions helped them to understand that the viscoelastic properties of the emulsions were a result of the segregation of oil droplets in protein network due to the incompatibility between the protein and the oil phase.

2.3 Continuous flow high pressure throttling (CFHPT)

As it is a relatively new technology, the review or research data are very limited for the continuous flow high pressure throttling (CFHPT) process. High pressure throttling was developed as a means of continuous microbial inactivation of fluid foods at The University of Georgia, Athens (Moorman 1997). Throttling valve (or micrometering valve) is a fine restriction such as a partially closed valve or porous plug to control the flow of the fluid through a micro-metering valve. CFHPT uses high pressure (up to 310 MPa) to pressurize liquid foods and to continuously throttle the foods from that high pressure to atmospheric pressure for the purpose of inactivating microbes and modifying proteins which should change the rheological properties of

the food (Moorman 1997). Thus, the name continuous flow high pressure throttling (CFHPT) aptly suits the process. Moorman (1997) reported considerable reduction in the targeted maximum pressure achieved (varies from 310 to 365 MPa for a target pressure of 310 MPa) between the strokes of the intensifier pistons even though the micrometering valve was set at a fixed position. This phenomenon was described as due to the alternate accumulation and dispersion of the fluid material at the constriction which alters the constriction opening and thereby changes the registered pressure. This was because the target pressure was achieved by pressure strokes of intensifiers and by the opening and closing of the micrometering valve. As the fluid material exits the throttling valve, temperature rise occurs and the product is then very quickly cooled to prevent over processing of the product which lead to a reduction in overall quality (nutrition and sensory).

The temperature rise occurred after throttling can be explained by the first law of thermodynamics (Amornsin 1999). Pressure difference between the inlet (higher pressure) and outlet (lower pressure) of the throttling valve represents the energy conserved in the form of heat energy at the exit of the throttling valve which is visible from the temperature rise of the product. This change in potential energy due to high pressure to heat energy due to reduction in pressure across the throttling valve can be given by the equations given below (Toledo 2007; Amornsin 1999):

$$q = C_p (T_{out} - T_{in}) = \frac{(P_{in} - P_{out})}{\rho} \quad \text{.....(1)}$$

$$(T_{out} - T_{in}) = \frac{(P_{in} - P_{out})}{\rho C_p} \quad \text{.....(2)}$$

$$T_{out} = T_{in} + \frac{(P_{in} - P_{out})}{\rho C_p} \quad \text{.....(3)}$$

where q = energy per unit mass (J/kg), C_p = specific heat of the fluid at constant pressure (J/kg.°C), T_{out} = temperature of the fluid at the inlet to the throttling valve (°C), T_{in} = temperature of the fluid at the inlet of the throttling valve (°C), P_{in} = Pressure of the liquid at the inlet of throttling valve (Pa), P_{out} = Pressure of the liquid at the outlet of throttling valve (Pa), assumed as atmospheric pressure, ρ = density of the fluid (kg/m^3). The initial temperature or the temperature to which the fluid is heated in the tubular heat exchanger (T_{in}) affects the exit temperature and thereby the temperature rises in all of the equations given above. If flow rate is constant at the exit for product collection, the temperature variation due to volumetric flow rate difference is avoided. However, the heat loss due to convection (to the surroundings) and conduction (through the connecting pipe to cooling heat exchanger) further reduces the measured temperature (experimental temperature) at the exit of the holding tube (after throttling valve) from the theoretical results (Amornsin 1999).

Amornsin (1999) studied the relation between pressure oscillation amplitude in the pressure gauge of a Microfluidizer Processor (model M-140K, Microfluidics International Corporation, Newton, Mass.) intensifier (which works similar to CFHPT with a throttling valve attachment) and the product flow rate. Reducing the intensifier hydraulic fluid flow pressure slowed down the rate of forward movement of the intensifier piston and thereby reduced the rate of delivery of the pressurized product and dampens the oscillation amplitude of pressure fluctuation as seen in the pressure gauge. To achieve the target pressure, a combination of intensifier pressure and throttling valve adjustment was used.

Pressurization was done upto 310 MPa using a pressure intensifier and conveyed through stainless steel tubing to throttling valve where it was throttled to atmospheric pressure. Even when the throttle valve was in completely closed position, the flow still occurred (Amornsin

1999). According to manufacturer's data for water, the targeted pressure (310 MPa) was attained by decreasing the flow rate by adjusting the throttling valve which alters the orifice diameter from a fully-opened position of 1.5748 mm to a nearly-closed position of 0.1556 mm. The maximum shear rate (-dV/dr) calculated (using equation 5) (Toledo 2007) with a mean fluid velocity (V_{av}) in the orifice of 284 m/s (using equation 4) (Toledo 2007), with nearly closed diameter (R/2) for throttling valve, and fluid flow rate (q) of 5.4×10^{-6} m^3/s was 14.6×10^{-6} s^{-1}.

$$V_{av} = \frac{q}{\prod R^2} \quad \text{.....(4)}$$

$$-\frac{dV}{dr} = \frac{4V_{av}}{R} \quad \text{.....(5)}$$

Advantages of CFHPT processing include simultaneous homogenization and sterilization which can benefit physical properties, texture, and stability of the sterilized product during storage. CFHPT treatment has a wide range of applications in the manufacture of functional ingredients (Moorman 1997). An exceptional result on particle size reduction and narrowing the distribution has been reported by Peck (2004) where the CFHPT processed blueberry-whey beverage showed no aggregation of particles during storage in addition to the significantly smaller size offered by the treatment compared with thermally processed beverage. Effects of various holding times during pressurization (310 MPa) and after depressurization (at elevated temperature) were studied by Moorman (1997) on ultra filtered skim milk and permeate. At the high pressure applied, the milk was held for 0.3 s and 1 s using the tube of length 7.6 cm or 176.5 cm. Two more treatment combinations were studied by changing the holding time (using different holding tubes) after the micrometering valve at the elevated temperature (80 °C) for 0 s and 10 s. Moorman (1997) reported increase in viscosities of CFHPT treated milk concentrations and increase in apparent viscosities and water holding capacity of yogurt made from CFHPT

treated milk. Apparently the combination treatment of lower pressurization dwell (0.3 s) and lower depressurization holding time (0 s) at elevated temperature, with milk concentrations of 0.19 and 0.24 kg s.s/L, produced even greater viscosity. These changes in the properties of milks and yogurt were due to the modification of dairy proteins by the CFHPT process. Therefore, the author suggested that CFHPT treatment of skim milk could be utilized for improving the "mouth feel" of skim milk without added ingredients such as polysaccharides.

The mechanism underlying the rheological changes of the CFHPT treated milk products were proposed by Moorman (1997) as the disruption of the casein micelle and protein denaturation due to shear in the constriction of the micrometering valve followed by protein stretching, configuration, and re-aggregation, which resulted in effectively bigger protein macromolecules (due to increased level of protein-protein interactions) and subsequently, a stronger matrix which bound more water and augmented the viscosity of the product. This proposal of mechanism for rheological changes occurred was according to the hypothesis for the texture alterations in extruded vegetable protein given by Shen and Mor (1979) which states "high shear first denatures and then stretches and aligns proteins, which results in increased protein-protein interactions". High shear rates can increase the surface area to volume ratio, which can increase viscosity of certain fluid materials leading to improved texture, taste, and flavor characteristics (Areekul 2003).

Earlier studies show that CFHPT treatment of milk gave darker color (lower L* value) to milk (Adapa and others 1997). The concentrated (by ultrafiltration) milks (18.00% total solids, 8.20-15.42% protein) were darker than unconcentrated milks (9.00% total solids, 3.28-3.69% protein). The decreased L* value in pressurized milks were attributed to the disruption of noncovalent bonds (hydrogen bonds, ionic interactions, and hydrophobic forces) which caused

separation of casein micelle fragments, individual caseins, and calcium phosphates thus resulted in reduced light scattering ability and the observed darker color. The increased protein concentration above a particular level decreased the foaming capacity in pressurized-concentrated milks due to the decrease in protein solubility. But the foaming stability was increased with increased protein content and thus increased viscosity and slow serum drainage. Areekul (2003) reported slight increase in total solids content (but not significant) on CFHPT treated honeys over the conventionally pasteurized (CP) honey due to moisture evaporation at the exit after throttling at a temperature of 125.2 °C. Other increases exhibited by CFHPT processed honey were HMF content and consistency index. It was further stated that the physicochemical properties of both CFHPT and CP honeys were dependent on time and temperature of storage. Consistency indices of CFHPT processed honeys (20.5 and 33.8 $Pa.s^n$ for buckwheat and clover honeys) were higher than that of CP honeys due to evaporation at the exit of throttling valve at temperature of 125.2 °C (Areekul 2003).

CFHPT treatment increased the viscosity of pressure-concentrated (concentrated with ultrafiltration) and pressure-unconcentrated milks (Adapa and others 1997). This increase was attributed to the formation of protein-protein linkage during CFHPT treatment. They explained further that due to increase in the effective volume due to the CFHPT treatment, protein aggregates became bigger, occupied more space and provided increased viscosity to the fluid system while the milk behaved like a Newtonian fluid. Newtonian behavior was shown by CFHPT processed buckwheat and clover honeys while a higher initial moisture content of buckwheat honey gave lower viscosity than the clover honey (Areekul 2003). Newtonian behavior was exhibited by CFHPT processed blueberry-whey beverage (after storage too) when power law model was used to fit the data (Peck 2004). No visible or significant change in

rheological properties in that fluid was considered due to the low concentrations of proteins, pectins, and soluble solids. The pressurized-unconcentrated milk showed enhanced emulsion stability with pressure treatment which indicated that an optimum amount of protein aggregation promoted emulsion stability. The increased surface area of new fat globules formed were not adsorbed by emulsifying material (protein) and showed decreased emulsifying property in CFHPT treated and ultrafiltration-concentrated milk. This lower emulsion stability of pressure-concentrated milk was attributed to the formation of protein-protein interactions and formation of large protein aggregates. But this protein-protein interaction at higher pressures was shown by whey proteins. Because of the globular aggregated structure of soy proteins, they do not unfold and adsorb at the interface, but rather form a thick interfacial layer, which acts as a physical barrier to coalescence (Molina and others 2001). Adapa and others (1997) describes the formation of large protein aggregates during CFHPT treatment of milk. This protein-protein interaction reduced the emulsifying property of the milk due to the inability of protein to bind as a thin layer around the single fat globule and prevent fat coalescence and form stable emulsion. Also they further stated that pressurization did not affect the interfacial tension and surface tension results of milk as expected due to increased protein-protein linkage rather than unfolding of protein at the interface. Due to the increased size of protein aggregates as a result of CFHPT treatment, they did not diffuse to the surface and reduced the surface tension. In spite of this result, the CFHPT treated milks gave no feathering (feathering is a phenomenon by which milk proteins coagulate when added to hot coffee), and resulted +5 on the scoring scale.

Areekul (2003) conducted research on buckwheat and clover honey processing in CFHPT system at 275.8 MPa, exit temperature of 125.3 °C and 15 s hold before exiting to a cooler resulted in zero microbial counts (at both day 1 and 6 months of storage) and slightly elevated

hydroxymethylfurfural (HMF) content, but HMF was at an acceptable level after storage at 6 months at 4 °C. Also, significant alteration was not detected in physicochemical properties during storage of CFHPT and conventionally pasteurized (CP) honey. Processing of milk was done using CFHPT by Adapa and others (Adapa and others 1997) and milk produced good foams with high stability at 310 MPa. Milk concentrates produced by CFHPT treatment resulted in increased surface tension and viscosity but decreased emulsion stability was thought to be due to increased protein-protein interactions, which result in larger protein aggregates and less protein migrating to the liquid-gas interface or adsorbing to the surface of fat globules.

Skim milk concentrate with 0.24 kg s.s/L treated with CFHPT (0.3 s pressurization dwell and 0 s depressurization holding time at elevated temperature) formed gels when stored at 4 °C for 24 h (Moorman 1997). The gels liquefied when warmed to 8 °C and were reversible for 7 days. An exponential model (equation 6) was fitted to the apparent viscosities (μ_a) versus temperature (T) data same treatment milk (R^2 = 0.87) and untreated control milk (R^2 = 0.99) with same solids content:

$$\mu_a = ae^{bT} \quad \text{.....(6)}$$

where a = 5.506, b = -0.0435 for untreated samples, and a = 15.999, b = -0.0674 for the CFHPT treated sample. Yogurt made from the same treatment milk exhibited higher mean apparent viscosity than control. Moorman (1997) proposed that shear is the main factor which contributes to the significant changes in secondary and tertiary protein structure. This conclusion was based on the comparison between the times required for the pressure treatment: HHP (30 min to 1 h) or CFHPT (0.3 to 1 s), so that gel formation occurred in the product. The time, 0.3 to 1 s, the product was exposed to high pressure in CFHPT was very less to bring about any significant alterations in secondary and tertiary protein structure.

Study on ascorbic acid, pectin esterase activity, and limonin content in CFHPT processed (0, 137.9, 206.8, and 275.8 MPa pressures) citrus juice (initial temperature of 20 °C) was reported by Amornsin (1999) and results showed no evidence in ascorbic acid destruction even with the highest pressure treatment, retain of cloud stability for at least 21 days with highest pressure treatment and also at a lower pressure of 206.8 MPa when adiabatic heating was utilized with 38.4 s holding time before cooling, and no increase in the bittering compounds represented by limonin content even at highest pressure level treatment with 118 s holding time before cooling as compared to the level present in the fresh grapefruit juice.

Areekul (2003) reported that CFHPT sterilization of honey (suitable for use in pharmaceutical and infant foods) and found that a combination of 255 MPa pressure and heating the pressurized fluid to 80 ºC followed by depressurization to 300 kPa and cooling was found to kill 7 log of inoculated *Bacillus megaterium* spores while producing commercially sterile honey having indistinguishable properties from conventionally heat pasteurized (60 ºC, 30 min., non-sterile) honey. The viability of native microflora in the milk was reduced by 2.5 to 4 log cycles when it was treated with CFHPT at 310 MPa, and neither the increase in hold time at the elevated temperature (>80 °C) nor the longer holding at the pressurization before throttling was effective in reducing the microbial count to a smaller number (Moorman 1997). Though the CFHPT processing reduced the number of viable *Pseudomonas putida* cells by 7 log cycles and the distinct spheroid bulges on cell surfaces and the amount of debris in the SEM images of the CFHPT treated samples suggested that the process was very disruptive to cell membranes, the author suggested more research on CFHPT treatment of thermally resistant organisms to demonstrate that whether the mechanical effect (by shear and explosive decompression) of

CFHPT helped in microbial inactivation above and beyond the heating effect (by enzyme denaturation) alone, or a combination of both.

2.4 Microfluidization

Microfluidization is a homogenization technique developed by Cook and Lagacé (1985) in which the liquid food in a high pressure chamber is split into two streams in the interaction chamber and collides with each other at high speed at a 180 ° angle (Lemay and others 1994; Tunick and others 2002). Smaller particle size and narrower particle size distribution was exhibited by the foods processed by this process. Paquin (1999) explained that the size reduction observed was due to the cavitation which resulted from collisions of the fat globules while the two streams were united. Microfluidizers are produced by Microfluidics, Inc., Newton, MA. Moorman (Moorman 1997) studied the microbicidal effect of CFHPT treatment, microfluidizer treatment, and microfluidizer/throttling treatment. The microfluidizer used a double–acting electro-hydraulic pressure intensifier to force the process fluid through the constriction which is in the interaction chamber with downstream back pressure module. This elevated the temperature of process fluids from an entrance temperature of 4 °C to ~85 °C, and an in-line cooling coil with built-in water cooling coils helped to lower the temperature to 40 °C within 3.4 s. The hybrid process of CFHPT and microfluidization introduced a micrometering valve (which was used in CFHPT process) in place of interaction chamber in the microfluidizer. However, the other parameters used such as flow rate (9.2 L/s), operating pressure 276 MPa maximum), cooling coil (12 °C water jacket), and cooling time (3.4 s) are the same as that of microfluidizer with interaction chamber. Minimum pressure between the strokes of the dual-acting intensifiers while it changed it directions was compared among the processes and it was registered as 151 MPa in

CFHPT (for a desired pressure of 310 MPa) and 220 MPa in both the microfluidizing treatments (for a desired pressure of 276 MPa). This difference was due to the difference in pressure intensifiers action: microfluidizer used Microfluidics pressure intensifiers in which pressure was controlled by piston stroke and cycle time to achieve the desired pressure whereas the CFHPT treatment used Hydropac pressure intensifiers where a constant stroke and cycle time was maintained and the desired pressure was achieved by varying the opening of throttling valve. The results showed that microfluidizer and microfluidizer/throttling treatments were more effective than the CFHPT process and the lower microbicidal effect of CFHPT process was attributed to the variation in the pressure attained between the intensifiers and the throttling valve. However, the author suggested improving the process by lowering this pressure variation, so that low pressure fluid flow through the throttling valve could be minimized.

Amornsin (1999) also studied the relation between pressure oscillation amplitude in the pressure gauge of a Microfluidizer Processor (model M-140K, Microfluidics International Corporation, Newton, Mass.) intensifier (which works similar to CFHPT with a throttling valve (model 60 VRMM Autoclave Engineer, Erie, PA 16512) attachment) and the product flow rate. One stroke of an intensifier delivered 12.1488 cm^3 (Amornsin 1999). If P_d is the lowest pressure in the cycle and P_t is the target pressure, the amplitude of pressure oscillation is given by $P_t - P_d$. Reducing the intensifier hydraulic fluid flow pressure slows down the rate of forward movement of the intensifier piston and thereby reduces the rate of delivery of the pressurized product and dampens the oscillation amplitude of pressure fluctuation as seen in the pressure gauge. To achieve the target pressure, a combination of intensifier pressure and throttling valve adjustment was used. A linear change was assumed to be involved in changing the pressure from P

(pressure, psi, at a specific time (t) in the pressure cycle) to P_t with time, t (s) and the equation (7) formulated by Amornsin (1999) is given below:

$$P = P_d + at \ldots..(7)$$

where a is the slope (psi/s) of the pressure increase with time. The coefficient of flow (C_v) for the valve is the volume of water (US gallons per minute) at room temperature which will flow through the valve. The values of C_v ranges from 0.0002 (closed position) to 0.004 (fully open position) USgal/min (from Autoclave Engineers Catalog 1500-2) and the flow of fluid of liquid across the valve is given by the equation 8:

$$Q = C_v \frac{\sqrt{P_{in} - P_{out}}}{\sqrt{S_g}} \ldots..(8)$$

where, Q = flow rate (USgal/min), C_v = Valve coefficient of flow when fully open (USgal/min), P_{in} = Inlet pressure (psia), P_{out} = Outlet pressure (psia), S_g = Liquid specific gravity (water = 1). Equations 7 and 8 were combined (equation 9) (Amornsin 1999) and integrated from time 1 (t_1) to 2 (t_2) and considering P_{in} as 0 due to its negligible value (atmospheric pressure) compared to P_{out}.

$$Q = \frac{2C_v}{3a\sqrt{S_g}} \left[(at_2 + P_d)^{3/2} - (at_1 + P_d)^{3/2} \right] \ldots..(9)$$

where Q = amount of fluid passing through throttling valve during t_1 to t_2 (mL), C_v = The experimental valve coefficient of flow (mL/min), and S_g = liquid specific gravity. Equation 9 showed that both the pressures P_d and P_t are equally important processing parameters in the system and this is to be considered while studying the effects of pressure on a liquid processed through the system. The results of the study showed that for deionized water at the flow rate of 350-360 mL/min, the measured temperature was lower than the calculated temperature for the

targeted pressure (at all pressures). However, this elevated temperature was higher than the temperature rise calculated for the mean of the target and minimum pressure. The results were attributed to the exponential rise of pressure in a cycle i.e., more liquid leaves the throttling valve at higher pressures in a cycle than that at lower cycles. Moreover, the inlet temperature of the liquid influences the temperature rise of the liquid as per the equation 3. At a flow rate of 300 mL/min of water, the dropping pressure was 103.4 MPa (15,000 psi) for a target pressure of 275.8 MPa (40,000 psi). This confirmed that the targeted pressure was not achieved during each stroke of intensifier piston. When flow rate and target pressure was increased, the pressure drop also increased. So, the amplitude of pressure oscillation and flow rates are to be carefully monitored so that the system will stay close to the target pressure and the processing of fluids could be done at the same target pressure.

Microstructure of Mozzarella cheeses made form milk microfluidized at various temperatures and pressures were studied by (Tunick and others 2000) using scanning electron microscope. Images showed that the temperature (10 °C, compared to control) did not liquefy the fat to an extent necessary for its complete microfluidization. However, at 54 °C processing temperature of milk, the fat droplets in cheese were more discrete and smaller, giving the casein matrix a spongy appearance. When the microfluidization pressure was increased from 34 MPa (the maximum used for dairy homogenization) to 172 MPa, fat droplet size was reduced in scanning electron micrograph showing an emulsion of fat and protein (Tunick and others 2000). The emulsification process allows small particle size distributions of 0.1-0.3 μm. Microfluidization was suggested as an alternative method for the production of milk fat microcapsules (Vuillemard 1991), alcoholized cream, and for milk homogenization.

Cobos and others (1995) investigated the rheological properties of acid milk gels made from recombined milks subjected to microfluidization and found that gelation time was reduced by low levels of solids, low level of fat, high heat treatment, high incubation temperature, and high concentration of acidulant, while the gelation pH was increased by heating the milk after homogenization, high heat treatment and high incubation temperature. The elastic modulus and viscous modulus of gels were increased by high level of solids and fat, high heat treatment and low incubation temperature. The rheological properties of acid gels made from milks subjected to microfluidization were very similar to those of acid gels made from milks subjected to HPH in a valve homogenizer, though microfluidizer produced smaller, less variable particles.

Microfllluidization of pasteurized whole milk and recombined milk was done by McCrae (1994) and observed that fat surfaces were covered with high amounts of casein but only minute amounts of serum protein. Also, the total amount of protein which covered the fat surfaces was higher than that predicted on basis of decreased globule size, and this was thought to account for the inhibition of fat cluster formation observed in the microfluidized milks. Mozzarella cheeses made with the control full fat milks (no microfluidizing) and milks microfluidized at 10 °C/34 MPa or 10 °C/103 MPa were softer and less rigid, and had the lowest visco-elastic properties and the highest meltabilities of all the cheeses (Van Hekken and others 2007). Microfluidization of the cheese milk did not improve the melt or rheology of low fat milk cheeses. Microfluidization of milk with fat in the liquid state at higher pressures resulted in smaller lipid droplets that altered the component interactions during the formation of the cheese matrix and resulted in low fat and full fat Mozzarella cheeses with poor melt and altered rheology.

2.5 Soymilk processing

Soymilk is one of the traditional nutritious beverages used for thousands of years in oriental countries. Dry and mature soybeans are used for producing soymilk. Increased growth rates of 31% in soy-based drinks during 2003-2004 shows its increasing consumer acceptability in Western countries also (Sloan 2005). Soy isoflavones have gained attention in the past decade due to their potential protective or preventive activity against a number of diseases such as cardiovascular diseases, cancers, and osteoporosis. The absence of lactose, in addition to its natural health benefits, can be counted as one of the major factors involved in increasing its popularity among the lactose-intolerant group. The traditional Chinese method utilizes bean soaking in cold water for hydration, fine grinding of the mixture of water and hydrated beans in stonemill, insoluble solids removal by slurry filtration, and finally boiling the mixture to get better soymilk flavor. Lo and others (1968) reports that this method yields only 65% of soybean solids. Huge amounts of spent residue (okara – having 27% protein, dry basis) as waste by-product is formed through soymilk and tofu manufacture and the clearance of the same creates severe environmental problems (Chan and Ma 1999). Enhanced soybean solids utilization in soymilk processing was reported by Hand and others (1964) in which dehulled bean was steam-dried, finely ground to make powder, and soymilk was produced by adding water. Another process with further improvement in full utilization of soy solids without soak water losses of soy soluble solids was reported which includes moisture-conditioning of dehulled soybean flakes, and high temperature, high pressure, and short time bean mixture extrusion (Mustakas and Mayberry 1964). Soymilk was produced by adding water in the puffed, cooked, dried, and finely ground material. One of the milestones in the soymilk processing with effective utilization of cotyledon solids is the Illinois process of producing soymilk which removed only 10-12% of

water-soluble bean solids in the final product which also avoided residue formation (Rosenthal and others 2003). But the main quality factors counteracted against its wide acceptance in the industry was the bean milk's chalkiness and low physical stability (Rosenthal and others 2003).

While considering commercialization of pressurized soymilk, viscosity is of utmost importance (Lakshmanan and others 2006; Cheng and others 2005). The viscosity of soymilk is affected by its solid content which in turn is influenced by processing method and variety of the bean used in processing (Nelson and others 1976; Kuntz and others 1978; Kwok and Niranjan 1995; Iwuoha and Umunnakwe 1997; Mullin and others 2001; Cheng and others 2005). Increased soymilk viscosity was observed with hull addition which decreased the flow behavior index and thereby deviation of soymilk from Newtonian behavior (Forster and Ferrier 1979). Information about variation in viscosity of a product with temperature and shear rates is imperative due to its exposure to diverse shear rates during industrial applications which will also facilitate in the design of equipments for its processing (Ditchfield and others 2004). An earlier study (Son and Singh 1998) on soymilk with different solids content showed the best fit with power law model was fitting best and the effect of measurement temperature on apparent viscosity could be expressed using Arrhenius relationship.

Prevention of settling in soymilk plays a very decisive role in improving the soymilk physical stability. This can be improved by the formation of phospholipids-protein complex which suspends the solids with reduction in density between the particle and the serum (Priepke and others 1980). Increasing the mutual attraction of hydrophilic phosphate groups and water is aided by this coating of the particle. CFHPT processing of blueberry-whey beverage had only soft feathery sediment (after 36 days of storage) which was dispersed during slight disturbance in contrast to the thickly packed chalk-like sediment which was difficult to agitate even after

vigorous agitation in the heat treated blueberry-whey beverage shows the ability of CFHPT treatment on stability of a product (Peck 2004). A comprehensive study on the protein-lipid complex using specific staining techniques for fat and protein in soymilk samples and its observation through confocal laser scanning microscope will spread more light on this topic. Effect of peptide profiles in thermal- and pressure-treated samples of soymilk were studied by Lakshmanan and others (2006) and results showed that the peptide profiles were not affected though aggregates were observed in the soluble protein fraction. Another major impact of high pressure processing of soymilk on the same study was increased emulsion stability and decreased hydrophobicity.

The beany flavor of soy in food products are still a constraint for its complete acceptance among the American consumer (Torres-Penaranda and others 1998; Wilson 1996). There were attempts to nullify this effect of lipoxygenase-produced off-flavor in soy food products by producing lipoxygenase-null soybeans and the results showed that cultural differences had effect in differences in perception of sensory attributes for the consumption of the soy food products in the sensory studies reported earlier (Wilson 1996; Torres-Penaranda and others 1998; Kobayashi and others 1995). Also, the need of using consumer from the targeted market is essential for the success of product development (Hollingsworth 1998).

Still, the standard soymilk process utilizes filters or centrifuges to remove the large-sized solids in the comminuted soy and these solids are discarded. Thus all the essential solids of the whole bean are not transferred into the final product. Commercially, soymilk is produced by thermal treatment to assure safety and extended shelf-life, and to inactivate unwanted biologically active compounds such as trypsin inhibitors and lipoxygenase (Kumar and others 2003; Liener 1981).

The utilization of soybean solids in the processing of soymilk and the soymilk properties can be improved by application of high pressure treatment. However, extensive research is required in this area to understand the properties of high pressure treated soymilk.

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CHAPTER 3

RHEOLOGICAL AND ULTRASTRUCTURAL PROPERTIES AND PARTICLE SIZE DISTRIBUTION OF SOYMILK AS AFFECTED BY PROCESSING METHODS

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Abstract

Soymilk was prepared from boiled and unboiled comminuted suspension of whole dehulled soybean and deionized water using homogenization at 96.53 MPa (one-pass and two-pass). Particle size distribution showed significant difference between soymilks homogenized by one-pass and two-pass. Apparent and total solid content showed significant difference between heated and unheated samples. Soymilks exhibited pseudoplastic and thixotropic behavior. Arrhenius model was fitted to express temperature dependence of apparent viscosity. Micrographs showed hydrated, separated, uniformly distributed, and homogeneous particles in boiled two-pass soymilk as they were disrupted easily and it was selected as the best treatment for processing soymilk containing all of the soybean solids.

Keywords: homogenization, soymilk, viscosity, particle size distribution, ultrastructure.

Introduction

Traditionally, oriental countries used soymilk as a nutritious beverage in their regular diet but now its consumption and popularity is increasing in Western countries as well among the lactose-intolerant group, vegetarian consumers and healthy diet lovers because the low-fat content soy-based foods are also an economical source of high quality protein (Kuntz and others 1978; Lakshmanan and others 2006). Soy-based drinks registered a whooping growth of 31% in the year 2003-2004 and this increased consumption can be attributed to its availability, health information regarding soy foods, and the approval of health claim by U.S. Food and Drug Administration in October 26, 1999 for soy protein (Min and others 2005; Lakshmanan and others 2006). Soy beverages are similar to cow's milk in their profile of essential amino acids, except for methionine, and usually contain from 1.5-3.0% protein (Liener 1981).

Soymilk is an aqueous extract of dry and mature soybeans which is similar to cow's milk in appearance (Lo and others 1968). The Chinese method of preparation utilizes soaking of soybeans in cold water until fully hydrated followed by fine grinding in stone mill with added water, filtration of the slurry formed to remove the insoluble solids, and boiling is done to obtain soymilk of better flavor. This process yields 65% of soybean solids (Lo and others 1968). Another soymilk manufacturing method with 90% utilization of soybean solids was reported by Hand and others (1964) which includes bean dehulling, fine grinding of steam-dried beans to make powder, and adding water to the powder to produce soymilk. Another process with no soak water losses of soybean soluble solids was developed by Mustakas and Mayberry (1964) which includes moisture-conditioning of dehulled soybean flakes, and extrusion of bean mixture through an orifice at high temperature, high pressure and short time. The puffed, cooked and dried material was finely ground to make soymilk by adding water. The Illinois process of

producing soymilk utilized all the cotyledon solids except for 10-12% of water-soluble solids in the final product which utilized all the proteins with high process yield in addition to an environmentally-friendly process which also avoided solids waste (Rosenthal and others 2003; Nelson and others 1976; Kuntz and others 1978) Even though there were immense advantages in the Illinois process, it is not widely accepted for manufacturing soymilk due to low physical stability and the presence of chalkiness in the soymilk (Rosenthal and others 2003) Chalkiness is a defect and is a sensory attribute of a liquid food which is related to the sensation of chalk powder in the mouth and throat after the ingestion of the liquid food. This defect in soymilk can be attributed to the presence of fine, grainy particles which fills the pores in mucous membranes of the mouth after its intake (Rosenthal and others 2003). The lack of suspension stability (clear separated of serum layer at the top and opaque layer of settled solids at bottom of soy beverage) can be prevented by filtration / centrifugation or by adding stabilizers. A separation index of 1 (means no visible separation) was obtained after 5 days of quiescent storage for soymilks processed at a homogenization pressure of 34.47 MPa (5000 psi) and for soymilk treated with temperature of 82 ºC prior to homogenization at 24.13 MPa (3500 psi) (Priepke and others 1980).

Thermal treatment utilized in commercial soymilk production gives assurance of food safety and extended shelf-life in addition to inactivation of trypsin inhibitors and lipoxygenase (and thus the enzyme-induced off-flavors) which are biologically active compounds present in soy (Kuntz and others 1978; Liener 1981; Wilkens and others 1967; Kumar and others 2003). The adverse effects of thermal processing were also reported as sensory and nutritional quality losses of soymilk (Kwok and Niranjan 1995; Chauhan and others 1998; Sancho and others 1999). Preservation of soymilk characteristics to some extent can be done using 34.47- 55.16 MPa

(5000-8000 psi) pressurized homogenization of cotyledons with colloid milling and centrifugal clarification to improve mouthfeel and suspension stability (Kuntz and others 1978; Priepke and others 1980; Mustakas and Mayberry 1974). Soymilk was pasteurized by heating to 85 ºC after the two homogenizations at 24.13 MPa (3500 psi) and prior to third homogenization with no pressure in the first stage and 3.44 MPa (500 psi) in the second stage (Kuntz and others 1978). The third homogenization was to break clumps formed by heating and the study showed that chalkiness was significantly reduced by heating. By keeping a minimum homogenization temperature of 82 ºC (for one of the two homogenizations) promoted the soymilk stability with formation of lipid-protein complex during processing (Priepke and others 1980). Homogenizing twice improved the sensory acceptability of the Illinois soy beverage but homogenizing more than twice added undesirable off-flavors to it (Kuntz and others 1978; Nelson and others 1976; Forster and Ferrier 1979).

The composition of soymilk differs with the variety of soybean used and the processing method (Iwuoha and Umunnakwe 1997; Kwok and Niranjan 1995; Mullin and others 2001). This variation in solid content and its increase imparted increased viscosity to soymilk (Nelson and others 1976; Kuntz and others 1978; Cheng and others 2005). Viscosity is an important functional property of soymilk and it is of vital importance when considering the commercialization of pressure treated soymilk (Lakshmanan and others 2006; Cheng and others 2005). Addition of hulls increased the viscosity of soymilk and dropped the flow behavior index which indicated a greater deviation from Newtonian behavior (Forster and Ferrier 1979). Son and Singh (1998) reported that the power law model was fitting best to the soymilk with different solids content and the effect of measurement temperature on apparent viscosity was expressed using Arrhenius relationship. A product will be exposed to different shear rates during industrial

applications and it becomes vital to know its variation in viscosity with temperature at those shear rates which will also help in the design of equipments for its processing (Lakshmanan and others 2006; Ditchfield and others 2004). Lakshmanan and others (2006) reported that the peptide profiles were not affected though aggregates were observed in the soluble protein fraction in thermal- and pressure-treated samples of soymilk. They also reported increased emulsion stability and decreased hydrophobicity for high pressure processed soymilk. The complexing of phospholipids and proteins in soymilk will aid in preventing settling of suspended solids by reducing density difference between the particles and the serum (Priepke and others 1980). This coating of the particle by lipids will help in strengthening the mutual attraction between hydrophilic phosphate groups and water. To learn more about the protein-lipid complex or film, an in-depth study using specifically stained soymilk samples observed through confocal laser scanning microscope is needed. The effect of heating at 82 ºC for 2 min before homogenization at 80 MPa on the rheological properties and microstructure of oil-in-water emulsions containing commercial fraction of soy protein concentrate and soybean oil was reported by Roesch and Corredig (2003). All emulsions displayed a gel-like behavior, stability to creaming and no separation during quiescent storage for 20 days at 4 ºC.

Very little information is available on full utilization of dehulled soy cotyledons, non-extracted pressurized soymilk, and its rheological and ultrastructural properties and particle size distribution even though the market of this increasingly accepted beverage was projected to reach $1 billion in 2006 (Wrick 2003). Comparison and correlation using real results has not been done yet on the multitude of influencing factors such as total solids, physical and ultrastructural properties, and particle size distribution of thermal-treated and pressurized soymilk, though several studies have reported assumptions of protein-lipid linkage based on data

from sensory, stability, and electrophoresis analysis (Priepke and others 1980; Nelson and others 1976; Lakshmanan and others 2006; Kuntz and others 1978). This study was undertaken with the objective to study the influence of number of passes of pressurized homogenization and preheating on the rheological and ultrastructural properties and particle size distribution of soymilk prepared from whole dehulled soybeans.

Materials and Methods

Materials

Soybeans were provided by the Georgia Seed Development Commission, 2420 South Milledge Avenue, Athens, GA 30605. The soybean (*Glycine max* [L.] Merrill) variety used for the study was Benning, Group VII cultivar, harvested in 2005 from Davisboro, Ga. Soybeans were stored in closed polyethylene-lined bags at 4 ºC and 20 %RH in the dark throughout the experiments (until they were processed into soymilk) to minimize the changes in composition. Deionized water (DW) was used throughout the experiments to prepare soymilk. Silk® Unsweetened (shelf-stable) soymilk (WhiteWave Foods Company, Broomfield, CO 800021) was purchased from the local grocery store.

Preparation of soymilk

The equipment for soymilk preparation was made of either stainless steel or plastic. Soybeans were dehulled by heating at 154.44 °C for 5 min on a pan in an impinger oven (Lincoln Impinger Model 1450, Lincoln Foodservice Products, Inc., Fort Wayne, Ind.). The tempered soybeans were coarse ground in a plate mill (Quaker City Mill Model 4-E, QCG Systems, LLC, Phoenixville, Pa.) with the plates set far enough apart to crack the hulls but not to break the cotyledons. The hulls and the cotyledons were separated by air classification. Soymilk was

prepared from whole dehulled soybeans by blanching in DW (1:5 w/w:: dehulled soybean: DW) at 60 °C for 2½ hours. The blanched cotyledons were drained and rinsed 3 times with DW and then adding DW (1:3 w/w :: blanched dehulled soybean: DW) the mixture was ground in a food processor (Robot Coupe Model RSI 10V, Robot Coupe USA, Inc., Jackson, Miss.) at 2500 rpm for 2½ min. and at 3000 rpm for 2½ min. Then comminution was done in a Fitzpatrick mill (Model JT, The Fitzpatrick Company, Elmhurst, Ill.) using 0.127 cm and 0.0508 cm screen. The comminuted suspension was homogenized at 96.53 MPa (14,000 psi) in a Gaulin one-stage homogenizer (Model 15MR-8TA, Gaulin, Everett, Mass.). The process designations for these unboiled soymilks are: one pass Gaulin homogenization: process A, and two pass Gaulin homogenization: process B. Two more treatments were done by heating the comminuted suspension at 98±2 °C for 15 min followed by Gaulin homogenization as described above. The process designations for these boiled soymilks are: one pass Gaulin homogenization: process C, and two pass Gaulin homogenization: process D. The whole experiment was triplicated at independent occasions.

Total solid content

The experimental soymilk samples and Silk® Unsweetened (shelf-stable) soymilk samples were analyzed for total solids using Halogen Moisture Analyzer (Model HR73, Mettler-Toledo, Inc., Columbus, Ohio). Three measurements were taken from the triplications. Proximate composition (protein, fat, crude fiber, moisture, ash, and carbohydrate (by difference)) of whole soybean and dehulled blanched bean were done at Feed and Environmental Water Laboratory, The University of Georgia, Athens, Ga. Duplicate measurements were taken in each sample.

Rheological properties

Rheological properties were measured using a dynamic stress controlled rheometer (model SR5000, Rheometric Scientific, Inc., Piscataway, N.J.) with concentric cylinder geometry. Apparent viscosity (μ_{app}) was measured using steady state shear test for all the treatments once. The boiled samples were made to an equal total solid content as that of the unboiled samples during analysis of each replication. Apparent viscosity measurements were done at 4, 10 and 25 ºC to simulate different storage conditions and Arrhenius equation (equation 2) was fitted to predict the apparent viscosity of soymilk at different temperatures. A new sample was used for each measurement. Temperature control at the measurement tool (couette) was done with a heating and refrigerating fluid in a water bath (model F25, Julabo USA, Inc., Kutztown, Pa.) attached to the rheometer and the heating / refrigerating fluid recirculates to the cup through the jacket in the rheometer for heating or cooling. Flow behavior and consistency indices at different temperatures were determined by fitting the power law model (equation 1). Time-dependence of the soymilk (for measuring settling during prolonged time period) was measured using transient shear rate ramp test with constant shear rate of 1000 s^{-1} at 10 ºC for a particular time period. The power law model (equation 1) (Toledo 2007) was used to describe the non-Newtonian behavior of the soymilk:

$$\tau = K(\gamma)^n \ldots\ldots (1)$$

where τ = shear stress (Pa), K = consistency coefficient (Pa.s^n), γ = rate of shear (s^{-1}), and n = flow behavior index. If n < 1 the fluid shows pseudoplastic behavior and if n > 1 the fluid shows dilatant behavior. For fluids having characteristics which fit in equation 1, the apparent viscosity (Pa.s), μ_{app}, can be expressed in log transformation as:

$$\ln \mu_{app} = \ln K + (n-1)\ln \gamma \ldots.. (2)$$

The apparent viscosity is dependent on temperature and can be expressed by the Arrhenius equation as given below (Toledo 2007):

$$\ln(\frac{\mu_{app}}{\mu_{app_1}}) = \frac{E_a}{R}(\frac{1}{T} - \frac{1}{T_1}) \ldots\ldots (3)$$

where μ_{app} stands for apparent viscosity (Pa.s) at absolute temperature T (K), μ_{app1} stands for apparent viscosity (Pa.s) at T_1 (K), E_a stands for the activation energy (J/gmole), and R stands for gas constant (8.314 J/gmole.K). The equation 3 can be rearranged (Toledo 2007) to obtain the following straight line equation:

$$\ln \mu_{app} = A + \frac{B}{T} \ldots\ldots (4)$$

where A and B are constants and are intercept and slope respectively for the plot of $\ln(\mu_{app})$ against (1/T). By equating the equations 3 and 4, we get the slope (B) as E_a/R, from which the E_a can be calculated after the model is obtained. Three observations obtained from three replications were used for the analyses.

Particle size distribution

Soymilk particle size distribution was measured using Malvern Laser Particle Size Analyzer, Mastersizer S with 300mm lens (Malvern Instruments, Southborough, Mass.). Soymilk samples were dispersed in deionized water until an appropriated obscuration point (10-20%) was obtained in the diffractometer cell at a pump speed of 2500 rpm. An optical model based on the Mie theory of light scattering by spherical particles was applied to calculate the predicted scattering pattern with the refractive indices of the soymilk and water as follows: real refractive index, 1.47; imaginary refractive index, 0.00; refractive index of water, 1.33. The instrument software calculated the average volume-weighted diameter, $D_{(4,3)} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$ (where n_i is the number of particles in a size class of diameter d_i), the surface-weighted mean diameter, $D_{(3,2)} = \Sigma n_i d_i^3 /$

$\Sigma n_i d_i^2$, the diameter below which 90% of the volume of particles are found, $D_{(v,0.99)}$, the diameter below which 90% of the volume of particles are found, $D_{(v,0.9)}$, the diameter below which 80% of the volume of particles are found, $D_{(v,0.8)}$, the diameter below which 60% of the volume of particles are found, $D_{(v,0.6)}$, the diameter below which 50% of the volume of particles are found, $D_{(v,0.5)}$, the diameter below which 40% of the volume of particles are found, $D_{(v,0.4)}$, the diameter below which 20% of the volume of particles are found, $D_{(v,0.2)}$, the diameter below which 10% of the volume of particles are found, $D_{(v,0.1)}$, from the size distribution were calculated by the instrument software. The measurement of particle size distribution of Silk Unsweetened (shelf-stable) soymilk was also done in the same way to compare it with the particle size distribution of the experimental soymilk samples. Six measurements from three replications were used for the analyses.

Ultrastructural properties

Ultrastructural observations were carried out for all treatments at the Center for Ultrastructural Research, University of Georgia, Athens, Ga. LEO 982 field emission scanning electron microscope (Zeiss SMT, Inc., Peabody, Mass.) equipped with an Oxford EDX detector (Oxford Intstruments, Inc. Concord, Mass.) and a Gatan Alto 2500 cryostage and cryoprep chamber (Gatan UK, Oxford, UK) was used for examining the structural network of the samples. One drop of sample was placed on a specimen stub and covered with a stub cap, then rapidly frozen by immersing it in liquid nitrogen slurry (approximately -206 °C). After moving to the cryoprep chamber, the sample was fractured with a knife to provide a fresh surface. Residual ice present on the frozen sample was removed by sublimation at -100 °C for 5 min, sputter-coated with gold to a thickness of approximately 20 nm, and placed in the sample chamber of the scanning electron microscope (SEM) on a cold stage maintained at -120 ºC.

Distribution of fat and protein droplets was also determined with a Leica- TCS SP2 confocal laser scanning microscope (CLSM, Leica Microsystems Inc., Exton, Pa.) used in reflectance, transmittance and fluorescence mode. Fluorescence dyes specific for lipid globules (lipid soluble nile red fluorescent dye- NR, Sigma-Aldrich, St. Louis, Mo.) and protein fraction (fluorescein isothiocyanate – FITC, Sigma-Aldrich, St. Louis, Mo.) were added to the samples separately. The preparation of the working solutions of dyes was done as described by Damodaraswamy (2005). NR stock solution was prepared with 0.5mg/ml in acetone. From this, a working solution for NR was made by combining 0.10 ml of stock solution to 100 ml of a 75:25 glycerol-water mixture. FITC stock solution was prepared with 0.25% FITC in water. This was further diluted to 1% in water to produce a working solution. The amount of each dye solution and soymilk sample used in the micro slide for analysis were: 25 μL NR, 10 μL FITC, and 25 μL soymilk. The sample was put on a pre-cleaned glass micro slide, stains were added to the sample, and a cover slip was placed over it. CLSM was equipped with a 63x (numerical aperture 1.4), oil immersion objective lens which provided good control in the z-direction in addition to optical sections in both xy- and xz-directions. Images were taken separately for each dye with a single sample. Excitation for both dyes was by argon/krypton laser with excitation wavelengths of 568 and 488 nm for fat and protein, respectively. Fat globules were pseudo-colored red and protein particles were colored green.

Statistical analysis

Statistical significance of treatment factors and its interactions were determined according to two-way factorial design with three replications of each treatment using SAS version 9.1 (SAS Inst., Inc., Cary, N.C.). The factors were homogenization temperature and number of passes;

each at two levels. Analysis of variance was used to study the effects of treatment factors on response variables (particle size diameter and total solids) quantifying soymilk properties.

Similarly, three-way factorial design with three replications was used to study the statistical significance of treatment factors and the interactions on the rheological properties (apparent viscosity, consistency index, and flow behavior index). The three factors were preheating, number of passes, and measurement temperature. Student's t-test was used further for pair wise comparison between the means of each variable. Silk® Unsweetened (shelf-stable) soymilk was used as a control for statistical comparison with the experiment samples for the response variables of total solids and particle size distribution.

Results and Discussion

Soymilk processing was done starting from whole bean with proximate composition of 36.62% protein, 19.66% fat, 9.31% crude fiber, 7.76% moisture, 5.53% ash and 21.12% carbohydrate (by difference). The dehulled blanched bean had proximate analysis of 18.08% protein, 6.17% fat, 5.40% crude fiber, 64.60% moisture, 1.13% ash, and 4.62% carbohydrate (by difference). The process B (unboiled two pass) soymilk had proximate analysis of 3.68% protein, 1.58% fat, 0.26% crude fiber, 92.51% moisture, 0.24% ash, and 1.73% carbohydrate (by difference). The process D (boiled two pass) had proximate analysis of 4.24% protein, 1.78% fat, 0.27% crude fiber, 91.49% moisture, 0.27% ash, and 1.95% carbohydrate (by difference).

Total solids content of soymilk

Total solids content is a key property which influences other properties such as sensory, physical, and rheological properties as well. Total solids content of the soymilk samples are given in Table 3.1. There was a significant difference ($p < 0.0019$) in solids content between

boiled and unboiled samples. The total solids content of heated soymilks were 0.79 times greater than that of unheated soymilks. This difference affected the apparent viscosity of these two samples. This relation agreed to the results of studies done on soymilk previously (Kuntz and others 1978; Lakshmanan and others 2006; Nelson and others 1976; Cheng and others 2005). Silk® Unsweetened (shelf-stable) soymilk displayed a similar total solids content of 7.55 ± 0.5% which was not significantly different from unheated soymilk samples. The results of Kuntz and others (1978) showed that chalkiness is a function of solid content. They reported that the least chalkiness score was obtained for the soymilks with 6-8% solid content. In the present study the solid content of the unboiled soymilk is near 8% and that of the boiled soymilk was higher solid content due to moisture evaporation during boiling. Priepke and others (1980) reported after testing the total solids top to bottom ratio for soymilks with total solid content 2.39-8.85% that after long term storage, settling is more likely to occur in beverages with lower solid content than with higher solid content. In addition to that, when the extracted total lipid was added back to the soymilk, it gave the same suspension stability (separation index of 1) as the unextracted Illinois soy beverage.

Rheological characteristics of soymilk

Starting from product development, product properties (appearance, stability, thickness, mouthfeel), process development, equipment design, and until commercialization of the product, rheological characteristics of soymilk plays a pivotal role. As expected, analysis of variance showed that the apparent viscosity (μ_{app}) decreased significantly (by a factor of 1.08 as temperature changed from 4 to 10 ºC change, and by a factor of 1.43 for 10 to 25 ºC temperature change) ($p < 0.0001$). Silk® Unsweetened (shelf-stable) had an apparent viscosity of 7.38 ± 0.23 mPa.s at 10 ºC which was significantly different from the soymilks obtained from the four

processes. Apparent viscosity of soymilk for 4 treatments is shown in Figure 3.1. Apparent viscosity decreased significantly ($p < 0.0005$) with increase in number of passes (Figure 3.1). The results agreed to those obtained by Forster and Ferrier (1979). Analysis of variance showed significant interaction between the factors boiling and number of passes. The interaction plot and t-test revealed that the boiled soymilk had significantly lower apparent viscosity than unboiled soymilk (by a factor of 1.27) when the soymilk was passed once through homogenizer at 96.53 MPa during preparation ($p < 0.0001$). However, this was not apparent in two pass homogenization (Figure 3.1) where the reduction factor was only 1.06. The reduction in μ_{app} may be due to the additional shearing during the second pass which also decreased the mean particle size. The quick orientation of smaller particles in the direction of shear force might have helped in lowering the apparent viscosity (Forster and Ferrier 1979). Similar results were reported by Lakshmanan and others (2006) for soymilk at pH 7 with no significant difference in viscosity between the controls and pressure homogenized soymilks, however thermal-treated soymilks displayed higher viscosity than controls. Puppo and others (2004) explained the differences in soymilk viscosity between the heat- and pressure-treated soymilks as due to the rupture in hydrogen-bonded networks that are affected by heating and not by pressure. Results of Kuntz and others (1978) showed that viscosity did not have any effect on perception of chalkiness in soymilk. This also agreed with results by Nelson and others (1976) that the stability of the soy beverage was not related to its viscosity. They reported a viscosity of 14 mPa.s (at 25 ºC for 4.6% protein concentration) for the Illinois soy beverage. Rosenthal and others (2003) reported similar results with enzyme treated soymilk as having the same apparent viscosity (approximately 10 mPa.s, measurement temperature and method not mentioned) as the doubly homogenized (34.47 MPa, 60 ºC) soymilk. The highest physical stability and the sensorial

attribute “body” of the enzyme treated soymilk were exhibited by the highest apparent viscosity obtained for a particular treatment. Iwuoha and Umunnakwe (1997) reported higher apparent viscosity of soymilk from blanched wet-dehulled seeds, soymilk from unblanched wet-dehulled, soymilk from toasted dry-dehulled, and soymilk from large flour particles as 38.00, 50.00, 45.00, 45.00 mPa.s respectively for freshly processed soymilks with measurement temperature at 30 ºC and moisture contents 91.56, 88.09, 90.51, 91.00% for the respective treatment samples. The rheological characteristics reported earlier were done using single shear rate (Kuntz and others 1978; Iwuoha and Umunnakwe 1997); for a Newtonian fluid the viscosity results obtained using single shear rate are appropriate but it may supply deficient or conflicting information on non-Newtonian fluids and its behavior (Forster and Ferrier 1979).

The flow behavior of soymilk was best explained by the power law model (equation 1). All soymilk treatments showed non-Newtonian pseudoplastic behavior. The values of K and n can be substituted in equation 2 from Table 3.1 for defining flow behavior of each soymilk sample. Data by Son and Singh (1998) showed similar results was obtained for 13% and 16% solids soymilk, although Casson and power law models fitted good for soymilk with 9% solids. Ease of pumping the beverage is the advantage of a pseudoplastic behavior as reduced power will be required to pump the beverage at higher speeds, an advantage in industrial applications (Son and Singh 1998; Forster and Ferrier 1979).

Analysis of variance for the consistency index showed significant effect of measurement temperature ($p < 0.0001$); however, the number of passes and boiling had significant interaction ($p < 0.0046$). The difference between consistency indices of boiled and unboiled samples was decreased with increase in number of passes. It was noticed that the number of passes significantly changed the consistency index of both boiled and unboiled products (p-values 0.03

and 0.05). In the viscometric evaluation of Illinois soy beverage, a consistency index of 0.67 $Pa.s^n$ and flow behavior index of 0.73 was obtained when the product was prepared without hulls and the total solids content was 7% (Forster and Ferrier 1979). The consistency index increased exponentially (0.02, 0.14, and 0.70 $Pa.s^n$ at 25 ºC for 9%, 13%, and 16% total solids) with increasing concentration and decreased with increasing temperature (0.02, 0.01, and 0.008 $Pa.s^n$ at 25, 45, and 65 ºC for 9% total solids) for aseptically processed soymilk under turbulent conditions (Son and Singh 1998).

Like consistency index the flow behavior index was also affected by the temperature of analysis ($p<0.0060$). Increasing the number of passes, significantly decreased the flow behavior index for boiled sample ($p<0.0001$), however, this was not evident for unboiled samples. There was no noticeable difference in flow behavior index between boiled and unboiled either at one pass or two passes. Son and Singh (Son SM 1998) reported decreasing flow behavior index with increasing total solids content (0.77, 0.67, and 0.58 at 25 ºC for 9%, 13%, and 16% total solids) and increased with increasing temperature (0.77, 0.77, and 0.77 at 25, 45, and 65 ºC for 9% total solids). Flow behavior index (0.82) did not appreciably change with measurement temperature, and it was considered that there was no influence of measurement temperature on the degree of non-Newtonian behavior of soymilk (Forster and Ferrier 1979). The values of n agreed with Lakshmanan and others (2006), who reported a more pronounced pseudoplastic behavior with n values ranging from 0.45 to 0.49 for 1:6 (soybean-to-water ratio) soymilk and 0.56 to 0.57 for 1:8 soymilk at pH 6 for the pressure- and thermal-treated soymilks.

Apparent viscosity changes of the soymilk over time were determined at a constant shear rate (1000 s^{-1}) at 10 ºC and the curve obtained is shown in Figure 3.2 for the soymilks obtained by processes C and D. During the first 200 s apparent viscosity decreased, showing that the soymilk

was thixotropic, and it became relatively constant later on when the shear rate was kept constant. Similar results reported earlier explained that the identical curve given by soymilk after a rest period of 900 s with a second analysis using same constant shear rate indicated that shear-induced structural changes on the soymilk were reversible (Forster and Ferrier 1979; Rosenthal and others 2003). For Priepke and others (1980), a separation index of 1 (means no visible separation) was obtained after 5 days of quiescent storage for soymilks processed at a homogenization pressure of 34.47 MPa (5000 psi). Same result was obtained for soymilks treated with temperature of 82 ºC prior to one pass homogenization at 24.13 MPa (3500 psi) with formation of lipid-protein complex during processing (Priepke and others 1980). The thixotropic behavior shown by soymilk may indicate that the beverage will have less tendency to settle, increasing its shelf life and improving mouthfeel by reducing the chalkiness (Forster and Ferrier 1979).

The temperature dependence of the soymilk apparent viscosity was correlated and expressed in terms of the Arrhenius model (Figure 3.3). Arrhenius relationship (equation 4) was examined for all treatments and it showed that apparent viscosity decreased exponentially with increase in temperature. This was in agreement with the results of Son and Singh (1998) who also reported that the exponential increase in apparent viscosity with increase in concentration at all measurement temperatures indicated protein-protein interaction. The activation energy, Ea, was calculated as 401.47 (0.096), 252.75 (0.060), 244.32 (0.058), and 245.58 (0.059) J/mol (kcal/mol) for processes A, B, C, and D respectively. Son and Singh reported activation energy for soymilks with 9%, 13%, and 16% solid content as 43542.72 (10.40), 29307.60 (7.00), and 28470.24 (6.80) J/mol (kcal/mol) respectively. Though substantiating earlier reports it can be correlated with the present rheological properties results that there seems to be no or little

chalkiness and no settling for the process D soymilk, however it is difficult to establish such a correlation that homogenization temperature and pressurized double homogenization reduced the chalkiness and settling of soymilk without testing the sensory and physical properties of soymilk.

Particle size distribution

Particle diameter decreased significantly ($p < 0.05$) with two passes (Table 3.2). There was no significant difference ($p > 0.05$) in the particle size between the boiled and unboiled soymilk samples. The soymilk particle size decreased by a factor of ~1.50 (Figure 3.4, Table 3.2) in the second homogenization pass. The results of Roesch and Corredig (2003) agreed with this and they attributed the presence of large particles in the image taken with integrated light scattering was partly due to large soy fiber structures. From microscopy observations, those fiber particles gave an average size between 50 and 100 μm and their size did not seem to be affected by heat treatment or by high-pressure homogenization. This partly agreed to the results of Kuntz and others (1978) which showed that homogenization temperature (~82 °C) had a significant effect on chalkiness of soymilk which might have resulted from the more extensive hydration and tenderization of the tissue during heating. This high temperature resulted in greater disruption of tissue particles during pressurized homogenization and reduced the particle size which, in turn, reduced the chalkiness of soymilk. Particle size measurements using Coulter Counter resulted with 80% of particles with size range 3.4-7.3 μm and 10% of the particles above 10 μm in the Illinois beverage (Nelson and others 1976). They further explained that particles sizes were well above the upper limit of colloidal particles and it was considered to be in the range of unhomogenized milk. The large particle size of the Illinois beverage does not represent a true colloidal system and so the particle size distribution was not the single factor for good stability of the beverage. From previous reports and by comparing our results to particle size distribution of

Silk® Unsweetened (shelf-stable) soymilk which is far below [$D_{(4,3)}$ was higher than Silk® Unsweetened (shelf-stable) soymilk by a factor of 12.03, 7.93, 11.83, and 7.91 for processes A, B, C, and D respectively] the particle size obtained for any of the treatments (Table 3.2). It seems that particle size reduction is of utmost importance to commercialize soymilk processed using any of these treatments. Roesch and Corredig (2003) reported a similar result for oil-in-water emulsion with soy protein concentrate (SPC) and soybean oil that after homogenization and/or heat treatment, the suspensions were still characterized by a wide distribution of large particles, but the mean particle size was smaller than that for untreated SPC. In that case, heat treatment alone seemed to produce a similar effect on the particle size distribution as homogenization alone. Our results showed that although the process D produced the smallest particle diameter [$D_{(4,3)}$ ~ 29 μm], only 40% of the particles were less than 25 μm. Thus, more research is needed on how to reduce particle size and narrowing the particle size distribution before soymilk from whole soybeans can be successfully commercialized.

Ultrastructural properties

Cryo-scanning electron microscope (cryo-SEM) images (Figures 3.5, 3.6, and 3.7) showed the homogeneity and hydration of the Process D sample (Figure 3.7). The preheating treatment before homogenization tenderized and hydrated the particles. All the other processes (image for Process C soymilk is not given) did not impart a uniform structure to the soymilk which was apparent from the aggregates of fat globules in the images (Figures 3.5 and 3.6). Magnification of the images was limited to 10,000× for all the process samples except for process D which allowed magnification of 100,000× (image not shown). This reiterates the minute particle size and the homogeneity of particles in process D soymilk. The result is also in agreement with the particle size distribution results obtained by laser light scattering which showed that process D

soymilk had the smallest particle size among the treatments. The agglomerated particles were not separated by one-pass homogenization (Figure 3.5). Aggregates were ruptured only by the combined heating and pressurized double homogenization (Figure 3.7). Similar result was obtained by Roesch and Corredig (2003) in which the network formed in the heated oil-in water emulsions containing SPC and soybean oil seemed to have a smoother surface than that present in unheated emulsions. They explained that it was due to the enhanced unfolding of the globular protein caused by the high pressure homogenization, the high shear in the presence of higher SPC or/and the heat treatment applied. Wagner and others (1992) explained that hydration in soymilk was due to thermal treatment which causes the proteins to open due to denaturation forming aggregates through disulphide bonds and hydrophobic interactions, which results in higher water binding capacity. Lakshmanan and others (2006) showed that at pH 6 pressurized soymilk exhibited higher water-binding capacity than thermal-treated samples. Previous reports (Priepke and others 1980; Nelson and others 1976; Kuntz and others 1978) assumed that hydrophilic protein-lipid complexes were formed during processing to tenderize the beans and when combined with pressurized homogenization the beverage was stable and had excellent mouthfeel. The microstructure exhibited by the cryo-SEM images, suggests will impart excellent mouthfeel and physical stability of the process D soymilk.

CLSM images obtained showed fat globules in fluorescent red color and protein particles in fluorescent green color. CLSM images (Figures 3.8 and 3.9) showed that in the heat-treated samples (image for Process C soymilk not shown; Figure 3.9 for Process D soymilk), fat globules (red) were distributed more uniformly than that in the other samples. When gelled proteins were adsorbed at the oil/water interface the droplets are protected against creaming, flocculation, and coalescence, while the stability of the emulsion increases (Lakshmanan and

others 2006). The fat globules seemed to coalesce to form big particle size in the two unboiled samples (Figure 3.8 for Process A soymilk; image for Process B soymilk not shown). Protein particles are not visible as separate particles and are not identifiable as they are distributed uniformly in the sample. Figure 3.8 shows a non-uniform distribution of fat particles and the particles are seen to have coalesced. The non-uniform particle size displayed by cryo-SEM image of process B (Figure 3.6) and the coalesced structure of process A (Figure 3.5) along with the results of particle size distribution obtained by laser light scattering collaborate the CLSM images of samples from the respecive treatments. Similar result was made by Lopez and others (2006) from the CSLM images of heated curd grains and the particle size obtained by laser light scattering. Process C and D soymilk images showed small sized fat globules (no coalescence) with homogeneous size and uniform distribution. The protein droplets (green) were very small and were difficult to identify due to the very small size it attained and uniform distribution in the image (Figure 3.9). Process D gave uniform particle size and uniform distribution of fat globules and protein particles and the fat globules can be seen separatly in this image. Thus better attachment of protein at the oil-water interface may have occurred due to protein unfolding and hydrophobic sites were exposed as a result of pressurized homogenization (Lakshmanan and others 2006). The results also agreed to the report by Puppo and others (2005) in which high pressure processing treatment of soy protein isolate, β-7S and A-11S polypeptides displayed increased ability to anchor at the oil-water interface. Similar results were reported by Priepke and others (1980) on Illinois soy beverage in which a protein-lipid film with an almost constant protein-lipid ratio was formed. They further assumed with this evidence that lipid- protein complex was formed in the Illinois soy beverage. Nelson and others (1976) made similar assumptions after testing the composition of freeze-dehydrated soy beverage which resulted in

no oil recovery by Soxhlet method and 1.7% oil recovery using sulfuric acid digestion. As the same fat-protein ratio as in soybeans (20:40) was obtained for the soy beverage (1.7:3.1), they assumed that the oil in that beverage was bound by complex chemical formation. CLSM images of mozzarella cheese made from homogenized and unhomogenized milk mixture variations (Rowney and others 2003) showed smaller and highly emulsified fat globules embedded in protein matrix for cheese from homogenized milk (image displayed similarity to Figure 3.9), and larger pools of fat within voids in the protein matrix for cheese from homogenized milk and for cheese from 1:1 mixture of the homogenized and unhomogenized milks (images similar to Figure 3.8). This confirms that the fat-protein network was formed in process D soymilk which was obvious from the CLSM and cryo-SEM images and the particle size distribution. So, in the present study, process D has been confirmed as the best process among the treatments and the soymilk with full essential solids, and excellent rheological ad ultrastructural characteristics can be obtained by this process.

Conclusion

Soymilk with all the essential soybean solids were produced by softening of the cotyledons by hydration and cooking, adequate comminution, and heating prior to pressurized homogenization. The product flow properties, ultrastructural properties, and particle size distribution were a function of the processing parameters. Heating the coarsely comminuted slurry prior to homogenization resulted in softening and hydration of soy particles. The size of particles in soymilk is reduced when the homogenization was done twice. Pressurized homogenization helped to decrease the size of solid particles and disperse it uniformly. The ultrastructural images helped immensely to define and verify the structure and particle size distribution obtained for

each treatment. The results of the rheological and ultrastructural properties and particle size distribution of soymilk validated that preheating and pressurized double homogenization are very important factors in producing excellent quality soymilk and its commercialization. The results may lead to the use of high pressure homogenization in the processing of soymilk with whole soybean solids in the milk.

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Table 3.1 - Effect of various processes on total solids and flow properties of soymilk.

Process	Total solids (%)	Consistency index, K (Pa-s^n)			Flow behavior index, n		
		4 ºC	10 ºC	25 ºC	4 ºC	10 ºC	25 ºC
A (unboiled/ one pass)	8.20 ± 0.45	0.91 ± 0.28	1.09 ± 0.15	0.46 ± 0.02	0.47 ± 0.07	0.43 ± 0.04	0.51 ± 0.01
B (unboiled/ two pass)	8.08 ± 0.29	0.65 ± 0.24	0.77 ± 0.07	0.42 ± 0.08	0.46 ± 0.05	0.42 ± 0.01	0.45 ± 0.05
C (boiled/ one pass)	10.35 ± 1.27	0.50 ± 0.05	0.64 ± 0.05	0.39 ± 0.02	0.52 ± 0.02	0.49 ± 0.02	0.50 ± 0.00
D (boiled/ two pass)	10.37 ± 1.27	0.65 ± 0.30	1.10 ± 0.12	0.40 ± 0.05	0.44 ± 0.03	0.37 ± 0.03	0.42 ± 0.03

The values are mean and SD from 3 independent experiments.

Table 3.2 - Effect of different processes on particle size distribution of soymilk.

Process	**$D_{(v,0.10)}$ (µm)**	**$D_{(v,0.20)}$ (µm)**	**$D_{(v,0.40)}$ (µm)**	**$D_{(v,0.50)}$ (µm)**	**$D_{(v,0.60)}$ (µm)**	**$D_{(v,0.80)}$ (µm)**	**$D_{(v,0.90)}$ (µm)**	**$D_{(v,0.99)}$ (µm)**	**$D_{(3,2)}$ (µm)**	**$D_{(4,3)}$ (µm)**
A (unboiled/ one pass)	12.71 (1.00)	21.53 (0.69)	34.89 (0.89)	41.30 (1.35)	48.11 (1.85)	65.18 (3.44)	79.83 (5.05)	113.60 (9.46)	25.54 (0.71)	44.43 (1.83)
B (unboiled/ two pass)	11.10 (1.27)	15.90 (1.69)	23.76 (1.91)	27.69 (1.85)	31.94 (1.74)	42.71 (1.14)	52.06 (1.04)	73.73 (3.00)	20.44 (1.57)	29.96 (1.16)
C (boiled/ one pass)	12.48 (0.99)	20.71 (0.92)	34.06 (1.01)	40.60 (1.44)	47.65 (2.00)	65.38 (3.80)	80.66 (5.57)	116.27 (10.28)	25.46 (0.99)	44.25 (2.08)
D (boiled/ two pass)	11.58 (1.13)	16.03 (1.05)	23.53 (0.75)	27.31 (0.76)	31.40 (1.04)	41.88 (2.51)	51.12 (4.18)	72.22 (8.61)	20.91 (1.12)	29.62 (1.30)
Silk® Unsweetened (shelf-stable)	0.63 (0.00)	0.71 (0.01)	0.84 (0.01)	0.93 (0.02)	1.04 (0.03)	3.03 (1.22)	5.68 (0.64)	40.16 (4.54)	1.02 (0.03)	3.18 (0.59)

The values are mean and SD (in parenthesis) from 3 independent experiments.

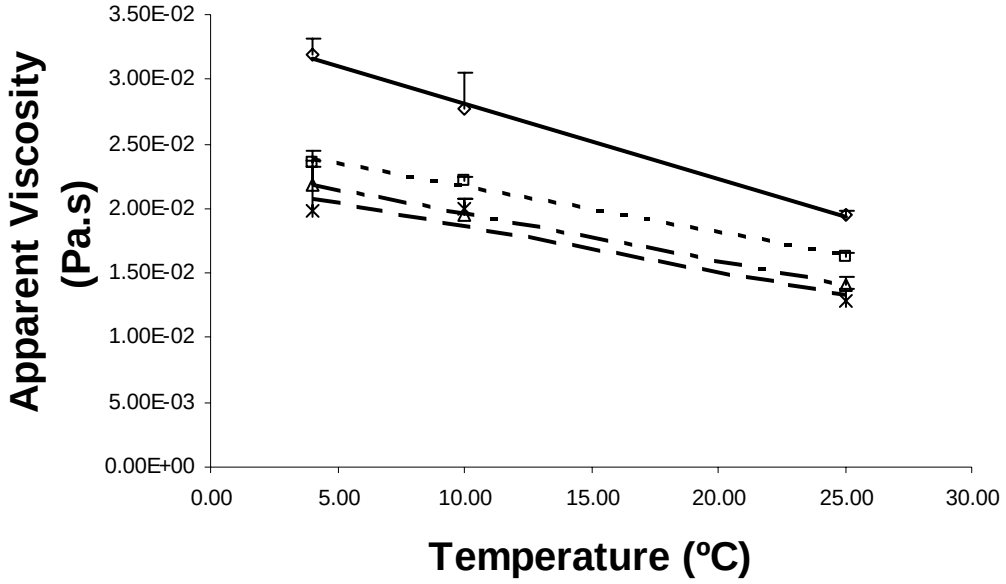

Figure 3.1 - Influence of various processes on apparent viscosity of soymilk. Legends are: process A (unboiled/ one pass) ____◊____, $R^2 = 1.00$; process B (unboiled/ two pass)___ _ _Δ_ _ ___, $R^2 = 1$; process C(boiled/ one pass) _ _ _ _□_ _ _ _, $R^2 = 0.99$; and process D (boiled/ two pass)__ __*__ __, $R^2 = 0.92$.

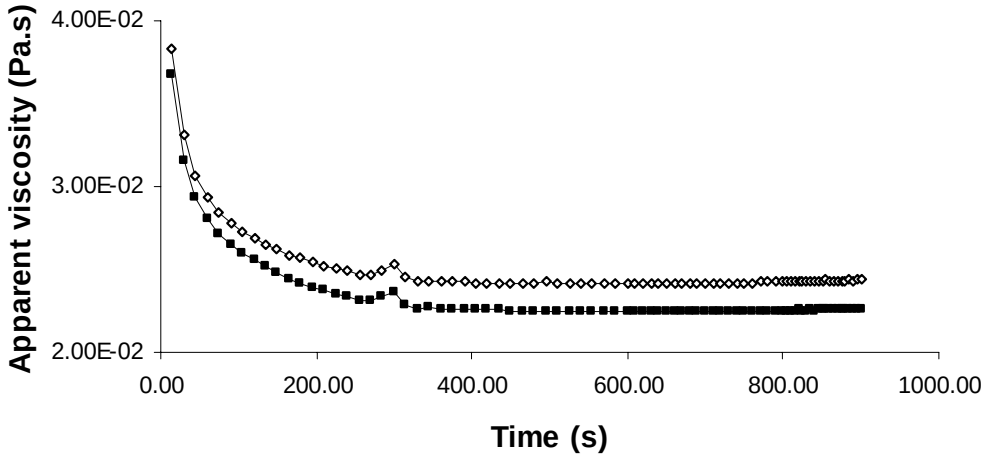

Figure 3.2 - Thixotropic behavior of soymilk, at constant shear rate of 1000 s^{-1} and 10 ºC, shows the resistance to settling by keeping constant apparent viscosity after 200 s. Legends are: process C (boiled/ one pass)___◊___ and process D (boiled/ two pass)___■___.

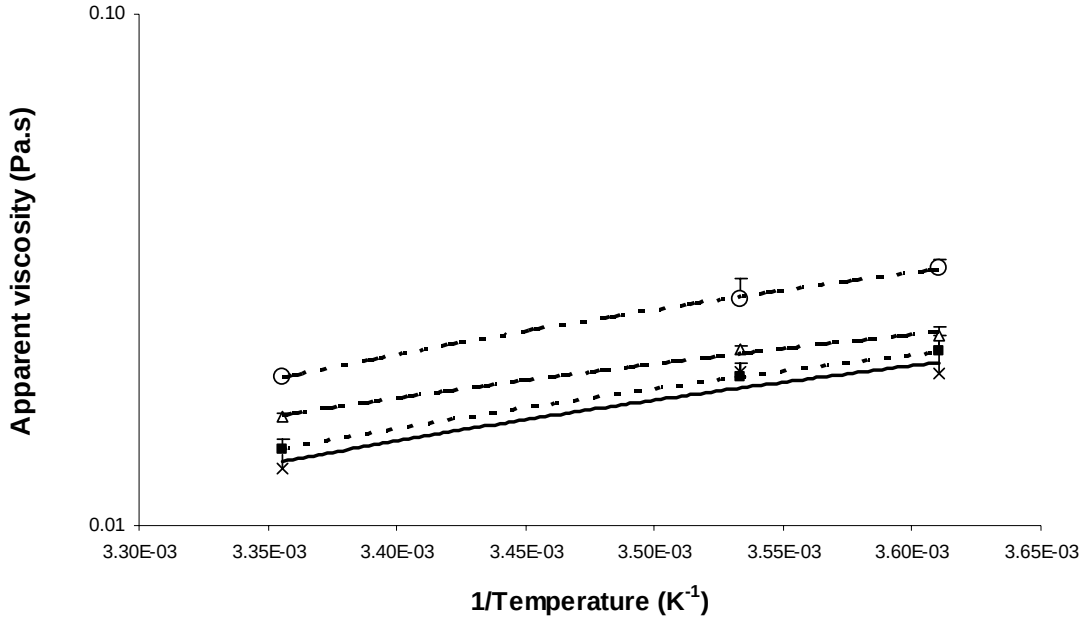

Figure 3.3 - Arrhenius model for soymilk showing temperature dependency of apparent viscosity. Legends are: process A (unboiled/ one pass)___ _ ¤_ ___, $y = 48.29x - 0.14$, $R^2 = 0.99$; process B (unboiled/ two pass)_ _■ _ _, $y = 30.40x - 0.09$, $R^2 = 1$; process C (boiled/ one pass)__ __Δ__ __, $y = 29.39x - 0.09$, $R^2 = 0.99$; and process D (boiled/ two pass) _____×_____, $y = 29.54x - 0.09$, $R^2 = 0.91$.

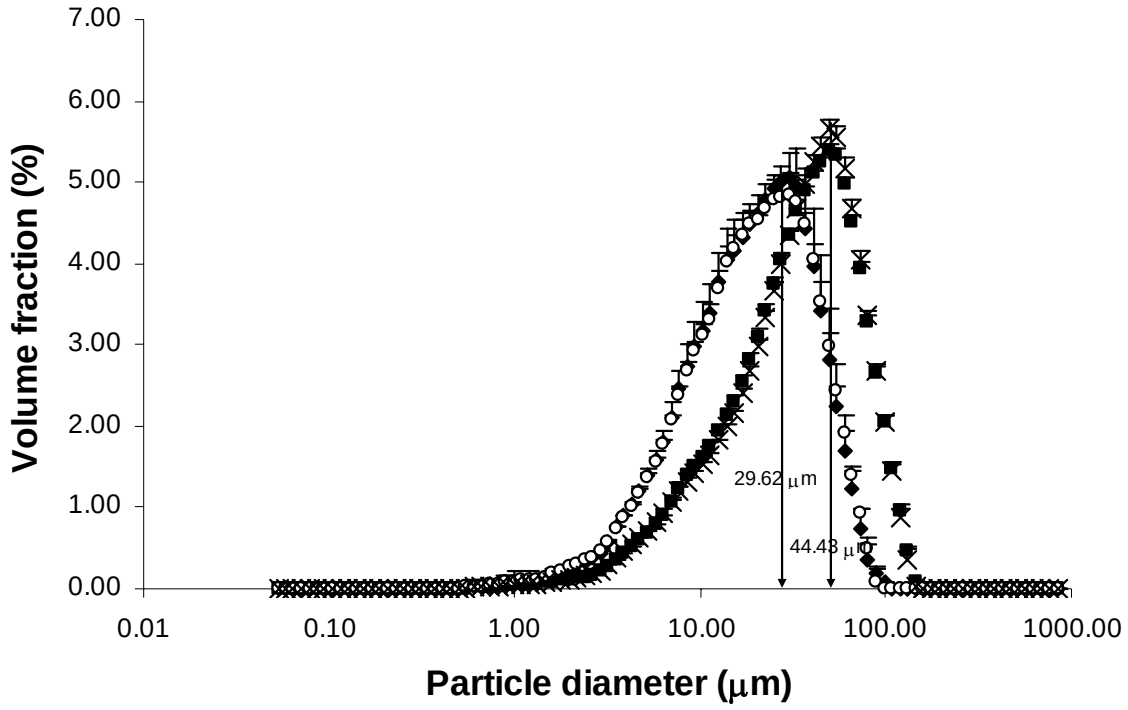

Figure 3.4 - Effect of processing methods on particle size distribution of soymilk. Process legends are: process A (unboiled/ one pass)- ×, process B (unboiled/ two pass)- ¤, process C (boiled/ one pass)- ■, process D (boiled/ two pass)- ♦.

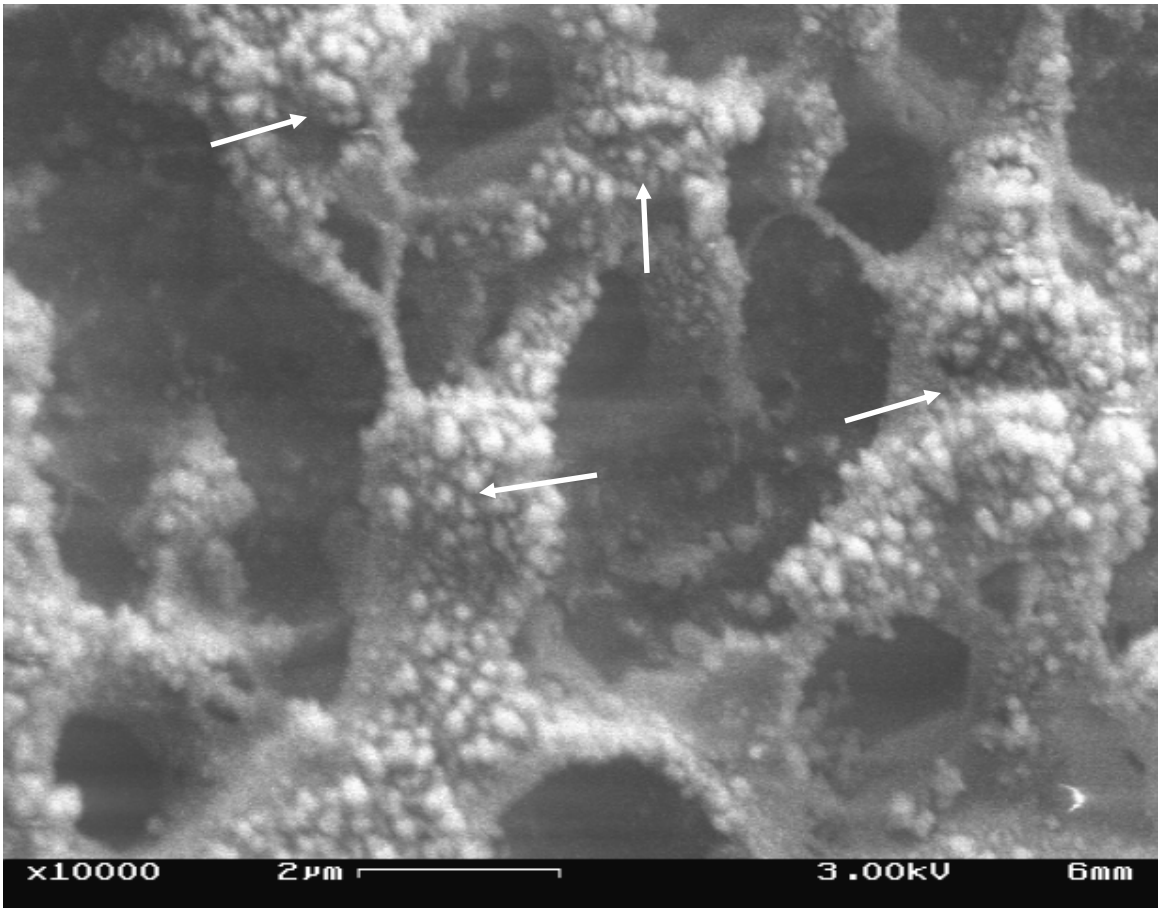

Figure 3.5 – Cryogenic scanning electron microscope image of Process A (single pass homogenization at 96.53 MPa with no preheating) soymilk, the arrows show that the particles are still coalescing and unhydrated without any pattern of distribution and large in size.

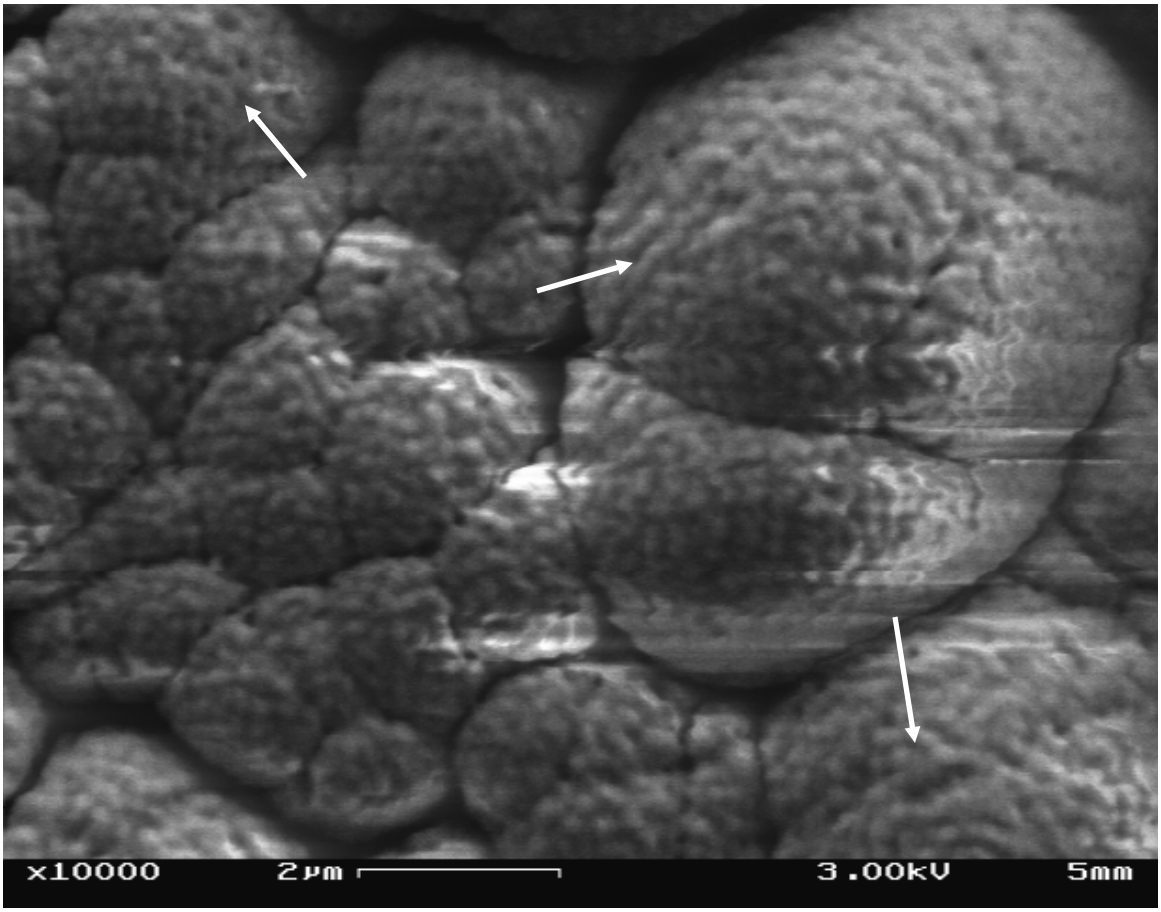

Figure 3.6 - Cryogenic scanning electron microscope image of Process B (double pass homogenization at 96.53 MPa with no preheating) soymilk, the arrows show that though the particles are separated, they are unhydrated with rough surface and not having uniform particle size. These larger particles may be fat globules coalesced and are not completely absorbed in the protein matrix.

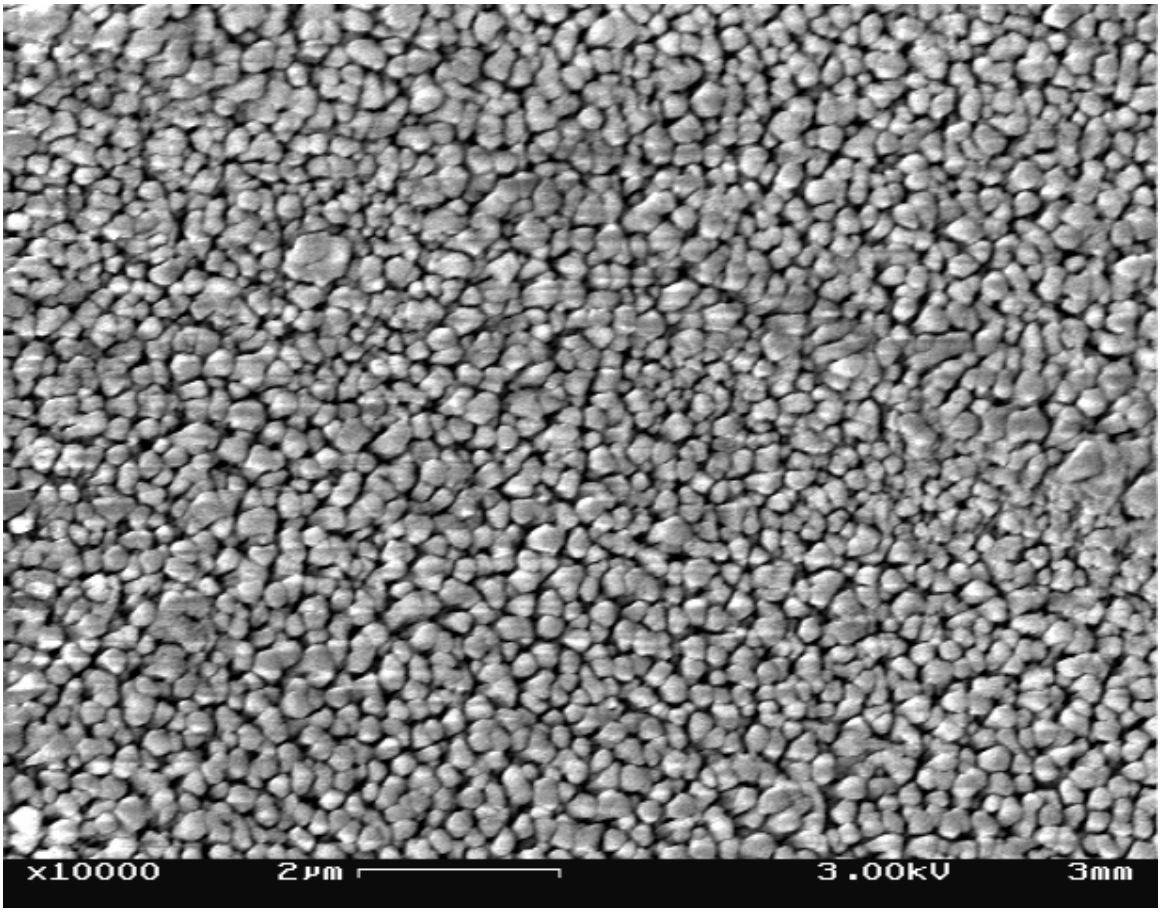

Figure 3.7 - Cryogenic scanning electron microscope image of Process D (double pass homogenization at 96.53 MPa with homogenization temperature of 98 ± 2 ºC) soymilk, shows that the particles are completely separated, hydrated with smooth surface and having uniform particle size and distribution. The fat and protein particles are difficult to be identified separately. Hydration due to heat treatment and small particle size due to pressurized double homogenization helped in dispersion and uniform arrangement of particles in the fat-protein matrix.

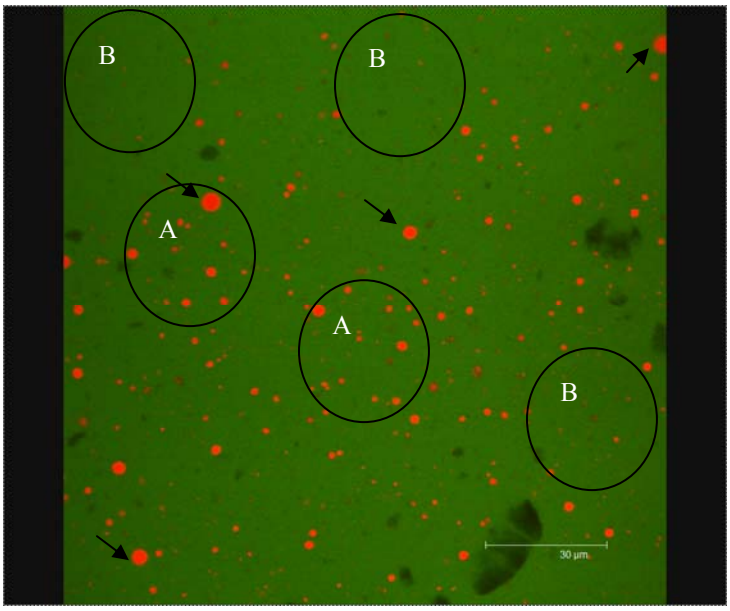

Figure 3.8 - Confocal laser scanning microscope images of Process A (single pass homogenization at 96.53 MPa with no homogenization temperature) soymilk; fat is colored red with nile red and protein is colored green with fluorescein isothiocyanate; the arrows show bigger sized fat globules; circles A show the clustering of fat globules; circles B show the spaces where fat globules are not present or are very few. Protein particles are not seen separately as they are distributed uniformly in the sample. This treatment did not give a uniform distribution of particles and the particles are seen coalesced in the image.

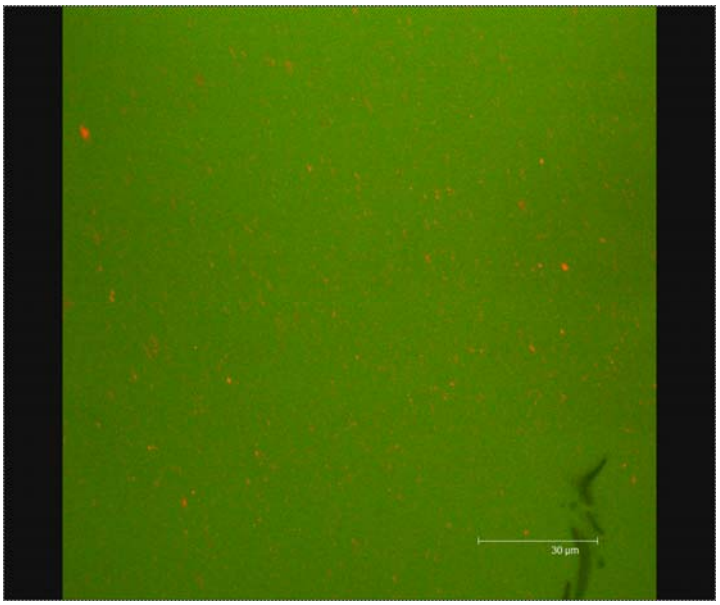

Figure 3.9 - Confocal laser scanning microscope images of Process D (double pass homogenization at 96.53 MPa with homogenization temperature of 98 ± 2 ºC) soymilk; fat is colored red with nile red and protein is colored green with fluorescein isothiocyanate; there is no clustering of fat globules and they are very small in size. This treatment gives uniform particle size and uniform distribution to fat globules and protein particles and the fat globules are seen separated in the image.

CHAPTER 4

EFFECT OF CONTINUOUS FLOW HIGH PRESSURE THROTTLING ON SOYMILK PROPERTIES AND ITS CONSUMER ACCEPTABILITY

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Abstract

Standard soymilk processing uses a filtration or centrifugation step to remove coarse solids in the comminuted soy. The objective was to determine the effect of soymilk sterilization by continuous flow high pressure throttling (CFHPT) in reducing particle size and narrow down particle size distribution. The rheological and ultra-structural properties of soymilk were also elucidated. To study the consumer acceptability of the best experimental sample, it was compared with the commercial soymilk sample (Silk® Unsweetened). A mixture of blanched whole dehulled soybeans and deionized water was ground in a food-processor before comminution in Megatron (process M); a Fitzmill (process F) or a Stonemill (process S). The comminuted slurry was homogenized at treatment pressures of 68.95, 103.42, 137.90, 206.84, and 275.79 MPa using a CFHPT system. The soymilk at high pressure was heated to 80 ºC in a tubular heat exchanger prior to depressurization and a holding tube held the product at elevated temperature after throttling. To avoid flashing, back pressure was applied after the holding tube and soymilk was cooled immediately. Process M samples showed the smallest particle size and the highest apparent viscosity. All soymilk samples showed non-Newtonian pseudoplastic flow behavior. Ultrastructural images showed a clear protein network and very small fat globules were entrapped in the protein matrix. Particles were uniformly distributed when the highest pressure treatment was used in the slurries of process M. This process M sample at the highest pressure was considered the best treatment. Results of consumer acceptability of soymilk showed that more research is needed to determine what properties of soymilk from whole dehulled soybeans are important to consumer acceptability.

Key words: high pressure throttling, soymilk, particle size distribution, rheological properties, ultrastructural properties.

Introduction

Conventional homogenization, developed by Gaulin in 1899, has been extensively adopted in food applications, especially in dairy industry (Zamora and others 2007). Non-thermal treatments, which maximize the microbial reduction while retaining the desirable physicochemical properties of the product, are in growing demand in dairy industry. Applications of ultrahigh pressure homogenization (pressures more than 200 MPa) are mainly found in pharmaceutical and biotechnology sectors (Floury and others 2000). Other applications include formulation of fine food emulsions, disruption of dense cell microbial cultures and subsequent recovery of intracellular metabolites, inactivation of bacteriophages, and modification of functional properties of hydrocolloids (Thiebaud and others 2003). High pressure treatment attained much attention in recent years and foods treated with high pressure were reported to have superior color, flavor, nutrient retention, lower microbial count, and functional properties due to its non-thermal and non-chemical application (Peck 2004; Moorman 1997; Adapa and others 1997; Amornsin 1999; Areekul 2003). High pressure treatment improves protein gelation properties and has been successfully used in the food industry (Hoover 1993) for guacamole and oyster. The protein denaturation which occurs with high pressure treatment is a result of hydrophobic and ionic bonds breakage as opposed to covalent bond disruption, which results in off-flavors (Zipp and Kauzmann 1973; Knorr 1993). Pressurization studies (392-980 MPa) conducted on egg and yolk resulted in firm gels with natural taste and these gels with no loss of vitamins and amino acids were softer, more elastic, and more digestible than heat-treated gels (Hayashi and others 1989; Hoover 1993).

The time of exposure is usually in order of few minutes or more in HHP treatments whereas the residence time in the dynamic high pressure treatments is in the order of seconds (Thiebaud

and others 2003). The CFHPT process has been developed at The University of Georgia, Athens, Ga. Unlike the HHP processing system, the CFHPT system does not need large volume vessels as comparable lower pressure is used in CFHPT and therefore the initial and operating costs are lower (Moorman 1997; Amornsin 1999; Areekul 2003). CFHPT involves smaller volumes of fluid material capable of pumping readily without particle restriction occurring through orifices with very small clearance. In HHP, sterilization is possible when the product is pressurized at elevated temperatures, but the problem of slow and exposure of the product to that high temperature minimizes the quality advantages of the high pressure treatment. In CFHPT, the instantaneous heating of the homogenized liquid due to conversion of pressure energy to kinetic energy which occurs on depressurization of a fluid at high pressure minimizes the total thermal exposure needed for sterilization.

High shear rates can increase the surface area to volume ratio of suspended particles, which can increase viscosity of the fluid suspension leading to improved texture, taste, and flavor characteristics (Areekul 2003). CFHPT treated blueberry-whey beverage was preferred by sensory panelists over the heat treated beverage indicating that there is higher retention of flavors in the CFHPT processed beverage as opposed to the thermally-processed product (Peck 2004). Also, the shelf-life of the CFHPT beverage was lower as evidenced by superior product quality on day 35 compared to visibly spoiled thermally-processed product at day 65. Areekul (2003) conducted research on buckwheat and clover honey processing in CFHPT system at 275.8 MPa, exit temperature of 125.3 °C and 15 s hold before exiting to a cooler resulted in zero microbial counts (at both day 1 and 6 months of storage) and slightly elevated hydroxymethylfurfural (HMF) content but HMF was at an acceptable level after storage at 6 months at 4 °C. In addition to that, there was no significant difference in the physicochemical properties during storage of

CFHPT sterilized and low temperature heat-pasteurized (conventional pastuerization) honey. Processing of milk was done using CFHPT at 310 MPa by Adapa and others (1997) and milk produced good foams with high stability. Milk concentrates produced by CFHPT treatment resulted in increased surface tension and viscosity but decreased emulsion stability was thought to be due to increased protein-protein interactions, which result in larger protein aggregates and less protein migrating to the liquid-gas interface or adsorbing to the surface of fat globules. Study on ascorbic acid, pectin esterase activity, and limonin content in CFHPT processed (0, 137.9, 206.8, and 275.8 MPa pressures) citrus juice (initial temperature of 20 °C) was reported by Amornsin (1999) and results showed no evidence of ascorbic acid destruction even with the highest pressure treatment. Cloud of CFHPT processed orange juice was stable at least 21 days at pressure treatment >206.8 MPa when the juice was held 38.4 s in a holding tube after adiabatic heating before cooling. The same study reported no increase in the bitter tasting compounds represented by limonin content even at the highest pressure level with 118 s holding time before cooling as compared to the level present in the freshly squeezed grapefruit juice. Advantages of CFHPT processing include simultaneous homogenization and sterilization which can benefit physical properties, texture, and stability of the sterilized product during storage.

Soymilk has immense health benefits and it is one of the traditional nutritious beverages used for thousands of years in oriental countries. Lately the product became Western countries because of the perceived health benefits (Sloan 2005). Dry and mature soybeans are used for producing soymilk. Huge amounts of spent residue (okara – having 27% protein, dry basis) as waste by-product is formed in the manufacturing of soymilk and tofu and the disposal of the waste creates severe environmental problems (Chan and Ma 1999). One of the milestones in soymilk processing is the effective utilization of all cotyledon solids in the milk. The Illinois

process of producing soymilk which removed only 10-12% of water-soluble bean solids (in the soaking step) avoided production of okara (Nelson and others 1976; Kuntz and others 1978; Rosenthal and others 2003). The beany flavor of soy in food products is still a constraint for its complete acceptance among American consumers (Wilson 1996; Torres-Penaranda and others 1998). There were attempts to nullify this effect of lipoxygenase-produced off-flavor in soy food products by producing lipoxygenase-null soybeans and the results of sensory studies reported earlier (Wilson 1996; Torres-Penaranda and others 1998; Kobayashi and others 1995) showed that cultural differences are responsible for differences in perception of desirable sensory attributes for the consumption of the soy food products. Also, the need of using consumer from a targeted market is essential in the success of product development (Hollingsworth 1998). CFHPT treated blueberry-whey beverage was preferred by sensory panelists over the heat treated beverage indicating a the higher retention of flavors in the CFHPT processed beverage as opposed to the thermally-processed item (Peck 2004). Also, the shelf-life of the CFHPT beverage was higher as it was visible by its superior quality on day 35 as against the visible spoilage of the thermally-processed product at day 65. Standard soymilk processing uses thermal treatment of fine soy slurry from which the coarser bean particles (okara – having 27% protein, dry basis) have been removed (Chan and Ma 1999; Kumar and others 2003; Liener 1981; Lakshmanan and others 2006) by filtration or centrifugation.

While considering commercialization of pressurized soymilk, viscosity is of utmost importance (Cheng and others 2005; Lakshmanan and others 2006). The viscosity of soymilk is affected by its solid content which in turn is influenced by processing method and variety of the bean used in processing (Nelson and others 1976; Kuntz and others 1978; Kwok and Niranjan 1995; Iwuoha and Umunnakwe 1997; Mullin and others 2001; Cheng and others 2005).

Increased soymilk viscosity was observed with hull additions which decreased the flow behavior index and thereby increase deviation of soymilk flow properties from Newtonian behavior (Forster and Ferrier 1979). Information about variation in viscosity of a product with temperature and shear rates is imperative due to its exposure to diverse shear rates during industrial applications. This knowledge will also facilitate the design of equipment for its processing (Ditchfield and others 2004). An earlier study (Son and Singh 1998) on soymilk with different solids content showed that the power law model fitted the best and the effect of temperature on apparent viscosity could be expressed using the Arrhenius relationship.

Prevention of settling of solids in the soymilk plays a vital role in perceived soymilk quality. Emulsion stability can be improved by the forming phospholipids-protein complex which will suspend the solids because of reduction in density difference between the particles and the serum (Priepke and others 1980). Increasing the mutual attraction of hydrophilic phosphate groups and water is aided by having the particles coated with the phopholipids. CFHPT processing of blueberry-whey beverage had only soft feathery sediment (after 36 days of storage) which was dispersed with slight agitation in contrast to the tightly packed chalk-like sediment which was difficult to disperse even after vigorous agitation in the heat treated blueberry-whey beverage. Thus CFHPT treatment can effectively control stability of a beverage containing dispersed particles (Peck 2004). Effect of peptide profiles in thermal- and pressure-treated samples of soymilk was studied by Lakshmanan and others (2006) and results showed that the peptide profiles were not affected by the pressures though aggregates were observed in the soluble protein fraction. Another major impact of high pressure processing of soymilk in the same study was increased emulsion stability and decreased hydrophobicity. Roesch and Corredig (2003) reported that heated oil-in-water emulsions containing commercial fraction of soy protein

concentrate and soybean oil at 82 °C for 2 min before homogenization at 80 MPa displayed a gel-like behavior, was stable to creaming and there was no separation of solids during quiescent storage for 20 days at 4 ºC. To understand the protein-lipid distribution, it is necessary to use specific staining techniques for fat and protein in soymilk samples and observing the particles and matrix through confocal laser scanning microscope. Better understanding of the protein network and particle size can be obtained if the soymilk sample is viewed through a cryogenic scanning electron microscope.

Though numerous processes were reported for full utilization of soy solids in soymilk manufacturing, the industry still utilizes heat treatment of filtered soy slurry to assure safety, extend shelf-life, and to inactivate unwanted biologically active compounds such as trypsin inhibitors and lipoxygenase (Liener 1981; Kumar and others 2003). By eliminating the filtration or centrifugation step in processing of soymilk, all the solids in the soybean are incorporated in the product. Therefore, the objective was to make soymilk containing all the soybean solids using the CFHPT process. The effect of this process on rheological and ultra-structural properties of soymilk including particle size distribution, apparent viscosity (μ_{app}) and flow constants (flow behavior index, n and consistency index, K) was also elucidated. To study the consumer acceptability of CFHPT processed soymilk, the best experimental soymilk sample selected by the objectives described earlier was compared with a commercial soymilk sample [Silk® Unsweetened (shelf-stable)].

Materials and Methods

Soybean supply and CFHPT processing of soymilk

The soybeans used were Benning variety, Group VII cultivar soybean (*Glycine max* [L.] Merrill), harvested in 2005 from Davisboro, Ga, supplied by the Georgia Seed Development Commission, 2420 South Milledge Avenue, Athens, Ga was used. Closed polyethylene-lined bags were used for storing soybeans at 4 ºC and 20 %RH in the dark until it was processed into soymilk to minimize the changes in composition. Deionized water (DW) was used throughout the experiments to prepare soymilk. Silk® Unsweetened (shelf-stable) soymilk (WhiteWave Foods Company, Broomfield, CO 800021) was purchased from the local grocery store.

Processing steps involved in soymilk preparation are shown in Figure 4.1. The equipments for soymilk preparation were made of either stainless steel or plastic. The details of the machines used in the process are as follows: impingent dryer (Lincoln Impinger Model 1450, Lincoln Foodservice Products Inc., Fort Wayne, Indiana, USA), plate mill (Quaker City Mill Model 4E, QCG Systems, LLC, Phoenixville, PA, USA), food processor (Robot Coupe Model RSI 10V, Robot Coupe USA Inc., Joliet, IL, USA), Megatron at 13,000 rpm for 15 min (Model MTK 5000Q, Kinematica Inc., Cincinnati, OH, USA), Fitzpatrick mill with 0.127 cm and 0.0508 cm screen (Model JT, Fitzpatrick Co., Elmhurst, IL, USA), Stonemill (Model MKCA6-3, stone#MKE6-46, Masuko Sangyo Co. Ltd., Saitama, Japan). The process designations are: Megatron-CFHPT: process M, Fitzpatrick mill-CFHPT: process F, Stonemill-CFHPT: process S. Each of the processes included 5 levels of pressure treatment. The diagram of the CFHPT system is shown in Figure 4.2. The comminuted suspension was pressurized at 68.95, 103.42, 137.90, 206.84, or 275.79 MPa using two alternately acting pressure intensifiers (Hydropac P60-03CXS, Stansted Fluid Power Ltd., Stansted, Essex, UK) driven by a hydraulic pump in the Stansted

CFHPT system (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, UK) (Figures 4.3 and 4.4). The pressure levels indicated above were read from the pressure gauge located in the CFHPT system. It is to be noted that this pressure is a combination of the intensifier pressure and the pressure generated due to the narrow clearance of the throttling valve (model 60VRMM4882, Autoclave Engineers, Fluid Components, Erie, PA 16506-2302) (Figure 4.5) adjustments for the desired flow rate at each intensifier pressure. The inlet temperature of soymilk to the CFHPT system was kept at 30 ± 2 ºC. The CFHPT system (Figure 4.2) consists of a feed pump that maintains a constant pressure to the fluid feed to the intensifier, dual intensifier pistons that work alternately to take in fluid while the other discharges high pressure fluid, a heat exchanger heated with steam to increase the temperature of the high pressurized fluid product prior to throttling, a throttling valve to drop the pressure, a hold tube to hold the fluid a designated time prior to cooling, and a tubular heat exchanger to cool the product using cold water and ice as the coolants. Thermocouples were connected at steam heated tubular heat exchanger and at the end of holding tube (located after the throttling valve). The outputs of thermocouples were recorded on a Fluke Hydra Data Bucket (PO Box 9090, Everett, WA 98206-9090).

In all the treatments, a tubular heat exchanger heated the fluid after pressurization to 80 ºC. After throttling a minimum back pressure of 350 kPa was applied to avoid flashing of vapors at the outlet by raising the boiling point of the fluid. The minimum back pressure needed for each applied pressure (adiabatic temperature rise varied with applied pressure) was estimated from the saturated steam table (Toledo 2007) using the saturation temperature of water at the applied pressure. Between throttling valve and back pressure valve a holding tube permitted the fluid to remain at the elevated temperature after depressurization for a minimum of 5 s. Thus the adiabatic temperature rise after the throttling valve is exploited for microbial, biochemical, and

physical effects on the product. This elevated temperature was measured using a thermocouple at the end of the holding tube. The sterilized soymilk was immediately cooled to 4 ºC or below by passing it through another heat exchanger coil immersed in ice bath. The soymilk samples were collected at a volumetric flow rate of 1 L/min. After collecting the sample, it is kept in iced water in a cooler (< 4 °C) to further reduce its temperature and to avoid additional heat-induced changes in the product. The whole experiment was repeated on 3 independent occasions.

Measurements

All the soymilk treatment samples were analyzed for total solids (HR73 Halogen Moisture Analyzer, Mettler-Toledo, Inc., Columbus, Ohio), rheological properties using a dynamic stress-controlled rheometer (Rheometric Scientific model SR5000, Rheometric Scientific, Piscataway, N.J.) with concentric cylinder (couette) geometry using water bath heating/cooling device and steady stress sweep test for measuring μ_{app} and transient rate ramp test at a shear rate of 1000 s^{-1} for evaluating time-dependence (resistance to settling for a prolonged time at 10 ºC) behavior, and particle size distribution by integrated light scattering using Malvern Laser Particle Size Analyzer (Mastersizer S with 300mm lens, Malvern Instruments, Southborogh, Mass.). Apparent viscosity was measured at 4, 10 and 25 ºC. The temperature control at the tool point (couette) which is in contact with the sample was controlled with heating and refrigerating fluid in a water bath (model F25, Julabo USA, Inc., Kutztown, Pa.) attached to the rheometer and the heating / refrigerating fluid recirculates to the cup through the jacket in the rheometer for heating or cooling accordingly. The couette tool had a 32 mm diameter cup and a bob of diameter 29 mm. The bob length was 44 mm and approximately 16.3 ml of sample was required in the cup to fill it so that the sample meniscus remains within 5 mm height from the top of the cup-bob level when

the bob has descended into the cup. Duplicate measurements were obtained from each of the three treatment replications.

The non-Newtonian behavior of the soymilk was described by the power law model (equation 1) (Toledo 2007):

$$\tau = K(\gamma)^n \ldots\ldots (1)$$

where τ = shear stress (Pa), K = consistency coefficient (Pa.s^n), γ = rate of shear (s^{-1}), and n = flow behavior index. Pseudoplastic behavior is shown by fluids having $n < 1$ and dilatant behavior is shown by fluids with $n > 1$. For fluids having characteristics which fit equation 1, the apparent viscosity (Pa.s), μ_{app}, can be expressed in log transformation as:

$$\ln \mu_{app} = \ln K + (n-1)\ln \gamma \ldots.. (2)$$

For particle size distribution measurement, deionized water (approximately 100 ml) was used as dispersing medium for soymilk samples and measurements were taken at an appropriate obscuration (15 ± 2%) point reached in the diffractometer cell at an impeller speed of 2500 rpm. The optical model used to calculate the predicted scattering pattern with the refractive indices of the soymilk and water was based on the Mie theory of light scattering by spherical particles and was applied as follows: real refractive index, 1.47; imaginary refractive index, 0.00; refractive index of water, 1.33. The full size distribution was obtained using a polydisperse analysis. All the values for the average volume-weighted diameter, $D_{(4,3)} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$ (where n_i is the number of particles in a size class of diameter d_i), the surface-weighted mean diameter, $D_{(3,2)} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$, the diameter below which 99% of the volume of particles are found, $D_{(v,0.99)}$, the diameter below which 90% of the volume of particles are found, $D_{(v,0.9)}$, the diameter below which 80% of the volume of particles are found, $D_{(v,0.8)}$, the diameter below which 60% of the volume of particles are found, $D_{(v,0.6)}$, the diameter below which 50% of the volume of particles

are found, $D_{(v,0.5)}$, the diameter below which 40% of the volume of particles are found, $D_{(v,0.4)}$, the diameter below which 20% of the volume of particles are found, $D_{(v,0.2)}$, the diameter below which 10% of the volume of particles are found, $D_{(v,0.1)}$, from the size distribution were calculated by the instrument software. Six measurement values were obtained from the three replications.

Ultra-structural observations were carried out for treatments with pressure levels of 68.95 and 275.79 MPa at the Center for Ultrastructural Research, University of Georgia, Athens, Ga. Scanning electron microscope with a cold stage attachment (cryo-SEM) was used for examining the structural network of the samples. LEO 982 field emission scanning electron microscope (Zeiss SMT, Inc., Peabody, Mass.) equipped with an Oxford EDX detector (Oxford Intstruments, Inc. Concord, Mass.) and a Gatan Alto 2500 cryostage and cryoprep chamber (Gatan UK, Oxford, UK) was used for this. Soymilk sample (one drop) was placed on a specimen stub, covered with a stub cap, and rapidly frozen by immersing it in liquid nitrogen slurry (approximately -206 °C). The frozen sample in the stub was moved to the cryoprep chamber (vacuum) and was fractured with a knife to provide a fresh surface. Residual ice present on the frozen sample was removed by sublimation at -100 °C for 5 min. The fresh sample surface was then sputter-coated with gold to a thickness of approximately 20 nm, and placed in the sample chamber of the scanning electron microscope (SEM) on a cold stage maintained at -120 ºC. Then the samples were observed and images were taken. Also, Leica- TCS SP2 confocal laser scanning microscope (CLSM, Leica Microsystems Inc., Exton, Pa.) used in reflectance, transmittance and fluorescence mode was used to determine the distribution of fat and protein droplets. Fluorescence dyes specific for lipid globules (lipid soluble nile red fluorescent dye-NR, Sigma-Aldrich, St. Louis, Mo.) and protein fraction (fluorescein isothiocyanate – FITC,

Sigma-Aldrich, St. Louis, Mo.) were added to the samples separately. Working solutions of dyes were prepared as described by Damodaraswamy (2005). NR stock solution was prepared with 0.5mg/ml in acetone. Working solution for NR was made by combining 0.10 ml of NR stock solution and 100 ml of 75:25 glycerol-water mixture. Stock solution for FITC was prepared with 0.25% FITC in deionized water. The stock solution on further dilution to 1% in water gave the FITC working solution. The sample with the dye solutions on it (25 μL NR, 10 μL FITC, and 25 μL soymilk) was put on a pre-cleaned glass micro slide and a cover slip was placed over it. CLSM was set with a 63x (numerical aperture 1.4), oil immersion objective lens which offered good control in the z-direction as well as optical sections in both xy- and xz-directions. Separate images were taken for each dye in the same sample. Excitation wavelengths of 568 and 488 nm for fat and protein respectively was used by argon/krypton laser for the respective dyes. Fat globules were pseudo-colored red and protein particles were colored green.

Sensory evaluation

The soymilk processed using CFHPT at the highest pressure (275.79 MPa) was used for consumer acceptability test. Silk® Unsweetened (shelf-stable) (WhiteWave Foods Company, Broomfield, CO 800021) was used as the commercial sample. The panelists were the students and staff of Food Science and Technology Department. In order to determine the differences between the soymilks, experimental and commercial samples, a triangle test was performed. Thirty panelists from the Department of Food Science and Technology of The University of Georgia, Athens, Ga who had previous experience with the triangle tests performed the evaluation. Prior to the test, panelists received an orientation to the triangle test and about the samples. Twenty mL of the samples were presented in 1.18×10^{-4} m^3 (4 oz) white plastic cups coded with 3-digit numbers with lids on and sample temperature ~10 °C. Panelists were

instructed to taste each sample by swirling it in their mouths for 3 s, then swallow, and select the odd sample out of the three samples presented. For the first 15 panelists, the samples presented included one cup of experimental sample and two cups of commercial sample. After the first 15 panelists, the 3-digit numbers were changed, the samples presented included one cup of commercial sample and two cups of experimental samples. An equal number of 6 possible combinations of the 3-digit numbers for both the samples were prepared and presented in random order.

To study the purchase intent of the consumer on the soymilk, a consumer acceptability test was performed. One hundred panelists participated in the consumer acceptability test. The panelists were the students and staff of Department of Food Science and Technology of The University of Georgia, Athens, Ga. The panelists received an orientation about the test and the samples prior to the test. The panelists were instructed to taste the twenty mL samples presented in 1.18×10^{-4} m^3 (4 oz) cups coded with 3-digit numbers. Two samples, one experimental and one commercial sample, were presented to the panelists. The panelists were instructed to taste the each sample by swirling it in their mouths for 3 s, then swallow it and select the one with better mouthfeel and the preferred sample based on taste. They were asked to taste each sample and rate each sample from 1 to 5 where 1 stands for definitely would not purchase, 2 stands for probably would not purchase, 3 stands for might or might not purchase, 4 stands for probably would purchase, and 5 stands for definitely would purchase. Panelists were instructed to taste the samples separately and not to compare the samples with one another while rating it. The 3-digit codes were changed after the first 50 panelists. The order of presentation of the samples was changed after every 5 panelists.

The sensory tests were performed in the sensory booth in the Food Processing Research and Development Laboratory of the Department of Food Science and Technology at The University of Georgia, Athens, Ga. As it consisted human subjects, an approval was obtained from the Institutional Review Board of The University of Georgia, Athens, Ga, prior to the entire study was performed.

Statistical analysis

The whole experiment was triplicated. Three measurement values were obtained for all the properties from three replicated samples of the treatments and the data were interpreted statistically using SAS version 9.1 (SAS Inst., Inc., Cary, N.C.). Analysis of variance was performed to determine the significance of various processes and variables in soymilk. Means of each value were compared pair wise further using Student's t test to see the significance among the pairs. The results of the difference (triangle test) test and the consumer acceptability tests were analyzed for significance using analysis of variance.

Results and Discussion

Total solids content

Total solids content of the soymilk samples are given in Table 4.1. There was no significant difference in total solid content among the treatment samples. This observation shows that the milling machines used did not influence the solids content as milling was the only difference in sample treatments prior to CFHPT. Therefore, it can be considered that the rheological properties due to total solid content would not be affected by the milling treatments. Also, the application of a back pressure to retain the fluid in the liquid state and the product was cooled immediately before sample collection at the exit, avoided loss of moisture.

Particle size distribution

Since all the samples had the same solids content, the of physical properties exhibited by the soymilk can be attributed primarily to particle size distribution. Particle diameter $D_{(4,3)}$ and $D_{(3,2)}$ decreased significantly with the increase of applied pressure (Table 4.2). Application of higher pressures narrowed down the distribution of particle size (Figure 4.6). For both the parameters analyzed, the interaction of milling procedure used for comminution (M, F, or S) and pressure was significant ($p < 0.05$). Also, contribution of milling procedure in addition to the pressure applied by CFHPT system in the particle size reduction is evident from the above said interaction and results. It can be generalized that the size of suspended particles in the liquid food before its entry into CFHPT may not affect its final particle size obtained after the CFHPT processing. Figure 4.6 shows the particle size distribution of soymilk samples treated by the three processes at 275.79 MPa. Process M shows the narrowest particle size distribution. Processes F an S gave particle size distribution results which were not significantly different. This re-emphasizes the importance of the milling procedure used. An exceptional result on particle size reduction and narrowing of the size distribution was earlier reported by Peck (2004) where the CFHPT processed blueberry-whey beverage showed no aggregation of particles during storage in addition to the significantly smaller size offered by the treatment compared with thermally processed beverage. The particle size reduction observed in the current study can be attributed to the weakening of membranes in the individual particle due to high pressure. The weakened membrane easily ruptured from shear in the throttling valve, and the particle size distribution was narrowed due to the restricted opening in the micrometering valve. The cavitation that occurred after depressurization also aided in facilitating reduction of the particle size; and the turbulence at depressurization helped in mixing up the ruptured particles and distributing it

evenly. The results show that the high pressure throttling helped to decrease the size of solid particles, homogenize the particles within the suspending fluid and disperse it evenly. Also, there was no visible separation of the particles of soymilk treated at 275.79 and 206.84 MPa after a month of storage at 4 °C. This shows that the emulsion formed was stable. Puppo and others (2005) reported that there was 25-30% decrease in particle size of the droplet size distribution (surface frequency) of high pressure processed soy protein isolate dispersions of 10 g/l protein at pH 8 (50mM Tris-HCl buffer), together with a shift in droplet populations towards smaller size. Also, the extent of high pressure treatment was effective in reducing the particle size distribution curve from 10 μm to 3-5 μm for the other treatment emulsion at pH 3 (Glycine-HCl buffer 50mM).

Rheological parameters

Apparent viscosity of soymilk for all treatments is shown in Figure 4.7. Apparent viscosity significantly increased ($p < 0.05$) with increase in pressure levels while it decreased significantly ($p < 0.05$) with increase in temperature of analysis. This was an expected result; however, there was a third order significant interaction ($p < 0.05$) for apparent viscosity among the parameters: milling procedure (M, F, or S), pressure applied, and temperature of analyses. This shows the importance of the type of milling procedure used (pretreatment) and its influence in particle size reduction together with the pressure of CFHPT processing. The process M soymilk, as seen by the smallest particle size obtained, gave the highest apparent viscosity. This can be attributed to the increased number and surface volume of particles which added to the resistance to flow. The results are similar to those obtained by Forster and Ferrier (1979) and Adapa and others (1997). The reason behind the increase in apparent viscosity observed can be explained as follows with the help of earlier reports (Sharma and Dalgleish 1993; Rowney and others 2003; Cano-Ruiz and

Richter 1997). The homogenization that occurred during shear, turbulence, and cavitation helped in generating new fat globules of smaller size and dispersed them uniformly in the protein matrix. It is already seen that the size reduction of soymilk was increased with increase in the applied pressure. This reduced the availability of free fat globules as it was coated with protein aggregates to stabilize the expanded surface area and thus increased the apparent viscosity and thereby improve emulsification of soymilk with the pressurization process. Higher apparent viscosity shows that no additives are needed to increase the "body" sensory perception of the beverage for improved product mouthfeel.

The flow behavior of soymilk can be best explained by the power law model (equation 2). Soymilks obtained from all the processes were pseudoplastic ($n < 1$) and non-Newtonian. The pseudoplastic behavior is good for industrial applications as it may facilitate pumping of the beverage by requiring less force to pump the beverage at high speed due to lowered viscosity of the beverage at higher shear rates (Forster and Ferrier 1979; Son and Singh 1998). The flow behavior index (n) obtained for different treatments analyzed at different temperatures is shown in Figure 4.8. Though it showed slight decrease with increase in temperature, n was relatively constant (in the range 0.63 – 0.83) with temperature showing that the soymilk did not undergo chemical changes at these temperatures (Toledo 2007). No visible rheological change was observed after storage at 4 °C for one month for the soymilks treated at 275.79 and 206.84 MPa. This again shows the emulsion stability obtained by high pressure treatment. Peck (2004) reported Newtonian behavior for high pressure throttled blueberry-whey beverage. The n value ranged from 0.93 – 0.97 (which is very closer to 1) for that beverage and did not show any significant difference during storage. Son and Singh (1998) reported similar result for 13% and 16% solids soymilk, although Casson and power law models fitted good for 9% solids content

soymilk. Consistency index, K increased significantly with applied pressure (Table 4.3). This is similar to the trend shown by apparent viscosity. Arrhenius model was established between K and temperature (T) and also between apparent viscosity and T (Table 4.3). A straight line eaquation was obtained for each of the treatments. Consistency indices of CFHPT processed honeys (20.5 and 33.8 $Pa.s^n$ for buckwheat and clover honeys) were higher than that of CP honeys due to evaporation at the exit of throttling valve at temperature of 125.2 °C (Areekul 2003). Arrhenius model (Table 4.3) was fitted to predict K at different temperatures of the fluid. Peck (2004) reported K values ranging from 0.0039 – 0.0054 $Pa.s^n$ (at day 6 after processing) and from 0.0046 – 0.0066 $Pa.s^n$ (for day 36 after processing) for high pressure throttled blueberry-whey beverage. Though the author reported significant changes in the K values after storage, a failure to observe any real changes in rheological properties was attributed to the low concentration of proteins, pectins, and soluble solids.

Apparent viscosity changes in the soymilk over time were determined at a constant shear rate (1000 s^{-1}) at 10 ºC and the curve obtained is shown in Figure 4.9 for the soymilk obtained by process M at 275.79 MPa. During the first 3 min viscosity decreased, showing that the soymilk was thixotropic (shear-thinning), and it became relatively constant later on when the shear rate was kept constant as already shown by other authors (Forster and Ferrier 1979; Roesch 2002). This thixotropic behavior shows the beverage's great resistance to settling, enhancing its shelf life and improving mouthfeel. After 900 s rest period and a second trial on the same sample with same constant shear rate produced an identical curve which indicated that the shear-induced structural changes on the soymilk were reversible. Roesch (2002) reported increasing viscosity with increase in soy protein concentrate (SPC) in 10% and 6% oil-in-water emulsions at 0 and 10 days of storage. The emulsions exhibited shear-thinning behavior and hysteresis and the author

attributed the shear-thinning behavior to the presence of aggregated protein-protein droplets due to phase separations at high concentrations of SPC.

Ultra-structural properties

Figures 4.10 (a) and (b) show the images of soymilk from process M using cryogenic scanning electron microscope (cryo-SEM). At 275.79 MPa of process M (Figure 4.10 (a)) particles are of uniform size throughout the area of the image. The continuous network can be attributed to the rearrangement of particles and formation of network structure due to CFHPT treatment at the highest pressure. Figure 4.10 (c) and (d) show the cryo-SEM image of process F soymilk at 275.79 MPa and 68.95 MPa respectively. The particles were still in suspension even in products subjected to the lowest pressure treatment (Figure 4.10(d)). For process F at the highest pressure (Figure 4.10 (c)) shows that the network structure is not uniform. There was no apparent coalescence of fat globules (as evidenced by fat separation) in any of the soymilk samples. This can be explained by the formation of interfacial layer of protein aggregates in the newly formed fat surface reducing its hydrophobicity and thus increasing emulsion stability. This explanation and results were in agreement with earlier reports (Dickinson and Stainsby 1988; Molina and others 2001) which explained that because of the globular aggregated structure of soy proteins, they do not unfold and adsorb at the interface, but rather form a thick interfacial layer, which acts as a physical barrier to coalescence. Increase in solubility and surface hydrophobicity of soy protein isolate emulsion at pH 3 was reported by Puppo and others (2004). In another study, a better potential of protein adsorption was suggested due to the displayed decrease in bridging flocculation (Puppo and others 2005). This bridging was described as the adsorption of proteins in two different droplets during emulsion formation and resulting reduction in bridging flocculation was due to increased adsorption of proteins in oil-water

interface with increased pressure application. Cryo-SEM images contributed in identifying the network structure of soymilk formed as a result of pressurization. From the images, process M processed at 275.79 MPa can be considered as the best treatment.

The theory of entrapped fat globules in the protein network is obvious in the confocal scanning laser microscope (CSLM) image (Figure 4.11) which shows the distribution of fat and protein in soymilk. The protein particles could not be identified individually due to the very small particle size and the even distribution of the protein network as observed in cryo-SEM images (Figures 4.10 (a), (b), (c) and (d). The fat globules were also very small in size as seen in the Figure 4.10. The image shows that the fat particles are entrapped in the network (which is obvious in cryo-SEM images) and the size of the fat particles was very small (~5 μm) at highest pressure applied. However, images of soymilk at the lowest pressure (images not shown) showed separated and bigger sized fat globules with uneven distribution. This is in agreement with the cryo-SEM images of the same treatments. Walstra (1983) explained that under the turbulent conditions that occur during homogenization, more dense materials are displaced by convection movements and then the adsorption of aggregates predominate. The microstructure observations of the different components of commercial soy protein concentrate (SPC) in oil-in-water emulsions helped them to understand that the viscoelastic properties of the emulsions were a result of the segregation of oil droplets in protein network due to the incompatibility between the protein and the oil phase. Puppo and others (2004) reported that the high pressure treatment induced physicochemical changes of soybean proteins at pH 8 with increase in surface hydrophobicity, a partial unfolding of 7S and 11S fractions, and an aggregation of protein (with 11S fraction). The physicochemical changes in soymilk due to pressurization and theory of protein aggregation were reinstated by Puppo and others (2005). They could explain that the

combined effect of increase in surface hydrophobicity, partial denaturation, and more disordered structure were giving a better opportunity for adherence at the oil-water interface. This gave a clearer picture to why size reduction and the increase of the percentage of adsorbed proteins increased with high pressure treatment levels in the present study. The emulsifying activity index of 7S and 11S fractions of soybean proteins at pH 7.5 was increased with high pressure treatment of 200-600 MPa (Molina and others 2001). It is assumed that, in the present study, there may be some drying of the carbohydrate molecules and interaction might have taken place between carbohydrate and the dye(s) used for staining. As the carbohydrate molecules were not specifically stained, this assumption still needs research before coming to a conclusion.

Difference test

The results showed that 20 panelists were able to identify the odd sample correctly. This was significantly different from the incorrect judgments. A similar result on difference test with soymilk was reported earlier by Torres-Penaranda and Reitmeier (2001) who reported 17 correct judgments out of 35 panelists.

Consumer Acceptability

Better mouthfeel and taste preference scores were significantly different ($p < 0.05$) between the samples (Figure 4.12). The experimental sample had no ingredients added to increase its apparent viscosity or flavorant added to mask its beany note. A beany flavor no matter how slight can be easily identified by consumers who are not used to soymilk flavor. This observations similar that reported earlier for soymilk Torres-Penaranda and Reitmeier (2001) in which a commercial sample was used to compare with the experimental soymilk samples prepared from normal and lipoxygenase-free soybeans. Torres-Penaranda and Reitmeier (2001) found a significant difference in mouthfeel (astringent) score with 7 for commercial soymilk

sample while it was 37 on a 0 – 100 intensity scale for the experimental sample made from normal soybean. Also, the beany flavor (grassy) was significantly different with a score of 1 for the commercial sample compared to the score 38 for the experimental sample from normal soybeans. In the Figure 4.12, the results are shown in which a purchase-intent score of 4 or 5 was considered acceptable. The experimental and commercial samples were both rejected by the consumer panel, but it favored the commercial sample. Future studies should focus on and would be unlikely to reject soy products.

Conclusion

The results suggest that soymilk containing all the solids in the soy can be produced by softening the cotyledons by hydration, comminution, and CFHPT processing. The product properties and particle size distribution were a function of the processing parameters. CFHPT processing helped to decrease the size of solid particles and disperse it evenly and thereby improving the rheological and ultra-structural properties of soymilk to prevent settling of solids during storage. The size of particles plays an important role in the homogeneity of soymilk and its properties; the particle size of soymilk is reduced and a narrow distribution was obtained when the pressure applied was increased. More number of particles created through CFHPT processing gave increased apparent viscosity to soymilk. This will help in improving the beverage's mouthfeel without additional ingredients for increasing the thickness of the fluid. The ultrastructural images showed the protein network and the entrapped small fat globules at the highest pressure. Thus process M soymilk at the highest pressure can be considered as the best treatment. Even though the experimental soymilk contain all of the health-functional compounds in the soy, flavor modifying ingredients in the commercial soy product used as a reference has affected panelists'

perception of the quality of product from the CFHPT process. Consumer acceptability test showed that more research is needed to appeal the taste of American consumer before venturing into the commercial scale production of soymilk. Soymilk with all the essential soy solids can be made available to the public and processors can benefit from the high processing yields since there is no waste in the process.

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Table 4.1 - Total solids of soymilk from different treatments.

Process	Pressure (MPa)	Total solids (%)	Process	Total solids (%)	Process	Total solids (%)
M	68.95	8.17 ± 0.06[a]	**F**	8.10 ± 0.06[a]	**S**	8.12 ± 0.11[a]
M	103.42	8.24 ± 0.07[a]	**F**	8.11 ± 0.08[a]	**S**	8.09 ± 0.13[a]
M	137.9	8.26 ± 0.11[a]	**F**	8.15 ± 0.06[a]	**S**	8.15 ± 0.06[a]
M	206.84	8.18 ± 0.02[a]	**F**	8.20 ± 0.06[a]	**S**	8.20 ± 0.11[a]
M	275.79	8.19 ± 0.05[a]	**F**	8.15 ± 0.06[a]	**S**	8.20 ± 0.03[a]

The values are mean and SD from 3 independent experiments. Mean values in a column not followed by the same letter were not significantly different ($p < 0.05$).

Table 4.2 - Particle size diameter obtained for different pressure level treatments of process M.

Pressure (MPa)	**$D_{(v, 0.10)}$ (µm)**	**$D_{(v,0.20)}$ (µm)**	**$D_{(v,0.40)}$ (µm)**	**$D_{(v,0.50)}$ (µm)**	**$D_{(v,0.60)}$ (µm)**	**$D_{(v,0.80)}$ (µm)**	**$D_{(v,0.90)}$ (µm)**	**$D_{(v,0.99)}$ (µm)**	**$D_{(3,2)}$ (µm)**	**$D_{(4,3)}$ (µm)**
275.79	7.19	9.01	13.37	15.85	18.75	27.17	34.97	54.04	12.94	18.73
	(0.54)	(0.60)	(0.98)	(1.22)	(1.44)	(2.45)	(3.67)	(7.75)	(1.03)	(1.66)
206.84	7.46	9.63	14.72	17.57	20.80	29.70	37.62	56.18	13.79	20.31
	(0.23)	(0.57)	(1.21)	(1.50)	(1.84)	(2.53)	(3.11)	(4.68)	(0.72)	(1.53)
137.90	8.75	12.05	18.49	22.00	25.99	36.54	46.07	68.35	16.87	25.07
	(0.86)	(1.43)	(2.01)	(2.27)	(2.47)	(2.86)	(2.99)	(3.25)	(1.67)	(2.11)
103.42	10.10	14.65	22.82	27.13	31.85	43.97	54.44	78.96	19.82	30.11
	(1.09)	(1.66)	(2.47)	(2.81)	(3.15)	(4.00)	(4.65)	(5.85)	(1.84)	(2.84)
68.95	10.35	15.66	25.52	30.69	36.36	51.06	63.69	92.08	20.91	34.30
	(1.95)	(2.74)	(3.69)	(4.14)	(4.67)	(6.02)	(7.27)	(10.01)	(3.00)	(4.38)

The values are mean and SD (in parenthesis) from 3 independent experiments.

Table 4.3 – Arrhenius equation obtained for soymilk for different CFHPT treatments.

Process	Pressure (MPa)	Arrhenius Equation	
M	**68.95**	lnK=7936.50(1/T)-21.20	$\ln(\mu_{app})$=53720.00(1/T)-173.40
M	**275.79**	lnK=26733.00(1/T)-73.70	$\ln(\mu_{app})$=79016.00(1/T)-253.30
F	**68.95**	lnK=7131.00(1/T)-21.10	$\ln(\mu_{app})$=42605.00(1/T)-136.60
F	**275.79**	lnK=13740.00(1/T)-35.60	$\ln(\mu_{app})$=64013.00(1/T)-206.30
S	**68.95**	lnK=8191.50(1/T)-25.10	$\ln(\mu_{app})$=49738.00(1/T)-161.70
S	**275.79**	lnK=10812.00(1/T)-25.70	$\ln(\mu_{app})$=64207.00(1/T)-206.80

The values are mean from 3 independent experiments.

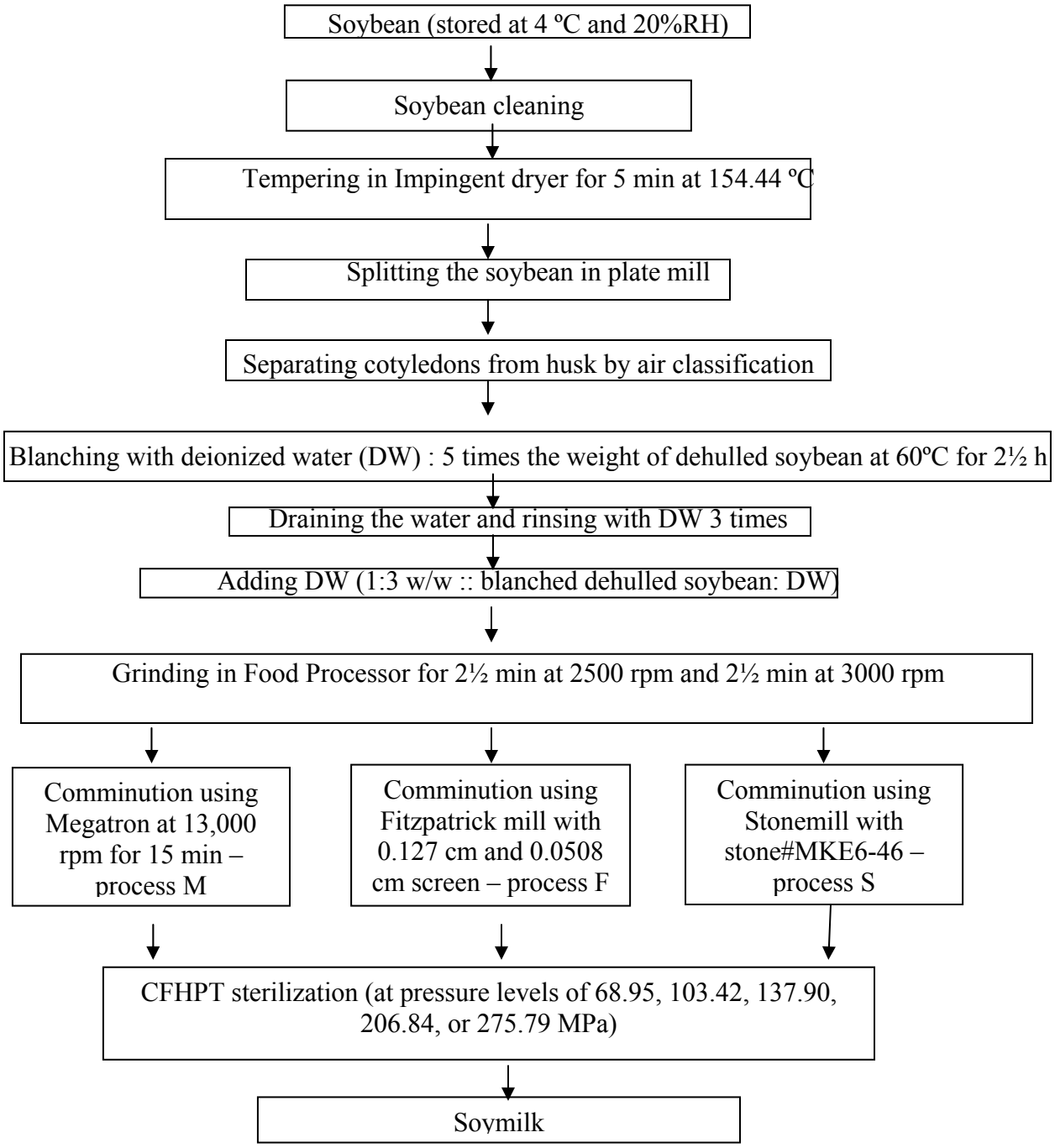

Figure 4.1 - Flow diagram for preparation of soymilk from whole dehulled soybeans.

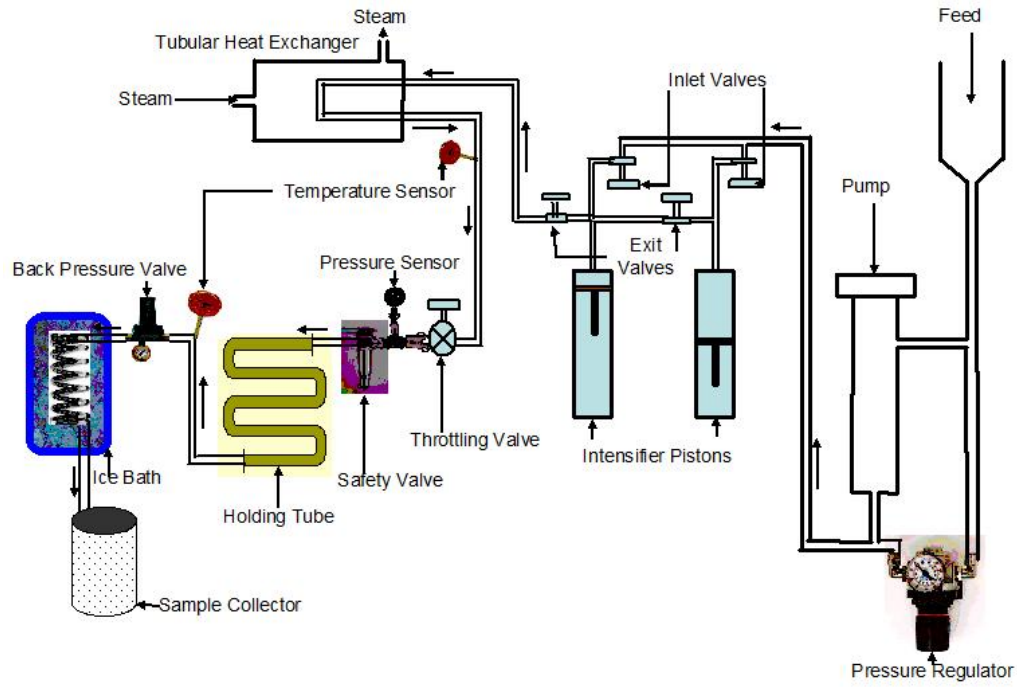

Figure 4.2 – Diagram of CFHPT system showing the flow direction of liquid food and parts.

Figure 4.3 – Inside view of Stansted CFHPT system (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, UK) with throttling valve (white arrow), intensifiers (dashed white arrows), inlet to CFHPT (black arrow), back pressure valve (light grey arrow), inlet valves to intensifiers (grey arrows), and outlet valves from intensifiers (dashed gray arrows).

Figure 4.4 – Outside view of Stansted CFHPT system (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, UK) with thermocouple attachment to Fluke Hydra Data Bucket (PO Box 9090, Everett, WA 98206-9090). Data bucket (white arrow), holding tube (light grey arrow), thermocouple connected after holding tube (black arrow), and ice bath (white dashed arrow) are shown with arrows.

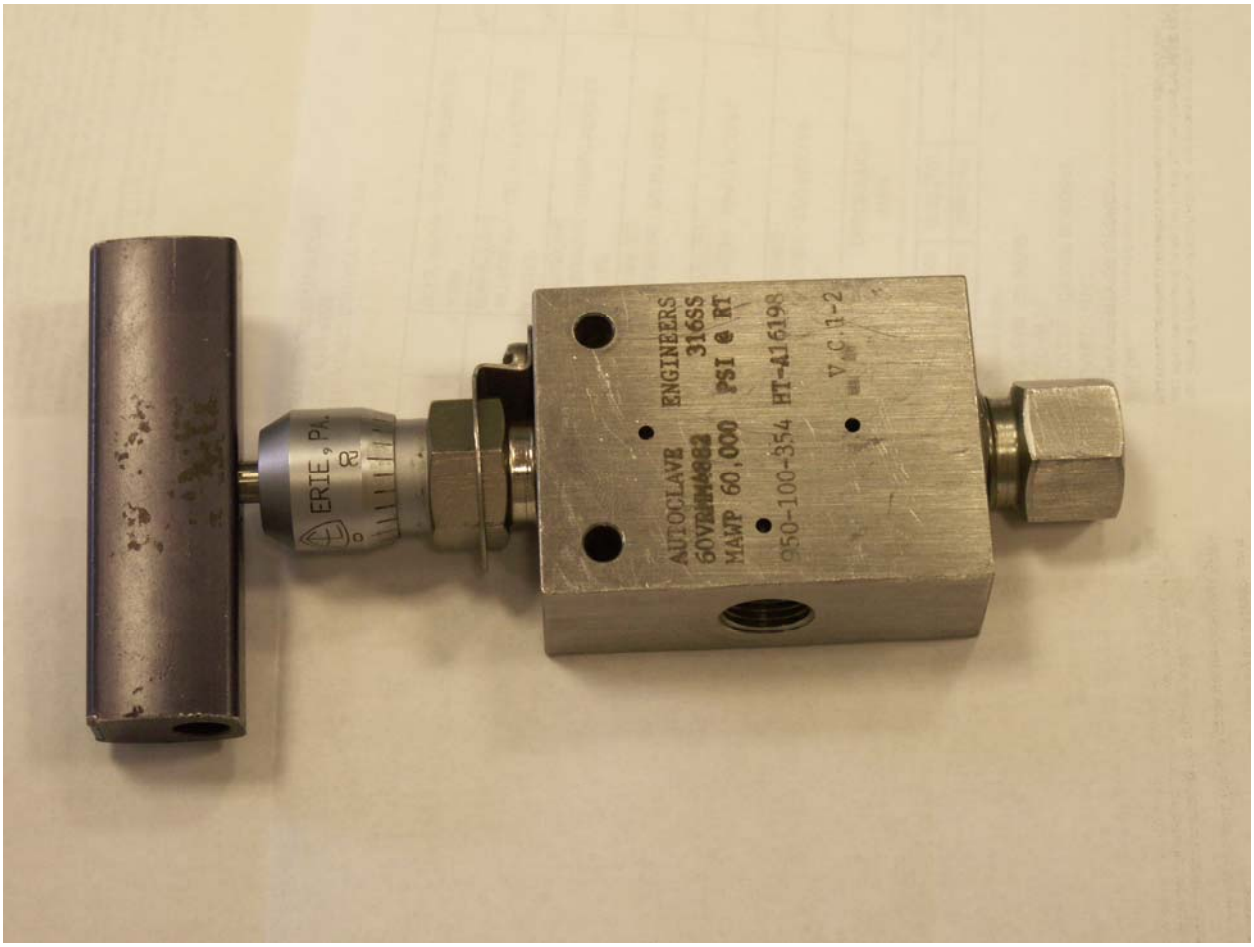

Figure 4.5 – Throttling valve (model 60VRMM4882, Autoclave Engineers, Fluid Components, Erie, PA 16506-2302).

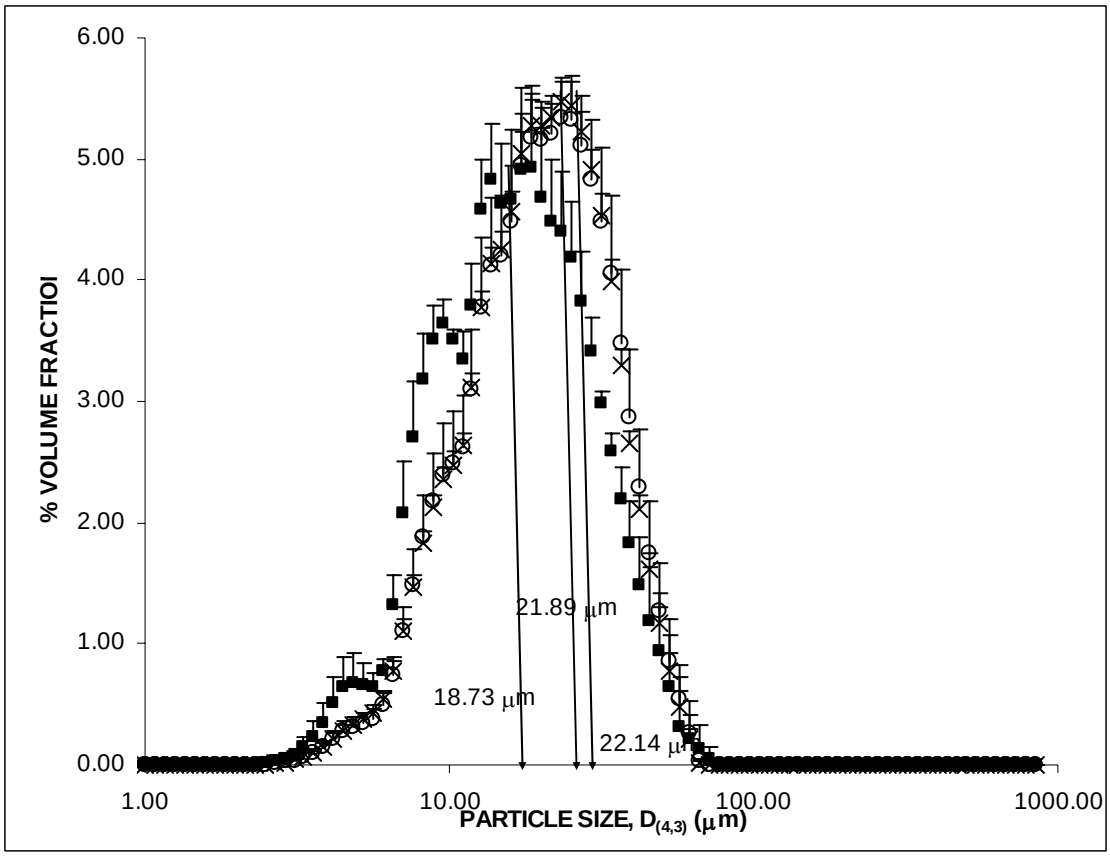

Figure 4.6 - Particle size diameter $D_{(4,3)}$ and distribution of soymilk processed at 275.79 MPa. Process symbols are M - ■, F - ×, S - ¤.

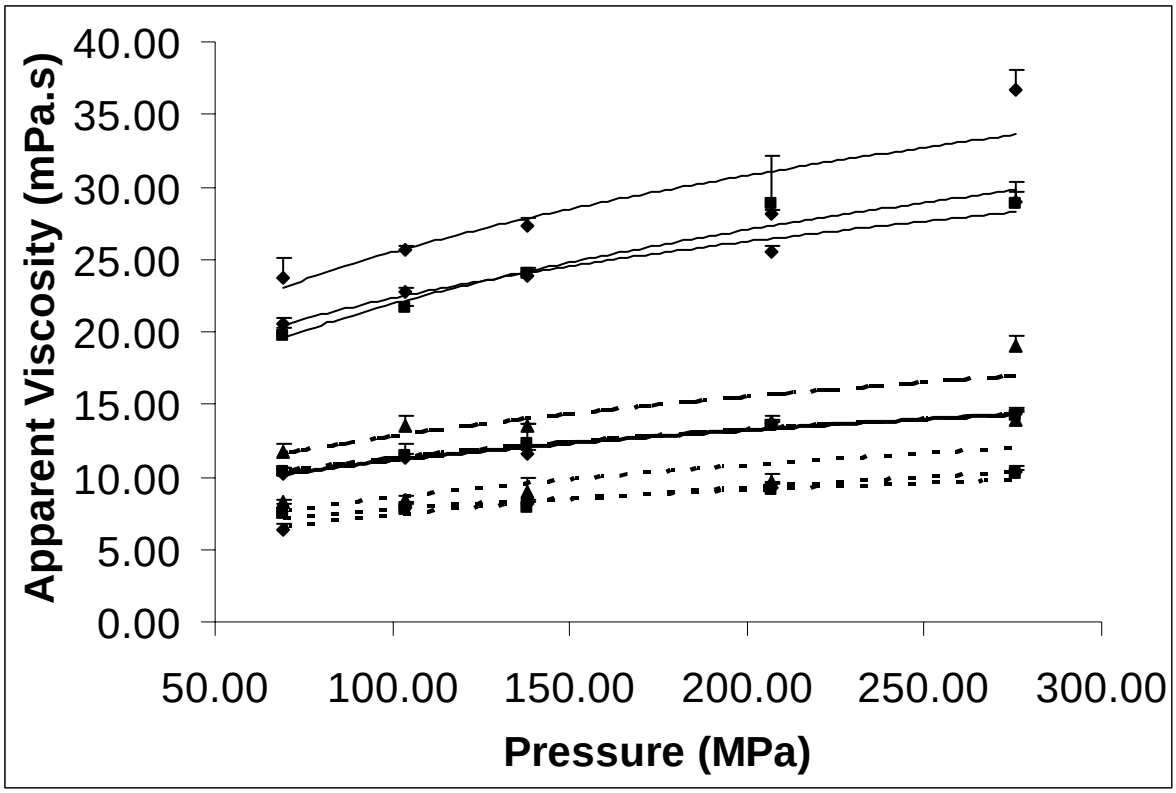

Figure 4.7 - Apparent viscosity of soymilk analyzed at different temperatures. At 25 °C: process M (- - ▲ - -), R^2 = 0.73, process F (- - ■ - -), R^2 = 0.89, process S (- - ♦ - -), R^2 = 0.97; at 10 °C: process M (__ __▲ __ __), R^2 = 0.73, process F(__ __ ■ __ __), R^2 = 0.99, process S (__ __ ♦ __ __), R^2 = 0.97; at 4 °C: process M (____▲____), R^2 = 0.83, process F (____■____), R^2 = 0.96, process S (____♦____), R^2 = 0.97.

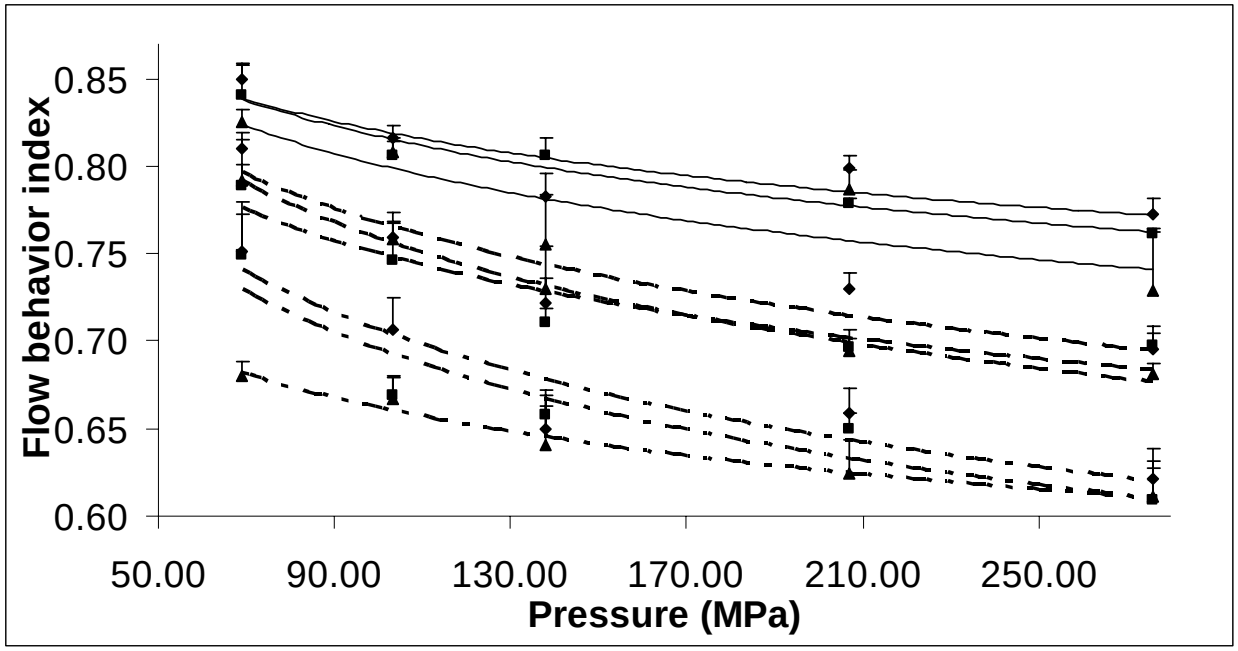

Figure 4.8 – Flow behavior index of soymilk analyzed at different temperatures. At 25 °C: process M (- . - . ▲ . - . -), R^2 = 0.98, process F (- . - . ■ . - . -), R^2 = 0.88, process S (- . - . ♦ . - . -), R^2 = 0.89; at 10 °C: process M (- - ▲ - -), R^2 = 0.99, process F (- - ■ - -), R^2 = 0.89, process S (- - ♦ - -), R^2 = 0.87; at 4 °C: process M (____▲____), R^2 = 0.70 process F (____■____), R^2 = 0.96, process S (____♦____), R^2 = 0.78.

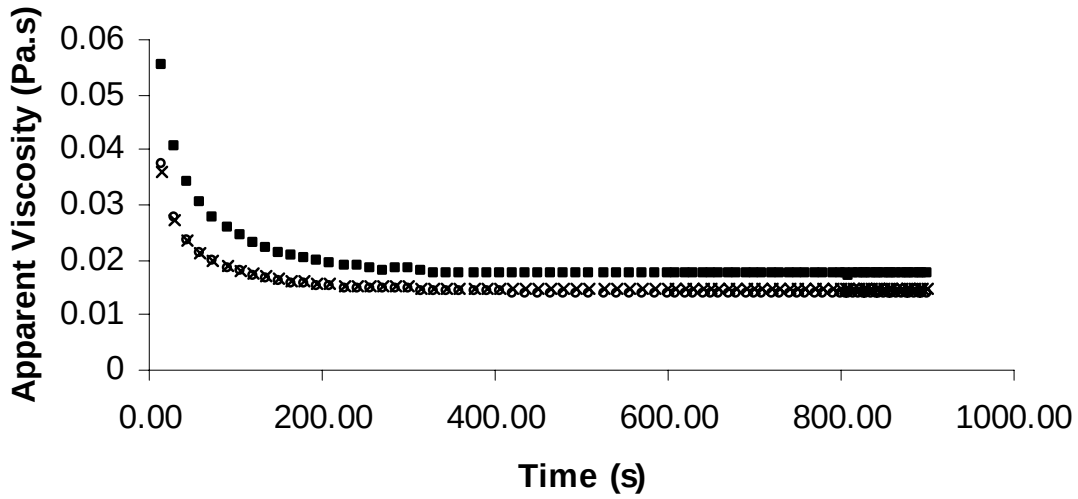

Figure 4.9 - Thixotropic behavior of soymilk at constant shear rate of 1000 s^{-1} from the three various processes at 275.79 MPa. Symbols represent the following: Process M - ■, process F - ×, and process S - ¤.

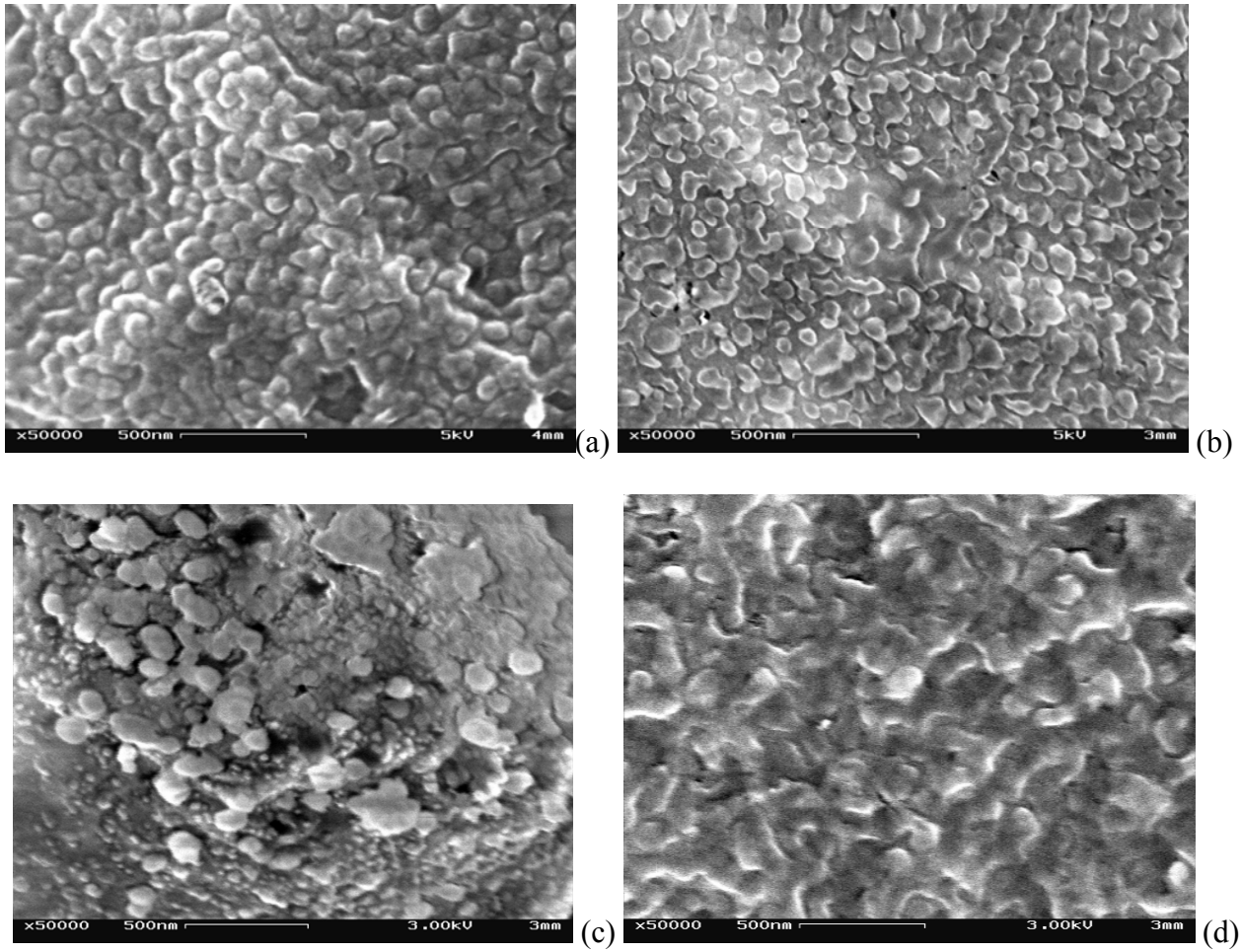

Figure 4.10 - Cryo-scanning electron microscope images of soymilk from process M, (a) 275.79 MPa (b) 68.95 MPa, and from process F (c) at 275.79 MPa (d) at 68.95 MPa. The protein-protein network structure is visible in image (a) while the particles were yet to form a protein matrix in image (b) which is process M at lowest pressure. The comparison of process F soymilk image at the lowest pressure shows that the particles were not having a network at this treatment whereas image at the highest pressure treatment of process F (c) shows the network is not uniform.

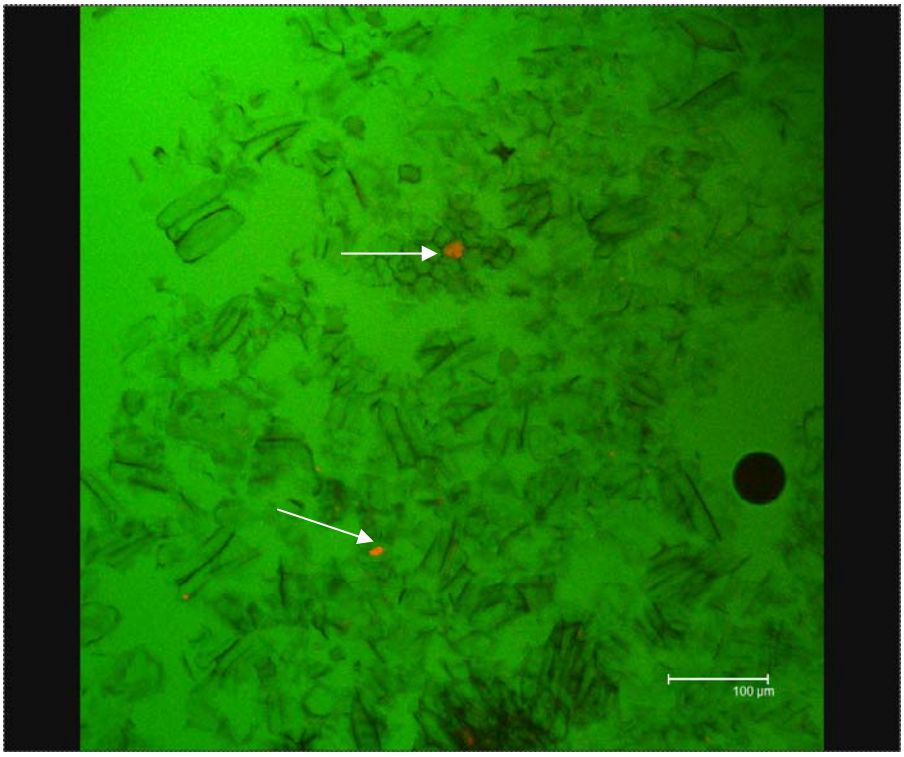

Figure 4.11 - Confocal scanning laser microscope images of soymilk from process M at 275.79 MPa – red particles (arrows) show fat globules and green particles show protein. The protein particles are unable to be identified from the image as the particles are homogeneously distributed with very small fat globules entrapped in the structure.

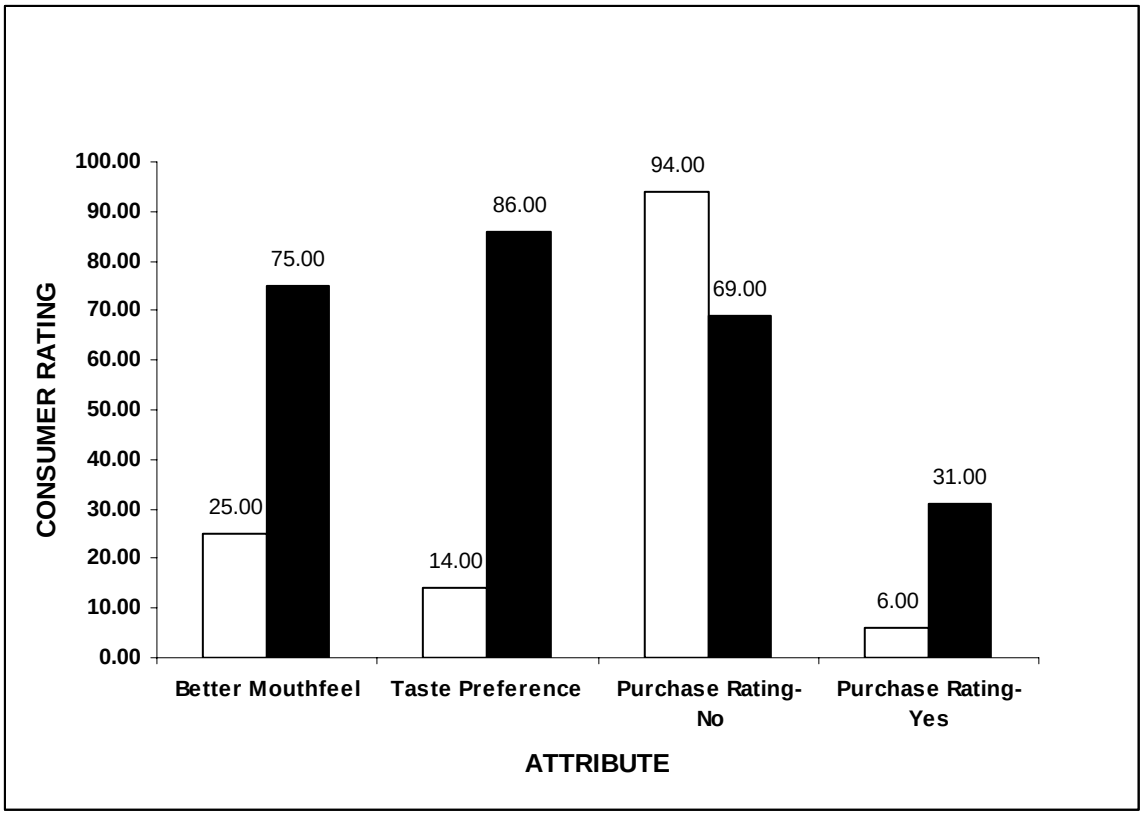

Figure 4.12 – Results of consumer acceptability of experimental soymilk (□) sample and control (Silk Unsweetened - ■).

CHAPTER 5

MODELLING THE EFFECT OF PROCESSING PARAMETERS OF CONTINUOUS FLOW HIGH PRESSURE THROTTLING SYSTEM AND MICROFLUIDIZER ON PARTICLE SIZE DISTRIBUTION OF SOYMILK

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Abstract

Texture, appearance, and emulsion stability of soymilk are affected by the size and distribution of suspended particles. To produce soymilk which contains all solids in the soy, whole dehulled beans were used in the study. A microfluidizer with throttling valve attachment was used to study the influence of various pressure levels on the particle size distribution of soymilk. Soymilk was processed with a continuous flow high pressure throttling (CFHPT) system to study the effects of different pressures and flow rates in the temperature rise and particle size distribution of soymilk. The results showed that there existed a significant effect of flow rate through the CFHPT on the particle size suspended in soymilk. Temperature rise was proportional to increase in pressure of the CFHPT system. Significant decrease in particle size of soymilk was obtained by increasing pressure for both CFHPT and microfluidizer. Empirical models were established between the pressure applied, volume fraction of particle size, and particle size diameter obtained for soymilk in each system.

Key words: high pressure throttling, microfluidizing, soymilk, particle size distribution.

Introduction

The consumer demand for non-thermally treated foods increased in last few years because of impaired physicochemical properties of the processed food while maximizing the microbial destruction (Zamora and others 2007; Rastogi and others 2007). Non-thermal processes were shown to retain or improve in the food properties such as color, flavor, nutrient retention, lower microbial count, and functional properties were reported earlier for foods treated with high pressure (Moorman 1997; Adapa and others 1997; Peck 2004; Amornsin 1999; Areekul 2003). The time of in-process exposure is less in dynamic high pressure treatments compared to the high hydrostatic pressure (HHP) processing. The continuous flow high pressure throttling (CFHPT) system uses a micrometering valve to restrict the flow of pressurized fluid. The CFHPT process has been developed at The University of Georgia, Athens, Ga (Moorman 1997). Unlike the HHP processing system, the CFHPT system does not need large volume vessels as comparable lower pressure is used in CFHPT and therefore the initial and operating costs are lower (Moorman 1997; Amornsin 1999; Areekul 2003). When the liquid food exits from the micrometering valve, it is depressurized to atmospheric pressure and instantaneous temperature rise occurs due to the conversion of pressure energy to heat energy. This temperature rise, in CFHPT, is proportional to the pressure applied to the material being processed (equations 1, 2, and 3). The combination of instantaneous temperature rise, impacts of pressure, high turbulence, exposure to hydrodynamic cavitations, impingement against static surfaces, and high shear rates contribute to microbial inactivation by means of disrupting cell membrane integrity (Lopez-Pedemonte and others 2006; Areekul 2003; Moorman 1997; Floury and others 2000; Amornsin 1999; Zamora and others 2007; Floury and others 2004). The product is very quickly cooled after holding to prevent over processing of the product which may lead to a reduction in overall

quality (nutrition and sensory). The temperature rise after throttling can be explained by the first law of thermodynamics (Amornsin 1999; Toledo 2007). Pressure difference between the inlet (higher pressure) and outlet (lower pressure) of the throttling valve represents the energy conserved in the form of heat energy at the exit of the throttling valve which is manifested in the temperature rise of the product. This change in potential energy due to high pressure to heat energy due to reduction in pressure across the throttling valve can be given by the equations given below (Toledo 2007; Amornsin 1999):

$$T_{out} = T_{in} + \frac{(P_{in} - P_{out})}{\rho C_p} \quad \text{.....(1)}$$

where q = energy per unit mass (J/kg), C_p = specific heat of the fluid at constant pressure (J/kg.°C), T_{out} = temperature of the fluid at the outlet of the throttling valve (°C), T_{in} = temperature of the fluid at the inlet of the throttling valve (°C), P_{in} = Pressure of the liquid at the inlet of throttling valve (Pa), P_{out} = Pressure of the liquid at the outlet of throttling valve (Pa), assumed as atmospheric pressure, ρ = density of the fluid (kg/m^3). The initial temperature or the temperature to which the fluid is heated in the tubular heat exchanger (T_{in}) affects the exit temperature and thereby the temperature rises in all of the equations given above. Flow rate can be maintained at the exit for product collection and therefore temperature variation due to volumetric flow rate difference is avoided. However, the heat loss due to convection (to the surroundings) and conduction (through the connecting pipe to cooling heat exchanger) further reduce the measured temperature (experimental temperature) at the exit of the holding tube (after throttling valve) from the theoretical results (Amornsin 1999).

Microfluidization is a homogenization technique developed by Cook and Lagacé (1985) in which the liquid food in a high pressure chamber is split into two streams in the interaction

chamber and collides with each other at high speed at a 180 ° angle (Lemay and others 1994; Tunick and others 2002). Smaller particle size and narrower particle size distribution was exhibited by the foods processed by this process. Paquin (1999) explained that the size reduction observed was due to the cavitation which resulted from collisions of the fat globules when the two streams were united. Microfluidizers are produced by Microfluidics, Inc., Newton, MA. Moorman (1997) studied the microbicidal effect of CFHPT treatment, microfluidizer treatment, and microfluidizer/throttling treatment. The microfluidizer used a double–acting electro-hydraulic pressure intensifier to force the process fluid through the constriction which is in the interaction chamber with downstream back pressure module. This elevated the temperature of process fluids from an entrance temperature of 4 °C to ~85 °C, and an in-line cooling coil with built-in water cooling coils helped to lower the temperature to 40 °C within 3.4 s. The hybrid process of CFHPT and microfluidization introduced a micrometering valve (which was used in CFHPT process) in place of interaction chamber in the microfluidizer. However, the other parameters used such as flow rate (9.2 L/s), operating pressure 276 MPa maximum), cooling coil (12 °C water jacket), and cooling time (3.4 s) are the same as that of microfluidizer with interaction chamber. Minimum pressure between the strokes of the dual-acting intensifiers while it changed it directions was compared among the processes and it was registered as 151 MPa in CFHPT (for a desired pressure of 310 MPa) and 220 MPa in both the microfluidizing treatments (for a desired pressure of 276 MPa). This difference was due to the difference in pressure intensifiers action: microfluidizer used Microfluidics pressure intensifiers in which pressure was controlled by piston stroke and cycle time to achieve the desired pressure whereas the CFHPT treatment used Hydropac pressure intensifiers where a constant stroke and cycle time was maintained and the desired pressure was achieved by varying the opening of throttling valve. The

results showed that microfluidizer and microfluidizer/throttling treatments were more effective than the CFHPT process and the lower microbicidal effect of CFHPT process was attributed to the variation in the pressure attained between the intensifiers and the throttling valve. However, the author suggested improving the process by lowering this pressure variation, so that low pressure fluid flow through the throttling valve could be minimized.

Amornsin (1999) studied the relation between pressure oscillation amplitude in the pressure gauge of a Microfluidizer Processor (model M-140K, Microfluidics International Corporation, Newton, Mass.) intensifier (which works similar to CFHPT with a throttling valve attachment) and the product flow rate. Reducing the intensifier hydraulic fluid flow pressure slowed down the rate of forward movement of the intensifier piston and thereby reduced the rate of delivery of the pressurized product and dampens the oscillation amplitude of pressure fluctuation as seen in the pressure gauge. To achieve the target pressure, a combination of intensifier pressure and throttling valve adjustment was used. So, the amplitude of pressure oscillation and flow rates are to be carefully monitored so that the system will stay close to the target pressure and the processing of fluids could be done at the same target pressure. Pressurization was done upto 310 MPa using a pressure intensifier and conveyed through stainless steel tubing to throttling valve where it was throttled to atmospheric pressure. Even when the throttle valve was in completely closed position, the flow still occurred (Amornsin 1999). According to manufacturer's data for water, the targeted pressure (310 MPa) was attained by decreasing the flow rate by adjusting the throttling valve which alters the orifice diameter from a fully-opened position of 1.5748 mm to a nearly-closed position of 0.1556 mm. The maximum shear rate (-dV/dr) calculated (using equation 3) (Toledo 2007) with a mean fluid velocity (V_{av}) in the orifice of 284 m/s (using

equation 2, (Toledo 2007)), with nearly closed diameter (R/2) for throttling valve, and fluid flow rate (q) of 5.4×10^{-6} m^3/s was 14.6×10^{-6} s^{-1}.

$$V_{av} = \frac{q}{\prod R^2} \text{ …..(2)}$$

$$-\frac{dV}{dr} = \frac{4V_{av}}{R} \text{ …..(3)}$$

High shear rates can increase the surface area to volume ratio, which can increase viscosity of certain fluid materials leading to improved texture, taste, and flavor characteristics (Areekul 2003).

Soymilk is in growing demand in Western countries too due to its immense health benefits and it is one of the traditional nutritious beverages used for thousands of years in oriental countries (Sloan 2005). Several processes were developed for soymilk processing to include the whole bean solids in the product (Nelson and others 1976; Hand and others 1964; Mustakas and Mayberry 1964). One of the milestones in the development of processes for producing soymilk from the whole soy is the use of CFHPT system at different pressures and flow rates. There is a need to estimate the particle size reduction obtained in whole bean milk and to establish an empirical relation between the processing parameters and particle size. Soymilk processing with effective utilization of cotyledon solids in the milk was first adopted in the Illinois process where only 10-12% of water-soluble bean solids have been removed. There is no waste by product in this process (Nelson and others 1976; Kuntz and others 1978; Rosenthal and others 2003).

The inclusion of whole bean cotyledons solids in the soymilk is essential for increasing the consumer demand of this healthy drink. It is necessary to establish a relation between the pressure applied and the size of suspended particles in the liquid food processed using high

pressure throttling. The objectives of this study were: (1) to study the effect of microfluidizer pressure levels on particle size distribution of soymilk and to establish an empirical relation between the processing pressure and particle size and (2) to process soymilk using CFHPT system at different pressures and flow rates to estimate the particle size reduction and temperature rise obtained in whole bean milk and to establish an empirical relation between the processing parameters and particle size.

Materials and Methods

Soybean supply and CFHPT processing of soymilk

Benning variety, Group VII cultivar soybean (*Glycine max* [L.] Merrill), harvested in 2005 from Davisboro, Ga, and supplied by the Georgia Seed Development Commission, 2420 South Milledge Avenue, Athens, Ga was used in this study. Closed polyethylene-lined bags were used for storing soybeans at 4 ºC and 20 %RH in the dark until it was processed into soymilk to minimize the changes in composition. Deionized water (DW) was used throughout the experiments to prepare soymilk.

Processing steps involved in soymilk preparation is shown in Figure 5.1. The equipments for soymilk preparation were made of either stainless steel or plastic. The details of the machines used in the process are as follows: impingent dryer (Lincoln Impinger Model 1450, Lincoln Foodservice Products Inc., Fort Wayne, Indiana, USA), plate mill (Quaker City Mill Model 4E, QCG Systems, LLC, Phoenixville, PA, USA), food processor (Robot Coupe Model RSI 10V, Robot Coupe USA Inc., Joliet, IL, USA), Megatron at 13,000 rpm for 15 min (Model MTK 5000Q, Kinematica Inc., Cincinnati, OH, USA), Fitzpatrick mill with 0.127 cm and 0.0508 cm screen (Model JT, Fitzpatrick Co., Elmhurst, IL, USA), Stonemill (Model MKCA6-3,

stone#MKE6-46, Masuko Sangyo Co. Ltd., Saitama, Japan). Each of the processes was having 5 pressure levels. The comminuted suspension of dehulled soybean slurry in DW was pressurized at 68.95, 103.42, 137.90, 206.84, or 275.79 MPa using two alternately acting pressure intensifiers (Hydropac P60-03CXS, Stansted Fluid Power Ltd., Stansted, Essex, UK) driven by a hydraulic pump in the Stansted CFHPT system (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, UK). The pressure levels indicated above were read from the pressure gauge located in the CFHPT system. It is to be noted that this pressure is a combination of the intensifier pressure and the pressure generated due to the narrow clearance offered to the fluid by the throttling (micrometering) valve (model 60VRMM4882, Autoclave Engineers, Fluid Compponents, Erie, PA 16506-2302) adjustments for the desired flow rate at each intensifier pressure. The microfluidizer (model M-140K, Microfluidics International Corporation, Newton, Mass.) (Figure 5.2) with micrometering valve attachment (Figure 5.3) was used for the second objective. The inlet temperature of soymilk to both systems was kept at 30 ± 2 ºC. Both the systems work in a similar way except that the CFHPT system has a tubular heat exchanger between the intensifiers and the throttling valve, a holding tube to hold the liquid food at the elevated temperature after depressurization, and a backpressure valve to keep the depressurized product at its saturation pressure to avoid flashing of vapors. Thermocouples were connected at steam heated tubular heat exchanger and at the end of holding tube (located after the throttling valve). The outputs of thermocouples were recorded on a Fluke Hydra Data Bucket (PO Box 9090, Everett, WA 98206-9090).

In the CFHPT treatment, the fluid was heated to 80 ºC and the fluid after throttling was held at a minimum back pressure of 350 kPa to avoid flashing of vapors from the liquid before it was cooled. The minimum back pressure needed for each applied pressure was estimated from the

saturated steam table (Toledo 2007) using the temperature of saturated steam at the applied pressure. Between throttling valve and back pressure valve a holding tube permitted the fluid to remain at the elevated temperature after depressurization for a minimum of 5 s. Thus the temperature rise due to adiabatic heating after throttling is exploited for microbial inactivation and alteration of the fluid physicochemical properties. This elevated temperature was measured using a thermocouple at the end of the holding tube. The sterilized soymilk was immediately cooled to 4 ºC or below by passing it through another heat exchanger coil immersed in ice bath. The soymilk samples were collected at a volumetric flow rate of 0.75, 1.00, 1.50 L/min from CFHPT system. There were a total of 15 treatments (5 pressure levels at 3 flow rates each) for CFHPT treated soymilk, and a total of 5 treatments (5 pressure levels) for microfluidized soymilk. For microfluidized soymilk, samples were collected thrice for each of the three replications. After collecting the sample, it is kept in iced water in a cooler (< 4 °C) to further reduce its temperature and to avoid the heat-induced changes of the product. The whole CFHPT experiment was repeated on 6 independent occasions. For theoretical temperature rise calculations (equation 1), Choi and Okos' (1987) equations as cited by Toledo (2007), were used for calculating the specific heat C_p, (J/kg.°C) and density, ρ (kg/m^3) of the soymilk used in the equation 1. Inlet temperature (P_{in}) used was 80 °C, inlet pressure (P_{in}, Pa) was the pressure applied, and the outlet pressure (P_{out}) was atmospheric (101325 Pa) pressure. The proximate composition of soymilk used in the calculations were taken from the chapter 3, in which the unboiled and two pass Gaulin homogenized soymilk did not show any significant difference from the total solids content of the CFHPT soymilks [unboiled and two pass Gaulin homogenized soymilk had proximate analysis of 3.68% protein, 1.58% fat, 0.26% crude fiber, 92.51% moisture, 0.24% ash, and 1.73% carbohydrate (by difference)].

Particle size measurements

All the soymilk treatment samples were analyzed for particle size distribution by integrated light scattering using Malvern Laser Particle Size Analyzer (Mastersizer S with 300 mm lens, Malvern Instruments, Southborogh, Mass.). For particle size distribution measurement, deionized water (approximately 100 ml) was used as dispersing medium for soymilk samples and measurements were taken at an appropriate obscuration point (15 ± 2%) reached in the diffractometer cell at an impeller speed of 2500 rpm. The optical model used to calculate the predicted scattering pattern with the refractive indices of the soymilk and water was based on the Mie theory of light scattering by spherical particles and was applied as follows: real refractive index, 1.47; imaginary refractive index, 0.00; refractive index of water, 1.33. The full size distribution was obtained using a polydisperse analysis. All the values for the average volume-weighted diameter, $D_{(4,3)} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$ (where n_i is the number of particles in a size class of diameter d_i), the surface-weighted mean diameter, $D_{(3,2)} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$, the diameter below which 99% of the volume of particles are found, $D_{(v,0.99)}$, the diameter below which 90% of the volume of particles are found, $D_{(v,0.9)}$, the diameter below which 80% of the volume of particles are found, $D_{(v,0.8)}$, the diameter below which 60% of the volume of particles are found, $D_{(v,0.6)}$, the diameter below which 50% of the volume of particles are found, $D_{(v,0.5)}$, the diameter below which 40% of the volume of particles are found, $D_{(v,0.4)}$, the diameter below which 20% of the volume of particles are found, $D_{(v,0.2)}$, the diameter below which 10% of the volume of particles are found, $D_{(v,0.1)}$, from the size distribution were calculated by the instrument software. The microfluidizer samples were analyzed twice. Therefore, 18 observations were obtained from triplicate experiments. Each CFHPT sample was measured thrice. Thus for a particular soymilk treatment using CFHPT, 18 observations were obtained from 6 replications. For temperature rise

observations, one measurement from each of the 6 replications was obtained and therefore a total of 6 observations were made for each CFHPT treatment. For modeling, volume fraction, V (%) below which the particles of diameter D (μm) was used with the processing parameters, pressure, P (MPa), and flow rate, F (L/min) for CFHPT system. Pressure, P (MPa), and volume fraction, V (%) were used for developing the model to predict particle size diameter of soymilk produced by microfluidizer.

Statistical analysis

Eighteen measurement values were obtained for CFHPT and microfluidizer replicated samples of the treatments and the data were interpreted statistically using SAS version 9.1 (SAS Inst., Inc., Cary, N.C.). Analysis of variance was performed to determine the significance of various processes and variables in soymilk. Means of each value were compared pair wise further using Student's t test to see the significance among the pairs.

Results and Discussion

Particle size distribution

Particle size diameter decreased significantly with the increase of applied pressure for both microfluidizer (Figure 5.4) and CFHPT treatments (Figure 5.5). Application of higher pressures reduced the particle size and narrowed down the particle size distribution in both microfluidizer treatments and CFHPT treatments. Figure 5.5 shows that the effect of flow rate was significant ($p < 0.0001$) in CFHPT when the flow rate increased from 0.75 to 1.50 L/min, and the particle size reduced with increase in flow rate. This reduction in particle size with flow rate can be attributed to the increase in shear and turbulence at the micrometering valve exit. An interaction of the factors pressure and flow rate was also significant ($p < 0.05$) in CFHPT. Therefore, it is

important to adjust the flow rate also, at a particular pressure treatment, to obtain desired particle size of soymilk. The particle size reduction observed in both high pressure throttling machines can be attributed to the weakening of membrane in individual particles due to the high pressure. The weakened membranes easily ruptured from shear in the throttling valve, and the narrow particle size distribution can be attributed to the restricted opening for fluid flow in the micrometering valve. The cavitation that occur after depressurization aided in further reducing particle size; and the turbulence at depressurization helped in mixing up the ruptured particles and distributing it evenly within the fluid matrix. The results show that the high pressure throttling helped to decrease the size of solid particles, and make the particle size more homogenize. An earlier study using CFHPT on particle size reduction and narrowing the distribution was reported by Peck (2004) where the CFHPT processed blueberry-whey beverage showed no aggregation of particles during storage in addition to the significantly smaller size offered by the treatment compared with thermally processed beverage.

A model was established, each for the soymilk processed using microfluidizer and CFHPT processes, between the particle size, D (μm), pressure, P (MPa), flow rate (for CFHPT), F (L/min), and volume fraction, V (%) (Table 5.1). The standard error (SE) for each of the models is also given in the Table 5.1. The models can be effectively utilized for predicting the particle size diameter (μm) at a particular pressure (MPa) and volume fraction (%) of particles. An earlier study reported (Walstra 1975) mean drop size was found to vary as $D_{(4,3)} \propto P_h^{-0.6}$ (where $D_{(4,3)}$ is the particle size diameter and P_h is the homogenizing pressure). This result was in agreement with drop breakage in a locally isotropic turbulent flow field (Floury and others 2000). Even though the relation $D_{(4,3)} \propto P_h^{-0.6}$ was obtained with low dispersed phase fractions,

the value of the exponent decreased at higher emulsion fat contents, leading to larger drop sizes, possibly due to the opposing effect of drop coalescence (Mohan and Narsimhan 1997).

Temperature rise

The flow rates at a particular pressure in CFHPT process did not result in a significant difference in temperature rise of the product (Figure 5.6). The observed temperature rise was directly proportional to the applied pressure. This temperature rise might have contributed to the particle size reduction by facilitating the in rupture of the particles at high turbulence, shear, and cavitation occurred at and after the micrometering valve. The temperature rise in CFHPT after throttling was calculated using the equation 3 and compared with the experimental results in Figure 5.7. The specific heat and density obtained for soymilk at 80 °C [calculated using Choi and Okos' (1987) equations as cited by Toledo (2007)] at different pressure levels for CFHPT treatments are 4042.58 J/kg.K and 998.32 kg/m^3 respectively. The theoretical temperature rise calculcated using the density and specific heat values (using equation 1) are shown in Figure 5.7. The figure shows that the experimental values of temperature rise were higher than the calculated values at lower pressures. This difference in temperature rise (between calculated and experimental values) decreased as the pressure increased, and finally it was lower than the calculated results at the highest pressure (275.79 MPa). This can be attributed to the increased pressure fluctuation as the pressure levels increased. This explanation was based on the results obtained for a previous study (Amornsin 1999). Due to time constraints, to undertake a study with the pressure fluctuation objective was not possible. The results of a previous study (Amornsin 1999) showed that for water at the flow rate of 350-360 mL/min, the measured temperature was lower than the calculated temperature for the targeted pressure. However, this elevated temperature was higher than the temperature rise calculated for the mean of the target

and minimum pressure. This was in addition to the assumption that the loss due conduction and convection cannot be avoided. The results were attributed to the exponential rise of pressure in a cycle i.e., more liquid leaves the throttling valve at higher pressures in a cycle than that at lower cycles. At a flow rate of 300 mL/min of water, the dropping pressure was 103.4 MPa (15,000 psi) for a target pressure of 275.8 MPa (40,000 psi). This confirmed that the targeted pressure was not achieved during each stroke of intensifier piston. When flow rate and target pressure was increased, the pressure drop also increased.

Conclusion

The high pressure throttling process helped in reduction of particle size of soymilk. In CFHPT process, increase of flow rate and pressure application are effective in reducing the particle size. The particle size can be predicted by using the empirical models developed in this study. The particles in soymilk were reduced to a level so that there was no apparent separation in the soymilk after a month of storage at 4 °C. Thus, the high pressure throttling process will help in utilizing the whole bean solid to produce excellent quality soymilk with high emulsion stability.

Acknowledgments

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Table 5.1 – Particle size diameter models obtained for the high pressure throttling treatments.

Process	Model	R^2	SE
CFHPT	$\ln(D) = 3.16 + 1.12 \times 10^{-5}(P^2) + 0.27(F^2) - 2.52 \times 10^{-4}(FP) - 6.42 \times 10^{-3}(P) + 2.17 \times 10^{-2}(V) - 0.68(F)$	0.97	0.12
Megatron-Microfluidizer	$\ln(D) = 2.38 + 8.87 \times 10^{-6}(P^2) - 1.33 \times 10^{-5}(PV) - 4.12 \times 10^{-3}(P) + 2.35 \times 10^{-2}(V)$	0.97	0.12
Fitzmill-Microfluidizer	$\ln(D) = 2.34 + 1.04 \times 10^{-5}(P^2) - 1.66 \times 10^{-5}(V^2) - 2.27 \times 10^{-5}(PV) - 4.20 \times 10^{-3}(P) + 2.67 \times 10^{-2}(V)$	0.97	0.12

Parameters are D = particle size diameter (μm), P = pressure applied (MPa), F = flow rate in L/min, and V = volume fraction (%). The model for CFHPT used 18 observations from six replications for each treatment combination of pressure level and flow rate. Microfluidizer model was established from 18 observations from 3 replications at each pressure level.
SE stands for standard error.

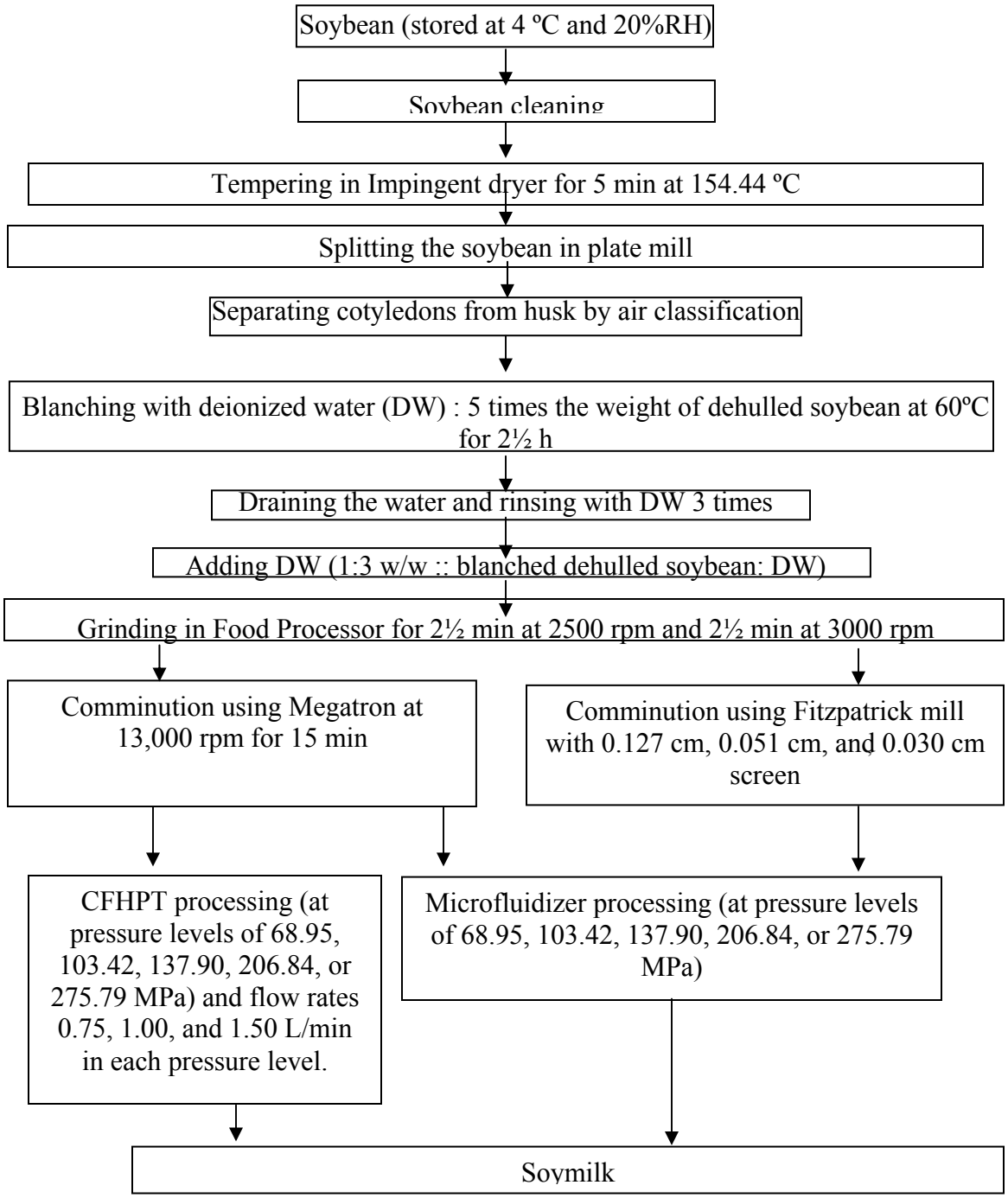

Figure 5.1 - Flow diagram for preparation of soymilk from whole dehulled soybeans.

Figure 5.2 – Microfluidizer (outside view) (model M-140K, Microfluidics International Corporation, Newton, Mass.).

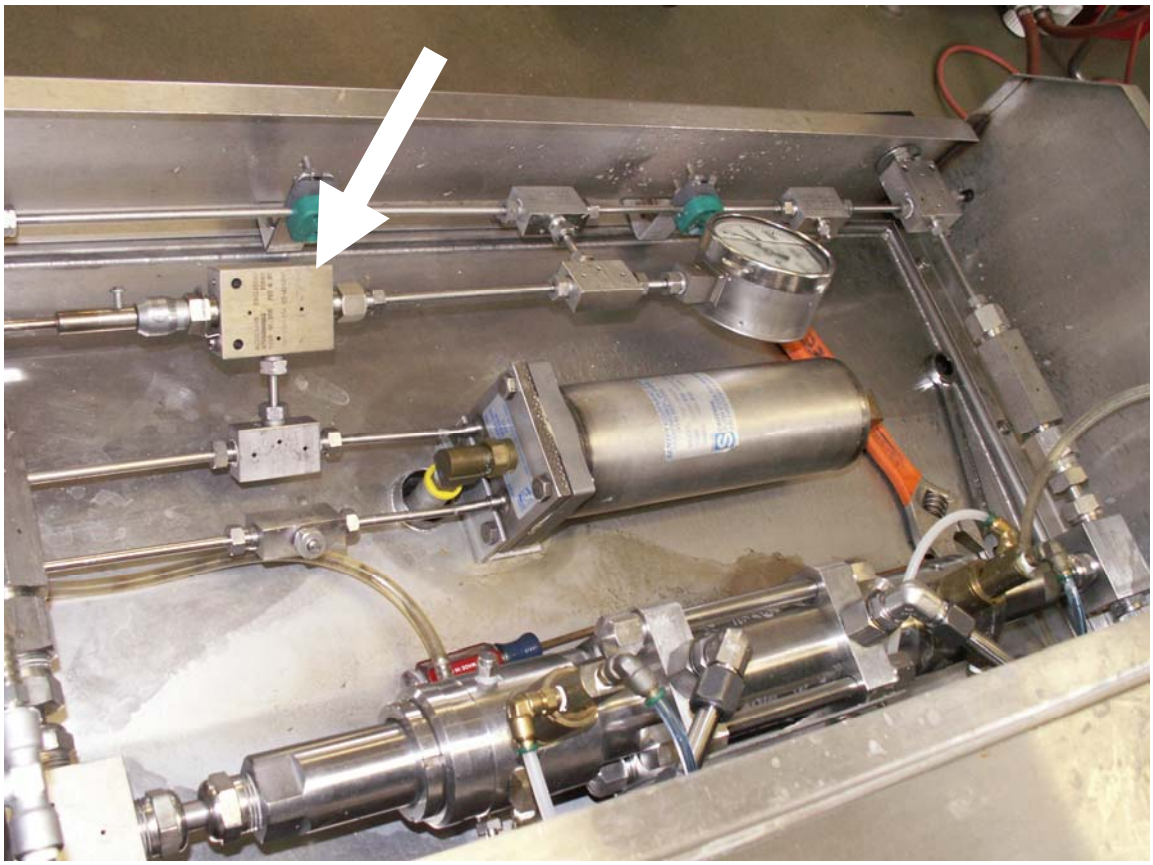

Figure 5.3 – Micrometering valve (shown by white arrow) (model 60VRMM4882, Autoclave Engineers, Fluid Components, Erie, PA 16506-2302) attached in the microfluidizer (inside view).

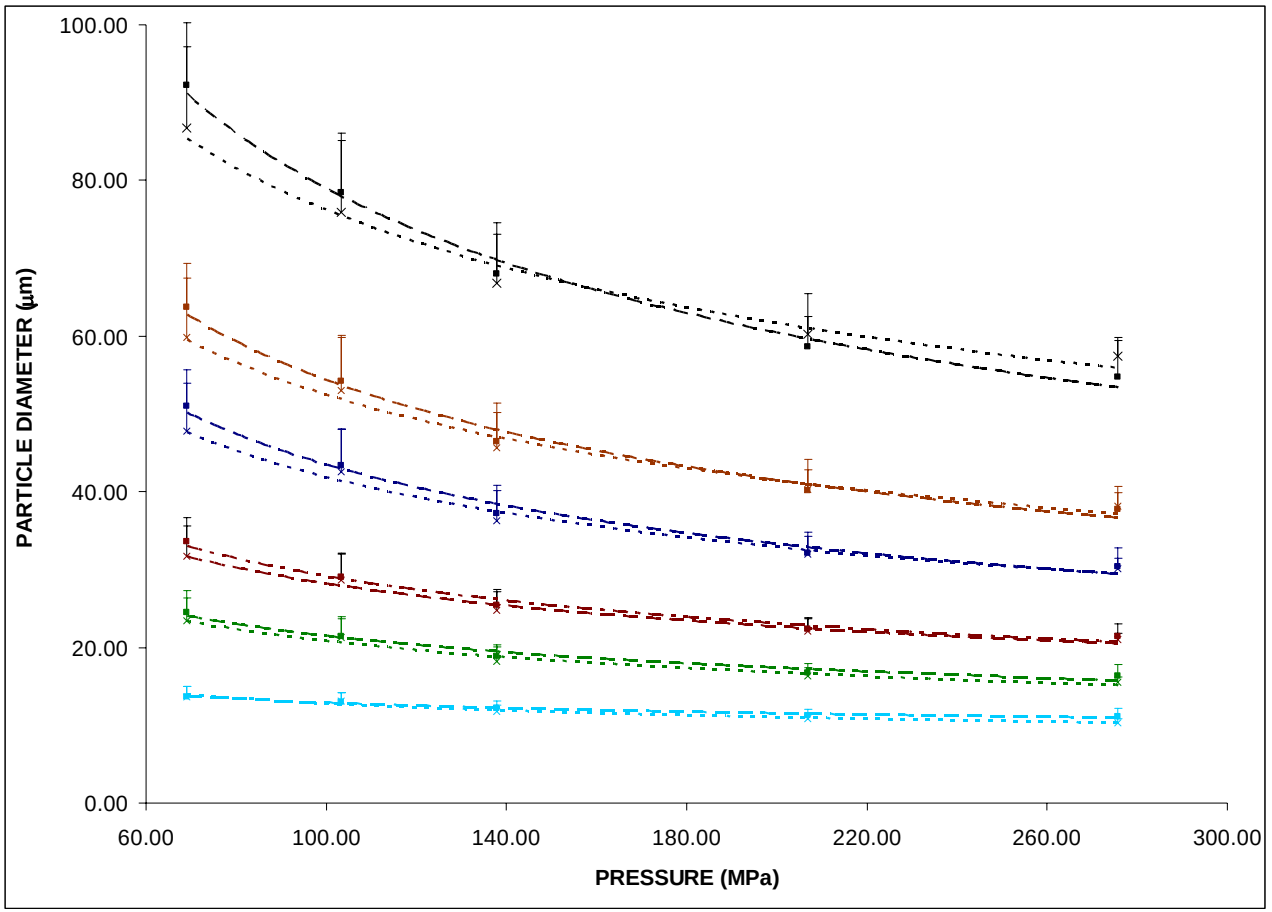

Figure 5.4 – Microfluidizer treated soymilk particle size reduction with pressure application. The pretreatment was either megatron or fitzmill prior to microfluidization as described below by the notations: megatron-microfluidizer treatment (MM) and fitzmill-microfluidizer treatment (FM). MM $D_{(v,0.99)}$: ----×----, $R^2 = 0.98$; FM $D_{(v,0.99)}$: __ __■__ __, $R^2 = 0.99$; MM $D_{(v,0.90)}$: ----×----, $R^2 = 0.98$; FM $D_{(v,0.90)}$: __ __■__ __, $R^2 = 0.99$; MM $D_{(v,0.80)}$: ----×----, $R^2 = 0.98$; FM $D_{(v,0.80)}$: __ __■__ __, $R^2 = 0.98$; MM $D_{(v,0.40)}$: ----×----, $R^2 = 0.98$; FM $D_{(v,0.40)}$: __ __■__ __, $R^2 = 0.97$; MM $D_{(v,0.20)}$: ----×----, $R^2 = 0.97$; FM $D_{(v,0.20)}$: __ __■__ __, $R^2 = 0.97$; MM $D_{(4,3)}$: ----×----, $R^2 = 0.98$; FM $D_{(4,3)}$: __ __■__ __, $R^2 = 0.98$.

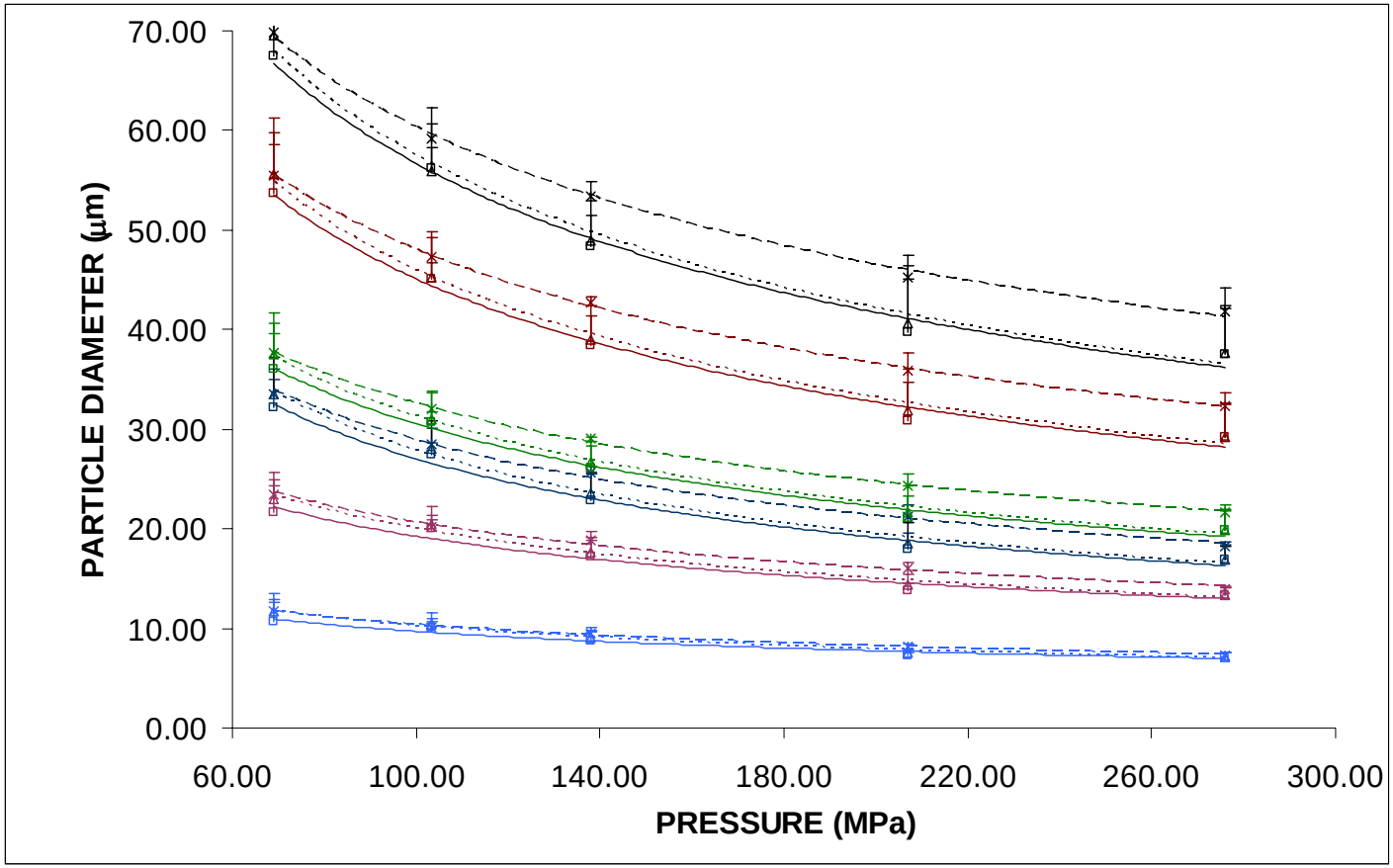

Figure 5.5 – CFHPT treated soymilk particle size reduction with pressure application at various flow rates. The particle diameter (D) at various flow rates (F in L/min as F0.75, F1.00, and F1.50) is described below. F0.75 $D_{(v,0.90)}$: _____*__ __, R^2 = 1.00; F1.00 $D_{(v,0.90)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(v,0.90)}$: ____□____, R^2 = 0.99; F0.75 $D_{(v,0.80)}$: __ __*__ __, R^2 = 98; F1.00 $D_{(v,0.80)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(v,0.80)}$: ____□____, R^2 = 0.99; F0.75 $D_{(4,3)}$: __ __*__ __, R^2 = 1.00; F1.00 $D_{(4,3)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(4,3)}$: ____□____, R^2 = 0.99; F0.75 $D_{(v,0.50)}$: __ __*__ __, R^2 = 99; F1.00 $D_{(v,0.50)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(v,0.50)}$: ____□____, R^2 = 0.98; F0.75 $D_{(3,2)}$: __ __*__ __, R^2 = 0.99; F1.00 $D_{(3,2)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(3,2)}$: ____□____, R^2 = 0.96; F0.75 $D_{(v,0.10)}$: __ __*__ __, R^2 = 0.99; F1.00 $D_{(v,0.10)}$: ----Δ----, R^2 = 0.99; F1.50 $D_{(v,0.10)}$: ____□____, R^2 = 0.95.

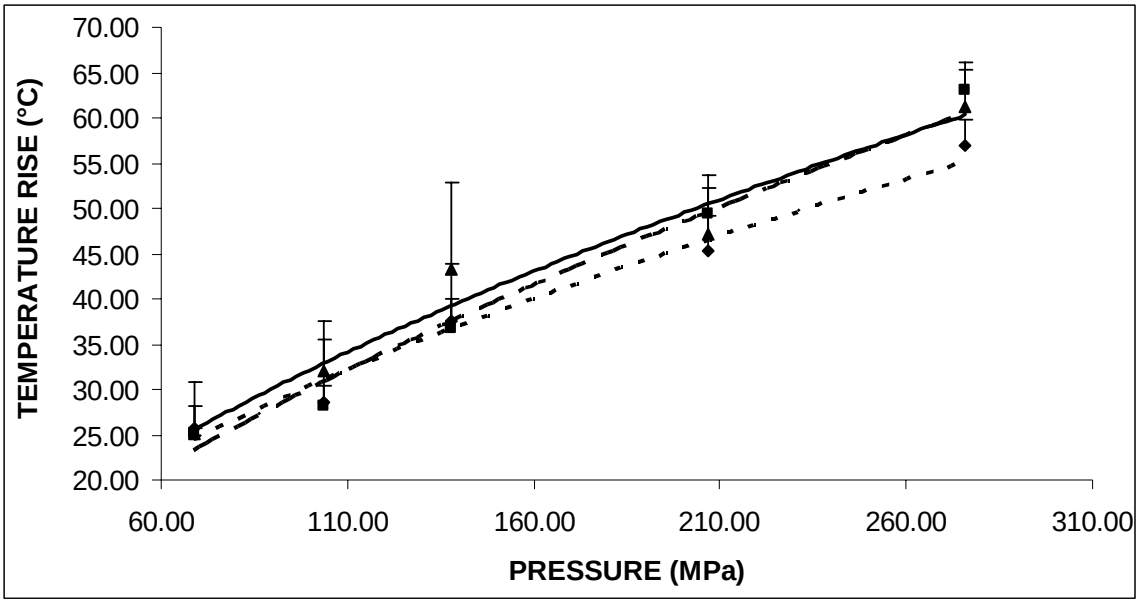

Figure 5.6 – Effect of CFHPT pressure and flow rates on temperature rise of soymilk at depressurization. Legends are: 1.50 L/min (____▲____), $R^2 = 0.97$; 1.00 L/min (___ ___■___ ___), $R^2 = 0.97$; and 0.75 L/min (_ _ _ _♦_ _ _ _), $R^2 = 0.97$.

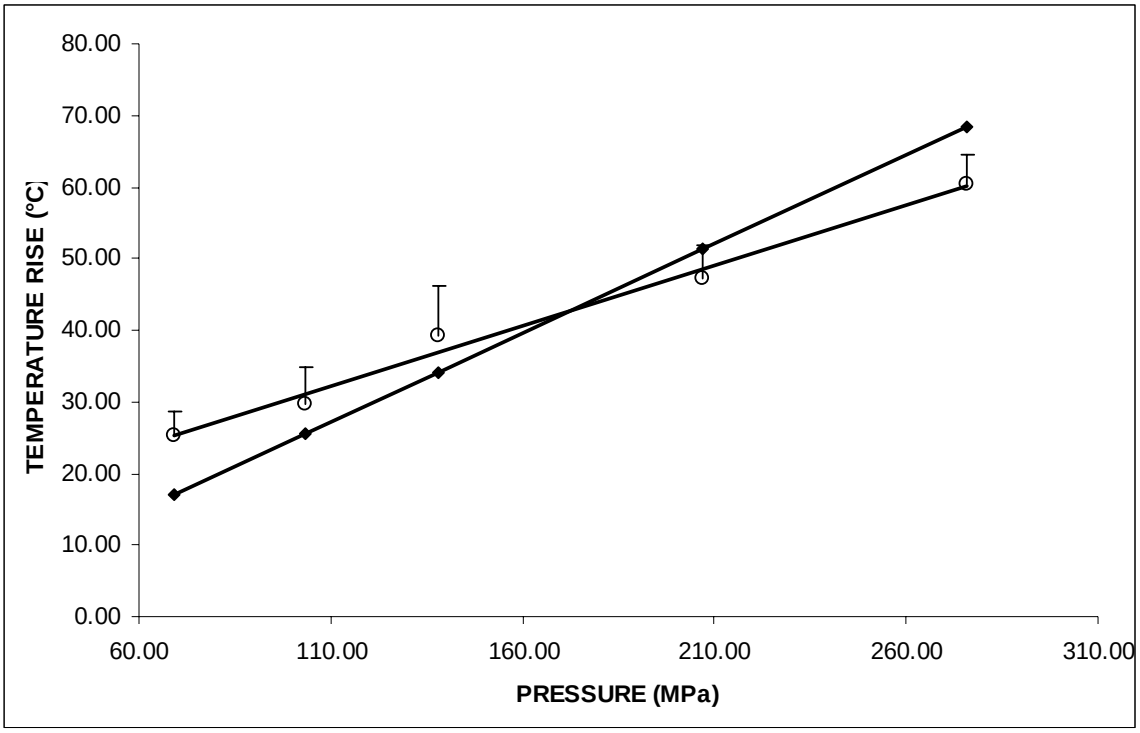

Figure 5.7 – Comparison of experimental (¤) and calculated (♦) values of temperature rise of soymilk after throttling in CFHPT system. The linear (-----) model of experimental curve obtained a $R^2 = 0.99$.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Soymilk was processed successfully using the Gaulin homogenizer and high pressure throttling systems. Evaluation of the particle size distribution, rheological, and ultrastructural properties showed excellent quality for soymilk produced with high pressure throttling. No significant difference was observed in the total solids content among the processes.

Particle size diameter, $D_{(4,3)}$, of soymilk produced using the best process M at the highest pressure (275.79 MPa) in CFHPT process was 18.73 ± 1.66 μm whereas the Gaulin homogenizer offered $D_{(4,3)}$ of 29.62 ± 1.30 μm with the boiled soymilk at 96.53 MPa. The particle size reduction with increased pressure application occurred in CFHPT can be attributed to the weakening of individual particle membrane due to high pressure, the easy rupture of the membrane resulted from shear in the throttling valve, and the narrow particle size distribution due to the restricted opening in the micrometering valve. The cavitation occurred after depressurization aided in increased reduction of the particle size; and the turbulence at depressurization helped in further particles size reduction and mixing up the ruptured particles and distributing it evenly. The high pressure throttling helped to decrease the size of solid particles, homogenize the particle size, and disperse it evenly. The smaller particle size and uniform distribution in CFHPT soymilk was confirmed by its increase in the apparent viscosity values with increase in pressure application. At 25 °C, the process M at 275.79 MPa soymilk obtained apparent viscosity of 13.9 ± 0.57 Pa.s. This can be attributed to the increased number and surface volume of particles obtained added to the resistance to flow. All samples showed non-Newtonian pseudoplastic and thixotropic flow behavior. Pseudoplastic behavior is important

in industrial applications, as the beverage can be pumped with reduced pump power at higher shear rates. Ultrastructural images of soymilks showed structural network in cryogenic-SEM images and the very small fat globules entrapped in the protein matrix with uniform distribution at highest pressure treatment of process M in confocal microscope images.

The increase in flow rate had significant effect on the particle size reduction of soymilk in CFHPT treatments. The empirical models established can be used to predict the particle size diameter of soymilk processed at different processing pressures and flow rates using high pressure throttling process. The models showed that the particle size reduced significantly with pressure application. The particles in soymilk were reduced to a level so that there was no apparent separation in the soymilks processed at 206.84 and 275.79 MPa after a month of storage at 4 °C. Thus, the high pressure throttling process will help in utilizing the whole bean solid to produce excellent quality soymilk with high emulsion stability. Consumer acceptability test showed that more research is needed to appeal the taste of American consumer before venturing into the commercial scale production of soymilk. Thus, soymilk with all the essential solids can be made available to the public and processors benefit from the high processing yields since none of the essential solids of the beans are discarded.

APPENDICES

APPENDIX – A

EFFECT OF FLOW RATES ON PRESSURE FLUCTUATION

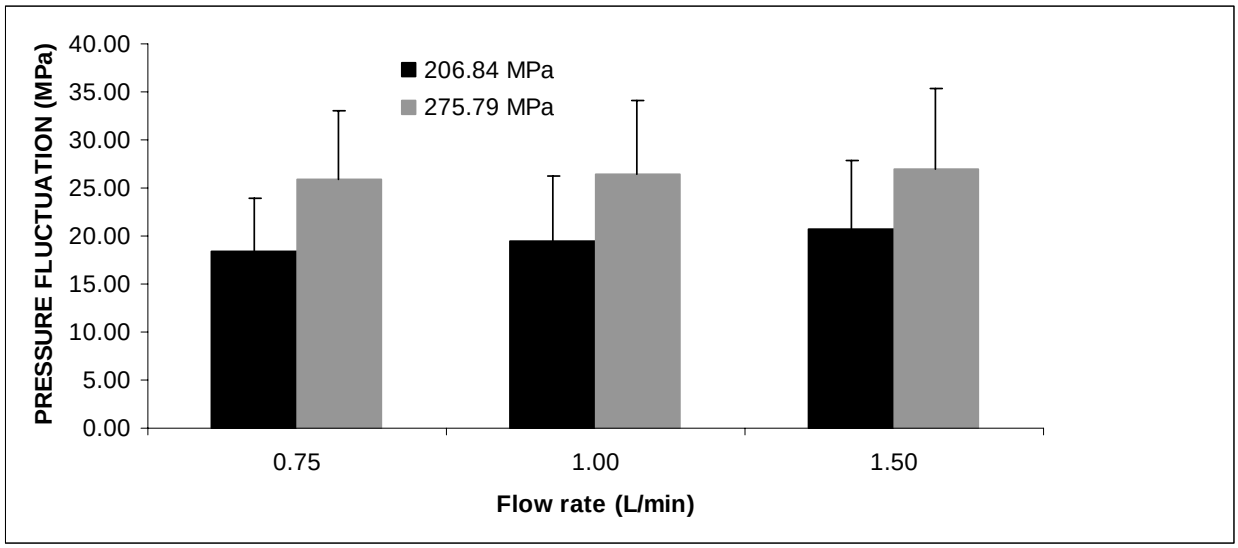

Figure A.1 – Pressure fluctuation in CFHPT system at different flow rates. The values are means (with SD) from 6 replications. Due to time constraints, the pressure fluctuations at 206.84 (30,000 psi) and 275.79 MPa (40,000 psi) only were done.

APPENDIX – B

PARTICLE SIZE DIAMETER OBSERVATIONS

Table B.1 - Particle size diameter obtained for different pressure level treatments of using CFHPT system explained in chapter 4.

Process	Pressure (MPa)	$D_{(v, 0.10)}$ (µm)	$D_{(v,0.20)}$ (µm)	$D_{(v,0.40)}$ (µm)	$D_{(v,0.50)}$ (µm)	$D_{(v,0.60)}$ (µm)	$D_{(v,0.80)}$ (µm)	$D_{(v,0.90)}$ (µm)	$D_{(v,0.99)}$ (µm)	$D_{(3,2)}$ (µm)	$D_{(4,3)}$ (µm)
Fitzmill/CFHPT	**68.95**	14.37	21.10	31.64	36.84	42.49	56.88	69.05	96.87	26.42	39.73
		(1.94)	(2.40)	(2.57)	(2.61)	(2.66)	(2.95)	(2.73)	(4.19)	(2.44)	(2.52)
	103.42	12.38	17.34	25.48	29.51	33.89	45.12	54.33	76.33	22.54	31.8
		(0.46)	(0.58)	(0.84)	(0.98)	(1.10)	(1.21)	(1.26)	(1.29)	(0.75)	(0.87)
	137.90	11.24	15.54	22.83	26.48	30.46	40.76	49.41	69.99	20.58	28.74
		(0.34)	(0.45)	(0.73)	(0.90)	(1.12)	(1.49)	(2.05)	(4.19)	(0.65)	(1.09)
	206.84	8.95	11.83	16.83	19.41	22.36	30.01	36.21	51.4	15.97	21.29
		(0.15)	(0.17)	(0.08)	(0.14)	(0.30)	(0.86)	(1.40)	(2.58)	(0.09)	(0.39)
	275.79	9.19	12.22	17.37	20.03	23.04	30.7	37.02	53.29	16.45	21.89
		(0.26)	(0.32)	(0.31)	(0.32)	(0.34)	(0.48)	(0.77)	(1.41)	(0.31)	(0.26)
Stonemill/CFHPT	**68.95**	17.59	25.16	36.88	42.75	49.06	65.1	79.07	109.88	31.03	45.87
		(1.47)	(1.44)	(0.90)	(0.59)	(0.56)	(1.80)	(2.95)	(7.09)	(1.05)	(0.65)
	103.42	14.45	20.6	30.15	34.86	40	53.32	64.56	91.05	26.05	37.62
		(0.30)	(0.34)	(0.46)	(0.54)	(0.64)	(0.86)	(1.23)	(1.28)	(0.46)	(0.61)
	137.90	11.27	15.86	23.47	27.21	31.29	42.02	50.68	71.86	20.82	29.48
		(0.14)	(0.10)	(0.17)	(0.24)	(0.32)	(0.55)	(0.81)	(1.64)	(0.09)	(0.31)
	206.84	10.26	14.2	20.98	24.46	28.35	38.57	47.22	68.55	19.01	26.99
		(1.49)	(2.51)	(3.97)	(4.57)	(5.14)	(6.52)	(7.43)	(8.97)	(2.86)	(4.53)
	275.79	9.28	12.27	17.49	20.22	23.26	31.17	37.66	53.54	16.6	22.14
		(0.57)	(0.92)	(1.33)	(1.55)	(1.73)	(2.11)	(2.46)	(3.82)	(1.05)	(1.47)

The values are mean from 3 independent experiments. Values in parenthesis show the SD for the mean value above it.

Table B.2 - Particle size diameter obtained for different pressure level treatments of using Microfluidizer/throttling valve system explained in chapter 5.

Process	Pressure (MPa)	$D_{(v, 0.10)}$ (µm)	$D_{(v,0.20)}$ (µm)	$D_{(v,0.40)}$ (µm)	$D_{(v,0.50)}$ (µm)	$D_{(v,0.60)}$ (µm)	$D_{(v,0.80)}$ (µm)	$D_{(v,0.90)}$ (µm)	$D_{(v,0.99)}$ (µm)	$D_{(3,2)}$ (µm)	$D_{(4,3)}$ (µm)
Megatron/Microfluidizer	**275.79**	10.37	15.51	8.01	21.47	30.15	18.28	57.40	38.16	14.65	20.96
		(0.45)	(0.65)	(0.29)	(0.94)	(1.30)	(0.74)	(2.49)	(1.68)	(0.58)	(0.85)
	206.84	10.85	16.35	8.21	22.74	31.93	19.34	60.25	40.28	15.29	22.10
		(0.51)	(1.05)	(0.18)	(1.81)	(2.91)	(1.41)	(5.22)	(3.83)	(0.69)	(1.69)
	137.90	11.77	18.23	8.47	25.72	36.24	21.76	66.74	45.61	16.41	24.72
		(0.86)	(1.86)	(0.23)	(3.06)	(4.60)	(2.46)	(7.80)	(5.74)	(1.07)	(2.75)
	103.42	13.18	21.29	8.89	30.34	42.54	25.59	75.94	52.95	18.10	28.64
		(1.03)	(2.42)	(0.26)	(3.77)	(5.47)	(3.07)	(9.17)	(6.86)	(1.20)	(3.35)
	68.95	13.72	23.38	8.72	33.84	47.77	28.40	86.76	59.85	18.77	31.79
		(1.28)	(3.05)	(0.41)	(4.45)	(6.13)	(3.75)	(10.40)	(7.59)	(1.33)	(3.84)
Fitzmill/ Microfluidizer	**275.79**	11.15	16.35	8.47	22.21	30.43	19.12	54.81	37.79	15.40	21.37
		(1.05)	(1.41)	(0.59)	(1.87)	(2.40)	(1.63)	(4.58)	(2.93)	(1.23)	(1.71)
	206.84	11.25	16.79	8.44	23.16	32.10	19.77	58.63	40.12	15.65	22.30
		(0.78)	(1.10)	(0.41)	(1.55)	(2.22)	(1.34)	(3.93)	(2.76)	(0.85)	(1.38)
	137.90	12.20	18.86	8.72	26.60	37.23	22.53	67.99	46.49	16.94	25.41
		(0.89)	(1.45)	(0.45)	(2.09)	(2.97)	(1.77)	(5.07)	(3.70)	(0.88)	(1.82)
	103.42	13.05	21.42	8.79	30.76	43.33	25.90	78.44	54.21	18.19	29.07
		(1.13)	(2.60)	(0.32)	(3.64)	(4.78)	(3.14)	(7.59)	(5.87)	(1.36)	(3.12)
	68.95	13.71	24.53	8.66	36.08	51.01	30.10	92.29	63.67	19.30	33.58
		(1.22)	(2.80)	(0.41)	(3.69)	(4.68)	(3.29)	(7.93)	(5.69)	(1.39)	(3.08)

The values are mean from 3 independent experiments. Values in parenthesis show the SD for the mean value above it.

APPENDIX – C

ULTRASTRUCTURAL IMAGES

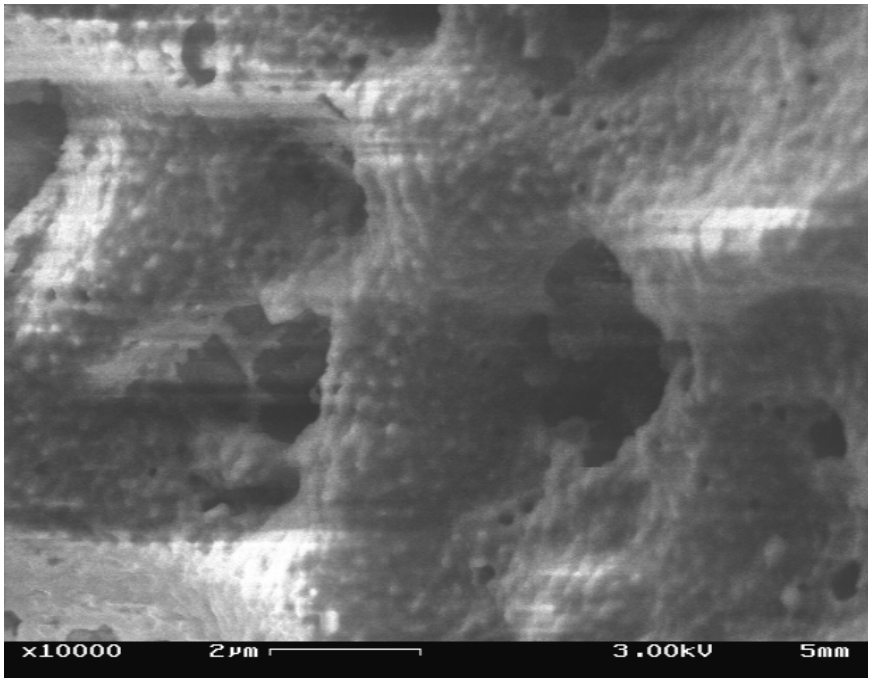

Figure C.1 – Cryogenic scanning electron microscope image of process C (boiled with one pass homogenization at 96.53 MPa). The image shows that the network structure was not broken with this process.

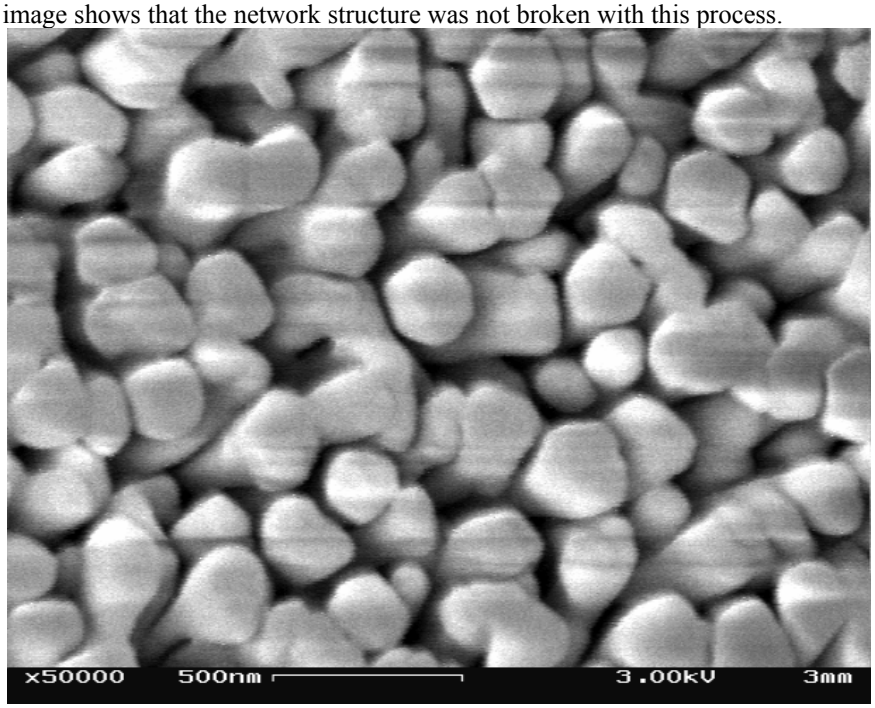

Figure C.2 – Cryogenic scanning electron microscope image of process D (boiled with two pass homogenization at 96.53 MPa) at the highest magnification (50,000). The image shows that the network structure was broken with uniform distribution of hydrated particles with this process.

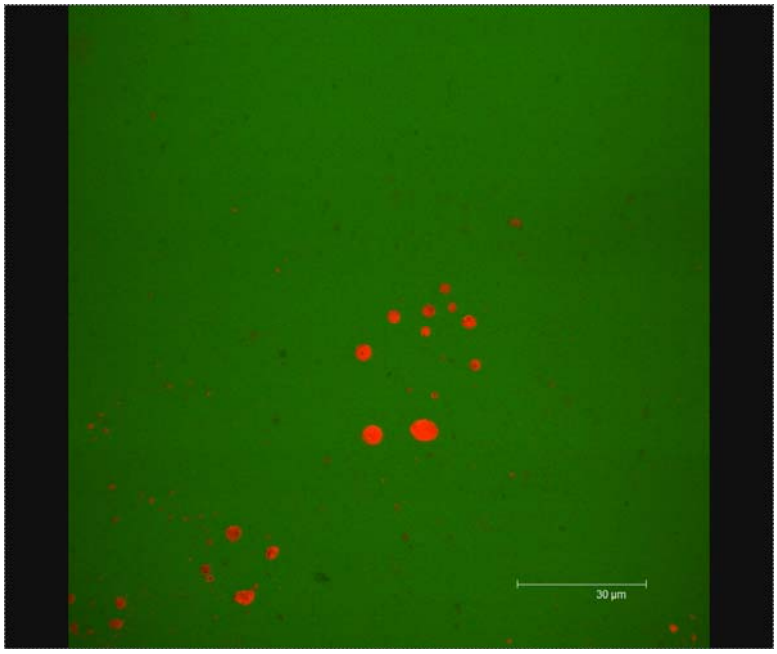

Figure C.3 – Confocal laser scanning microscope image of process B (unboiled with two pass homogenization at 96.53 MPa). Fat globules are fluorescent red in color and protein particles are fluorescent green.

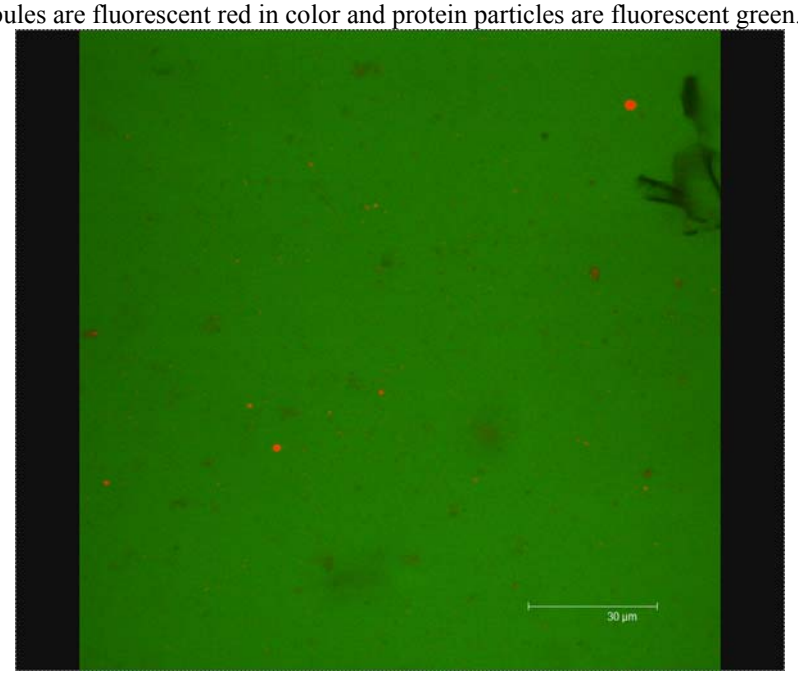

Figure C.4 – Confocal laser scanning microscope image of process C (boiled with one pass homogenization at 96.53 MPa). Fat globules are fluorescent red in color and protein particles are fluorescent green.

APPENDIX – D

SAS CODES AND OUTPUTS FOR THE MODELS DEVELOPED FOR MICROFLUIDIZER AND CFHPT TREATMENTS

MICROFLUIDIZER-FITZMILL MODEL

```
dm 'log; clear; output; clear';
options nodate nonumber linesize=80;
data lab;
do P = 275.79, 206.84, 137.90, 103.42, 68.95;
do rep = 1 to 3;
        do m= 1 to 6;
        do DV = 10, 50, 90, 20, 40, 60, 80, 99;
   input lpsize @@;
        b= P*DV;
        sqp = P*P;
        sqDV= DV*DV;
        psize=Log(lpsize); /* log base e */
   output;
        end;
  end;
end;
end;
cards;
```

7.645	16.929	34.662	9.72	14.39	19.73	27.53	52.52
7.785	17.423	35.471	10.02	14.86	20.23	28.13	50.88
7.671	17.395	35.102	9.89	14.77	20.29	28.19	49.89
7.938	18	36.378	10.28	15.32	20.96	29.07	54.08
9.191	20.876	39.886	12.42	17.93	24.06	32.45	57.01
9.255	21.16	40.264	12.55	18.15	24.38	32.88	57.06
9.234	21.41	42.23	12.57	18.28	24.89	33.99	61.71
9.239	21.485	41.708	12.6	18.35	24.95	33.87	61.05
9.47	21.798	41.99	12.89	18.66	25.24	34.14	61.43
8.119	17.811	34.448	10.44	15.29	20.55	27.92	49.34
8.173	17.855	34.438	10.49	15.32	20.7	28.09	48.85
8.101	17.676	34.006	10.37	15.17	20.4	27.68	48.49
8.367	18.591	37.173	10.87	15.92	21.68	29.75	54.85
8.661	19.405	38.802	11.44	16.64	22.6	30.98	57.03
8.276	18.022	34.694	10.64	15.49	20.89	28.3	49.15
8.379	19.3	40.349	11.02	16.41	22.68	31.78	58.91
8.742	20.235	39.417	11.78	17.29	23.51	32.02	56.43
8.279	18.907	39.233	10.8	16.09	22.2	30.99	58
7.868	18.798	39.952	10.29	15.79	22.31	31.69	57.9
8.249	20.294	43.826	11.07	17.02	24.04	34.34	65.45
8.215	19.915	42.035	10.98	16.77	23.52	33.25	63.17
9.077	21.008	40.328	12.34	17.98	24.27	32.87	57.26
9.115	20.862	39.989	12.34	17.88	24.09	32.59	56.88
8.924	20.311	39.559	11.99	17.41	23.55	32.01	57.02

8.554	21.941	45.43	11.95	18.37	25.9	36.37	65.41
8.614	21.857	44.947	12	18.34	25.76	36.05	64.6
9.225	22.31	43.989	12.76	18.93	25.98	35.57	63.76
8.425	18.944	38.721	10.99	16.18	22.17	30.73	57.31
8.258	18.585	37.97	10.72	15.86	21.76	30.16	56.45
8.062	18.09	37.101	10.37	15.4	21.19	29.47	55.39
8.401	19.123	37.824	11.04	16.34	22.34	30.57	54.87
8.533	19.577	38.786	11.32	16.72	22.85	31.3	56.31
7.762	18.389	39.037	10.16	15.51	21.72	30.66	58.08
8.25	18.583	36.771	10.72	15.87	21.7	29.74	53.21
8.345	18.946	38.662	10.92	16.17	22.18	30.71	57.2
8.216	18.469	37.253	10.66	15.77	21.58	29.75	55.16
8.528	24.286	52.326	12.31	19.96	29.08	41.57	76.45
8.298	23.934	51.668	12.01	19.64	28.68	41.06	75.53
8.399	24.189	52.334	12.19	19.87	28.99	41.52	76.61
9.37	24.908	49.094	13.63	21.03	29.15	39.91	70.25
9.58	25.604	50.258	14	21.65	29.93	40.88	72.04
9.006	24.784	51.239	13.11	20.68	29.32	41.11	73.64
9.34	22.773	44.412	13	19.33	26.45	36.04	64
9.164	22.234	43.419	12.71	18.88	25.86	35.25	62.48
9.343	23.144	45.285	13.12	19.64	26.88	36.68	65.38
8.241	20.065	41.778	11.03	16.88	23.77	33.41	61.63
8.344	20.336	43.13	11.24	17.11	24.08	34	65.11
8.09	19.737	41.269	10.77	16.59	23.42	32.98	60.87
8.647	22.098	46.465	11.99	18.45	26.13	36.85	69.66
8.586	22.099	46.319	11.92	18.43	26.14	36.85	67.72
8.546	21.898	45.799	11.79	18.24	25.94	36.6	67.12
8.501	21.088	43.847	11.59	17.69	24.93	35.01	64.87
8.583	21.451	44.493	11.79	17.99	25.33	35.52	65.68
8.425	20.97	43.774	11.46	17.58	24.83	34.92	64.85
9.048	30.833	64.726	14.55	25.33	36.71	51.71	92.5
9.319	32.081	64.656	15.21	26.6	37.74	52.08	92.48
9.283	31.987	64.127	15.16	26.54	37.61	51.77	91.89
8.691	25.815	54.325	13.05	21.37	30.69	43.33	78.37
8.893	26.413	55.575	13.39	21.9	31.36	44.27	79.58
8.897	26.418	55.432	13.39	21.91	31.36	44.21	79.4
9.029	26.57	55.632	13.56	22.07	31.5	44.36	79.56
9.071	26.583	55.749	13.59	22.09	31.51	44.42	79.7
8.929	26.401	55.302	13.43	21.91	31.32	44.11	79.28
8.564	22.954	48.168	12.06	19.01	27.29	38.57	70.73
8.364	22.73	47.589	11.76	18.78	27	38.12	69.75
8.23	22.632	47.596	11.57	18.65	26.93	38.09	69.88
9.174	26.375	54.909	13.36	21.8	31.38	44.16	79.54
8.692	25.993	55.956	12.78	21.31	31.11	44.31	80.47
8.59	25.273	53.255	12.51	20.72	30.24	42.8	77.02
8.606	22.633	47.933	12	18.76	26.93	38.27	70.73

```
8.488  22.352  47.53  11.81  18.52  26.57  37.78  70.69
8.444  22.262  47.405 11.72  18.43  26.49  37.7   70.42
9.058  36.381  73.288 15.78  29.93  42.97  59.22  105.64
9.139  36.475  73.553 15.98  30.04  43.06  59.37  105.99
9.092  36.587  74.09  15.88  30.09  43.23  59.72  106.65
8.982  28.769  60.319 13.94  23.69  34.34  48.39  86.77
8.822  28.777  60.212 13.86  23.69  34.34  48.34  86.47
8.761  29.004  61.188 13.86  23.83  34.66  48.97  88.43
9.091  29.856  62.047 14.4   24.63  35.51  49.81  89.2
8.783  29.162  61.193 13.88  23.96  34.82  49.06  88.2
8.986  29.769  62.423 14.24  24.5   35.48  49.97  90.03
8.096  29.817  67.244 12.55  23.67  36.54  53.33  98.23
8.508  32.544  66.93  14.03  26.5   38.89  54.19  96.57
7.568  30.973  68.644 12.24  24.57  37.82  54.68  99.43
8.664  26.624  56.678 12.81  21.74  32.01  45.47  82.97
8.684  27.111  57.57  12.91  22.12  32.6   46.21  84.38
8.285  26.382  56.053 12.33  21.43  31.69  44.99  81.9
8.797  27.893  59.339 13.11  22.69  33.59  47.6   87.21
8.455  27.793  62.341 12.64  22.32  33.87  49.24  91.23
8.229  27.896  63    12.36  22.29  34.09  49.68  91.98
;

proc print;
title 'Table.1.The Data';
var P rep m DV psize;
run;
proc reg;
model psize = sqP sqDV b P DV/clb;
model psize = sqP sqDV b P DV/cli;
output out=result r=residuals p=yhat;
run;
proc boxplot data=result;
plot residuals; /* Ploting box plot*/
run;
proc gplot data=result;
plot residuals*yhat;
plot residuals*sqP;
plot residuals*sqDV;
plot residuals*b;
plot residuals*P;
plot residuals*DV;
title 'Graph between observed and predicted values for fitzmill-microfluidizer';
plot psize*yhat /vref=0 href=0;
run;
Proc univariate normal plot data=result;
var residuals;
```

```
run;
proc rank normal=bloom;
var residuals;
ranks rresiduals;
proc gplot;
title 'NPP';
plot residuals*rresiduals /vref=0 href=0;
run;
proc glm data = lab;
class  rep;
model psize = sqP sqDV b P DV /solution;
lsmeans P /pdiff out = int1;
run;
quit;
```

OUTPUT

****

The GLM Procedure

Class Level Information

Class	Levels	Values
rep	3	1 2 3

Number of Observations Read 720
Number of Observations Used 720

The GLM Procedure

Dependent Variable: psize

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	314.2747946	62.8549589	4747.84	<.0001
Error	714	9.4523990	0.0132387		
Corrected Total	719	323.7271935			

R-Square	Coeff Var	Root MSE	psize Mean
0.970801	3.570008	0.115059	3.222944

Source	DF	Type I SS	Mean Square	F Value	Pr > F

sqp	1	11.9690281	11.9690281	904.10 <.0001
sqDV	1	279.8062617	279.8062617	21135.6 <.0001
b	1	0.0050622	0.0050622	0.38 0.5365
P	1	3.4961214	3.4961214	264.08 <.0001
DV	1	18.9983212	18.9983212	1435.06 <.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
sqp	1	1.42627288	1.42627288	107.74	<.0001
sqDV	1	0.11398108	0.11398108	8.61	0.0035
b	1	1.86884426	1.86884426	141.17	<.0001
P	1	1.71514409	1.71514409	129.56	<.0001
DV	1	18.99832117	18.99832117	1435.06	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	2.341295745	0.03454902	67.77	<.0001
sqp	0.000010412	0.00000100	10.38	<.0001
sqDV	-0.000016574	0.00000565	-2.93	0.0035
b	-0.000022711	0.00000191	-11.88	<.0001
P	-0.004198872	0.00036890	-11.38	<.0001
DV	0.026685535	0.00070443	37.88	<.0001

The REG Procedure
Model: MODEL2
Dependent Variable: psize

Number of Observations Read	720
Number of Observations Used	720

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	314.27479	62.85496	4747.84	<.0001
Error	714	9.45240	0.01324		
Corrected Total	719	323.72719			

Root MSE	0.11506	R-Square	0.9708
Dependent Mean	3.22294	Adj R-Sq	0.9706

Coeff Var 3.57001

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.34130	0.03455	67.77	<.0001
sqp	1	0.00001041	0.00000100	10.38	<.0001
sqDV	1	-0.00001657	0.00000565	-2.93	0.0035
b	1	-0.00002271	0.00000191	-11.88	<.0001
P	1	-0.00420	0.00036890	-11.38	<.0001
DV	1	0.02669	0.00070443	37.88	<.0001

The REG Procedure
Model: MODEL1
Dependent Variable: psize

Number of Observations Read 720
Number of Observations Used 720

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	314.27479	62.85496	4747.84	<.0001
Error	714	9.45240	0.01324		
Corrected Total	719	323.72719			

Root MSE 0.11506 R-Square 0.9708
Dependent Mean 3.22294 Adj R-Sq 0.9706
Coeff Var 3.57001

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.34130	0.03455	67.77	<.0001
sqp	1	0.00001041	0.00000100	10.38	<.0001
sqDV	1	-0.00001657	0.00000565	-2.93	0.0035
b	1	-0.00002271	0.00000191	-11.88	<.0001

P 1 -0.00420 0.00036890 -11.38 <.0001
DV 1 0.02669 0.00070443 37.88 <.0001

Parameter Estimates

Variable	DF	95% Confidence Limits	
Intercept	1	2.27347	2.40913
sqp	1	0.00000844	0.00001238
sqDV	1	-0.00002766	-0.00000548
b	1	-0.00002646	-0.00001896
P	1	-0.00492	-0.00347

DV
1 .02530 .02807

Graph between observed and predicted values for fitzmill—microfluidizer

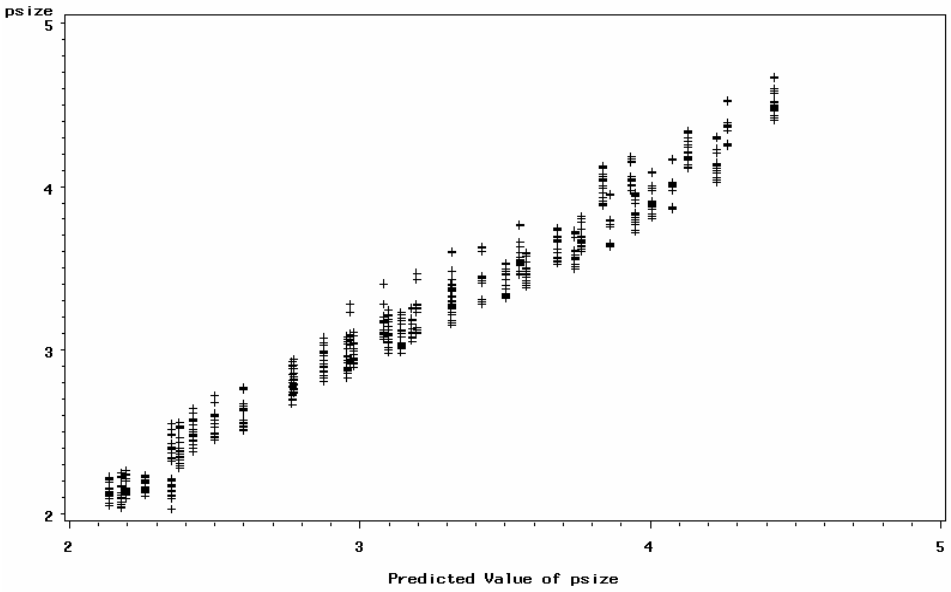

Figure D.1 – Observed versus predicted values for the particle size diameter model developed for soymilk produced by Microfluidizer-Fitzmill processing.

MICROFLUIDIZER-MEGATRON MODEL

OUTPUT

****

The GLM Procedure

Class Level Information

Class	Levels	Values
rep	3	1 2 3

Number of Observations Read	720
Number of Observations Used	720

The GLM Procedure

Dependent Variable: psize

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	313.9611296	78.4902824	5082.82	<.0001
Error	715	11.0412288	0.0154423		
Corrected Total	719	325.0023584			

R-Square	Coeff Var	Root MSE	psize Mean
0.966027	3.886564	0.124267	3.197347

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sqp	1	11.2157471	11.2157471	726.30	<.0001
b	1	225.2245232	225.2245232	14584.9	<.0001
P	1	12.4877450	12.4877450	808.67	<.0001
DV	1	65.0331143	65.0331143	4211.37	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
sqp	1	1.03411555	1.03411555	66.97	<.0001
b	1	0.63936075	0.63936075	41.40	<.0001
P	1	1.65149132	1.65149132	106.95	<.0001

DV 1 65.03311428 65.03311428 4211.37 <.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	2.380468264	0.03505379	67.91	<.0001
sqp	0.000008866	0.00000108	8.18	<.0001
b	-0.000013284	0.00000206	-6.43	<.0001
P	-0.004120220	0.00039842	-10.34	<.0001
DV	0.023459352	0.00036150	64.90	<.0001

Table.1.The Data

The REG Procedure
Model: MODEL1
Dependent Variable: psize

Number of Observations Read	720
Number of Observations Used	720

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	313.96113	78.49028	5082.82	<.0001
Error	715	11.04123	0.01544		
Corrected Total	719	325.00236			

Root MSE	0.12427	R-Square	0.9660
Dependent Mean	3.19735	Adj R-Sq	0.9658
Coeff Var	3.88656		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.38047	0.03505	67.91	<.0001
sqp	1	0.00000887	0.00000108	8.18	<.0001
b	1	-0.00001328	0.00000206	-6.43	<.0001
P	1	-0.00412	0.00039842	-10.34	<.0001
DV	1	0.02346	0.00036150	64.90	<.0001

Parameter Estimates

Variable	DF	95% Confidence Limits	
Intercept	1	2.31165	2.44929
sqp	1	0.00000674	0.00001099
b	1	-0.00001734	-0.00000923
P	1	-0.00490	-0.00334
DV	1	0.02275	0.02417

Graph between observed and predicted values for megatron—microfluidizer

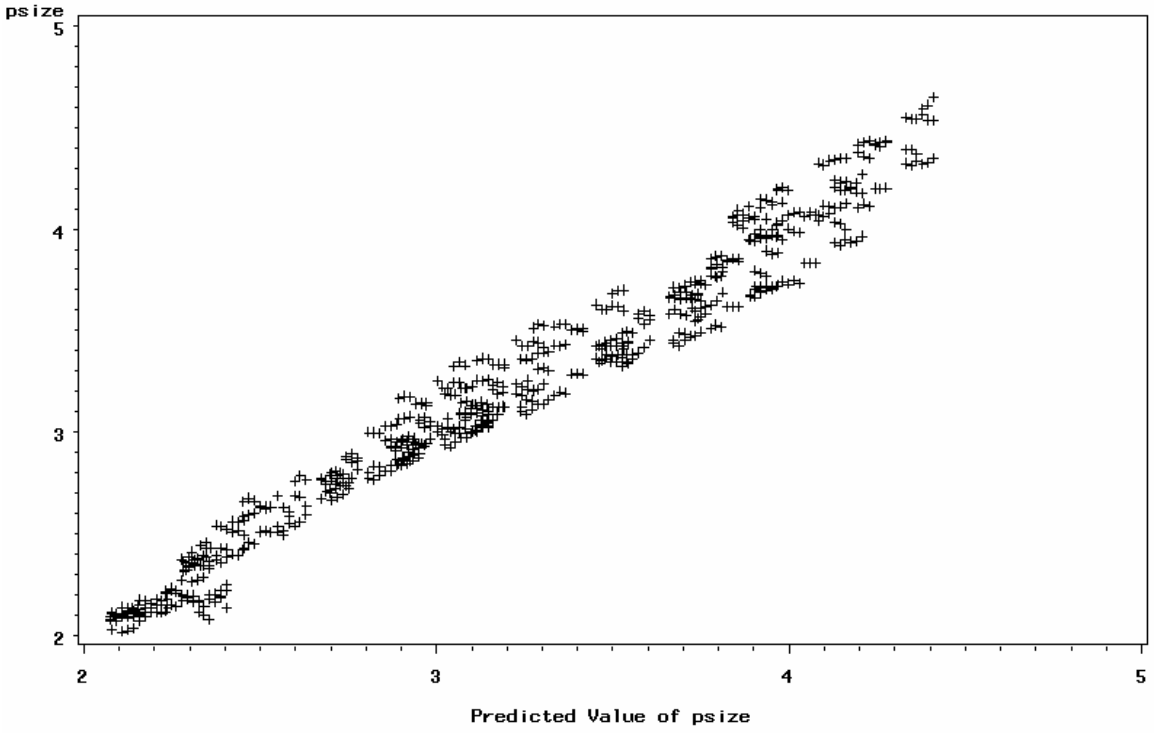

Figure D.2 – Observed versus predicted values for the particle size diameter model developed for soymilk produced by Microfluidizer-Megatron processing.

MODEL OBTAINED WITH CFHPT SYSTEM AT DIFFERENT FLOW RATES AND PRESSURES

OUTPUT

Table.1.The Data

The REG Procedure
Model: MODEL1
Dependent Variable: psize

Number of Observations Read 2160
Number of Observations Used 2160

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1027.72824	171.28804	11716.7	<.0001
Error	2153	31.47513	0.01462		
Corrected Total	2159	1059.20338			

Root MSE 0.12091 R-Square 0.9703
Dependent Mean 3.26069 Adj R-Sq 0.9702
Coeff Var 3.70810

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	3.16051	0.06060	52.15	<.0001
sqp	1	0.00001117	6.085942E-7	18.36	<.0001
sqF	1	0.27050	0.04496	6.02	<.0001

c	1	-0.00025197	0.00011236	-2.24	0.0250
P	1	-0.00642	0.00024632	-26.06	<.0001
DV	1	0.02167	0.00008612	251.63	<.0001
flow	1	-0.68431	0.10463	-6.54	<.0001

Parameter Estimates

Variable	DF	95% Confidence Limits	
Intercept	1	3.04166	3.27935
sqp	1	0.00000998	0.00001237
sqF	1	0.18233	0.35867
c	1	-0.00047231	-0.00003163
P	1	-0.00690	-0.00594
DV	1	0.02150	0.02184
flow	1	-0.88950	-0.47912

The GLM Procedure

Dependent Variable: psize

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1027.735233	114.192804	7802.00	<.0001
Error	2150	31.468143	0.014636		
Corrected Total	2159	1059.203376			

R-Square	Coeff Var	Root MSE	psize Mean
0.970291	3.710276	0.120981	3.260694

Source	DF	Type III SS	Mean Square	F Value	Pr > F
sqp	1	4.92886958	4.92886958	336.76	<.0001
sqF	1	0.52917805	0.52917805	36.16	<.0001
sqDV	1	0.00062873	0.00062873	0.04	0.8358
a	1	0.00304611	0.00304611	0.21	0.6483
b	1	0.00331646	0.00331646	0.23	0.6341
c	1	0.07351906	0.07351906	5.02	0.0251
P	1	9.36769793	9.36769793	640.03	<.0001
DV	1	25.51772909	25.51772909	1743.45	<.0001
flow	1	0.59929778	0.59929778	40.95	<.0001

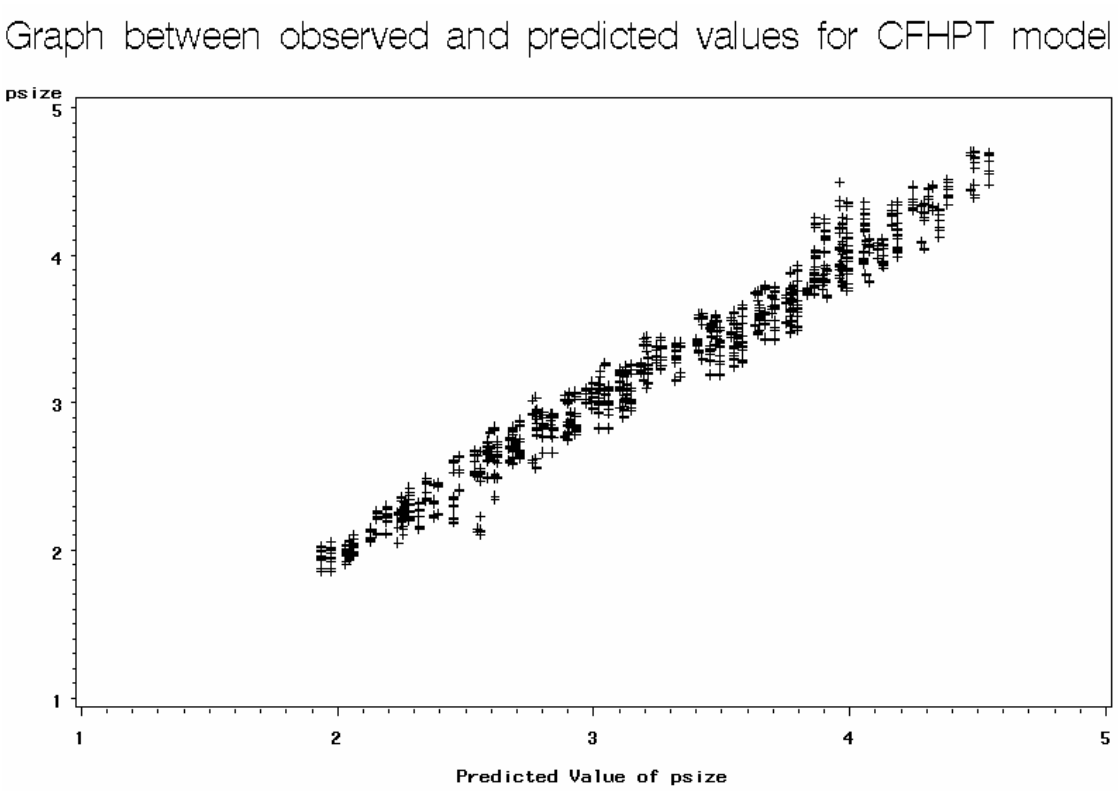

Figure D.3 – Observed versus predicted values for the particle size diameter model developed for soymilk produced by CFHPT-Megatron processing.

APPENDIX – E

DETAILS OF MICROFLUIDIZER AND THROTTLING VALVE

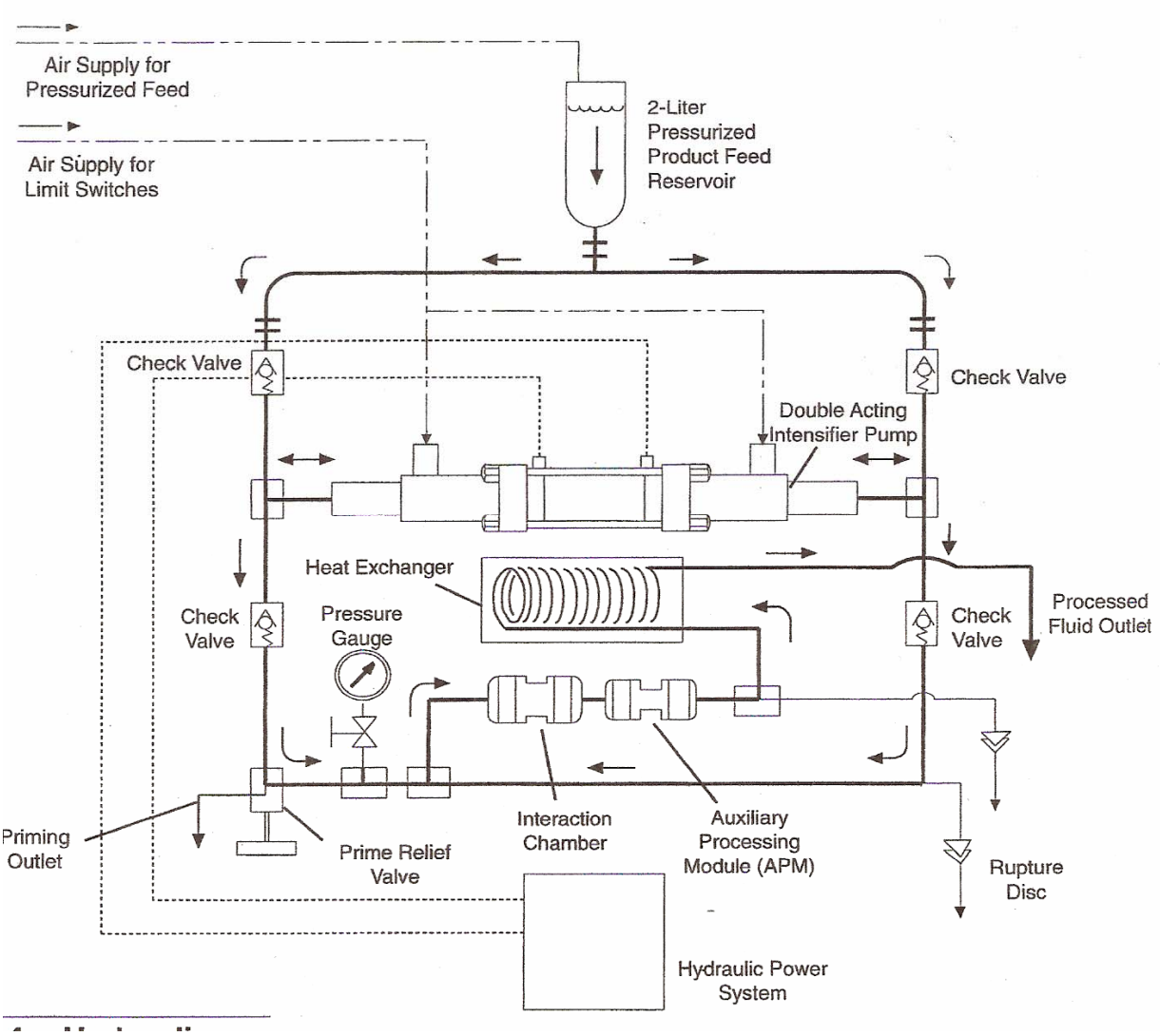

Figure E.1 – Microfluidizer (M-140K) flow diagram. Source: Microfluidizer M-140K user manual 140o-1.

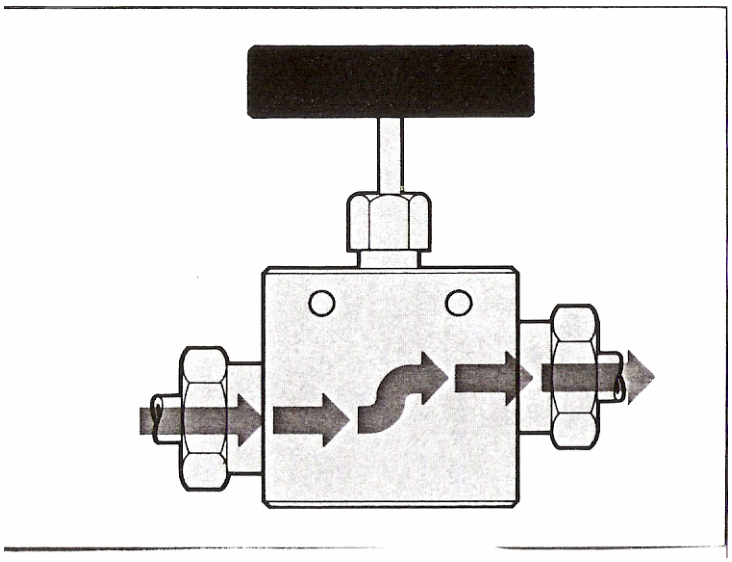

Figure E.2 – Micrometering (throttling) valve flow direction. Source: Autoclave Engineers Catalog 1500-2.

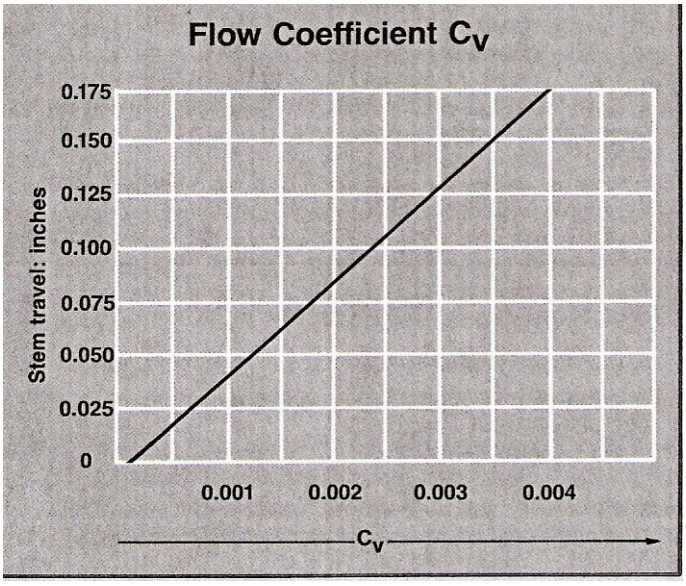

Figure E.3 – Flow coefficient curve for throttling valve. Source: Autoclave Engineers Catalog 1500-2.

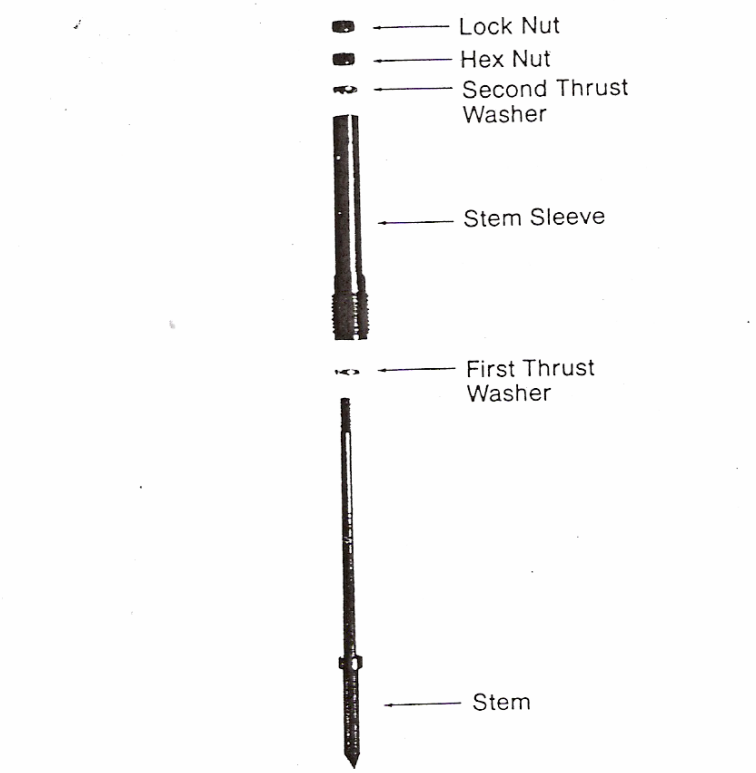

Figure 1
Two-Piece Non-Rotating Stem Assembly (Exploded)

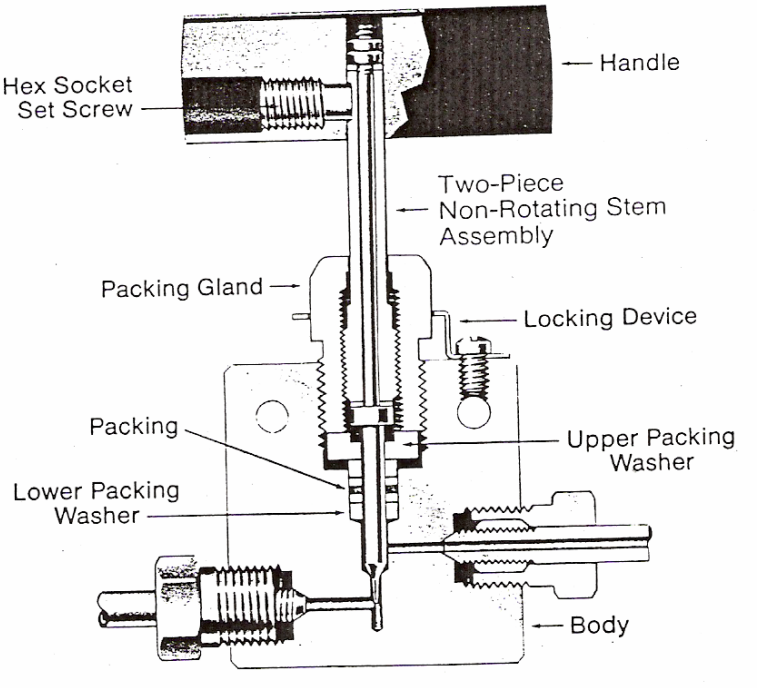

Figure E.4 – Throttling valve assembly. Source: Autoclave Engineers Catalog 1500-2.

APPENDIX - F

CALCULATIONS FOR TEMPERATURE RISE AFTER THROTTLING VALVE IN CFHPT SYSTEM

For theoretical temperature rise calculations (equation 1)(Toledo 2007; Amornsin 1999), Choi and Okos' (1987) equations as cited by Toledo (2007), were used for calculating the specific heat C_p, (J/kg.°C) and density, ρ (kg/m^3) of the soymilk used in the equation 1. Inlet temperature (P_{in}) used was 80 °C, inlet pressure (P_{in}, Pa) was the pressure applied, and the outlet pressure (P_{out}) was atmospheric (101325 Pa) pressure. The proximate composition of soymilk used in the calculations were taken from the chapter 3, in which the unboiled and two pass Gaulin homogenized soymilk did not show any significant difference from the total solids content of the CFHPT soymilks [unboiled and two pass Gaulin homogenized soymilk had proximate analysis of 3.68% protein, 1.58% fat, 0.26% crude fiber, 92.51% moisture, 0.24% ash, and 1.73% carbohydrate (by difference)].

$$T_{out} = T_{in} + \frac{(P_{in} - P_{out})}{\rho C_p} \quad \text{.....(1)}$$

Table F.1 – Density and specific heat calculations for soymilk using Choi and Okos (1987) equation as cited by Toledo (2007).

Protein,P (kg)	3.68*10^-2	**ρ_p=**	1288.45	**ρ_p*P=**	47.41	**Cpp=**	2096.51	**P*Cpp=**	77.15
Fat,F (kg)	1.58*10^-2	**ρ_f=**	892.18	**ρ_f*F=**	14.10	**Cpf=**	2071.34	**F*Cpf=**	32.73
Crude fiber, Fi (kg)	2.60*10^-3	**ρ_{fi}=**	1282.23	**ρ_{fi}*Fi=**	3.33	**Cpfi=**	1970.58	**Fi*Cpfi=**	5.12
Water,W (kg)	9.25*10^-1	**ρ_w=**	973.38	**ρ_w*W=**	900.48	**Cpw=**	4211.22	**W*Cpw=**	3895.80
Ash,A (kg)	2.40*10^-3	**ρ_a=**	2401.35	**ρ_a*A=**	5.76	**Cpa=**	1220.21	**A*Cpa=**	2.93
Carbohydrate, C (kg)	1.73*10^-2	**ρ_c=**	1574.26	**ρ_c*C=**	27.23	**Cpc=**	1667.79	**C*Cpc=**	28.85
				ρ_{avg} (kg/m^3)=	**998.32**			**Cavg (J/kg.K)=**	**4042.58**

Table F.2 – Temperature rise calculations for soymilk using equation (1) (Toledo 2007; Amornsin 1999).

P_1(Pa)	**Pressure after throttling, P_2 (Pa)**	**T_1 (°C)**	**T_2 (°C)=T_1+((P_1-P_2)/(ρ_{avg}*C_{avg}))**	**Temperature rise (°C)=T_2-T_1**
68947570	101325	80	97.06	17.06
103421355	101325	80	105.60	25.60
137895140	101325	80	114.14	34.14
206842710	101325	80	131.23	51.23
275790280	101325	80	148.31	68.31

References

Amornsin A. 1999. Effect of high pressure throttling on ascorbic acid, pectin esterase activity and limonin content in citrus juice [MS Thesis], Athens: University of Georgia 92 p. Available from: Athens: University of Georgia [MS Thesis].

Choi Y, Okos MR. 1987. Effects of temperature and composition on thermal properties of foods. In: IeMaguer M & Jelen P, editors. Food engineering and process applications. New York: Elsevier Applied Science Publishers.

Toledo RT. 2007. Fundamentals of food process engineering,3rd ed. New York: Springer 579 p.

APPENDIX - G

ISOFLAVONE PROFILE ANALYSIS RESULTS

Table G.1 – Isoflavone profile of soymilk before pressure treatment.

Isoflavone profile	Amount (ppm)
Diadzin	<1.00
6-OMal-Diadzin	1.00
6-OAc-Diadzin	<1.00
Diadzein	4.00
Total Diadzein Compounds	5.00
Genistin	1.00
6-OMal-Genistin	31.00
6-OAc-Genistin	<1.00
Genistein	10.00
Total Genestein Compounds	14.00
Glycitin	<1.00
6-OMal-Glycitin	1.00
Glycitein	2.00
Total Glycitein Compounds	3.00
Total Isoflavones	22.00
Diadzin (aglucon units)	<1.00
6-OMal-Diadzin (aglucon units)	1.00
6-OAc-Diadzin (aglucon units)	<1.00
Diadzein (aglucon units)	4.00
Total Diadzein (aglucon units)	5.00
Genistin (aglucon units)	1.00
6-OMal-Genistin (aglucon units)	2.00
6-OAc-Genistin (aglucon units)	<1.00
Genistein (aglucon units)	10.00
Total Genistein (aglucon units)	13.00
Glycitin (aglucon units)	<1.00
6-OMal-Glycitin (aglucon units)	1.00
Glycitein (aglucon units)	2.00
Total Glycitein (aglucon units)	3.00
Total All Forms (aglucon units)	21.00

Source: Analyzed at Nestle' Purina PetCare Company Checkerboard Square, St. Louis, MO 63164.

Table G.2 – Isoflavone profile of soymilk produced by CFHPT treatment at 68.95 MPa.

Isoflavone profile	Amount (ppm)
Diadzin	1.00
6-OMal-Diadzin	2.00
6-OAc-Diadzin	1.00
Diadzein	2.00
Total Diadzein Compounds	6.00
Genistin	7.00
6-OMal-Genistin	5.00
6-OAc-Genistin	<1.00
Genistein	4.00
Total Genestein Compounds	16.00
Glycitin	3.00
6-OMal-Glycitin	1.00
Glycitein	1.00
Total Glycitein Compounds	5.00
Total Isoflavones	27.00
Diadzin (aglucon units)	1.00
6-OMal-Diadzin (aglucon units)	1.00
6-OAc-Diadzin (aglucon units)	1.00
Diadzein (aglucon units)	2.00
Total Diadzein (aglucon units)	5.00
Genistin (aglucon units)	4.00
6-OMal-Genistin (aglucon units)	3.00
6-OAc-Genistin (aglucon units)	<1.00
Genistein (aglucon units)	4.00
Total Genistein (aglucon units)	11.00
Glycitin (aglucon units)	2.00
6-OMal-Glycitin (aglucon units)	1.00
Glycitein (aglucon units)	1.00
Total Glycitein (aglucon units)	4.00
Total All Forms (aglucon units)	20.00

Source: Analyzed at Nestle' Purina PetCare Company Checkerboard Square, St. Louis, MO 63164.

Table G.3 – Isoflavone profile of soymilk produced by CFHPT treatment at 275.79 MPa.

Isoflavone profile	Amount (ppm)
Diadzin	<1.00
6-OMal-Diadzin	<1.00
6-OAc-Diadzin	1.00
Diadzein	4.00
Total Diadzein Compounds	5.00
Genistin	2.00
6-OMal-Genistin	<1.00
6-OAc-Genistin	<1.00
Genistein	11.00
Total Genestein Compounds	13.00
Glycitin	1.00
6-OMal-Glycitin	<1.00
Glycitein	2.00
Total Glycitein Compounds	3.00
Total Isoflavones	21.00
Diadzin (aglucon units)	<1.00
6-OMal-Diadzin (aglucon units)	<1.00
6-OAc-Diadzin (aglucon units)	1.00
Diadzein (aglucon units)	4.00
Total Diadzein (aglucon units)	5.00
Genistin (aglucon units)	1.00
6-OMal-Genistin (aglucon units)	<1.00
6-OAc-Genistin (aglucon units)	<1.00
Genistein (aglucon units)	11.00
Total Genistein (aglucon units)	12.00
Glycitin (aglucon units)	1.00
6-OMal-Glycitin (aglucon units)	<1.00
Glycitein (aglucon units)	2.00
Total Glycitein (aglucon units)	3.00
Total All Forms (aglucon units)	20.00

Source: Analyzed at Nestle' Purina PetCare Company Checkerboard Square, St. Louis, MO 63164.

Table G.4 – Isoflavone profile of soymilk produced by CFHPT treatment at 103.42 MPa.

Isoflavone profile	Amount (ppm)
Diadzin	<1.00
6-OMal-Diadzin	1.00
6-OAc-Diadzin	1.00
Diadzein	4.00
Total Diadzein Compounds	6.00
Genistin	1.00
6-OMal-Genistin	1.00
6-OAc-Genistin	<1.00
Genistein	11.00
Total Genestein Compounds	13.00
Glycitin	<1.00
6-OMal-Glycitin	<1.00
Glycitein	2.00
Total Glycitein Compounds	2.00
Total Isoflavones	21.00
Diadzin (aglucon units)	<1.00
6-OMal-Diadzin (aglucon units)	1.00
6-OAc-Diadzin (aglucon units)	1.00
Diadzein (aglucon units)	4.00
Total Diadzein (aglucon units)	6.00
Genistin (aglucon units)	1.00
6-OMal-Genistin (aglucon units)	1.00
6-OAc-Genistin (aglucon units)	<1.00
Genistein (aglucon units)	11.00
Total Genistein (aglucon units)	13.00
Glycitin (aglucon units)	<1.00
6-OMal-Glycitin (aglucon units)	<1.00
Glycitein (aglucon units)	2.00
Total Glycitein (aglucon units)	2.00
Total All Forms (aglucon units)	21.00

Source: Analyzed at Nestle' Purina PetCare Company Checkerboard Square, St. Louis, MO 63164.

APPENDIX - H

SCORE SHEETS USED IN DIFFERENCE TEST AND CONSUMER ACCEPTABILITY

Difference Test

Instructions: Clear your palate with water and cracker between the samples.

Taste each of the three soymilk samples and write the sample code which tastes odd.

Odd sample code _____

Consumer Test

Instructions: Clear your palate with water and cracker between the samples.

Taste each of the two soymilk samples and write the sample code which gives better mouth feel.

Code _____

Taste each of the two soymilk samples and write the preferred sample code.

Code _____

Taste each of the two soymilk samples and rate each as follows:

5 – definitely would purchase, 4 – probably would purchase

3 – might or might not purchase, 2 – probably would not purchase

1 – definitely would not purchase

Evaluate each sample separately. Do not compare one sample with another.

Code	Rating
_____	_____
_____	_____

Consent Form

I _____, agree to participate in the research study entitled "Effect of high pressure homogenization and sterilization on properties of soymilk from whole dehulled soybeans" which is being conducted at The University of Georgia by Litha Sivanandan of the Department of Food Science and Technology, telephone number (706)-542-1079. This study will be done under the guidance of Dr. Robert L. Shewfelt (Faculty member and Co-Investigator) and Dr. Romeo T. Toledo (Faculty Advisor). I understand that participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of the participation, to the extent that it can be identified as mine, returned to me, removed from the research records, or destroyed.

The following points have been explained to me:

1. The reason for the research study is to determine the consumer acceptability of high pressure processed soymilk by sensory evaluation.
2. My participation in this study will help identify the characteristics that are most important to consumers in soymilk flavor. With this help, we hope to provide manufacturers with information that will help improve the quality of soymilk available to consumers. I will not benefit from my participation.
3. This consent applies strictly to today's session, which should last no longer than 10 minutes. Participation in future tests will require the completion of a new consent form.
4. The procedures are as follows: I will be served soymilk samples which I will evaluate for acceptability using standard procedures. I must indicate in the evaluation sheet about my preference and associated comments. All procedures are methods published in guidelines by

the American Society for Testing Materials (ASTM) and the Sensory Evaluation Division of the Institute of Food Technologists (IFT).

5. Participation entails the following risk: allergenic reaction to soymilk. This risk is considered minimal. In the event of an allergic reaction, the investigator will follow standard emergency procedures, such as calling 911.
6. It is my responsibility to make known to the investigators any allergic reaction I may develop towards the samples presented when they occur. I have known allergies towards _____.
7. The results of this participation will be confidential and will not be released in any identifiable form without my prior consent unless required by law.
8. I understand that I will receive a small reward, such as a candy or toffee, for participation in this study.
9. The investigator will answer any further questions about the research either now or during the course of the study. I can reach her at (706)-542-1079.
10. My signature below indicates that the researchers have answered all of my questions to my satisfaction and that I consent to volunteer for this study. I have been given a copy of this form.

_____ _____

Signature of Investigator Signature of Participant

(706)-542-1079

Date: _____

Additional questions or problems regarding your rights as a research participant should be addressed to IRB chairperson, Human Subjects Office, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411, Telephone (706)-542-3199.

APPENDIX – I

NUTRITION FACTS OF WHOLE DEHULLED SOYMILK

Nutrition facts			
Serving size 1 cup (240 mL)			
Servings per container 1			
Amount per serving			
Calories			97
Calories from fat			42
			%Daily Value*
Total Fat 4.6 g			6%
Total Carbohydrate 5.3 g			2%
Protein 8.4 g			13%
*Percent Daily Values are based on a 2,500 calorie diet. Your daily values may be higher or lower depending on your calorie needs.			
	Calories:	2,000	2,500
Total Fat	Less than	65g	80 g
Total Carbohydrate	Less than	300 g	375 g
Protein	Less than	50 g	65 g
Calories per gram:			
Fat 9.	Carbohydrate 4.	Protein 4.	
"Not a significant source of cholestrol".			

This was calculated on the basis of the chemical composition (in %, moisture-free basis) of soybean cotyledons as follows:

Crude protein	-	43.4
Crude fat	-	24.3
N-free extract + fiber	-	27.4
Ash	-	5.0

Soymilk, having 8% non-volatile solids, was produced from the cotyledons by adding deionized water, (1:3 w/w) to the dehulled blanched cotyledons.

APPENDIX – J

EFFECT OF MICROFLUIDIZER-INTERACTON CHAMBER PROCESSING ON PARTICLE SIZE DIAMETER OF SOYMILK

Table J.1 - Particle size diameter obtained for different pressure level treatments using Microfluidizer-Interaction chamber (MI).

Process	Pressure (MPa)	$D_{(v, 0.10)}$ (µm)	$D_{(v,0.20)}$ (µm)	$D_{(v,0.40)}$ (µm)	$D_{(v,0.50)}$ (µm)	$D_{(v,0.60)}$ (µm)	$D_{(v,0.80)}$ (µm)	$D_{(v,0.90)}$ (µm)	$D_{(v,0.99)}$ (µm)	$D_{(3,2)}$ (µm)	$D_{(4,3)}$ (µm)
Fitzmill 3-screens/MI	68.95	6.40	19.70	41.03	49.24	57.63	78.41	96.48	138.62	19.88	51.46
		(0.86)	(2.43)	(0.80)	(0.59)	(0.40)	(0.50)	(0.97)	(2.22)	(1.91)	(0.56)
	103.42	8.50	17.03	29.47	35.43	41.80	57.82	71.53	101.69	13.24	38.36
		(0.18)	(0.17)	(0.12)	(0.09)	(0.08)	(0.23)	(0.45)	(1.14)	(0.11)	(0.10)
	137.9	9.79	15.22	24.54	29.35	34.61	47.99	59.39	84.89	20.02	32.42
		(0.12)	(0.18)	(0.29)	(0.36)	(0.44)	(0.76)	(1.26)	(4.57)	(0.26)	(0.59)
	206.84	8.78	12.07	18.31	21.87	25.83	36.41	45.71	66.40	16.95	24.87
		(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.04)	(0.05)	(0.07)	(0.02)	(0.02)
	275.79	8.14	10.11	15.30	18.19	21.59	31.01	39.77	60.57	14.87	21.38
		(0.03)	(0.02)	(0.02)	(0.01)	(0.01)	(0.10)	(0.21)	(1.57)	(0.04)	(0.05)
Fitzmill 2-screens/MI	68.95	7.09	14.17	36.08	44.78	53.76	75.56	94.17	136.3	19.75	48.23
		(0.06)	(0.92)	(0.41)	(0.32)	(0.08)	(0.95)	(1.94)	(3.12)	(0.18)	(0.46)
	103.42	7.24	15.71	29.26	35.44	41.96	58.37	72.60	104.57	18.28	38.41
		(1.04)	(1.26)	(0.23)	(0.23)	(0.34)	(0.98)	(1.58)	(1.73)	(1.29)	(0.79)
	137.9	10.5	16.15	25.77	30.71	36.04	49.69	61.37	87.00	21.14	33.74
		(0.20)	(0.16)	(0.10)	(0.08)	(0.06)	(0.04)	(0.04)	(0.08)	(0.35)	(0.10)
	206.84	8.89	12.36	18.78	22.47	26.51	37.36	46.93	67.69	17.31	25.50
		(0.06)	(0.09)	(0.10)	(0.10)	(0.09)	(0.07)	(0.04)	(0.06)	(0.10)	(0.08)
	275.79	8.27	10.44	15.96	19.00	22.65	32.72	42.23	67.39	15.38	22.56
		(0.06)	(0.14)	(0.21)	(0.23)	(0.26)	(0.33)	(0.39)	(0.50)	(0.16)	(0.22)
Stonemill/MI	68.95	11.60	25.23	41.69	49.58	58.00	79.85	98.99	141.14	24.88	53.57
		(0.35)	(0.09)	(0.04)	(0.06)	(0.04)	(0.04)	(0.17)	(0.49)	(0.22)	(0.05)
	103.42	10.10	18.39	30.74	36.82	43.31	60.09	74.74	106.51	21.50	40.24

		(0.12)	(0.06)	(0.01)	(0.01)	(0.04)	(0.16)	(0.30)	(0.44)	(0.12)	(0.05)
	137.9	10.40	15.84	25.34	30.28	35.68	49.59	61.61	88.21	21.02	33.58
		(0.03)	(0.00)	(0.04)	(0.05)	(0.05)	(0.06)	(0.07)	(0.07)	(0.03)	(0.03)
	206.84	8.97	12.51	19.15	23.03	27.27	38.99	49.44	74.34	17.66	26.53
		(0.03)	(0.05)	(0.05)	(0.05)	(0.04)	(0.05)	(0.06)	(0.10)	(0.06)	(0.04)
	275.79	8.30	10.48	15.97	19.00	22.66	32.82	42.45	68.11	15.44	22.64
		(0.04)	(0.09)	(0.16)	(0.19)	(0.24)	(0.38)	(0.50)	(0.61)	(0.13)	(0.23)
Megatron/MI	68.95	6.99	18.69	39.89	48.77	58.05	81.87	102.80	146.97	20.58	52.77
		(0.05)	(1.88)	(0.97)	(0.91)	(0.86)	(1.03)	(1.22)	(0.80)	(0.38)	(0.95)
	103.42	6.89	15.74	29.75	36.01	42.56	59.04	73.46	105.93	18.15	38.84
		(0.55)	(0.40)	(0.26)	(0.22)	(0.19)	(0.80)	(1.45)	(1.63)	(0.65)	(0.44)
	137.9	7.58	13.48	24.21	29.59	35.42	50.33	63.31	91.62	17.55	33.02
		(0.66)	(0.73)	(0.40)	(0.32)	(0.25)	(0.13)	(0.05)	(0.11)	(0.74)	(0.33)
	206.84	8.96	12.43	18.92	22.73	26.91	38.42	48.73	74.08	17.57	26.21
		(0.04)	(0.06)	(0.10)	(0.12)	(0.14)	(0.20)	(0.23)	(1.12)	(0.09)	(0.12)
	275.79	8.23	10.26	15.67	18.68	22.32	32.48	42.14	67.85	15.23	22.37
		(0.02)	(0.05)	(0.06)	(0.06)	(0.07)	(0.10)	(0.13)	(0.20)	(0.04)	(0.07)

The values are mean from 3 observations of single experiment. Values in parenthesis show the SD for the mean value above it.
MI stands for Microfluidizer-Interaction chamber. The fine grinding machines used here are Fitzmill with 3 screens (0.127 cm, 0.051 cm, and 0.030 cm), Fitzmill with 2 screens (0.127 cm and 0.051 cm), Stonemill (with stone#E6-46), and Megatron (13,000 rpm for 15 min). The process of producing soymilk until grinding was the same as used in chapters 4 and 5. Pressure levels used in MI is given in the table.