EFFECT OF CONTINUOUS FLOW HIGH PRESSURE THROTTLING (CFHPT) ON QUALITY ATTRIBUTES OF SOYMILK AND CHANGES DURING STORAGE

by

JAIDEEP SINGH SIDHU

(Under the Direction of Rakesh K. Singh)

ABSTRACT

We investigated the effect of continuous high pressure processing on the physical, chemical, microbiological and sensory qualities of soymilk prepared form whole dehulled soybeans. Changes in quality were also investigated by storing the soymilk at 4 °C for 28 days. The soymilk remained stable without any separation at the end of storage period. The particle size was reduced significantly ($\alpha<0.05$) and it did not change during storage. No lipoxygenase activity was detected. The pH value and microbial load decreased significantly after processing. During storage, the pH value reduced while the microbial load increased. The intensities of beany aroma, beany flavor and cooked flavor were generally higher as compared to commercial soymilk samples. The intensity of beany flavor was found to reduce during storage. However, there was no significant difference in the astringency, bitterness and chalkiness of the samples prepared in this study and the commercial samples.

INDEX WORDS: Continuous, High Pressure, Particle Size, pH, Lipoxygenase, Microbiology, Sensory, Storage

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B.E., Panjab University, India, 2007

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

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August 2013

# DEDICATION

I dedicate this work to my mom and dad; their love and support has been pivotal.

# ACKNOWLEDGEMENTS

My work would be incomplete without the mention of those who have helped bring it to fruition. First, I want to thank my parents, Saranjit Kaur and Amarjit Singh Sidhu, who encouraged me to pursue my master's degree and have supported me throughout. Big thanks to my advisor, Dr. Rakesh K. Singh, for his guidance, experience, as well as his patience. The poise with which he helped me overcome the hurdles always left me awestruck. I am grateful that he let me be independent in my project and think about things on own first. I am gratified that whenever needed, he took out the time to discuss my progress, and also my life. Thanks to my committee: Dr. William L. Kerr, for trusting me with using the equipment and instruments in his lab, and for his valuable technical expertise; and Dr. Robert L. Shewfelt, whom I cannot thank enough for helping me further my critical thinking and reasoning abilities. Thanks to him for helping me understand the American way of life and encouraging me to explore it. Huge thanks to Carl Ruiz, whom I also consider as my *compadre*, in helping me run the experiments. My research would not have been possible without his assistance. Thanks to him for his lessons on life and for enabling me to persevere. Thanks to Bilal Kirmaci for also helping me run the experiments, and for not losing his calm even though his was the toughest job. Thanks to Vijendra Sharma for teaching me how to conduct the experiments. I also want to thank Vikramaditya Yandapalli for his very valuable guidance on statistics.

Thanks to our graduate coordinator, Dr. Mark A. Harrison and to the entire faculty of the Food Science department; I have learnt something from everyone. Thanks

to Vicki Wentzel, Ruth Ann Morrow and Danny Morris for helping me with my work in their labs. I am also grateful to Lisa Cash, Karen Simmons, Lisa Porterfield, Beth Knight and Eve Mayes for their support and help. Thanks to our former librarian, Monica Pereira, for helping me understand the university's library system and for making it easier for me to search for literature. Thanks to the Food Science Club, International Student Life and the Indian Student Association for helping me in my extra-curricular development. Thanks to all those unnamed individuals who have been a part of my journey at University of Georgia.

Finally, thanks to my close friends: Manoj Manjare, Rahi Kachole, Nita Verma, Winnie Lim, Deepali Pavnaskar, Sanhita Bhargava, Jeff Thompson, Adriana Rincon Villegas, Bilal Kirmaci, Venessa Chandra, Chris Sharps, Rumit Singh, Nidhi Singla, Prakarsh Singh, Supakana Nagachinta, Todd Fisk, Tracy Jaico and actually, to all my friends. It is because of them that my time in Athens has been absolutely enjoyable and pleasantly memorable. Thank you!

*Go Dawgs!*

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# CHAPTER 1

# INTRODUCTION

Continuous flow high pressure throttling (CFHPT) is a novel food processing technique developed around 15 years ago by researchers at University of Georgia, GA, USA (Adapa et al., 1997). A variety of foods, processed by this technique have since been studied. CFHPT falls under the broad category of high pressure processing (HPP) and more specifically under high pressure homogenization (HPH), also known as continuous high pressure (CHP) processing. As the name implies, it is a continuous method and is thus used for foods that can be pumped. The other type of HPP is the high hydrostatic pressure (HHP) processing which is a batch process. HHP technique can be used on solid foods without loss of their shape (Balasubramaniam, 2006). In both techniques, the food is subjected to high pressure (100 – 1000 MPa), but the working principle is different. CHP relies on flow rate and mode of pressure release for its action while in the case of HHP, duration of pressure application is the primary controlling factor. Pressure may be supplemented with mild heat treatments to achieve better results (San Martin et al., 2002). Some of the advantages of HPP over conventional food processing methods such as thermal treatments etc. include better retention of sensory qualities of food and a lesser impact on nutrients (Tewari, 2007). Further, some of the advantages of CHP over HHP are that lower pressures are needed (Popper & Knorr, 1990); CHP being a continuous method can have a higher throughput; the pressure

release device (homogenizing/ throttling valve) helps reduce the size of suspended particles (Cruz et al., 2007) thereby stabilizing solutions (especially emulsions), and also aids in the rupture of microbial cells (Shirgaonkar et al., 1998) achieving a reduction in microbial load. In addition, there is a sudden increase in temperature at the point of pressure release which can be exploited for microbial reduction and enzymatic inactivation (Sharma et al., 2009; Datta et al., 2005).

Soymilk, though not native to the Western world, has gained popularity in the past few decades (Savitry & Prakash, 2004). It is an easy way to incorporate soybeans into the diet which are a good source of high quality proteins as well as unsaturated fatty acids (Smith & Circle, 1972; USDA 2013). Soymilk, apart from being easy to digest, is a suitable dairy substitute for those with lactose intolerance since it is naturally absent in this disaccharide (Xu & Chang, 2009). In simple terms, soymilk can be described as the water extract of soybeans (Kwok & Niranjan, 1995). However, since the Western population is not used to the flavor of soymilk, which is typically described as beany, even slight hints of this note can turn consumers off (Liu, 1997a). Soybeans contain an anti-nutritional factor known as trypsin inhibitor (Guerrero-Beltran et al., 2009b) which must be inactivated prior to the consumption of soybeans in any form as it can inhibit the digestion of proteins. This is usually accomplished be heat. Similarly, heat is employed to inactivate a naturally occurring enzyme in soybeans known as lipoxygenase, which can oxidize the fatty acids, creating off flavors (Baysal & Demirdoven, 2007). Chalkiness, which is defined as the sensation of grittiness or sandiness in the mouth or on the tongue, is another quality parameter which has to be controlled in soymilk. It is caused by soybean solids that are big enough to be discerned by the tongue (Kuntz et al., 1978). A

lot of research has focused on improving the flavor and mouthfeel of soymilk to increase its acceptability. Secondly, the conventional method of soymilk processing requires that the water extract be filtered to remove the coarse soybean solids which could lead to chalkiness as well as destabilize the beverage. However, this leads to about 35% of soybean solids remaining unutilized (Hand et al., 1964).

To alleviate the issues associated with conventional soymilk processing methods and improve the consumer acceptability of soymilk, Sivanandan (2007) developed a soymilk processing method employing CFHPT. This method enabled the use of practically all the soybean solids into the final product which meant a higher yield of soymilk. Different soymilk elaboration techniques in combination with CHP were studied to come up with a method that gave the best particle size distribution of soybean solids in the soymilk, and the most homogenous and stable product. This work was continued by Sharma (2008) who looked at the effect of CFHPT on the inactivation of microbial spores in soymilk and their resuscitation during storage.

The current work was undertaken to study some quality attributes of CFHPT soymilk and is a furtherance of the work already done by Sivanandan (2007) and Sharma (2008). The objectives of our research were:

- to study the effect of continuous flow high pressure throttling on particle size distribution, lipoxygenase activity, pH, microbial quality (aerobic plate count and total psychrotrophs), and sensory qualities
- to investigate the changes occurring in these parameter during 4 weeks of quiescent storage at 4 °C.

# CHAPTER 2

# REVIEW OF LITERATURE

An examination of past studies on high pressure processing of soymilk and related topics is presented in this chapter. This includes studies related to thermal processing as well. The emphasis is on processing parameters which have an effect on the yield, physical, enzymatic, sensory, and microbiological properties.

## Soybeans and their nutritional value

Soybeans are not native to the U.S. and were first brought to Georgia in 1765 (Hymowitz & Harlan, 1983). Apart from its significance in the developing world, soy is now gaining popularity in the developed countries as well. This can be attributed to the consumers' awareness of nutraceutical compounds naturally present in soybeans like isoflavones which are known to reduce the risk of diseases such as heart-attack, osteoporosis, kidney stones, etc., (Messina, 1997). In addition, some studies have linked soybean consumption to the reduction of breast, lung, colon, rectal, stomach, and prostate cancers along with reduction in cholesterol levels (N'Kouka et al., 2004). Hypolactasia (a low level of the enzyme lactase in the body) affects a large fraction of the world population (Nelson et al., 1978) resulting in lactose intolerance and preventing them from consuming dairy foods as they are rich in lactose (the primary milk sugar). Thus, soy (in

particular, soymilk) is a suitable substitute for bovine milk and other dairy products for the lactose intolerant as it does not contain lactose.

The Asian population has used soybeans in their diet for centuries (Nelson et al., 1976) and soybeans [*Glycine max* (L.) Merr.] are regarded as one of the principal food crops in the world (Sharma, 2008). It is the balanced amino acid profile of soybeans that make them an excellent source of protein (Smith & Circle, 1972). Compared to kidney beans, which have about 25% proteins and 1% lipids, soybeans have about 40% proteins and 20% lipids (USDA, 2013). With the supplementation of sulfur containing amino acids from cereals like wheat and rice, the overall amino acid profile confirms to FAO standards. Moreover, the lipids in the soybeans are almost 85% unsaturated, and being from a plant source, naturally absent in cholesterol. As of today, millions around the world don't get enough nutrition. As soybeans provide for a very inexpensive food, they can thus be utilized to overcome the nutrition problem (Nelson et al., 1978).

**Soybeans and their derived foods in the USA**

Traditionally, soy has contributed very little protein to the Western diet. In the US, soybeans were initially used as forage for animals (Gibson & Benson, 2005). Although the first commercial use of soybeans in the US was for the production of soybean oil (University of Kentucky, 2009), at present, soybeans are prized for their protein content. Herbicide resistant soybeans were developed in the 1990s which improved yields and greatly increased soybean production (North Carolina Soybean Producers Association, 2013). Soybeans now rank second in the US crop cash sales.

About 80% of the total edible fats consumed in the country come from soybeans (Gibson & Benson, 2005).

In the 1980s, use of soy extenders in ground beef and the use of soy as partial meat substitute in school-lunches were approved (Erdman & Fordyce, 1989). The FDA in 1999 approved the soy health claim that it may lead to a reduction in heart diseases (FDA, 1999). As a result, soy based foods (for example soymilk, tofu, soy yogurts, spreads, etc.) and ingredients (such as flour, concentrate, protein isolate, texturized protein, lecithin, etc.) have found increased prevalence in the market (Achouri et al., 2007).

**Soymilk: Processing methods and the yields**

One of the pragmatic ways to incorporate soybeans into the diet is in the form of soymilk (Nelson et al., 1978). In fact, soymilk sales have increased manifold in the US and they stood at over $600 million in 2003 (Savitry & Prakash, 2004). In 2011, 37% of Americans reported consuming soy-foods or beverages once or more a month and the percentage of those who never consumed soy declined (Soyfoods Association of North America, 2013).

Soymilk, also known as tonyu in Japanese, is an easily digestible soy food (Kaneko et al., 2011). It is basically a water extract of soybeans (Kwok & Niranjan, 1995) and is free of lactose, gluten, and cholesterol (Xu & Chang, 2009). The classical method of soymilk processing starts with soaking the beans, usually at room temperature (Mizutani & Hashimoto, 2004). This hydration step is followed by wet grinding of the soaked soybeans with the ratio of soybeans to water varying from one manufacturer to

another. The ground slurry is then filtered or centrifuged to remove the larger solids and the residue is known as okara. Finally, the filtered soymilk is cooked to improve flavor (Kwok & Niranjan, 1995; Sivanandan, 2007). However, this process results in a loss of about 35% of the soybean solids in the form okara and in those leached out during soaking (Lo et al., 1968). In addition, soymilk produced this way has a painty odor and flavor (Nelson et al., 1976). It was realized early on that this off flavor would stand in the way of soymilk acceptability (Hand et al., 1964). This has been confirmed by Morr & Ha (1991) and Liu (1997a).

Several studies have investigated the modification of the conventional method to either improve flavor, or yield, or both. Wilkens et al. (1967) used dehulled soybeans, a high temperature, along with a rapid grinding process and achieved an almost bland flavored soymilk. Their primary aim was to inactivate a naturally occurring and off-flavor causing enzyme, lipoxygenase before it could have a chance to act on its substrates, which are naturally present in soybeans. In another study (Kon et al., 1970), the researchers ground dry soybeans under acidic conditions to obtain a bland soymilk. However, acidic pH can lead to protein denaturation as well as increase the salt content of the product (Li, 2006). On the other hand, Ha et al. (1992) boiled soybeans in hot $NaHCO_3$ and noted a reduction in the total headspace volatile organic compounds, which can cause off flavors. Iwuoha & Umunnakwe (1997) compared soymilk made from blanched vs. unblanched soybeans and found that the blanched soybeans produced a blander soymilk. Mizutani & Hashimoto (2004) noted that low temperature grinding of soybeans helped reduce off flavors in addition to being more cost-effective. Although Carrao-Panizzi et al. (1999) concluded that pre-soaking of soybeans resulted in an

increased beany flavor in the product, they also suggested pre-heating of soybeans to improve the flavor. A recent study (Ly et al., 2011) looked at the effect of blanching soybeans at high temperature followed by grinding with nearly boiling water. They compared the effect of this process on the beany flavor as well as non-beany flavors in soymilk. It was discovered that methods used to reduce beany flavors also reduced the non-beany flavors. Some other methods to reduce off-flavors in soymilk include supercritical $CO_2$ extraction, enzyme treatment, and genetic modification, in particular to produce varieties which lack the enzyme lipoxygenase (Li, 2006).

Since the yield of the traditional method of soymilk manufacture is not very high, several studies have improved upon it by trying to include greater soybean solids in the soymilk as well a higher protein recovery. In addition, the waste generated from the discarded okara during soymilk manufacture can cause environmental issues (Chan & Ma, 1999). Hand et al. (1964) prepared soymilk from steam-dried and powdered soybeans thereby bypassing the soaking step. They achieved yields ranging from 80% to 90%. Some other studies also made slight modifications to the standard process. Miles (1966) removed the hulls of soybeans followed by grinding them into flakes. These flakes were then made into slurry, and homogenized at 35 – 55 MPa, and the slurry was then centrifuged and cooked. It was claimed that this process required 15% fewer soybeans to produce the same amount of soymilk although a stabilizer was required. In a patented process, Mustakas et al. (1972) used chiefly full-fat flour as the starting material instead of whole soybeans and performed wet grinding of 10-20% slurry of the flour. Their primary aim was to achieve finer particles in the final beverage rather than improving yields.

An often cited study, known as the Illinois Process (Nelson et al., 1976) sought to overcome both, the flavor, and the yield issues. Their method involved blanching of soaked beans in $NaHCO_3$ solution to inactivate lipoxygenase and tenderize the soybeans. Grinding of these blanched beans was followed by heating them to 93.3 °C, and a two stage homogenization (24 MPa, 3.4 MPa). The soymilk then had to be neutralized with HCl after which a second, two stage homogenization was done at 82.2 °C. They claimed a 90% recovery of soybean solids, 99% inclusion of soybean proteins into the milk and no separation after storing the soymilk for about 60 days in a refrigerator. Sucrose and other flavorings were also added to the beverage. Even though this work was done in the 1970s, it is still not widely used, perhaps because of the prevalence of chalkiness in the beverage (Rosenthal et al., 2003), generation of significant amounts of waste water and the process being energy intensive (Johnson et al., 1981). Another research method (Johnson et al., 1981) studied direct steam-infusion cooking of soymilk achieving higher temperatures (99 - 154 °C). A maximum of 86% soybean solids could be recovered by this method which also involved a filtration step. Finally, the variation of yield with the type of soybean cultivar has also been studied. Poysa & Woodrow (2002) found the yield of soymilk to be dependent upon the seed protein content as well the processing method.

**Food Processing**

Subjecting foods to various processing methods is essential to increase their shelf life, reduce negative changes in their quality, and make it viable for them to be transported over long distances (Luning et al., 1995; Norton & Sun, 2008). Foods with taste described as “natural” (by consumers), having fresh appearance and without

additives are preferred by consumers (Rastogi et al., 2007). Minimally processed, and shelf-stable are the two other important qualities that consumers favor in the foods they buy (Yaldagard et al., 2008). Some of the conventional food processing methods like drying, pasteurization, sterilization, freezing, etc. suffer from low rate of heating as well cooling which has undesirable effects on the product quality (Matser et al., 2004). Food processing by heating alone, albeit economical and efficient, has damaging effects on heat labile compounds and the food structure (Diels & Michiels, 2006). As a result, the color, flavor and texture of such foods gets affected (Yaldagard et al., 2008).

Non-thermal food processing methods are thus of special interest within the food industry (Tewari, 2007) and there has been a lot of interest in developing novel food processing technologies which achieve the desired processing requirements by means of either totally non-thermal, or mildly thermal methods (Welti-Chanes et al., 2005). Among these are Pulsed Electric Field (PEF) Pasteurization, High Intensity Pulsed Lights, High Intensity Laser, High Intensity Pulsed Magnetic Field, High Voltage Arc Discharge, Ozone Treatment, Ionizing Radiation (Gamma Irradiation), UV Decontamination, Manothermosonication (combination of pressure, heat and ultrasound), Membrane Separation, Streamer Plasma, High Hydrostatic Pressure (HHP), High Pressure Homogenization (HPH)/ Dynamic High Pressure and High Pressure Steriliztion (HPS) (Meyer et al., 2000; Ortega-Rivas, 2007; Tewari, 2007; Yaldagard et al., 2008). The last three technologies- HHP, HPH, and HPS have received considerable attention and collectively may be referred to as High Pressure Processing (HPP), which is a more commonly used term.

**High Pressure Processing (HPP)**

An important advancement in food processing during the past century has been the work on High Pressure Processing. It is a viable technology which produces fresh-tasting and minimally processed foods. Depending on the product and process, pressure in the range of 100 – 1000 MPa may be applied to the food. Accompanying heat may or may not be used (San Martin et al., 2002; Balasubramaniam, 2006). The food product could be a solid, or a liquid and it could be either in a package or handled directly (Farkas & Hoover, 2000). As HPP involves minimal heat, and inactivates microbes, maintains nutritional and sensory qualities of food, it has found favor in the food industry (Tewari, 2007). Some of the advantages of HPP are as follows:

- Food processing at room or cooler temperatures and retention of color, flavor, texture, and freshness
- Retentions of the crispy texture of high pressure processed fresh vegetables with no Vitamin C loss
- Microbial population reduction, without the use of excessive heat or preservatives
- The size & geometry of the food (in HHP) doesn't affect pressure transmittance
- Favorable changes in the functional properties of foods such as desirable changes in starch-gelatinization, and inactivation of quality abating enzymes
- Particle size reduction and improved colloidal stability
- Homogenization and emulsification of liquid foods
- No toxic substances or off flavors are produced
- Crystallization of lipids at room temperature

- Non-covalent bonds (hydrogen, ionic, and hydrophobic) are affected while low molecular weight molecules (which impart nutritional and sensory characteristics) are left largely unchanged

(Meyer et al., 2000; Stang et al., 2001; Diels & Michiels, 2006; Rastogi et al., 2007; Tewari, 2007; Sivanandan et al., 2008; Poliseli-Scopel et al., 2012; Dumay et al., 2012).

Although the market is small, there exist several HPP foods in the worldwide as well as the US markets. Balasubramaniam (2006) estimates that about 50 companies worldwide process over 100 food products using HPP. These products include fruit juices, smoothies, jams, jellies, salsa, guacamole, sauces, rice cake, ham, chicken strips, ready to eat meals with meats and vegetables, oysters and some other seafood (Hugas et al., 2002; Matser et al., 2004; Balasubramaniam, 2006; Tewari, 2007; Yaldagard et al., 2008). As has been mentioned before, high pressure processing can either be a batch, or a continuous process and these are described in detail below.

**High Hydrostatic Pressure (HHP) Processing**

The era of high pressure processing was essentially heralded by Hite (1899). In a first of its kind study, he applied pressures of up to 1378 MPa. Depending on time of pressure application, he was able to extend the shelf life of milk to 3-7 days, when stored at room temperature. After this initial word, another study was conducted by the same author along with some others (Hite et al., 1914) where they tried HPP on fruit juices, vegetables, pure cultures of bacteria and yeasts, and on culture media, obtaining mixed results. In some experiments, they also applied varying temperature along with pressure. This method of pressure application is known as hydrostatic pressure. Even though this

technology was conceived and implemented of at the end of 19th century and there were numerous other studies on various food throughout the 20th century, the first commercial HHP processed foods appeared in Japan only in 1991 (Yaldagard et al., 2008).

HHP processing requires an enclosed space which forms the pressure vessel. A fluid, called the pressure transfer fluid (usually water, or a suitable oil), which is responsible for the transmittance of pressure to the food is added to this space. The food, sealed in a flexible package is loaded into the pressure vessel and the whole assembly sealed. Pressure is then applied to the fluid which in turn transmits pressure to the food, and the pressure is held for a specific duration. The pressure gets applied equally from all directions (isostatically) which thus maintains the food product's integrity and shape (San Martin et al., 2002; Balasubramaniam, 2006). The pressure application is rapid and the foods don't implode (Kadam et al., 2012). However, in case of delicate foods, like those with large volumes of entrapped air (for example, leafy vegetables) the structure can get altered and there could occur a distortion of the cells (Rastogi et al., 2007).

As a food product is pressurized, an adiabatic rise in temperature is observed. The rise, dependent on the applied pressure, initial food temperature, and product characteristics can range from 0.03 – 0.09 °C/ MPa of applied pressure. This rise can be exploited for the food's simultaneous pasteurization, or sterilization (de Heij et al., 2003). Adiabatic heating is the homogenous rise in the temperature of a product as the pressure is increased, and is mathematically described as (Hoogland et al., 2001):

$$dT / dp = \alpha T / \rho C_p$$

where, $T$- temperature (K), $p$- pressure (Pa), α- volumetric expansion co-efficient (1/K), ρ- density (kg/ $m^3$), $C_p$- specific heat (J/kg K)

As the properties of all foods are not known, the temperature rise can be difficult to calculate with accuracy numerically (Matser et al., 2004). Water, which is usually the most abundant component of most food, exhibits a temperature rise of 0.03 °C/ MPa of applied pressure. Rasanayagam et al. (2003) found that for foods with a high fat content, the temperature rise increases to 0.08 – 0.09 °C/ MPa. Under pressure, the volume of gaseous components practically approaches zero while the solid and liquid components, depending on their compressibility, may or may not reduce in volume (Yaldagard et al., 2008). Also, under ideal conditions, if there is no external addition or subtraction of heat energy, the food product cools down to the initial temperature upon decompression (Rastogi et al., 2007).

Some other effects of HHP on foods such as: influence on bio-membranes, effect on microorganisms and spores, changes in enzyme activity, residual vitamin content, changes in color & texture, protein gelation and denaturation properties, rheology, particle size reduction, freezing and thawing properties, tenderization of meats, and as these relate to specific have been extensively studied and covered in some comprehensive review articles (San Martin et al., 2002; Rastogi et al., 2007; Yaldagard et al., 2008).

As with any technology, HHP too has some limitations and challenges. One of the foremost hurdles to the widespread acceptance of this technology is the high initial capital cost. It is also the rate limiting step in most food processing operations. HHP requires thick walled, high precision equipment with complex construction to prevent any leaks (Kadam et al., 2012). There is a lack of data on the thermophysical properties of most foods making it difficult to model the adiabatic temperature invariably associated with HHP. Even though the pressure application is largely isostatic, Minerich & Labuza

(2003) found that all foods may not get pressurized homogeneously. The inconsistency in pressurization gives rise to problems with assuring microbial sterility in all parts of a food. Specialized packaging material is needed, especially in batch processes so that its integrity doesn't get compromised at high pressures. Headspace reduction is essential to the attainment of pressure in the shortest time possible. The results of various studies are generally difficult to reproduce across laboratories (Rastogi et al., 2007). A detailed description of the methods used in a HHP study thus become essential, especially because of the number of process variables involved: vessel size and dimensions, pressure transmitting fluid, power of various equipment, and temperature distribution to name a few (Balasubramanian & Balasubramaniam, 2003). Being a batch process, the throughput is low (Moorman, 1997). Pressure resistant microbes are of a concern in HHP but there is hardly any information about their presence or load in foods. It is hence difficult to calculate processing parameters with confidence. The validation of HPP in general is lacking and presently there are no FDA regulations or specifications for this method (Sizer et al., 2002).

Food enzymes and bacterial spores are extremely pressure resistant, sometimes even up to 1200 MPa (Sivanandan, 2007). As a result, the lingering enzyme activity combined with the dissolved oxygen can lead to reactions which can affect the food constituents (Yaldagard et al., 2008). In particular, browning of vegetables, and oxidation of triglycerides in meats becomes hard to control (Cheftel, 1995). The other type of HPP, namely- High Pressure Homogenization or Dynamic High Pressure has recently come into the limelight and is believed to have the potential to address some of these limitations.

**High Pressure Homogenization (HPH) or Dynamic/ Continuous High Pressure (CHP) Processing**

Homogenization basically employs turbulence, cavitation, and shear forces, which are generated as the food under pressure flows through a highly constricted opening (in the order of microns). This narrow opening is housed inside a homogenizing valve which has some form of a needle and seat arrangement (Datta et al., 2005). The prevalent form of homogenization, which is used extensively in the dairy industry, was invented by Auguste Gaulin in 1899 (Peck, 2004; SPX, 2008), which coincidentally is the same year in which Hite first published his results on high hydrostatic pressure processing of milk (Hite, 1899). This form of homogenization however, uses low pressure, about 20-60 MPa. It is critical to the particle size reduction of milk fat globules and it helps arrest milk fat flocculation which can lead to creaming (Guamis et al., 2003). CHP and HPH processing are novel extensions of this method. CHP involves a continuous pumping of unprocessed food and collection of the treated product. Ergo, the foods mostly tend to be liquids, or pastes. The mechanical work done by the pressurized food at the point of decompression is exploited for homogenization. Ideally, the treated product is collected aseptically (Farkas & Hoover, 2000). At variance with high hydrostatic pressure processing, CHP employs lower pressures (Popper & Knorr, 1990) and depending on the pressure used, Dumay et al. (2012) have classified it as High Pressure Homogenization (HPH, 150-200 MPa) or Ultra High Pressure Homogenization (UHPH, 350-400 MPa).

**Elements of CHP Processing and the Equipment**

A simple layout for CHP processing is shown in Figure 2.1. A pumping system is required to continuously feed the food product into the pressurization unit which typically consists of intensifiers. There could be either one intensifier, or multiple intensifiers working in tandem to create a continuous flow, and at the same time raise the pressure of the food to the desired level very rapidly (Cavender & Kerr, 2011). The intensifiers (Figure 2.2) and they resemble a piston-cylinder arrangement and operate on the principle of Pascal's Law which states that pressure applied to a liquid at one point is transmitted equally throughout the liquid (Rodrigo et al., 2010). Upon pressurization, the fluid is pushed forward towards the homogenizing valve. An important differentiation from HHP occurs at this point in that the total residence time at high pressure is much shorter (Datta et al., 2005), usually in the order of seconds. In HHP, the time of pressure application is the key variable, whereas in CHP, the geometry of the decompression (homogenizing) valve, and the rate of pressure release are the two key controlling factors.

The most important element in CHP, the decompression valve, can be considered as the heart of the process. Some of the other names by which this valve is known as are: pressure release valve, throttling valve, micro-metering valve, needle valve, interaction chamber (used mostly in the case of Microfluidizer, Microfluidics Corporation, USA), homogenizing valve, etc. These valves come in several kinds of internal geometries, flow patterns, and the material of construction. Cavender & Kerr (2011), in a recent study, compared two different kinds of valves for their effect on microbial inactivation and found a significant difference in their microbial inactivation capabilities. The main aim of a homogenizing valve is to provide a highly restricted flow path to the fluid. The size of

opening can usually be adjusted thereby changing the flow rate. As the fluid passes through the constricted opening, it undergoes extremely rapid pressure release with an accompanying increase in its velocity. The velocity in some cases can even exceed the speed of sound. This sudden increase in velocity leads to tremendous shear and turbulence. It generates an elongational flow along with some stretching effects (Luning et al., 1995; Floury et al., 2004b; Dumay et al., 2012). There is an increase in the volume available to the fluid for flowing as the fluid exits the opening (Cavender, 2011). This increase in volume, in combination with the reduction in pressure makes the gaseous pockets increase in size (Guerzoni et al., 1999) since the solubility of gases in liquids decrease with decreasing pressure (Diels & Michiels, 2006). In addition, as the temperature rises (discussed in the next paragraph), the water in the food begins to turn into vapors. These vapors can also expand upon decompression. However, as the fluid decompresses and expands, there is an increase in the pressure on these gas pockets and the vapor bubbles causing them to implode. An enormous amount of energy is discharged at their point of collapse generating high intensity pressure waves, localized extreme temperature and pressure zones. This phenomenon of rapid bubble formation and collapse, thereby forming cavities, is known as cavitation (Gogate, 2011). The energy released affects the fluid surface, particles and even microbial membranes (Sivanandan, 2007). Impact of food against the walls of the static surfaces and amongst the food particles themselves also ensues (Laneuville et al., 2000). Cavitation, although an inescapable problem in flow systems (Gogate, 2011), is believed to play an important role in particle/ droplet disruption (Floury et al., 2004b) as well as cell disruption (Shirgaonkar et al., 1998) during homogenization of foods. This process of passing fluids

under high pressure though an extremely narrow orifice is sometimes also referred to as throttling (Adapa et al., 1997; Shirgaonkar et al., 1998; Toledo & Moorman, 2000; Sivanandan et al., 2008).

Another noteworthy aspect of HPH is the rise in temperature as the food throttles. This temperature rise is in addition to the adiabatic rise in temperature which occurs when the food is pressurized in the intensifiers (de Heij et al., 2003). The rise in temperature at the point of throttling is due to the frictional heat that is generated as a result of very high fluid velocities (Popper & Knorr, 1990). This temperature rise is significant and is directly proportional to the homogenization pressure. The mechanical energy gets transmuted into thermal energy. In some instances it might be necessary to cool the product immediately after throttling so as to protect the heat-sensitive biomolecules (Dumay et al., 2012). While most researchers (Floury et al., 2000; Datta et al., 2005; Hayes et al.; 2005; Donsi et al., 2009) have used cooling contraptions immediately after the homogenizing valve to cool the product, Cavender & Kerr (2011) used a modified micro-metering valve with a provision for its internal cooling ensureing that the product was cooled at the point of decompression and minimizing thermal effects. If the product however is not cooled immediately upon pressure release, the temperature rise can be exploited for microbial reduction (Toledo & Moorman, 2000; Areekul, 2003; Sharma et al., 2009; Cavender & Kerr, 2011) or for enzymatic inactivation (Datta et al., 2005) by holding the food at the elevated temperature for a suitable time. Different researchers have reported various levels of temperature rise with changing homogenization pressure. This incoherence is conceivably because of the differences in flow rates, homogenizing valve geometry, inlet temperature, pressure

maintained after throttling, and the food product used by the researchers. These differences notwithstanding, some of the reported values of rise in temperature per 100 MPa of homogenizing pressure are: 15 °C (Thiebaud et al., 2003), 17.6 °C (Hayes & Kelly, 2003), 18 °C (Donsi et al., 2009), 20-23 °C (Cortes-Munoz et al., 2009), 22 °C (Floury et al., 2004a), 26 °C (Sivanandan et al., 2008) all the way up to about 35 °C (Cavender & Kerr, 2011). The final step is cooling and collection (Figure 2.1) of the treated food product. One or more heat exchangers might be used for the cooling.

A special type of homogenizing device is used by Microfluidics Corporation, Newton, MA in their homogenizer called- The Microfluidizer, and it was invented by Cook & Lagace (1985). An interaction chamber (Figure 2.3) is used instead of valves, which work on the basis of constriction to path of fluid flow. The interaction chamber splits the high pressure fluid steam into two streams and these two streams then collide head on (at a 180° angle) to achieve homogenization. McCrae (1994) postulated that the mechanism of homogenization in Microfluidizer differs from that in conventional HPH. Geciova et al. (2002), in a review, compared the Microfluidizer to other homogenizers for their effect on disruption of microbial cells. Sivanandan (2007) compared the Microfluidizer with CFHPT (discussed in the following section) with respect to soymilk processing and found that CFHPT produced soymilk with smaller particle size and a higher apparent viscosity. A comprehensive review of studies involving Microfluidizer is beyond the scope of the present literature analysis.

**Continuous Flow High Pressure Throttling (CFHPT)**

Throttling, in the general sense, is achieved by the introduction of an inline restriction (such as a porous plug or a partially closed valve), obstructing the flow of a fluid thereby causing a significant pressure drop. It is assumed that no work is done on the valve and that there is no net change in enthalpy during the process (Huang & Gramoll, 2013). This technique, as it relates to food is, in principle, a variation of HPH and the term CFHPT was used first by the researchers at the University of Georgia, Athens, USA (Adapa et al., 1997). They used it to accomplish a continuous inactivation of microorganisms in liquid foods. A micro-metering valve, utilizing a needle-seat configuration was used as the homogenizing valve. Figure 2.4 shows the geometry of one such valve. This method was later patented in 2000 (Toledo & Moorman, 2000). CFHPT is a relatively new technique and some data is available on its effects on food processing. Researchers have applied CFHPT to foods such as skim milk, skim milk concentrate, citrus juices, oil-in-water emulsions, honey, pectin-casein dispersions, blueberry-whey beverage, soymilk, functional foods (beverages) production, ice cream mixes and achieved promising results (Adapa et al., 1997; Moorman, 1997; Amornsin, 1999; Roesch, 2002; Areekul, 2003; Wicker et al., 2000; Peck, 2004; Sivanandan, 2007; Sharma, 2008; Corey, 2009; Cavender, 2011). The remainder of this review will concentrate on the effects of high pressure processing (HPP) (high pressure homogenization (HPH) in particular) on soymilk so as to do justice to the research that was undertaken.

**Effect of HPP on the Particle Size Distribution (PSD) of Soymilk**

The texture and taste of food, among other factors, is also dependent on the particle size distribution (Pathomrungsiyounggul et al., 2010). PSD determines the stability and quality of colloidal systems such as soymilk (Malaki Nik et al., 2009). As a result, PSD is an important property of soymilk and related products which can be an indicative of the changes that take place during processing (Malaki Nik et al., 2008) as well as indicative of the formation of particle agglomerates (Poliseli-Scopel et al., 2012). High hydrostatic pressure has limited effect, and a much less drastic, or even the opposite effect on particle size (Galazka et al., 1996; Gaucheron et al., 1997; Galazka et al., 2000; Trujillo et al., 2002; Anema et al., 2005). The classical two-step homogenization has been used by the dairy industry throughout the last century to prevent creaming of milk as well as to improve the texture, flavor, and the consumer acceptability of dairy products (Dickinson & Stainsby, 1998). Recently, high pressure homogenization of bovine milk has been explored and success achieved in fat globule size reduction (Cruz et al., 2007). As the interest in applying HPH to soymilk and related products increases, numerous studies have been published of late on this topic.

Poliseli-Scopel et al. (2012) performed UHPH on soymilk (okara filtered out) using a high pressure homogenizer (model: FPG11300, Stansted Fluid Power Ltd., Essex, UK) fitted with a ceramic valve (Figure 2.5), at pressures of 200 and 300 MPa, and at varying inlet temperatures (55 – 75 °C). A particle size distribution with two peaks (majority of particles in the range of 0-0.6 μm and the second peak around 3 μm) was observed for all treatments. It was concluded that UHPH significantly reduced the average particle size and also checked the formation of aggregates. In their experiments,

they noticed that pasteurization with conventional single pass homogenization gave rise to aggregates. During UHPH although the temperature affected agglomerate formation, the combination of temperature and pressure did not point to any pattern. The temperature after throttling was observed to vary from 105.7 to 135.7 °C. In a very similar study by some of the same researchers (Cruz et al., 2007), effect of UHPH on soymilk was studied. The soymilk was prepared and treated in the same way, although the inlet temperature being lower, the maximum temperature achieved after homogenization was about 100 °C (at 300 MPa). It was found that UHPH significantly reduced the average particle size. However, when the pressure was raised from 200 MPa to 300 MPa, the mean particle size actually increased from $D_{[4,3]}$ = 0.13 µm to $D_{[4,3]}$ = 4.36 µm. The reason for the increase in size was thought to be due to coalescence of particles forming agglomerates. The formation of agglomerates has been attributed to conformational changes of proteins leading to denaturation. These can also intermingle with other protein molecules or fat globules (Thiebaud et al., 2003). The formation of aggregates was found by Hayes et al. (2005) during UHPH of bovine milk as well.

Sivanandan et al. (2010) used whole dehulled soybeans (without any filtration) to prepare soymilk, and homogenized it using a Gaulin homogenizer (96.53 MPa, Model 15MR-8TA, Gaulin, Everett, MA, USA). The particle size of soymilk with respect to the number of passes through the homogenizer, and the difference between soymilk prepared from boiled versus non-boiled soybeans was compared. Two-pass homogenization produced soymilk with significantly smaller particle size ($D_{[4,3]}$ = 29.62 µm) as compared to a single pass ($D_{[4,3]}$ =44.25 µm). The second pass, it was found, reduced the particle size further by a factor of 1.5. It was postulated that agglomerates were formed after the

first pass which were broken during the second pass. The boiling of soybeans did not affect the particle size. In an earlier study, the same authors (Sivanandan et al., 2008) used CFHPT (Model nG7900, Stansted Fluid Power Ltd., Essex, UK attached with a micro-metering valve, Figure 2.4) to process soymilk at varying pressures (higher pressures meant higher temperature after throttling). They investigated the difference in particle size of soymilk as affected by the grinding method. Three different grinders, using different mechanism of grinding were used: Megatron (15 min at 13000 rpm, Model MTK 5000Q, Kinematica, Inc., Cincinnati, Ohio,U.S.A.), Fitzpatrick mill (0.1270 and 0.0508 cm screens, Model JT, Fitzpatrick Co., Elmhurst, Ill., U.S.A.) and Stonemill (stone MKE6-46, Model MKCA6-3, Masuko Sangyo Co. Ltd., Saitama, Japan). It was observed that soybeans ground using the Megatron produced soymilk with the narrowest particle size distribution. In addition, higher pressures (276 MPa and 148 °C) during CFHPT further narrowed the PSD ($D_{[4,3]}$ = 18.73 µm). The authors ascribed the reduction in particle size to rupturing of particles as a result of shear, cavitation and turbulence in the micro-metering valve.

Roesch & Corredig (2003) examined the role of HPH on oil-in-water emulsions prepared using varying amounts of soy protein concentrate (2g to 8g) and 20g of soybean oil. A valve homogenizer (Emulsiflex C5, Avestin, Ottawa, Canada) was employed and two passes were given to the emulsions at room temperature: first at 5 MPa, and the second at 70 MPa. Some of the samples were pasteurized for 2 min at 82 °C prior to homogenization. Mixed results were obtained as they discovered that the PSD was not narrow even after homogenization and/or pasteurization. However, the average particle size for the treated samples was smaller. In addition the heat treatment alone produced

PSD similar to that after HPH. The researchers postulated the occurrence of flocculates of oil droplets in the emulsions.

In a similar study by Floury et al. (2002), UHPH of soy protein-stabilized emulsions was investigated using a Stansted high pressure homogenizer (Stansted Fluid Power Ltd., Essex, U.K.). The emulsions were prepared using 11S globulin protein (1% or 2% by weight) and sunflower oil. The pressure applied varied from 20 to 350 MPa. As in other studies, the presence of aggregates in globulin stabilized emulsions was confirmed and these aggregates increased with the homogenization pressure. Also, the mean diameter of droplets was greater in 1% globulin emulsions as compared to the 2% emulsions over the entire pressure range. It was concluded that as the pressured increased, soy proteins such as 11S globulin unfolded and there was a greater protein-protein interaction leading to the formation of flocculates. Interestingly, we could not find a study which investigated the change in particle size distributed of CHP soymilk over a period of storage.

**Effect of HPP on Lipoxygenase (LOX) Activity in Soymilk**

The enzyme lipoxygenase (EC 1.13.11.12) is an iron containing oxidoreductase which catalyzes the oxidation of molecules containing cis, cis-1,4-pentadiene units (Hildebrand, 1989). LOX is found in copious amounts in legumes such as soybeans and can constitute 1-2% of the total seed protein content. LOX, among other functions, plays a role in growth regulator manufacture, storage of nitrogen, and plant defense mechanism (Gaffney, 1996). In soybeans, LOX exists in the form of four isoenzymes- L-1, L-2, L-3a and L-3b (Draheim et al., 1989) although L-3a and L-3b are collectively referred to as L-

3 because of the similarity in their properties (Axelrod et al., 1981). LOX catalyzes the oxidation of polyunsaturated fatty acids (PUFAs) with linoleic acid being its principle substrate (Robinson et al., 1995), although linolenic acid is also a typical substrate (Bugg, 2003). The structure of linoleic acid is shown in Figure 2.6 with the cis, cis-1,4-pentadiene portion marked. The linoleic content of soybeans is quite high and in soybean oil, it can constitute as much as 54% of the total fatty acids (Howell & Collins, 1957). One of the major products of this oxidation reaction is hexanal which even at extremely low concentrations gives the food a very unpleasant odor (Hildebrand, 1989). This odor is characterized as beany, grassy, green, painty, etc. (Torres-Penaranda et al., 1998). Moreover, Baur et al. (1977) have related LOX to the formation of bitter compounds. Genetic removal of LOX from certain foods notwithstanding, it is important to inactivate this enzyme during food processing. Even though it is possible to inactivate LOX by a heat treatment, other protein present in food may also get denatured in the process (Ludikhuyze et al., 1998a). As a result, many studies have been published on the inactivation of LOX by pressure, especially in soy and related foods.

Guerrero-Beltran et al. (2009a) subjected soymilk and soybeans to various combinations of high hydrostatic pressure (275 – 620 MPa), temperature (19 – 80 °C) and holding time (0 – 30 min) with an aim to inactivate LOX. The treated soybeans were then processed into soymilk and analyzed for LOX. No LOX activity was found in samples subjected to the highest temperature and pressure. Though the inactivation of LOX was high at higher pressures, temperature significantly influenced LOX inactivation in the soymilk samples at all pressures. Interestingly, in case of soybeans pressurized at 345 MPa and 19 - 70 °C, an increase in LOX activity was observed and the increase was

due to the liberation of LOX isoenzymes. At higher pressures and suitable holding times however, the activity declined. Also, the inactivation followed first order kinetics. Another study (Rodrigo et al., 2007) examined tomatoes for LOX inactivation. At pressures below 400 MPa (20 °C) LOX activity increased as in the previously mentioned study. However, at pressures over 550 MPa, the enzyme was fully inactivated. Wang et al. (2008) studied the HHP inactivation of LOX in soymilk and crude soybean extract (soymilk centrifuged at 30,000 g, 4 °C for 30 min). They applied pressure over a wide range, 0.1 – 650 MPa in conjunction with temperature (5 – 60 °C). Thermal treatment alone at 63 – 71 °C was also examined. LOX inactivation was found to increase with increasing temperature. The inactivation of LOX in combined pressure-temperature conditions was irreversible. It was noted that at a constant temperature, the inactivation increased with pressure and that LOX was most stable at 20 °C. For all the cases studied, it was observed that LOX was more prone to inactivation in the crude extract as compared to soymilk. In this study also, the inactivation kinetics followed a first order reaction for all combinations of pressure and temperature. These results resonate extremely well the results of a prior study by Ludikhuyze et al. (1998b) although these researchers had used the enzyme LOX itself in a lyophilized form.

Inactivation of LOX in soymilk and raw soybeans by pressure alone was researched by van der Ven et al. (2005). However, even at 500 MPa, more than half of LOX remained active in soymilk while the levels were even higher in the soybeans. It is important to note that the holding times were less (1 – 2 min) than those used by Guerrero-Beltran et al. (2009a). To inactivate the enzyme completely, it was necessary to raise the pressure to 800 MPa. However, if heated along with a lower pressure (600

MPa), LOX was completely inactivated and the inactivation was found to be instantaneous. The final soymilk temperature, including the rise due to adiabatic heating was about 84 °C.

Studies of LOX inactivation under pressure have been performed on other foods such as green peas extract and juice (Indrawati et al., 2000; Indrawati et al., 2001), tomatoes (Shook et al., 2001; Rodrigo et al., 2007), carrots (Akyol et al., 2006), and green beans (Indrawati et al., 2000). Some other researchers have investigated the inactivation of the enzyme in soymilk by means of Pulsed Electric Field (Li et al., 2008; Riener et al., 2008; Li et al., 2012). Review of these studies is out of the purview of the present topic. As per our knowledge, so far only one study has been performed to investigate the effects of continuous high pressure processing on lipoxygenase in soymilk. Poliseli-Scopel et al. (2012) performed high pressure homogenization of soymilk using two pressure levels (200 and 300 MPa) in combination with heat (105 – 135 °C at the point of homogenization). However, it was found that LOX got inactivated during the soymilk processing stages itself as the processing involved grinding soaked soybeans for 20 min at 80 °C.

**Effect of HPP on Trypsin Inhibitor (TI) in Soymilk**

Legumes (for example, soybeans) typically contain large quantities of protease inhibitors which are essentially low molecular weight proteins. The term protease refers to the protein digesting enzymes in organisms (Wilson, 1988). In addition, cereals, tubers like potatoes, and eggs contain them (Liener, 1986). These inhibitors help protect the plant against microbes, pests, insects as well as regulate some physiological functions

(Rackis et al., 1986). Although soybeans contain a multitude of these proteolytic inhibitors which can inhibit the action of enzymes such as trypsin, chymotrypsin, elastase and serine, there are two principle inhibitors which are referred to as trypsin inhibitors (TIs) (Guerrero-Beltran et al., 2009b). These are: Kunitz Trypsin Inhibitor (mol. wt. 20,000), and Bowman-Birk Trypsin Inhibitor (mol. wt. 8000), the latter being more heat stable because of a greater number of di-sulfide linkages and it can inhibit chymotrypsin as well (Liener, 1986; Prawiradjaja, 2003). The Bowman-Birk TI is also the main type of TI present in cooked soymilk (Rouhana et al., 1996). Trypsin is a protein digesting enzyme produced by the pancreas in the human body (ThinkQuest, 2013) and as a result the amount of TI in a protein rich food is one of its important nutritional quality limiting factors (Smith et al., 1980). The inhibition of trypsin, apart from causing a reduction in digestion of ingested proteins, also causes the pancreas to produce more trypsin, leading to its enlargement (Prawiradjaja, 2003). It is thus necessary to inactivate TI completely although over-processing could denature other valuable proteins (Wallace et al., 1971) since TIs are relatively heat stable (owing to presence of disulfide bonds). According to Liu (1997b), they require 30 min at 100 °C, or 22 min at 110 °C for about 90% inactivation. Treatments at higher temperatures cause the amino acids lysine and cystine to become denatured (Kumar et al., 2003). As it is necessary to inactivate as much TI as possible whilst maintaining adequate protein quality, many commercial soybean derived foods may contain up to 20% of the total initial TI activity present in the soybeans used to process the food (Rackis & Gumbmann, 1981). Although Kwok et al. (2002) obtained a satisfactory TI inactivation while retaining adequate protein quality in soymilk using

ultra high temperature (UHT) processing, there has been an interest in using high pressure to achieve TI inactivation.

Poliseli-Scopel et al. (2012) compared pasteurized (95 °C, 30 s), UHT (42 °C, 6 s) and UHPH (200 & 300 MPa, 105 – 135 °C at the point of homogenization) soymilk for the effects on TI activity among other quality factors. Even though the soybeans were ground at 80 °C for 20 min, the untreated soymilk had a TI content of 9.25 g/L. Pasteurization and UHPH processing, both reduced the TI activity to about 63% of the initial activity. The UHT processed soymilk had the lowest residual TI activity at 40%. As the researchers have highlighted, it is important to note that the holding time at any temperature during UHPH was less than a second. In our knowledge, this is the only study which studied TI inactivation in soymilk by using CHP processing. Guerrero-Beltran et al. (2009b) applied high hydrostatic pressure instead, along with temperature for inactivation of TI in soymilk. The conditions were: 250 – 550 MPa at 50 – 80 °C for 0 – 15 minutes. Very little TI could be inactivated at 250 MPa even after 15 minutes while at 550 MPa and 65 °C at all holding times, the most inactivation occurred. A maximum of 76% TI inactivation could be achieved at 550 MPa, 80 °C for 15 min. Holding time in general, had a significant effect on the inactivation.

In a similar study (van der Ven et al., 2005) even higher pressures were used to inactivate TI in soymilk as well as raw soybeans. The treatment times, 0 – 2 min, were shorter though. At room temperature very little TI could be inactivated, even at 800 MPa. As the initial temperature (plus adiabatic heating) increased to 60 °C, up to 40% of the initial TI activity could be destroyed. The results were similar for soybeans as well as the soymilk. Treatment time was again found to have a significant effect on the residual TI

activity. The researchers also attempted to optimize TI inactivation and found that 90% of the total TI activity can be eliminated in 1 min at 77 °C initial temperature and 750 MPa, or 90 °C initial temperature and 525 MPa (the maximum temperature reached was 110 °C). As compared to UHT soymilk with similar TI activity, these are milder conditions. Yuan et al. (2008) also achieved a 90% reduction in TI activity but by using only heat treatments, which were more severe. This level of inactivation was possible only by heating the soymilk to 150 °C for 50 s in addition to first blanching the soybeans for 2 min at 80 °C. Inactivation of TI by thermal treatments is well documented and will not be discussed further. The variety of soybeans as well as the geographical location for their farming also affects the TI activity (Kumar et al., 2003).

**Effect of HPP on the pH of Soymilk and the Change during Storage**

The pH of soymilk can vary during processing. The pH of freshly prepared soymilk ranges from about 6.5 to 7.71 (Nelson et al., 1976; Iwuoha & Umunnakwe, 1997; Cruz et al., 2007; Guerrero-Beltran et al., 2009b' Sharma et al., 2009' Smith et al., 2009). For some commercial samples, Bai et al. (1998) reported the pH ranging from 6.41 to 7.31. However, it is known that pH has a marked influence on the solubility and viscosity of soy proteins (Torrezan et al., 2007). The pH of foods usually changes with pressure, although the direction and intensity of change is different for different food products. In case of acidic foods, the pH decreases with increasing pressure in general (Patterson, 2005). The physical quality aspects of a food are affected by pH as well and Guiavarc'h et al. (2005) observed an improvement in cloud stability in grapefruit juice at pH 7 during HPP. At lower pH values, microorganisms become more vulnerable to

pressure (Farkas & Hoover, 2000) but are highly resistant at neutral pH values such as those of soymilk (Smelt, 1998). Bovine milk is a neutral pH food product. HPH at 200 MPa and 30 °C reduced the pH of milk very slightly, but at 300 MPa and 30 °C, and during regular pasteurization (15s at 90 °C) and homogenization (18 MPa and 2 MPa), there was no significant change in pH (Pereda et al., 2007). This reduction in pH was due to the action of a milk enzyme lipoprotein lipase which remains active at 200 MPa, 30 °C but becomes inactivated at 300 MPa, 30 °C (Walstra et al., 1999).

High hydrostatic pressure, 400 to 600 MPa for 1 and 5 min at a temperature of 25 and 75 °C was applied on soymilk by Smith et al. (2009). The pH of soymilk that was prepared was 6.7 which the researchers adjusted to 7.0. No change in pH of soymilk was found after any treatment. The pH of untreated soymilk dropped to 4.8 after 28 days at 4 °C while that of the treated samples changed by only 0.6 units. A change in pH, in general, can be an indicator of microbial growth. A smaller change in pH could mean either that not many acid producing bacteria are present or that the growth of microbes is stunted. In another study (Malaki Nik et al., 2008), soymilk was subjected to heating (7 min at 95-100 °C) and some heated soymilk samples were further subjected to a 4-pass high pressure homogenization at 69 MPa at room temperature using a valve homogenizer (Emulsiflex C5, Avestin, Ottawa, Canada). The pH of soymilk, which was 6.7 prior to any treatment, did not change after either heating or homogenization.

Lakshmanan et al. (2006) adjusted the pH of soymilk from 6.6 to two different values- pH 6.0 using HCl and pH 7.0 using NaOH. These samples were either heat treated at 95 °C for 30 min, or given a HHP treatment of 500 and 600 MPa at room temperature for 10 min using a high-pressure food processor (Food-Lab 900, Stansted

Fluid Power Ltd, Essex, U.K.). Even in this study, no significant change in pH was observed in either soymilk sample after any treatment. pH was checked again after 2 days of refrigerated storage and no significant change was noted. A study (Iwuoha & Umunnakwe, 1997) investigating the effect of soymilk extraction method and the effect of blanching & de-hulling method on various characteristics of soymilk found that although the soymilk extraction methods influenced the pH, the other variables did not significantly affect it. Upon storage at 29 °C, there was a reduction in pH of all samples and an increase in the titratable acidity.

**Effect of HPP on the Microbiological Qualities of Soymilk and related Shelf Life**

When Bert Hite (Hite, 1899) first used high pressure on foods, his aim was to prevent milk from becoming sour for a longer period. He achieved an increase in the shelf life of milk by reducing the microbial population of milk as result of pressure application. Over the past century, one of the primary purposes of HPP has been to improve the microbial quality of foods, be it by HHP or by CHP. The inactivation of microorganisms by means of pressure is known as “pascalization” (Adapa et al., 1997). Research has shown that some microbes are killed, some injured, while some simply survive HPP (Rodrigo et al., 2010). Bacterial spores are the most resilient microbial entity under pressure and usually require a combination of pressure and temperature for their inactivation (Diels & Michiels, 2006; Black et al., 2007). The response of viruses to HPP is highly varied (Rodrigo et al., 2010).

The microbicidal effect of HHP is due to the damage it does to cell membranes, making them more permeable and causing the intracellular material to leak out (Tewari,

2007). In addition, biochemical reactions get affected, DNA replication may stop (Patterson, 2005), and the intracellular pH may drop (Smelt, 1998). CHP however, affects microbes in a different manner although there is still a debate regarding the underlying mechanisms. As mentioned earlier, cavitation and the collapse of these cavities, which results in a localized high pressure pulse, is one of the modes of microbial cell disruption in HPH (Shirgaonkar et al., 1998). In addition the pounding of bacterial cells against stationary surfaces causes disintegration of their cell walls (Engler & Robinson, 1981). Another mode by which CHP exerts its microbicidal effect is by the formation of free radicals which cause oxidation (Lander et al., 2000). Also, the fluid regions near impingement walls are under stress. If a microbial cell stays long enough in these regions, its cell wall can get ruptured (Ayazi Shamlou et al., 1995). As there is a substantial rise in temperature at the point of homogenization, some researchers have exploited it for simultaneous homogenization and sterilization (Meyer, 2000; Wilson & Baker, 2000; Sharma et al., 2009; Cavender & Kerr, 2011). Low acid foods (foods with pH greater than 4.6) such as soymilk, are susceptible to spoilage by several organisms (Sharma, 2008). High pressure sterilized low-acid foods are not yet currently available in the US but extensive research is underway across the world on the topic (Sizer et al., 2002; Rastogi et al., 2007). Some of the pertinent studies on soymilk have been reviewed in the remainder of this section.

Poliseli-Scopel et al. (2012) performed UHPH (200 and 300 MPa) on soymilk at 105 – 135 °C and compared it to pasteurized (95 °C, 30 min), and UHT (142 °C, 6 s) soymilk. They checked for mesophilic aerobes, mesophilic spore-forming aerobes, enterobacteria, yeasts and molds, *Staphylococcus aureus*, *Bacillus cereus*, and

*Salmonella sp.* It was found that UHPH outperformed pasteurization in general. The 200 MPa treatments at 105 and 117 °C were the least effective against all microbes although they were still better than pasteurization against aerobic spores. The authors did not find yeasts and molds, or any of the other tested organisms in any of the treated samples. After 20 days storage at 30 °C, only the 300 MPa, 135 °C and UHT samples remained stable. All 200 MPa samples spoiled within 2 days. Temperature and pressure were thus significant factors in determining the stability of UHPH soymilk. Sharma et al. (2009) executed CFHPT on soymilk using two pressure levels: 207 and 276 MPa, at 121 – 145 °C to determine the resistance of *C. sporogenes*. A holding tube was attached immediately after the throttling valve to make use of these high temperatures (after throttling) for microbial inactivation before the soymilk was cooled. The holding times varied from about 10 s to 20 s (compare this time to a holding time of 0.7 s in the previous study). Once again, pressure, temperature and holding time, all had a significant effect on the survival of *C. sporogenes*. A pressure of 207 MPa caused more injury than destruction of the spores. Temperature had a greater effect on spore inactivation than pressure. Overall, a complete destruction of entire spore population could not be achieved and the spores were able to resuscitate in about 10 days (4 °C) even after the severest treatment (276 MPa and 145 °C). Additionally, as compared to 0.1% peptone water, soymilk accorded a protective effect to the spores.

Another study (Smith et al., 2009), using high hydrostatic pressure instead (400 – 600 MPa), observed the microbial shelf life (total aerobic count, total psychrotrophs and *Enterobacteriaceae*) of soymilk when stored at 4 °C for 28 days. The soymilk was treated for 1 – 5 min at 40 °C to 85.5 °C (including the adiabatic heating). The spoilage criterion

was the counts reaching a level of 7 log CFU/ mL. According to their results, the pressure treated soymilk samples had 2 weeks of extra shelf life under refrigeration. The samples treated at 600 MPa, 48 °C for 5 min did not spoil for 25 days. An interesting aspect of their study was that increased pressure or longer holding time did not significantly affect the reduction in the total aerobic counts. However, treatments at 75 °C had lower counts than those at 25 °C (2.5 – 4.5 logs on average), and regardless of pressure, time, and aerobic/ anaerobic conditions, these samples did not reach the spoilage level even after 28 days. The researchers mention that the initial microbial load (3.7 – 4.6 log CFUs/ mL) may not have had been enough to fully capture the extent of log reduction. No *Enterobacteriaceae* were detected in any treated sample.

The comparison between the microbial qualities of UHPH and UHT milk was done by Cruz et al. (2007). The UHPH soymilk was treated at 200 and 300 MPa with an inlet temperature of 40 °C. The UHT soymilk was heated to 142 °C for 2 s. They found that UHT rendered the soymilk samples sterile. The UHPH, depending on the pressure, reduced the microbial load by 2.42 to 4.24 log CFU/ mL, but could not achieve total sterility. Somewhat similar results were obtained by Smiddy et al. (2007), who applied UHPH to bovine milk instead. Milk was homogenized at 200 and 250 MPa after being pre-heated to 55 or 70 °C. Although the initial total bacterial counts of about 5 log CFU/ mL was reduced to below detection immediately after UHPH, the counts rose to about 8 log CFU/ mL after 14 days storage at 5 °C.

Thus, even though UHPH alone or in conjunction with mild temperatures doesn't sterilize soymilk, it can nonetheless increase its refrigerated shelf life.

**Sensory Qualities of Soymilk**

The Asian population has been consuming soy derived foods, including soymilk for centuries and thus have become habituated to the distinctive flavor of these foods. However, in other parts of the world, especially the West, this flavor comes in the way of soymilk acceptability (Liu, 1997a) and the flavor of soymilk is a crucial parameter governing its consumption (Chambers et al., 2006). The majority of soymilk manufacturers in the US resort to the addition of flavor, and mouth-feel modifying/improving agents such as sugar, cocoa powder, vanilla flavor, malt extract, gums, etc. to improve its overall appeal (Wang et al., 2001). It is necessary to monitor off-flavor generation during processing. Researchers have been attempting to ameliorate the off-flavor of soymilk for about four decades (Li, 2006). These attemps includes developing novel food processing technologies which not only help obtain a product with superior flavor but also help achieve a better yield as well as a longer shelf-life (Lozano et al., 2007). Sensory analyses can be used to gauge the effect that food processing has on food quality (Kwok et al., 2000).

Descriptors used to evaluate Soymilk

Although numerous soymilk descriptors can be found in the literature, some of the most pertinent and commonly used one's have been highlighted hereafter.

**Beany:** This general term refers to the unpleasant beany or grassy flavor of soymilk and is widely studied. Apart from flavor, this descriptor can also be studied as beany aroma. Some authors tend to use more specific descriptors such as green, grassy, painty, and raw soy. As a result, it has been suggested that beany is possibly an aggregate term, which is

composed of several sub descriptors. Depending on the panel and the product, the panel leader may choose to either use beany, or one or more of the more specific terms. Generally, lipoxygenase is believed to cause this flavor in soy foods. However, several researchers have also reported the presence of beany attribute in soybeans containing no lipoxygenase (Kobayashi et al., 1995; Wilson, 1995; Liu, 1997b; Torres-Penaranda & Reitmeier, 2001; N'Kouka et al., 2004; Mizutani & Hashimoto, 2004; Sivanandan et al., 2010; Iassonova et al., 2009).

**Astringency:** Like beany flavor, astringency is another unfavorable attribute of soymilk. Chemically, astringent compounds precipitate salivary proteins. The precipitation reduces the lubrication provided by saliva and increases the friction between the mucosal surfaces. This descriptor is marked by puckering or a tingling sensation in the mouth accompanied by a feeling of dryness. Alum solution elicits astringency in the mouth. In soymilk, phenolic compounds such as phenolic acids and isoflavones as well as saponins are believed to be responsible for astringency. Sometimes, astringency can manifest itself as an aftertaste. Sugar addition has been reported to reduce astringency (Okubo et al., 1983; Ireland et al., 1986; Courregelongue et al., 1999; Torres-Penaranda & Reitmeier, 2001; Sivanandan et al., 2010; Min et al., 2005; Chambers et al., 2006).

**Bitterness:** Bitterness, like astringency, is present either as a basic taste or as an aftertaste. Bitterness in soymilk is undesirable and is caused by the saponins as well as isoflavones such as diadzin and genistin. It is also believed that certain bitter peptides from soy proteins, which remain unexposed in unprocessed soybeans, get released during

processing and lead to bitterness. In addition, certain microbes and enzymes can act on soy proteins which also release these bitter peptides. Bitterness can be masked by the addition of sugar. Interestingly, some researchers have found that soymilk prepared from LOX free soybeans as having a higher intensity of bitter and metallic attributes (Matsuura et al., 1989; Okubo et al., 1992; Torres-Penaranda et al., 1998; Torres-Penaranda & Reitmeier, 2001; Li, 2006).

**Cooked Flavor/Aroma**: Usually, cooking at high temperatures (such as HTST and UHT processing) will generate a cooked note in soymilk. Maillard reaction products are the chief causative agents of this descriptor. This descriptor may also be referred to as burnt flavor. Some researchers have also used the terms such as cooked beany aroma/flavor, roasted, or processed. Some heterocyclic compounds cause this flavor or aroma. A related aroma, caramelized aroma/taste has also been sometimes used to describe soymilk (Torres-Penaranda et al., 1998; Kwok et al., 2000; Chambers et al., 2006; Lozano et al., 2007).

**Color**: Color is a very important characteristic of soymilk as it is one of the first attributes that a consumer judges. As is the case with cooked notes, high temperature processing causes changes in color leading to a light yellow or light brown colored soymilk. Color changes may also be measured as change in lightness/ value (light reflecting capacity), chroma (degree of saturation), and hue angle (color change in terms of red, orange, yellow, green, and blue) of soymilk. Maillard reaction is usually

responsible for changes in color (Torres-Penaranda et al., 1998; Kwok et al., 2000; Lozano et al., 2007).

**Chalkiness:** This term is related to the texture of soymilk. It is the grainy or sandy sensation on the tongue and is the opposite of smoothness. Chalkiness is considered a defect in soymilk. It is caused by the fine particles which are big enough for the tongue to discern. Also, some proteins are not highly soluble in saliva and tend to stay on the tongue longer leading to the sensation of chalkiness (Kuntz et al., 1978; Torres-Penaranda et al., 1998; Sivanandan et al., 2010; Li, 2006).

**Rancid/Oily Aroma:** This attribute resembles the odor of stale or oxidized oil. If present, this attribute is another strong factor that can put off consumers. Once formed, it is hard to rid the soymilk of this aroma as rancid aroma causing compounds tend to remain bound to the soy proteins. Generally, inactivation of LOX enzyme leads to lower scores of rancid notes. Other terms that may be used in place of rancid aroma include cardboard, painty, or fish aroma (Torres-Penaranda et al., 1998; Min et al., 2005; Chambers et al., 2006).

Numerous researchers have studied the sensory qualities of soymilk in general, as well as the impact that conventional processing methods have on it. However, in our knowledge, no study has conducted a descriptive sensory analysis on high pressure (hydrostatic, or continuous) processed soymilk. Sivanandan (2007) conducted a consumer acceptability test on CFHPT soymilk. The mouthfeel and taste of CFHPT milk

were found to be superior. However, the treated soymilk had a greater intensity of beany flavor although the milk was still considered acceptable. Some researchers have looked at odor and taste causing compounds using analytical methods while others have tried to quantify the color of HPP soymilk. Descriptive sensory analysis of CHP processed soymilk is nevertheless lacking.

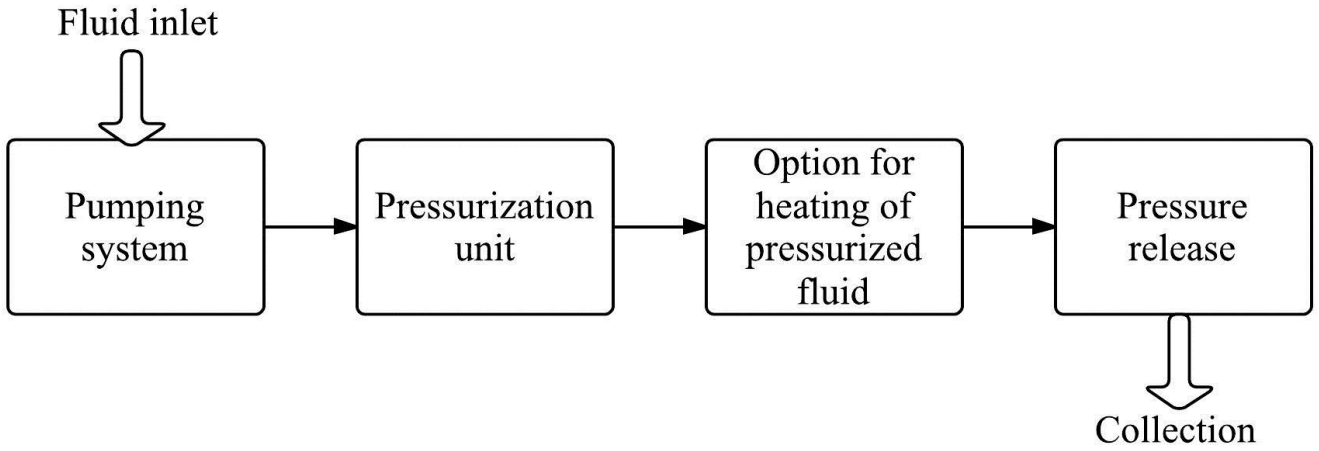


**Figure 2.1:** Schematic of a Continuous High Pressure (CHP) Processing Unit

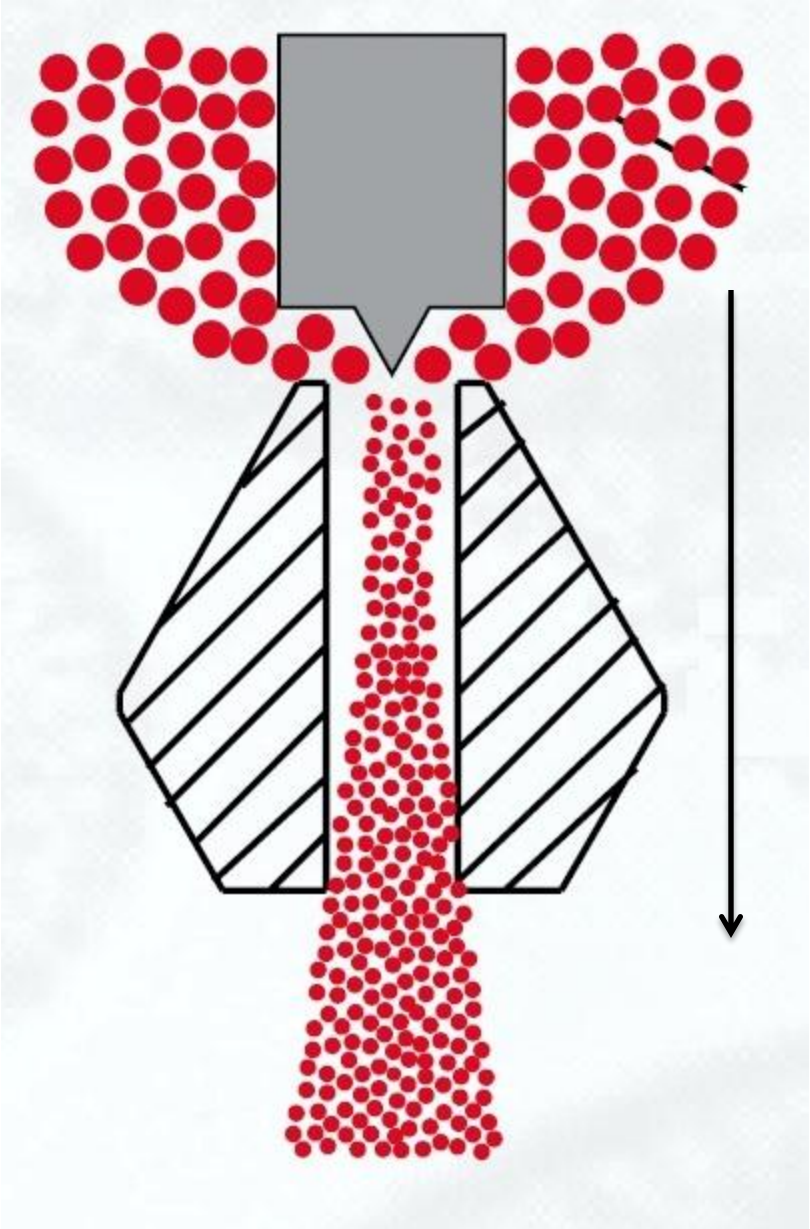

**Figure 2.2:** Schematic of the Stansted Fluid Power ceramic seat valve. The direction of flow of the fluid is in the direction of the arrow.

(Source: Stansted Fluid Power Ltd., UK)

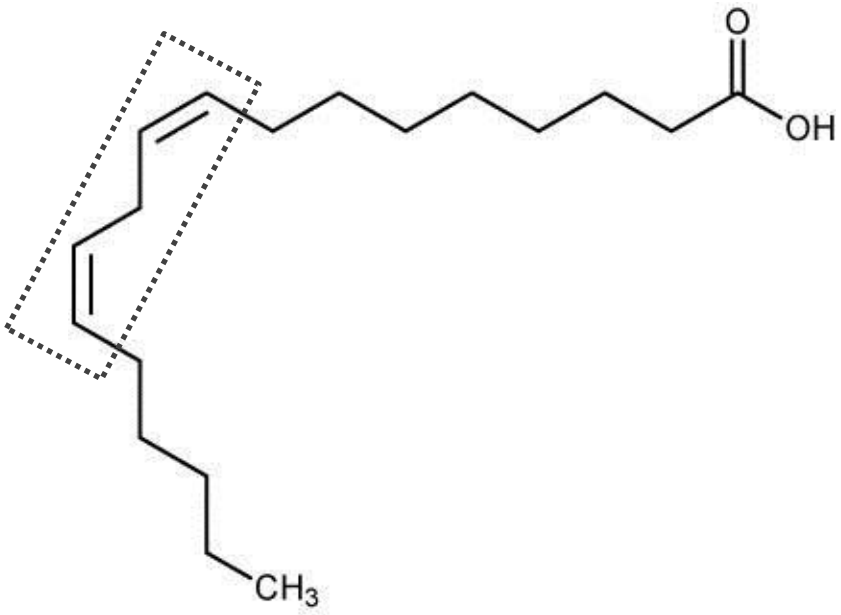


**Figure 2.3:** Structure of linoleic acid with the cis, cis-1,4-pentadiene portion enclosed in the dotted region.

# CHAPTER 3

# MATERIALS AND METHODS

## Preparation of soymilk

The soybeans used were provided by the Georgia Seed Development Commission, 2420 South Milledge Avenue, Athens, GA 30605. Woodruff variety of soybeans was used. The soybeans were stored in 22.68 kg bags at 4 °C and 20% relative humidity (RH) in dark. The required quantity of soybeans was dispensed from the bags for each experiment. Deionized water (DW) was used for making soymilk throughout the study unless otherwise stated. All the equipment used were made of stainless steel (SS).

Soymilk was prepared according to the method developed by Sivanandan (2007) with some modifications. Soybeans were left overnight (16 h) in loosely covered HDPE buckets before each run to let them equilibrate to room temperature (23 – 28 C°). The beans were put into perforated SS trays (1 kg beans per tray) and roasted at 154 °C for 5.5 min in a hot air impingement oven (Lincoln Impinger Model 1450, Lincoln Foodservice Products, Inc., Fort Wayne, IN). The soybeans were allowed to cool for 15 - 20 min and dehulled in a plate mill (Quaker City Mill Model 4-E, QCG Systems, LLC, Phoenixville, PA). The distance between the plates was adjusted so as to cause cracking of the hulls and separate the two cotyledons while preventing excessive breakage. The cotyledons were separated from the hulls by air classification. These whole dehulled soybeans were blanched in DW (1:5:: kg dehulled soybeans: kg DW) at 60 °C for 2.5 h.

The water was drained following blanching and the blanched soybeans were rinsed 3 times with DW. The blanched and rinsed soybeans were then transferred to HDPE buckets, covered and stored overnight at 4 °C, 20% (RH).

The following day, deionized water (3 times the mass of blanched soybeans) was weighed and divided into 2 equal parts. The first part was used to grind the blanched soybeans in a food processor (Robot Coupe Model RSI 10V, Robot Coupe UGA, Inc., Jackson, MS) for 2.5 min at 3000 rpm followed by 2.5 min at 3500 rpm. The soybean paste was then ground in a super mass-collider (Super Mass Collider Model MK CA6 - 3, Masuko Sangyo Co. Ltd., Japan) using a sanitary stone pair (E6 - 46). The electrical current of the equipment was kept between 2-3 amperes as this controlled the speed of grinding. The paste was passed through the mass collider 8 times after which the remaining water was added, mixed and the soymilk was passed through the equipment 4 more times.

To prevent clogging of the extremely small opening of the throttling valve during high-pressure processing, the soymilk was filtered using a 254 μm filter. The residue so obtained from a batch of about 20 L of soymilk weighed only about 200-250 g. The final step was the de-aeration of soymilk. During the elaboration of soymilk, air gets incorporated into the milk and this trapped air can cause pressure fluctuations during CFHPT (discussed in the following section). De-aeration was accomplished by applying vacuum to the soymilk for 20 min. This soymilk was the control. The soymilk preparation procedure is summarized in Figure 3.1.

**Continuous Flow High Pressure Throttling (CFHPT) of soymilk**

The filtered and de-aerated soymilk was processed in the CFHPT system. Soymilk, at room temperature (23 – 28 C°) was pneumatically fed into the Stansted high pressure equipment (Model nG7900, Stansted Fluid Power Ltd., Stansted, Essex, UK). Soymilk was kept stirred using a magnetic stir bar throughout the experiment to prevent settling of solids. The inlet pressure (to the high pressure equipment) was maintained around 700 kPa to ensure complete filling of the intensifiers. Soymilk was pressurized by the equipment to 207 or 276 MPa using two alternately acting pressure intensifiers (Hydropax P60-03CXS, Stansted Fluid Power Ltd., Stansted, Essex, UK) driven by a hydraulic pump in the Stansted high pressure equipment. The movement of the pistons in the intensifiers was controlled and synchronized by a microprocessor which also controlled the opening and closing of the intake and discharge valves connected to the intensifiers. A gauge displayed the applied pressure. It is important to note that the narrow clearance in the throttling valve (model 60VRMM4882, Autoclave Engineers, Fluid Components, Erie, PA) helped achieve high pressures and opening the valve caused a reduction in pressure. If a greater flow rate was required then the intensifiers generated higher pressure. The throttling valve (Figure 3.2) is basically a micro-metering needle valve and the flow rate of soymilk through the high pressure equipment was regulated by altering the gap between the needle and its seat.

A two-pass tubular heat exchanger was installed between the intensifiers and the throttling valve. Steam was used to preheat the pressurized soymilk to suitable temperatures which corresponded to the required exit temperatures (121 °C and 145 °C) after the holding tube (mentioned in the following). The temperature of soymilk after

preheating and after throttling was monitored. The difference between the two was calculated as the temperature rise after throttling. The temperature of preheated soymilk required to achieve the two exit temperatures changed with the applied pressure. Lesser preheating was required when soymilk was at 276 MPa. Also, the exit temperatures fluctuated by ±3 °C during the collection of soymilk samples. A holding tube was placed after the throttling valve, which allowed the soymilk to remain at the elevated temperature for the desired time. Thus, the temperature rise after throttling was exploited for microbial and physical effects on the soymilk. Two flow rates, 0.75 L/min and 1.25 L/min were studied and they corresponded to a residence time of 20.8 s and 12.48 s, respectively in the holding tube. Type K (chromel-alumel) thermocouples were connected at the outlet of heat exchanger and at the end of the holding tube. Another thermocouple (type K) was put immediately after the throttling valve (and thus before the holding tube) to observe the temperature rise after depressurization (or throttling).

Since the temperature of soymilk after throttling was above its boiling point, a back pressure valve was placed at the end of holding tube to prevent flashing. A minimum back pressure of 400 kPa was maintained in the holding tube which raised the boiling point of the soymilk and hence prevented flashing of soymilk. A coil-in-tube type heat exchanger was used to cool the soymilk back to room temperature. Tap water was used as the cooling medium. Soymilk was collected either in sterile tubes (Corning 15 mL Centrifuge Tubes, Polypropylene, Sterile, Corning, Inc., Corning, NY) or, for sensory analysis, in 946 mL HDPE jugs with lined caps. The samples were stored at 4 °C until analyzed.

A total of 12 treatments, as shown in Table 3.1, were investigated: 3 temperatures (no pre heating, and pre heating to achieve exit temperatures of 121 °C and 145 °C), 2 pressures (207 MPa and 276 MPa) and 2 flow rates (0.75 L/min and 1.25 L/min). The treatment conditions required finite time to be achieved during which the soymilk had to be used. As a result, only 4 treatments could be done from one batch (about 20 L) of soymilk. Thus, to complete one full trial and collect all 12 treatments, three separate batches of soymilk were prepared on separate occasions. So, the treatments were randomized between the three runs for each trial. The entire experiment was duplicated.

**Analyses**

All the analyses were done on day 1 and after weeks 1, 2, 3 and 4. Accordingly, the samples were collected in separate containers for each time interval. However, the sensory analysis was conducted only for 3 weeks for safety reasons. As mentioned before the de-aerated filtered soymilk served as the control.

(a) Microbiology

Aerobic plate counts (APC) and number of psychrotrophs were determined. The method of Smith et al. (2009) was followed. Duplicate samples were analyzed for each treatment and for the control. One mL soymilk samples were serially diluted in peptone water of the following concentration: 0.1g peptone (Bacto™ Peptone, Becton, Dickinson and Company, Sparks, MD) per 100 mL DW. The dilution factor was 1/10 per dilution. 0.1 mL aliquots of appropriate dilutions were spread plated onto tryptic soy agar (Difco Tryptic Soy Agar, Becton, Dickinson and Company, Sparks, MD) plates. If the number

of colonies at these dilutions were too few to detect, 0.1 mL aliquots of soymilk samples were plated directly onto the plates. The plates were incubated at 30±1 °C for 48 h and at 4 °C for 7 - 10 days to estimate the APC and number of psychrotrophs, respectively. Results were recorded as colony forming units per mL (CFUs /mL).

(b) pH

Triplicate pH readings of samples using a (Accumet Basic AB 15, Fisher Scientific Company L.L.C., Pittsburgh, PA) were taken on each day of analysis and the averages reported.

(c) Dry Solids Content

As there was no loss of water or of soybean solids during CFHPT, the total solids should be the same in the untreated soymilk. Thus, the de-aerated and filtered soymilk samples were analyzed for total solids using Halogen Moisture Analyzer (Model HR73, Mettler-Toledo, Inc., Columbus, OH). The temperature of the analyzer was set to 115 °C. The total solid content also served as an indicator of the control over soymilk preparation across the two trials.

(d) Particle Size Distribution

The particle size distribution of the control sample and CFHPT processed soymilk samples was measured using Malvern Laser Particle Size Analyzer, Mastersizer S with 300mm lens (Malvern Instruments, Southborough, MA). The soymilk samples were dispersed in about 150 mL DW in the samples dispersion unit connected to the

instrument. The pump speed was kept at 2100 rpm. The obscuration in the diffractometer cell was maintained at 16±0.5 % for all samples. The software controlling the equipment (Version 2.18, Malvern Instruments) works on an optical model based on Mie theory of light scattering and assumes all the particles in soymilk to be spherical. The calculation of the predicted scattering was done based on the following refractive index (RI) information fed into the software: real refractive index- 1.47; imaginary refractive index- 0.00; refractive index of water- 1.33. The 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 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 $D_{(v,0.9)}$ value which is the diameter below which 90 % of the particles (by volume) were found. All soymilk samples were analyzed in duplicate and averages calculated for each of the parameter.

(e) Visible Layer Separation/ Sedimentation

During the 4 week quiescent refrigerated storage period, all the samples were inspected twice a week for any visible layer separation.

(f) Lipoxygenase Activity

The method of Wang et al. (2008) was followed. First, 0.2 M borate buffer, pH 9.0 was prepared using sodium borate (Sodium Borate, 10-Hydrate, Crystal, A.C.S. Reagent, J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ) and boric acid (granular, A.C.S. Reagent, J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ). Next, the substrate solution was made by mixing 0.01 mL linoleic acid (TCI America, Portland,

OR), 0.01 mL Tween 20 (Fisher Scientific, Fair Lawn, NJ) and 4.0 mL of the borate buffer at 25 °C. This was homogenized using a Pasteur pipette by repeatedly taking in and pushing out the solution. 0.55 mL of 0.5 N NaOH (pellets, F.C.C, J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ) was added for clarification and the volume made up to 60 mL using the borate buffer. If the solution was not going to be used immediately following preparation, it was stored until used. To obtain the enzyme solution, the soymilk samples were centrifuged at 30,000 X g for 30 min at a temperature of 4 °C in a Sorvall RC6 Plus Centrifuge (Thermo Fisher Scientific, Inc., Waltham, MA) and a Sorvall SM-24 rotor. Both were allowed to equilibrate to 4 °C for 1 h prior to centrifugation. Since the supernatant was cloudy, it was filtered through a 0.1 µm syringe filter. Prior to the assay, the filtered supernatant was diluted 5 times with DW. This comprised the enzyme solution. If some samples could not be analyzed at the designated time interval, they were frozen and kept at -65 °C until analyzed.

The assay mixture consisted of 2.0 mL of the substrate solution, 0.9 mL of borate buffer and 0.1 mL of enzyme solution. This mixture was prepared in a quartz cuvette and the cuvette shaken to start the reaction. The increase in absorbance at 234 nm was monitored immediately after shaking the cuvette for 5 min using an Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, CA). The temperature of the lab and the reagents was maintained at 25 °C. The lipoxygenase activity was calculated from the linear portion of the absorbance-time curve as provided by the instrument software (UV-Visible Chem Station, Rev. B.04.01, Agilent Technologies). A blank was also prepared. It contained 2.0 mL of the substrate solution and 1.0 mL of the borate buffer.

(g) Sensory Analysis

A descriptive sensory analysis was conducted on two treatments- T6 (121 °C, 12.48 s, 276 MPa) and T8 (121 °C, 12.48 s, 207 MPa). During the training, treatment T9 (heated to 145 °C) was also evaluated. However, it was found to have excessive cooked flavor and not favored by the panelists. Thus, keeping in the mind that excessive cooked flavor could lead to problems in commercialization, it was decided not to evaluate any treatment combination that was heated to 145 °C. An electronic communication was sent out within the department for recruiting the panelists. A total of 16 panelists were initially on the panel, out of which 5 were identified as outliers and thus, the responses of these 5 panelists were not used in any analysis. All the panelists were food science graduate students, experienced in sensory analysis. There were seven 1-hour training sessions. A 150 mm unstructured line scale, with indents marked at 12.5 mm from either end, was used for each attribute. No words were put next to these indents but the panelists were informed that the region of the scale near the indents signified low/absent/weak or high/extreme/strong concentrations depending on if the indent was on the left part of the scale or the right, respectively. The panelists were free to place a vertical mark anywhere on the line depending on their perception of that attribute's intensity. The response was then converted into millimeters for calculation and statistical purposes. Six attributes were evaluated and these, in the order as evaluated by the panelists were- Beany Aroma, Beany Flavor, Astringency, Cooked Flavor, Bitterness and finally Chalkiness.

For the first few training sessions, the panel was calibrated for low and high intensity concentrations of each attribute. Solutions of reference samples with corresponding concentrations were prepared and served to the panel. Panelists were asked

to rinse their mouths with crackers (Unsalted Top Premium Saltine Crackers, Kraft Foods Global, Inc., Northfield, IL) and water (Crystal Springs Natural Spring Water, DS Waters of America, Inc., Mableton, GA). The panel, as a whole, came up with the intensities for each of these two concentrations. After calculating these consensual intensities in millimeters (based on the marks put on the scale by the panelists), boxes with 'x' marks were put on the scales at appropriate distances signifying the low and high concentrations. Next, a medium intensity concentration of the reference sample for each attribute was chosen and presented to the panel. Then, taking the help of the previously decided low and high intensities (and marked on the scale), the panel was calibrated for the medium concentration of each attribute. After the training, the final scales had the two indentation marks and only one box with 'x' mark corresponding to the medium intensity of the attributes. No words or numbers were present on the scale. Actual samples were also given during the final training session along with the reference samples of medium intensity concentration. The reference samples, their concentration and their final consensual intensity values are presented in Table 3.2.

Evaluation of the soymilk samples was held in individual sensory booths located in the Food Processing Lab at the Department of Food Science and Technology, University of Georgia, Athens. Fluorescent lighting was used and the sample trays were prepared in the kitchen adjoining the booths area. The soymilk samples were served at refrigeration temperature and the panelists were asked to cleanse their palates with crackers and water between samples. White plastic trays were used to arrange the soymilk samples, reference samples (of medium concentration intensity), crackers, water and the evaluation sheets. The trays were passed to the panelists through a window

between each booth and the kitchen area. Clear plastic cups (59.15 mL) with lids (Solo Cup Company, Highland Park, IL) were used for serving the soymilk samples and the reference samples. For each evaluation session, the soymilk samples were coded with three digit random numbers. The order of presentation of the 2 samples was also randomized. For each evaluation session, half the panelists received treatment T6 first and the other half received T8 first. This order was reversed during the second repetition. The evaluation sessions were held on days 1, 6, 13 and 20 after preparation & refrigerated storage of soymilk. The entire study was replicated. Three commercially available soymilk samples were also evaluated twice on separate occasions but no storage study was performed on them. These were Silk Organic Unsweetened Soymilk (White Wave Foods, Broomfield, CO), SoyCow Unsweetened Soymilk (Well Luck Co., Inc., Jersey City, NJ), and Vita Soy Unsweetened Authentic Asian Fortified Soy Beverage (Vitasoy USA, Inc., Ayer, MA).

All the data thus generated was analyzed statistically using JMP Pro Version 10.0.0 (SAS Institute Inc., Cary, NC). The results were considered to be significantly different if $\alpha < 0.05$.

**Table 3.1: Treatment Combinations**

| Treatment Number | Exit Temperature (°C) | Residence Time (s) | Pressure (MPa) |
|---|---|---|---|
| UT[A] | - | - | - |
| T 1 | (no pre-heating)[B] | 20.80 | 207 |
| T 2 | (no pre-heating) | 12.48 | 207 |
| T 3 | (no pre-heating) | 20.80 | 276 |
| T 4 | (no pre-heating) | 12.48 | 276 |
| T 5 | 121 | 20.80 | 276 |
| T 6 | 121 | 12.48 | 276 |
| T 7 | 121 | 20.80 | 207 |
| T 8 | 121 | 12.48 | 207 |
| T 9 | 145 | 20.80 | 276 |
| T 10 | 145 | 12.48 | 276 |
| T 11 | 145 | 20.80 | 207 |
| T 12 | 145 | 12.48 | 207 |

A: control sample
B:The exit temperature of treatments T1 – T 4 was not controlled by pre-heating

**Table 3.2: Sensory Attributes:** Reference samples, their preparation method, and the intensity of each reference as decided by the panel

| Attribute | Reference Sample | Preparation Method | Intensity (mm) |
|---|---|---|---|
| Beany Aroma | Raw soybeans soaked in deionized water for 16 h (1:12:: w/w) | Drained and ground with deionized water (1:4:: w/w) | 60 |
| Beany Flavor | same as Beany Aroma | same as Beany Aroma | 60 |
| Astringency | Alum Powder[A] | 0.01 % solution in water[B] | 20 |
| Cooked Flavor | Evaporated Milk[C] | Diluted with water (1:6:: w/w) | 45 |
| Bitterness | Caffeine[D] | 0.03 % solution in water | 20 |
| Chalkiness | Protein Juice[E] | Diluted with water (1:4:: w/w) | 55 |

A: Alum, McCormick & Co., Inc., Hunt Valley, MD, USA
B: Crystal Springs Natural Spring Water, DS Waters of America, Inc., Mableton, GA, USA
C: Carnation Evaporated Milk with Vit. D, Nestle, Glendale, CA, USA
D: Caffeine, Anhydrous, FCC, ScienceLab.com, Inc., Houston, TX, USA
E: Naked Protein Juice Smoothie, Naked Juice Company, Monrovia, CA, USA

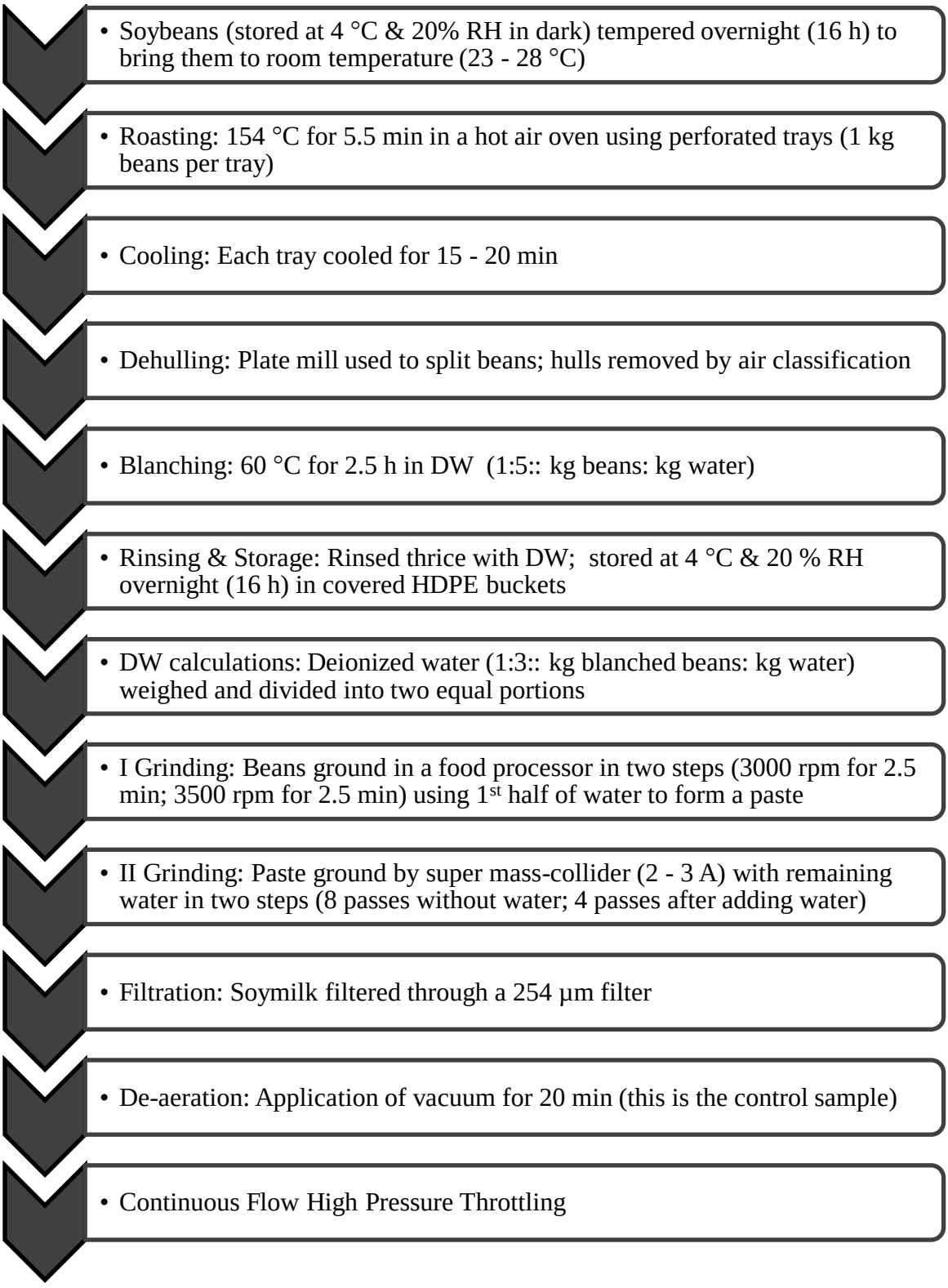


**Figure 3.1:** Soymilk preparation flowchart

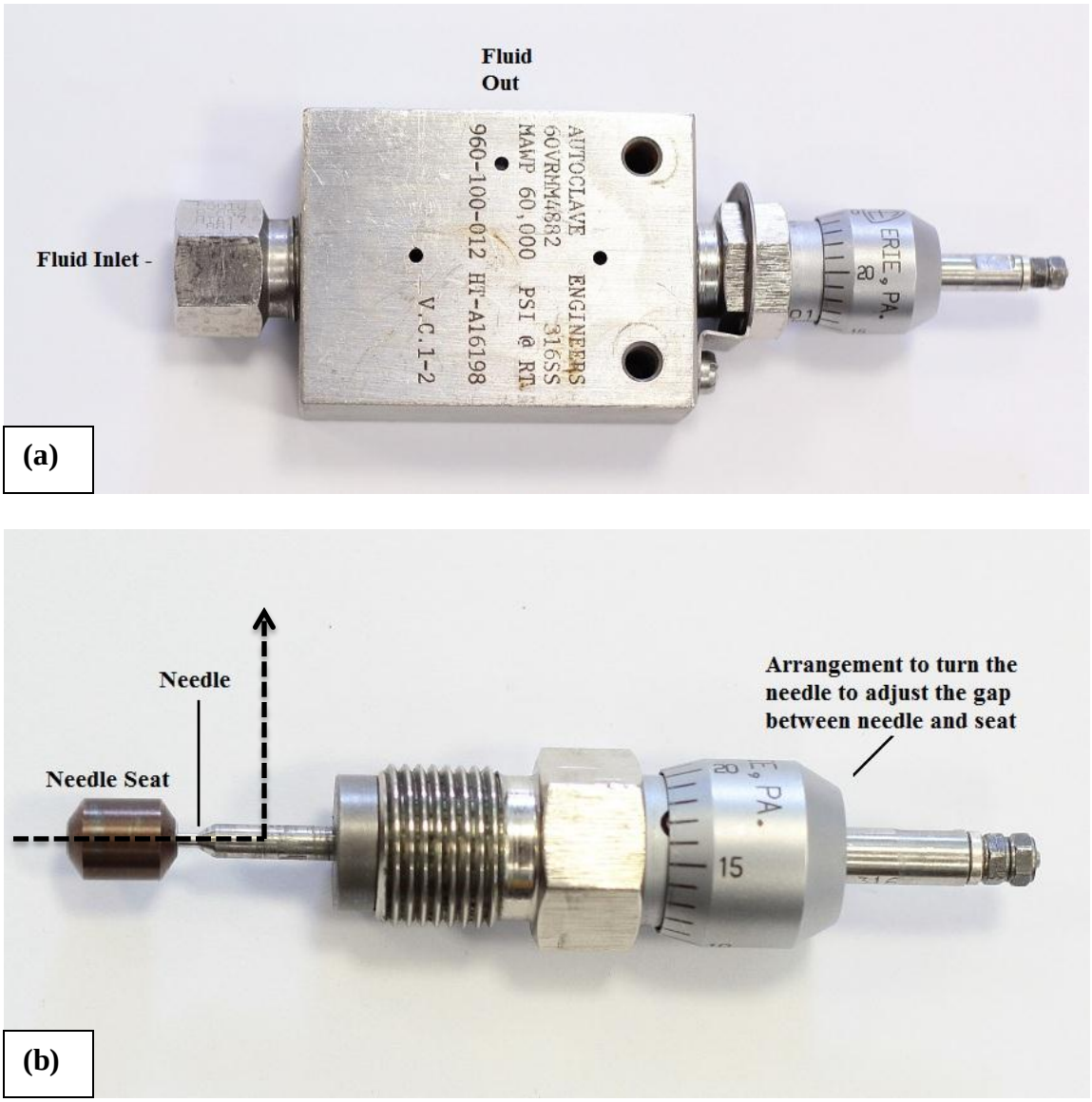


**Figure 3.2:** Throttling valve (Autoclave Engineers, Erie, PA) with – (a) the needle assembly fitted in; (b) the needle and its seat shown separately. The dotted line represents the direction and path of fluid flow.

# CHAPTER 4

# RESULTS AND DISCUSSION

As this research involved a storage study, for each of the parameters discussed, first the changes following CFHPT are discussed which are then followed by a discussion on changes during the storage of soymilk at 4 °C.

## Visible Layer Separation

All the samples were stored upright and undisturbed, in sterile tubes in a 4 °C chamber. These were periodically checked for any visible separation of the soymilk into two phases. The control sample separated into two layers with a well-defined boundary within 1 – 2 days (Figure 4.1) of storage. All the treated samples did not show any separation even after 4 weeks of storage. Other researchers (Guerrero-Beltran et al., 2009b; Cruz et al., 2007) have also reported similar stability results. In the present method of soymilk processing, since there is no filtration/ centrifugation step, there is a greater amount of soybean solids suspended in the soymilk as compared to the soymilk in other studies.

## Effect of CFHPT on Temperature Rise

The data for the sharp increase in temperature as a result of throttling is presented in Table 4.1. The temperature of the soymilk as it entered the equipment was around 23 -

28 °C. The average outlet temperature for the samples which were not pre-heated was 72.33 °C and 86.74 °C corresponding to 207 MPa and 276 MPa, respectively. For the remaining samples, the temperature to which the samples were pre-heated was adjusted to achieve the two outlet temperatures, 121 °C and 145 °C.

Only pressure had a significant effect on this rise. The average temperature rise (across all treatments) at 207 MPa was 51.48 °C while at 276 MPA it was 59.92 °C. The temperature rise per unit applied pressure was calculated to be: 0.25 °C/MPa and 0.22 °C/MPa at 207 MPa and 276 MPa, respectively. However, for the treatments with no pre-heating, the average temperature rise per unit applied pressure was 0.22 °C/MPa and this was not significantly different for either pressure level. Thus, when soymilk was pre-heated, the rise in temperature with increasing pressure (in the 200 – 300 MPa pressure range) did not follow a linear relation. However, at a constant pressure, as the soymilk was pre-heated to a higher temperature, the temperature of soymilk after throttling increased correspondingly. Thus, it is possible that the increase in temperature due to increased pre-heating occluded some of the temperature rise due to increased pressure. Using a setup that was similar to the one used in this study Sivanandan et al. (2008) and Poliseli-Scopel et al. (2012) observed a similar temperature rise in soymilk. Floury et al. (2004a) also reported a temperature rise of 0.22 °C/MPa although they used a throttling made out of a different material and of a different geometry. In a related study, Floury et al. (2002) subjected soy-protein stabilized emulsions to ultra-high pressure processing (UHPH) and found the same value for temperature rise.

As mentioned before, this temperature rise is chiefly due to the turbulence, shear and cavitation experienced by the fluid in the pressure release device, although some of it

may be a result of adiabatic heating (Hayes & Kelly, 2003). In milk, a linear temperature rise as a result of UHPH has been reported (Datta et al., 2005; Hayes et al., 2005) although the temperature rise is lesser (0.166 °C/MPa) as compared to soymilk. This could be attributed due to the higher viscosity of soymilk as well as to the presence of harder suspended particles.

For high hydrostatic pressure (HHP) processing, the rise in temperature is only due to adiabatic heating as the food is pressurized. This temperature rise has been reported to be only about 0.028 to 0.085 °C/MPa depending on the product (de Heij et al., 2003) and is about one-tenth to one-third of that observed for continuous high pressure (CHP) processing. Thus, to achieve similar temperatures, much greater pressure has to be used in HHP which leads to greater energy input. Additionally, the temperature rise is gradual in HHP while in CHP, the temperature rise is sudden (0.7 s as reported by Poliseli-Scopel et al., 2012) which could be beneficial from the microbial inactivation as well as quality point of view.

**Effect of CFHPT on the Particle Size Distribution (PSD) of Soymilk**

The D[4,3] (also known as Volume or Mass Moment Mean, or De Brouckere Mean Diameter) and D[3,2] (also known as Surface Area Moment Mean, or Sauter Mean Diameter) are the two most commonly reported mean values and their mathematical formulae are $\Sigma n_i d_i^4/\Sigma n_i d_i^3$ and $\Sigma n_i d_i^3/\Sigma n_i d_i^2$, respectively. In these formulae, $n_i$ is the number of particles in a class of diameter $d_i$ and this diameter is actually the equivalent diameter. As is known, not all food particles are spheres. However, sphere is the only shape which can be described by one unique number, its diameter. So, to report the mean

diameter of food particles of different shapes, the volume and the surface area of a food particle is converted into the volume and surface area of an equivalent sphere. The diameter of this equivalent sphere is used to calculate the D[4,3] and D[3,2] values. To do these calculations, the instrument generates a volume distribution. The D(v,0.9) represents the $90^{th}$ percentile and is the diameter below which 90% of the particles are found, based on the total volume of all the particles (Rawle, 2011). The D[4,3] value is highly sensitive to the presence of large particles and is a representative of those particles which make up most of the sample volume, or in other words, the coarse particles. The D[3,2] value is better suited to observe the proportion of finer particles (Horiba Scientific, 2012; Malvern Instruments, 2012).

The PSD data of all the soymilk samples post CFHPT are presented in Table 4.2. There was a significant reduction the particle size of all the treated samples as compared to the control. Amongst the treated samples however, there was no significant effect of pressure, temperature or residence time on the D[4,3] values. Nonetheless, the volume mean diameter was generally lower for the higher pressure level. Interestingly it was seen that this value was generally higher for higher temperatures. For the surface area mean diameter, pressure and temperature did have a significant effect. The average D[3,2] at 207 MPa was 13.45 µm and this was significantly lower at 276 MPa with an average of 12.19 µm. Increasing the exit temperature of soymilk caused a significant increase in this value and the averages were 10.38 µm, 12.80 µm and 15.29 µm for the samples without any pre-heating, samples with an exit temperature of 121 °C and samples with an exit temperature of 145 °C, respectively. Thus, it can be inferred that, increasing the pressure significantly reduced the particle size of finer soybean solids present in the soymilk while

the size of coarser particles was reduced to a level that did not differ significantly between the two pressure levels. The D(v,0.9) value did not differ significantly between any of the treatment combinations.

In a very similar setup, Sivanandan et al. (2008) observed a significant reduction in both, D[4,3] and D[3,2] values with increasing pressure as well as narrowing of the distribution. They attributed the reduction in particle size to the weakening of membranes of the particles as the pressure was increased. As a result, the particles disintegrated easily during the throttling process. On the contrary, Cruz et al. (2007) observed an opposite effect and found that the soymilk that was homogenized at 300 MPa had higher values of D[4,3] and D[3,2] as compared to the soymilk treated at 200 MPa. They attributed this to the coalescence of soybean particles. The results of Poliseli-Scopel et al. (2012) agree with the result of our study in that they also did not find a significant effect of either pressure or temperature on the mean diameters. The diameter of majority of the particles was below 1 μm which is much lower than the particle size of the soymilk processed in the current study. The low particle size could be attributed to the fact that they filtered out the okara during soymilk preparation. The okara being hard may have caused the average mean diameters to be higher. Nonetheless, as has been mentioned earlier, there was no visible separation of soymilk after 4 weeks of quiescent storage at 4 °C. Also, as has been discussed in the following sections, the chalkiness of the soymilk samples was not objectionable, rather it was comparable to that of market samples. Thus, even though the average particle size of soymilk particles was higher in our study compared to soymilk made by filtration, it did not affect its physical stability or sensory qualities. A lot of researchers have performed high pressure homogenization on bovine

milk and the particle size of milk fat globules after high pressure homogenization is much smaller, in the nanometer range (Hayes & Kelly, 2003; Thiebaud et al., 2003; Pereda et al., 2007) as compared to soymilk. The smaller particle size is probably due to the absence of hard, plant cell well type materials in bovine milk and also, the average particle size of raw milk is generally reported to be around 3 μm.

We did not find any significant change or a trend in any of these parameters during storage of soymilk samples at 4 °C for 4 weeks (data not shown). The stable particle size means that no aggregation or flocculation of soybean solids suspended in the soymilk occurred. Thus, all the samples remained physically stable during storage.

**Effect of CFHPT on the Lipoxygenase (LOX) Activity in Soymilk**

We did not find any lipoxygenase activity in the control sample. Since all the treatments involved further heating and application of pressure, it was deduced that no other treatment combination would have any lipoxygenase activity and thus we did not check the for LOX activity in these samples. The absence of LOX activity is in agreement with the results of Poliseli-Scopel et al. (2012) in which no LOX activity was found in the control soymilk samples. In this study, the soybeans were ground at 80 °C for 20 min which clarifies why there was no activity. In our study, the soybeans were blanched at 60 °C for 2.5 h and this explains the lack of any LOX activity.

**Effect of CFHPT on pH**

The pH data is summarized in Table 4.3. The UT sample represents the control and it did not undergo any processing. CFHPT caused a reduction in the pH of samples.

The pH of the control sample was 7.08. The pH of soymilk samples which were not preheated was significantly lower than that of the control, but the pH value did not differ across the two pressure levels and the average was 6.96. The pH of all pre-heated samples (which meant a much higher final temperature) was significantly lower than those without any preheating but again, the value did not differ across the different temperature and pressure levels. The average pH of samples with preheating was 6.89. There was a significant interaction effect between temperature and pressure and the lowest value of pH was 6.86, observed for the 121 °C, 276 MPa and 145 °C, 207 MPa treatment combinations regardless of the flow rate. It is possible that the application high pressure and temperature changes the confirmation of certain proteins affecting their charge and/or solubility which leads to a change in pH. As the soymilk is throttled, there is a tremendous reduction in particle size (discussed later). The reduction in particle size means that there is a large increase in the surface area of soybean solids thereby exposing more surfaces to the surrounding liquid. If there are charged molecules on the exposed areas, the pH could be affected. Secondly, as practically all the solids of dehulled soybeans are incorporated in our method of soymilk preparation, the additional solids may cause its pH value to be different from that of soymilk prepared by other researchers and also to change during processing. Nonetheless, the actual reasons for the difference pH remain to be investigated and could be taken up in a future study.

We could not find any study which compared the pH of soymilk before and after continuous high pressure (CHP) processing. However, one study (Malaki Nik et al., 2008) pasteurized soymilk at 95 – 100 °C for 7 min followed by homogenization, albeit at a lower pressure of 69 MPa. No change in the pH value of 6.7 was found after either

pasteurization or homogenization. However, Hayes & Kelly (2003) studied the high pressure homogenization of bovine milk and the pH level was found to reduce with increasing homogenization pressure. Another study (Pereda et al., 2007) performed UHPH on milk and again found a small reduction in pH from 6.74 to 6.72 at 200 MPa. The drop in pH with pressure has been highlighted by Farkas & Hoover (2000) as well. CHP notwithstanding, some studies have investigated the effect of high hydrostatic pressure (HHP) processing of soymilk on pH. Interestingly, no change in pH is generally observed with HHP processing (Smith et al., 2009; Lakshmanan et al., 2006; Zhang et al., 2005) even when soymilk only thermally treated without the application of pressure. However, it is important to note here that in all these studies, the preparation of soymilk involved a filtration step which changes the composition of suspended matter in soymilk.

Change in pH of CFHPT soymilk upon storage

The change in pH over four weeks of refrigerated (4 °C) is shown in Figure 4.2. There was a significant drop in the pH of the control (UT) sample after 2 weeks where it fell to 6.74. After 4 weeks, the pH further fell to 5.88. The pH of soymilk samples which were not pre-heated (T1 – T4) significantly changed only at the 3 week mark although by the end of 4 weeks, their pH was around that of the control. The soymilk samples which were pre-heated to achieve a final temperature of 121 °C or 145 °C remained stable for 3 weeks and there was a drop in their pH value only at the 4 week mark. Their final pH nonetheless was higher than that of all other samples. Neither pressure, nor the final temperature after throttling affected the change in pH upon storage for these samples. It

can thus be inferred that pressure, when combined with temperature helps prevent a drop in pH for a longer period.

Smith et al. (2009) using a high hydrostatic pressure system for processing soymilk reported similar results although the pH of their control sample dropped to 4.8 after 4 weeks of storage. Lakshmanan et al. (2006) stored homogenized and pasteurized soymilk for up to 2 days and did not observe any change in pH of the processed samples. Achouri et al. (2007) only used thermal treatment (116 °C for 6 min) to process soymilk. They found that the pH did not change much during the first 3 weeks and dropped by 0.6-0.7 units at the end of weeks. Referring to their results, we see that even though the soymilk samples in our study were treated at high temperatures (121 – 145 °C) for only about 20 seconds at the most, the pH stability is almost identical, and the maximum pH drop in the pre-heated pressurized samples is about 0.5 units. The change in pH is probably due to chemical changes occurring in the soymilk mediated perhaps by the growth of microbes.

**Effect of CFHPT on the Microbiological Quality of Soymilk**

The microbiological data are presented in Table 4.3. The initial total microbial counts in the control (UT) sample were 5.00 log CFU/ mL and 3.12 log CFU/ mL for aerobic plate count (APC) and psychrotrophs, respectively. All the treated samples had significantly lower counts. Pre-heating the soymilk, leading to higher exit temperatures (121 & 145 °C) resulted in significantly lower APC counts although there was no significant difference in counts of samples treated to either 121 °C or 145 °C (T5 – T12). The average count for these pre-heated samples was 0.32 log CFU/ mL. Similarly, there

was no significant difference in the APC counts amongst the soymilk samples that were not pre-heated (T1 – T4) and this average was 2.18 log CFU/ mL. For some of the treatment combinations (T6, T8 and T11), no microbial growth was detected. The results are similar for psychrotrophs. There was no significant difference amongst the non pre-heated samples or amongst the pre-heated samples. However, these two sets of treatments differed from each another significantly. The average psychrotrophs count for the samples receiving no pre-heating was 0.27 log CFU/ mL although only one treatment combination (T1) had an average count of 1.09 log CFU/ mL while in others, the counts were below detection. These small counts may be because of the soymilk getting contaminated in the equipment since not all parts of the equipment could be taken apart for extensive cleaning after each experiment. CIP (cleaning in place) could not be performed as it was not recommended by the manufacturer of the high pressure equipment. For all the samples receiving pre-heating, no psychrotrophs could be detected. It can be inferred that, continuous high pressure, by itself, causes an almost 3 log reduction in the aerobic bacteria population and at least a 3 log reduction in the psychrotrophs. Supplementing pressure with high temperature for short durations can lead to an almost sterile product. It should be mentioned that the collection of samples was not done in a laminar flow and this may have led to some contamination at the point of sample collection.

Poliseli-Scopel et al. (2012) treated soymilk with continuous high pressure (200 – 300 MPa) at exit temperatures ranging from about 105 – 135 °C. In contrast to our results, they concluded that the reduction in microbial population was also pressure dependent and reported a higher reduction at 300 MPa. In some instances, they observed

higher temperatures leading to lower counts. Cruz et al. (2007) also performed high pressure homogenization of soymilk at 200 – 300 MPa but in their study, the exit temperatures were lower, 88 – 100 °C. This might explain why they observed lower log reduction, 2.42 log CFU/ mL and 4.24 log CFU/ mL at 200 MPa and 300 MPa respectively. On the other hand, Smith et al. (2009) studied the effect of high hydrostatic pressure (HHP) on soymilk at a pressure range of 400 – 600 MPa at different initial temperatures and dwell times ranging from 1 – 5 min. They did not find the log reduction to be dependent upon pressure but to be significantly affected by the temperature. At the higher initial temperature of 75 °C, they observed a 4.5 log reduction. This reduction is comparable to the one that was achieved in this study. This shows that, as compared to HHP, lower pressures combined with short time high temperatures in CHP processing leads to similar or better microbial reduction.

Change in the microbial population of CFHPT soymilk upon storage at 4 °C

Changes in the APC and psychrotrophs are presented in Figures 4.3 and 4.4, respectively. During storage, only the time interval (D1, W1, W2, W3 and W4) had a significant effect. For the control, there was a significant increase in microbial counts after 1 week of storage, the counts being 6.43 log CFU/ mL and 6.17 log CFU/ mL for APC and psychrotrophs, respectively. By the end of 4 weeks, these had risen to 7.97 log CFU/ mL and 8.01 log CFU/ mL, respectively and there was no significant difference between the two counts. For treatments 1 to 4 (no pre-heating), there was no significant increase in the two counts for the first week of storage. After 1 week, there was a continuous rise in counts which reached 7.53 log CFU/ mL (APC) and 6.46 log CFU/ mL

(psychrotrophs) at the end of 4 weeks. Treatments 5 – 12 (pre-heated to achieve 121 °C or 145 °C at exit) also did not show a significant increase in APC until 1 week of storage while the psychrotrophs remained non-detectable. After 1 week however, both the counts began to rise, reaching 6.43 log CFU/ mL (APC) and 4.96 log CFU/ mL (psychrotrophs) after 4 weeks. Smith et al. (2009) have mentioned the spoilage detection level to be 7 log CFU/ mL and treatments 5 – 12 did not reach this level, even at the end of the storage period.

Other authors have also noted an increase in microbial counts upon storage of high pressure processed (HPP) milk. This signifies that HPP causes injury of many cells especially at lower pressures (Sharma et al., 2009). Poliseli-Scopel et al. (2012) observed an increase in microbial counts of soymilk samples upon storage even though no counts were detected immediately after high pressure processing. They stored the samples at 30 °C rather than 4 °C and in case of samples treated at 300 MPa and 135 °C there was no growth even after 20 days of storage. As mentioned before, their sample collection method (laminar flow) ensured no contamination at the point of sample collection. Wang et al. (2001) only pasteurized the soymilk (82 °C, 1 min) and even then observed no microbial growth after 4 weeks of storage at 3 °C. However, their soymilk contained flavoring, gum and sugar which may have acted as the preservative. In a similar study (Smiddy et al., 2007) on bovine milk with initial APC of 5 log CFU/ mL, no microbial counts could be detected after high pressure homogenization. However, the counts increased to 8 log CFU/ mL after only 14 days of storage at 5 °C. Thus, the effectiveness of high pressure processing on microbial inactivation varies widely. Nonetheless, it still holds promise and could be improved with further research.

**Effect of CFHPT on the Sensory Attributes of Soymilk**

Six sensory attributes related to soymilk were evaluated by a trained panel and the results are summarized in Figure 4.5. Two samples (T6 and T8) were prepared in the lab while three plain-unsweetened commercial soymilk samples were used for comparison. Two of the commercial samples (SoyCow and Vita Soy) were products made in Asia and bought from local Oriental stores while one sample (Silk) was made in the USA. It is important to note that we did not use any additives, sweeteners, flavors, viscosity modifiers, etc. in the processing of soymilk in our lab. However, all the three commercial samples, in addition to water and soybeans, had the following added to them (based on the ingredient statement on the packages):

Silk: Calcium Carbonate, Sea Salt, Flavors, Gum, Vitamins

SoyCow: Emulsifier

Vita Soy: Tricalcium Phosphate, Salt, Zinc Oxide, Vitamins

The addition of flavors and salt could improve the flavor perception of soymilk while the addition of emulsifiers etc. could improve the mouthfeel.

Silk had an average beany aroma intensity of 16.9 (on the 150 mm scale) which was significantly lower than the remaining four samples, which did not differ significantly amongst themselves. The beany aroma intensities for these samples ranged from 32.5 for SoyCow to 40.3 for T8. For beany flavor, Silk again had the lowest intensity (26.7) while Vita Soy had highest (50.7). The beany flavor of other samples ranged from 40 – 45. Iwuoha & Umunnakwe (1997) commented on the beany flavor of soymilk and wrote, “Generally, a slight beany flavor is usually perceptible which confers on soymilk a unique identity”. Even though we did not detect any lipoxygenase activity

in the soymilk, the presence of beany aroma and flavor indicates that some non-enzymatic reactions gave rise to the beany notes.

There was no significant difference in the astringency of the samples and its intensity ranged from 14.1 for Silk to 20.9 for T8. The samples processed in the lab had significantly higher cooked flavor intensity (63.9 for T6 and 65.2 for T8) as compared to the commercial samples, which had a cooked flavor intensity in the 33 – 35 range. There was not much difference in the bitterness of the samples and only T6, which had the highest bitterness intensity (21.2) was significantly different from Silk, which had the lowest (12.3). Finally, there was no significant difference in the chalkiness of the samples and it ranged from 20 – 23 for all the samples. Thus, even though we did not add any gum or emulsifier to the soymilk processed in the lab, the CFHPT process made the chalkiness of samples highly comparable to the commercially available samples. Chalkiness (sometimes referred to as sandiness) is considered to be a defect in soymilk and is not liked by the consumers. Thus, a processor can incorporate all the soybean solids into the soymilk without the issue of chalkiness, and this translates into a higher yield. In addition, it is imperative to mention that in general, the astringency, bitterness and chalkiness were quite low in intensity (less than 25) for all the samples, especially considering that a 150 mm scale was used.

Change in Sensory Qualities of CFHPT soymilk upon storage at 4 °C

Only the samples processed in the lab were used in the storage study to elucidate the effects of refrigerated storage on the sensory attributes. The samples were stored for 20 days (roughly 3 weeks). These results are presented in Figure 4.6. There was no

significant difference between the two samples in any attribute throughout the storage. However, there was significant change in the intensity of only beany flavor at the day 20 mark. The average beany flavor reduced from 41.6 on day 1 to 30.5 on day 20. This value is close to the beany flavor intensity of the Silk soymilk sample. Achouri et al. (2007) noticed a general decrease in the total volatile content of soymilk after storage at 4 °C. If some of these volatiles contribute to beany flavor, then the intensity of beany flavor will also reduce over a period. There was no significant change in any of the other attributes.

**Total Solids Content (%) and pH comparison with Commercial Samples**

We also measured the dry solids content of these samples and the summary is presented in Table 4.4. As can be seen, the samples prepared in the lab had the highest total solids content while the SoyCow sample had the lowest. Thus, there is some margin to further dilute the soymilk produced in this study to make the dry solids content comparable to that of commercial samples. The opportunity for further dilution again means a greater yield. Secondly, since the samples processed in this study had higher soybean solids, it is likely that this caused these samples to have greater intensities of the sensory attributes. A dilution of the soymilk may help lower some of these intensities.

The average pH of SoyCow samples was 6.69 ± 0.005 and that of Vita Soy samples was 6.43 ± 0.020. These values are comparable to the pH of T6 and T8 samples (Table 4.3). Interestingly, the Silk soymilk samples were alkaline with an average pH of 8.24 ± 0.010 and this may be due to the addition of calcium carbonate which is alkaline when in solution.

**Table 4.1: Temperature Rise during Soymilk Throttling**[A]

| T.No.[B] | Temp.[C] (°C) | Residence Time (s) | Pressure (kPa) | Temperature Rise (°C) | Rise per unit Applied Pressure (°C/ MPa) |
|---|---|---|---|---|---|
| 1 | No heating[D] | 20.80 | 207 | 46.23 (0.325) | 0.22 (0.002) |
| 2 | No heating | 12.48 | 207 | 44.90 (2.300) | 0.22 (0.011) |
| 3 | No heating | 20.80 | 276 | 59.35 (0.550) | 0.22 (0.002) |
| 4 | No heating | 12.48 | 276 | 58.05 (0.950) | 0.21 (0.003) |
| 5 | 121 | 20.80 | 276 | 53.50 (7.800) | 0.19 (0.028) |
| 6 | 121 | 12.48 | 276 | 64.85 (13.050) | 0.23 (0.047) |
| 7 | 121 | 20.80 | 207 | 53.35 (3.350) | 0.26 (0.016) |
| 8 | 121 | 12.48 | 207 | 61.25 (6.250) | 0.30 (0.030) |
| 9 | 145 | 20.80 | 276 | 63.95 (2.750) | 0.23 (0.010) |
| 10 | 145 | 12.48 | 276 | 59.80 (7.000) | 0.22 (0.025) |
| 11 | 145 | 20.80 | 207 | 50.85 (1.550) | 0.25 (0.008) |
| 12 | 145 | 12.48 | 207 | 52.30 (1.900) | 0.25 (0.009) |

A: The values are mean and SD (in parentheses) from 2 independent experiments. Temperature Rise was calculated by subtracting the temperature of pre-heated soymilk from the temperature of soymilk measured at the exit point of throttling valve.

B: Treatment Number

C: Exit Temperature, measured at the exit of throttling valve

D: Treatments 1 – 4 received no preheating while treatments 5 – 12 we preheated so as to obtain the required temperature after throttling.

**Table 4.2: Effect of CFHPT on Particle Size Properties**[A]

| T.No.[B] | Temp.[C] (°C) | Residence Time (s) | Pressure (kPa) | D[4,3][D] (µm) | D[3,2][E] (µm) | D(v,0.9)[F] (µm) |
|---|---|---|---|---|---|---|
| UT[G] | - | - | - | 129.86 (10.659) | 17.04 (0.690) | 335.90 (30.196) |
| 1 | No heating | 20.80 | 207 | 23.34 (3.903) | 10.60 (0.940) | 46.90 (8.560) |
| 2 | No heating | 12.48 | 207 | 25.21 (3.076) | 10.63 (0.820) | 51.13 (6.247) |
| 3 | No heating | 20.80 | 276 | 20.91 (1.131) | 10.75 (0.757) | 40.90 (2.885) |
| 4 | No heating | 12.48 | 276 | 19.74 (1.294) | 9.54 (0.350) | 39.21 (3.002) |
| 5 | 121 | 20.80 | 276 | 22.44 (2.517) | 12.63 (0.841) | 43.70 (5.469) |
| 6 | 121 | 12.48 | 276 | 23.47 (0.240) | 12.31 (1.039) | 46.70 (0.106) |
| 7 | 121 | 20.80 | 207 | 22.60 (1.584) | 12.38 (0.686) | 43.51 (3.140) |
| 8 | 121 | 12.48 | 207 | 26.24 (2.058) | 13.91 (1.336) | 51.98 (4.179) |
| 9 | 145 | 20.80 | 276 | 23.63 (6.039) | 13.59 (1.937) | 46.53 (12.459) |
| 10 | 145 | 12.48 | 276 | 28.25 (8.775) | 14.34 (1.648) | 56.39 (19.958) |
| 11 | 145 | 20.80 | 207 | 30.19 (3.543) | 17.08 (1.478) | 58.35 (7.266) |
| 12 | 145 | 12.48 | 207 | 30.10 (7.6374) | 16.14 (0.778) | 58.65 (14.711) |

A: The values are mean and SD (in parentheses) from 2 independent experiments
B: Treatment Number
C: Exit Temperature, measured at the exit of throttling valve
D: D[4,3] = average volume-weighted diameter ($\Sigma n_i d_i 4 / \Sigma n_i d_i 3$)
E: D[3,2] = surface-weighted mean diameter ($\Sigma n_i d_i 3 / \Sigma n_i d_i 2$)
where, $n_i$ is the number of particles in a size class of diameter $d_i$
F: D(v,0.9) = the diameter below which 90% of the particles (based on volume) are found
G: Untreated/ Control Sample

**Table 4.3: Effect of CFHPT on pH and Microbial Load**[A]

| T.No.[B] | Temp.[C] (°C) | Residence Time (s) | Pressure (kPa) | pH | log CFU/ mL | |
|---|---|---|---|---|---|---|
| | | | | | APC[D] | Psychrotrophs |
| UT[E] | - | - | - | 7.08 (0.032) | 5.00 (0.142) | 3.12 (0.699) |
| 1 | No heating | 20.80 | 207 | 6.96 (0.014) | 2.82 (0.262) | 1.09 (0.552) |
| 2 | No heating | 12.48 | 207 | 6.97 (0.042) | 2.17 (0.445) | ND[F] |
| 3 | No heating | 20.80 | 276 | 6.97 (0.085) | 1.72 (0.587) | ND |
| 4 | No heating | 12.48 | 276 | 6.93 (0.007) | 2.02 (0.092) | ND |
| 5 | 121 | 20.80 | 276 | 6.85 (0.021) | 0.87 (1.230) | ND |
| 6 | 121 | 12.48 | 276 | 6.86 (0.021) | ND | ND |
| 7 | 121 | 20.80 | 207 | 6.93 (0.057) | 0.35 (0.495) | ND |
| 8 | 121 | 12.48 | 207 | 6.95 (0.035) | ND | ND |
| 9 | 145 | 20.80 | 276 | 6.90 (0.049) | 0.35 (0.495) | ND |
| 10 | 145 | 12.48 | 276 | 6.91 (0.057) | 0.35 (0.495) | ND |
| 11 | 145 | 20.80 | 207 | 6.91 (0.057) | ND | ND |
| 12 | 145 | 12.48 | 207 | 6.81 (0.007) | 0.65 (0.919) | ND |

A: The values are mean and SD (in parentheses) from 2 independent experiments
B: Treatment Number
C: Exit Temperature, measured at the exit of throttling valve
D: Aerobic Plate Count
E: Untreated/ Control Sample
F: Not Detected

**Table 4.4: Dry Solids Content Comparison[A]**

| Sample | Mean (%) | SD (%) |
|---|---|---|
| T6[B] | 8.71 | 0.045 |
| T8[C] | 8.78 | 0.170 |
| Silk | 7.03 | 0.085 |
| SoyCow | 3.77 | 0.030 |
| Vita Soy | 6.89 | 0.045 |

A: The values are from 2 independent experiments
B: 121 °C, 12.48 s, 276 MPa
C: 121 °C, 12.48 s, 207 MPa

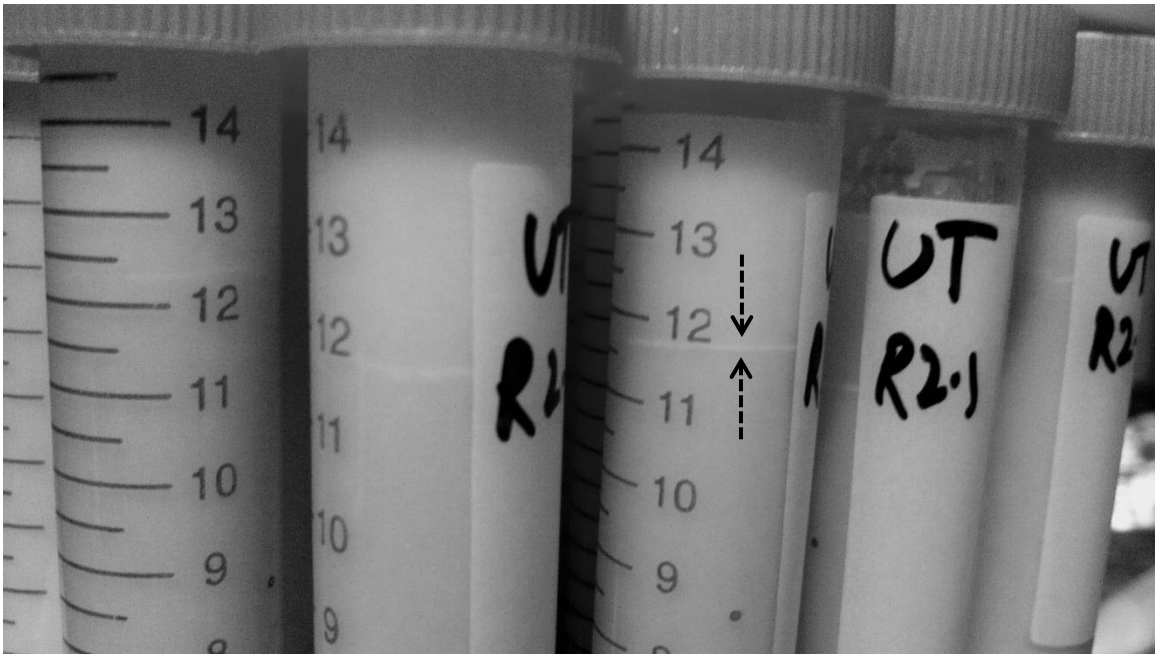


**Figure 4.1:** Visible separation (shown by the dotted arrows) of the control sample (UT) into two layers within 1 – 2 days of quiescent storage at 4 °C.

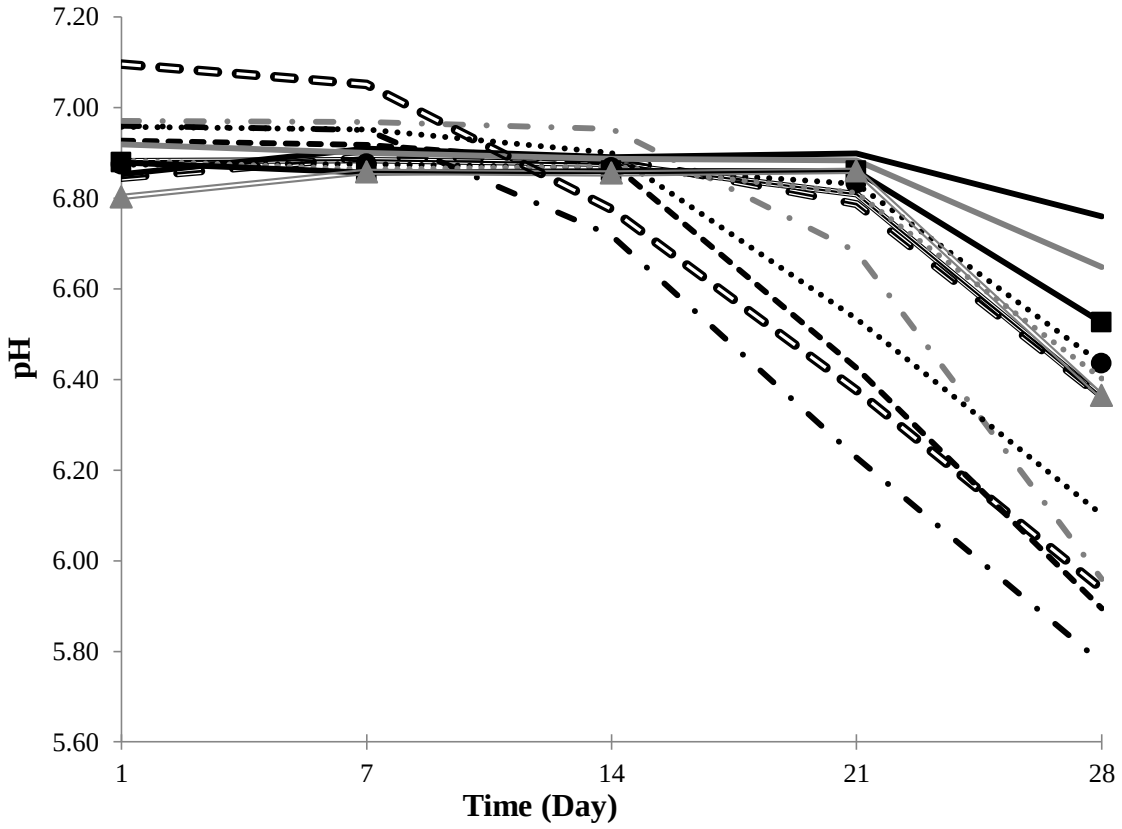


**Figure 4.2:** Change in the pH of soymilk over 4 week of storage at 4 °C. Treatments 1 – 4 received no pre-heating while treatments 5 – 12 were preheated (UT – control sample)

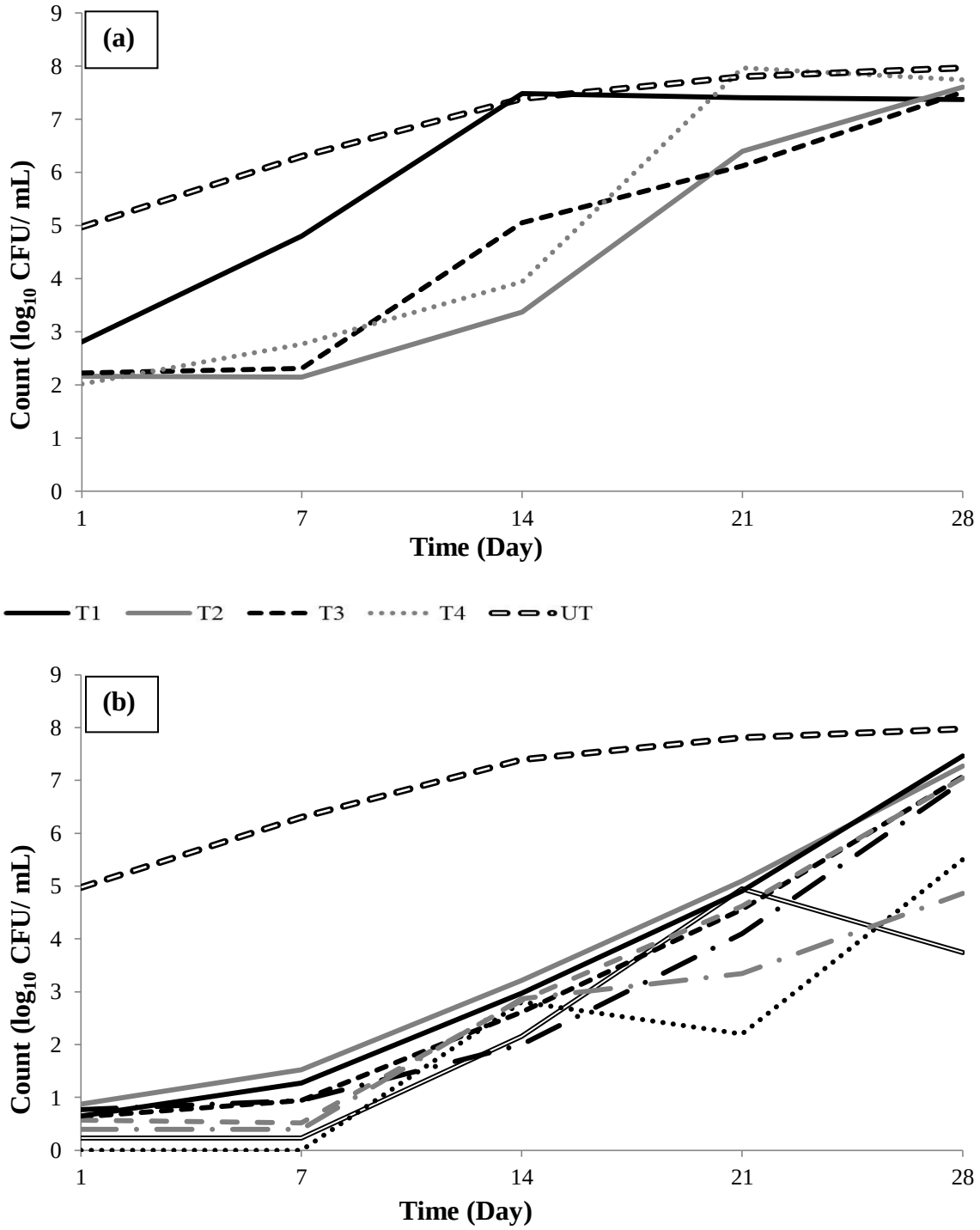


**Figure 4.3:** Changes in the aerobic plate counts over 4 weeks of storage at 30 °C in: (a) treatments receiving no pre-heating, and (b) treatments with pre-heating (UT – control sample)

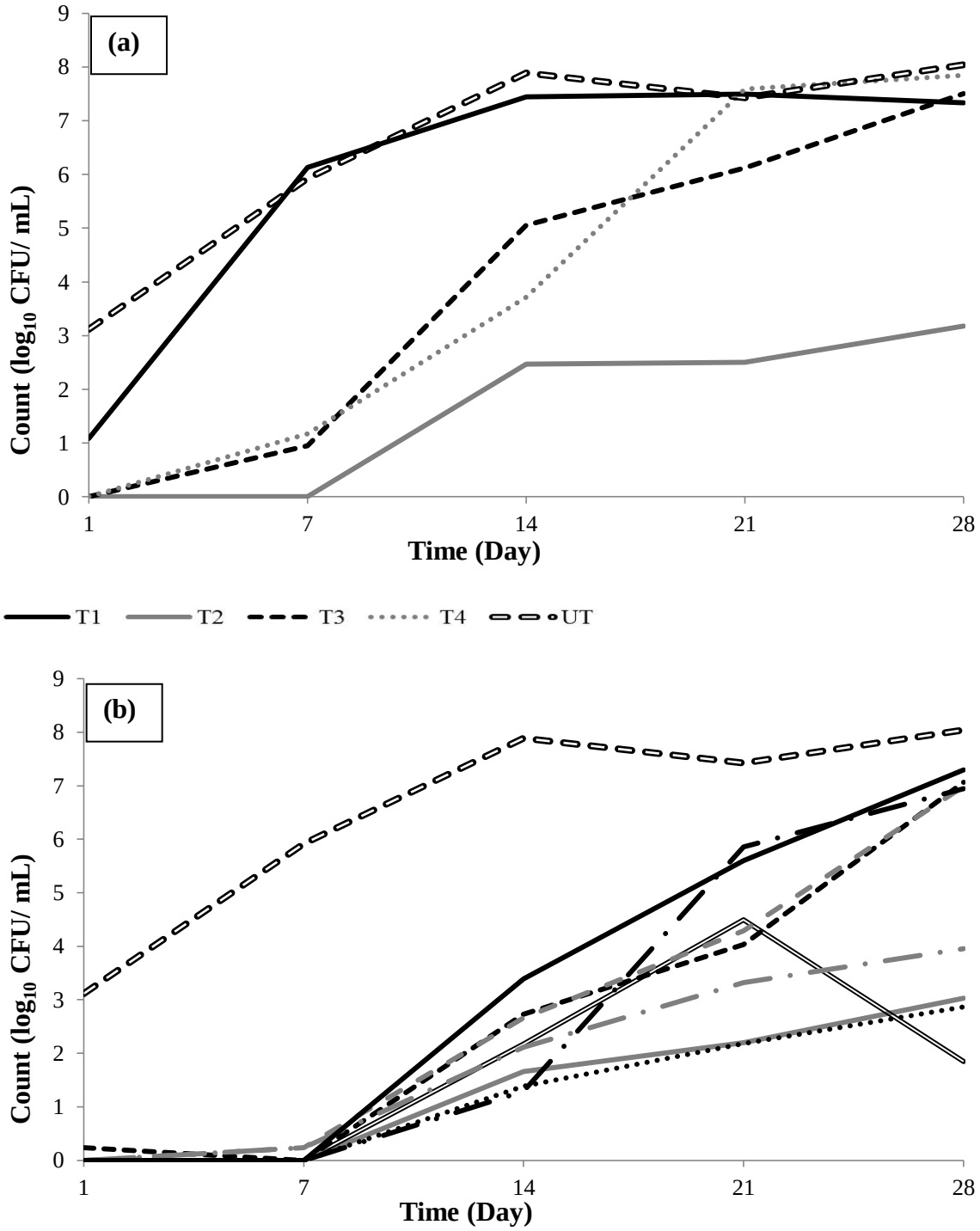


**Figure 4.4:** Changes in the psychrotrophs counts over 4 weeks of storage at 4 °C in: (a) treatments receiving no pre-heating, and (b) treatments with pre-heating (UT – control sample)

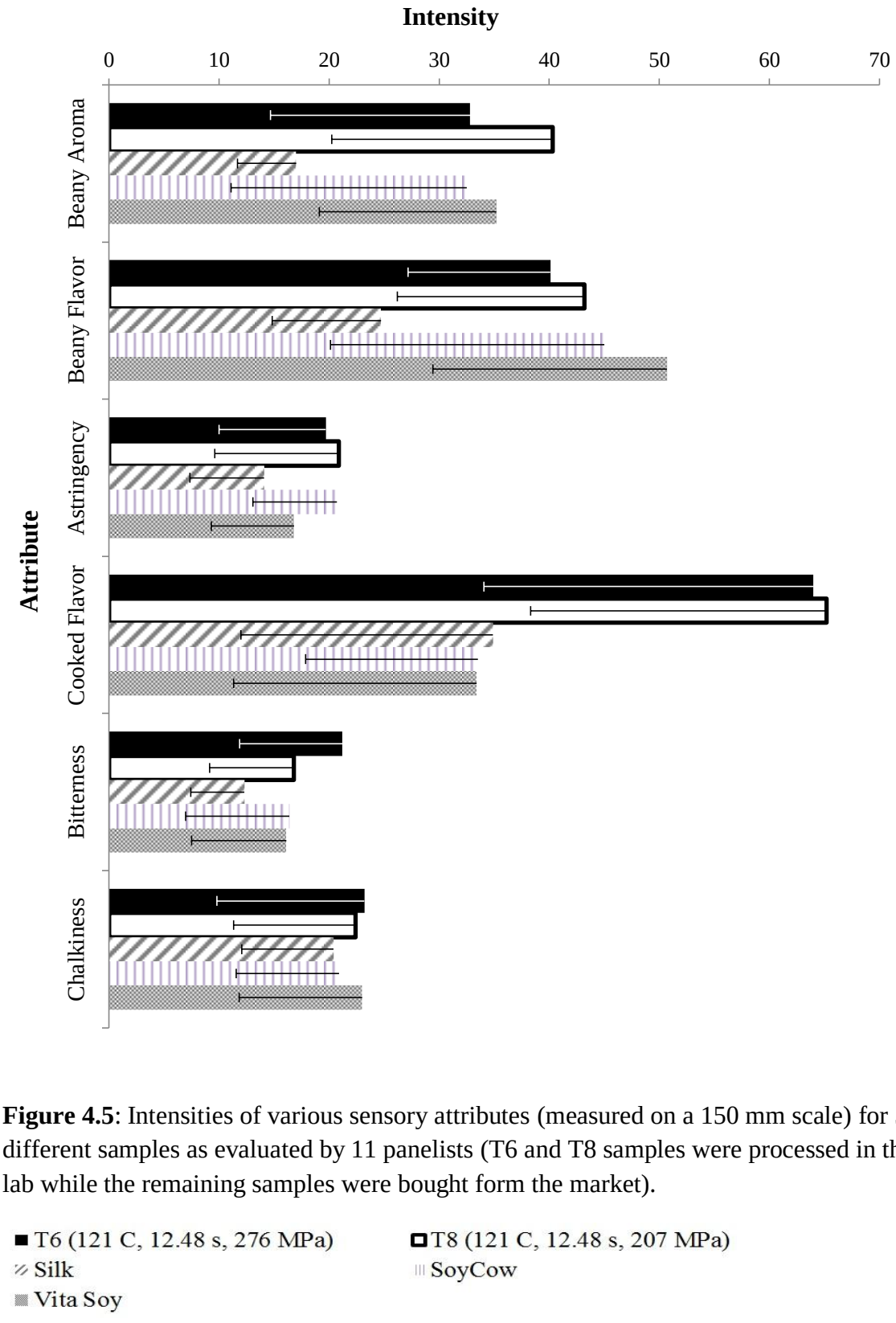


**Figure 4.5**: Intensities of various sensory attributes (measured on a 150 mm scale) for 5 different samples as evaluated by 11 panelists (T6 and T8 samples were processed in the lab while the remaining samples were bought form the market).

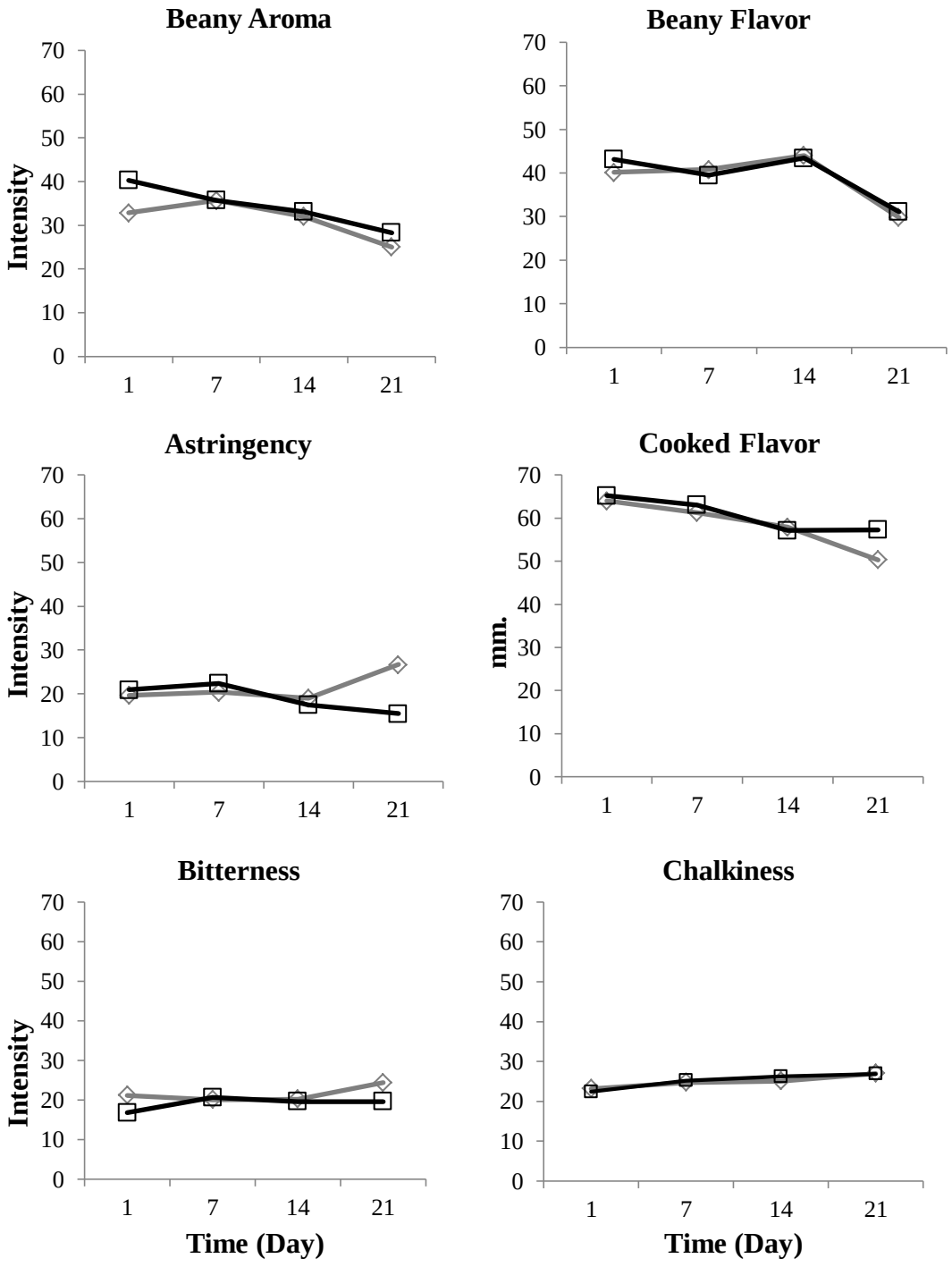


**Figure 4.6:** Change in the intensities of sensory attributes over 20 days of storage at 4 °C (T6: 121 °C, 12.48 s, 276 MPa; T8: 121 °C, 12.48 s, 207 MPa

# CHAPTER 5

# CONCLUSIONS

In the current study, we processed soymilk by continuous flow high pressure throttling (CFHPT) using two levels of pressure, three temperature levels and two holding times, thus making 12 possible combinations. We were able to use whole dehulled soybeans to process the soymilk without filtering out any okara. The effect on some of the physical, chemical, microbiological and sensory attributes was investigated. We found that all the CFHPT treated soymilk samples were stable and did not separate into visibly distinct phases even after 28 days of quiescent storage at 4 °C, whereas the control sample showed separation within 1-2 days of storage. During processing, it was observed that when soymilk was depressurized in the throttling valve, the accompanying temperature increase was significantly affected by pressure. The average temperature rise was 59.92 °C at 276 MPa and 51.48 °C at 207 MPa. This increase was primarily due to the tremendous shear, friction, turbulence and cavitation experienced by the soymilk as it throttled. No lipoxygenase activity was detected in the control sample and this was probably due the fact that blanching of dehulled soybeans at 60 °C for 2.5 h inactivated this enzyme.

CFHPT treatment significantly reduced the mean particle size of soymilk samples. Even though there was no significant difference in the average particle size of soymilk samples processed at the two pressure levels, the average diameter was generally

found to be lower at higher pressures. The particle size did not change significantly over the storage period which meant the soybean solids suspended in the soymilk did not coalesce and the beverage remained stable. Further, there was a significant decrease in the pH of the treated samples and it dropped from 7.08 for the control to 6.96 for the samples without any preheating (thus, lower exit temperatures) and falling further to 6.89 for the samples with an exit temperature of 121 °C or 145 °C. The pH level dropped significantly upon storage across all samples although the reduction was least for the samples which were preheated. CFHPT led to a reduction in the microbial counts of the soymilk samples. For the samples not preheated, we observed a 3 log reduction in the counts and when heat augmented pressure, an almost sterile product was obtained. The use of clean room/ laminar flow sample collection techniques in future studies could lead to a totally sterile product. Upon storage, the counts increased in all samples although the counts in the pre-heated samples did not reach the spoilage detection level even at the end of the storage period. In the sensory study, it was found that the soymilk samples prepared in this study had a significantly higher intensity of the beany aroma and beany flavor attributes when compared to only one of the three commercial samples. There was no significant difference in these attributes when compared to the other two commercial samples. However, the cooked flavor of our samples was significantly higher as compared to all the market samples. For the other attributes (astringency, bitterness and chalkiness) no significant difference was found between any of the samples. During storage, there was a significant reduction in the beany flavor intensity of the samples prepared in this study while the intensities of the other attributes did not significantly change.

Thus, we were able to prepare soymilk from whole dehulled soybeans without any substantial wastage of soybean solids. We processed the soymilk with continuous high pressure and minimal heating to obtain a physically stable product which remained below the microbial spoilage level for 4 weeks and with sensory characteristics which were not much different from those of some commercial soymilk samples. For the future, the nutritional characteristics as well as the consumer acceptability of CFHPT soymilk could be compared to conventionally prepared and processed soymilk. Finally, the difference in the capital and operating costs of CFHPT vs. conventional methods could be evaluated.

# REFERENCES

Achouri A, Boye JI & Zamani Y (2007) Changes in soymilk quality as a function of composition and storage. Journal of Food Quality, 30(5), 731-744.

Adapa S, Schmidt KA & Toledo R (1997) Functional properties of skim milk processed with continuous high pressure throttling. Journal of Dairy Science, 80(9), 1941-1948.

Akyol C, Alpas H & Bayındırlı (2006) Inactivation of peroxidase and lipoxygenase in carrots, green beans, and green peas by combination of high hydrostatic pressure and mild heat treatment. European Food Research and Technology, 224(2), 171-176.

Amornsin A (1999) Effect of high pressure throttling on ascorbic acid, pectin esterase activity and limonin content in citrus juice. MS Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Anema SG, Lowe EK & Stockmann R (2005) Particle size changes and casein solubilisation in high-pressure-treated skim milk. Food Hydrocolloids, 19(2), 257-267.

Areekul V (2003) High pressure sterilization of honey: physicochemical changes, sensory attributes and shelf-life. PhD Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Axelrod B, Cheesbrough TM & Laakso S (1981) Lipoxygenase from soybeans. Methods in Enzymology, 71, 441-451.

Ayazi Shamlou PA, Siddiqi SF & Titchener-Hooker NJ (1995) A physical model of high-pressure disruption of bakers' yeast cells. Chemical Engineering Science, 50(9), 1383-139l.

Bai Y, Wilson LA & Glatz BA (1998) Quality of commercial shelf-stable soymilk products. Journal of Food Protection, 67(9), 1161-1164.

Balasubramanian S & Balasubramaniam VM (2003) Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. Food Research International, 36(7), 661-668.

Balasubramaniam VM (2006) High pressure processing of foods. Ohio's Country Journal 15(4), 33-34.

Baur C, Grosch W, Wieser H & Jugel H (1977) Enzymatic oxidation of linoleic acid: formation of bitter tasting fatty acids. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 164(3), 171-176.

Baysal T & Demirdoven A (2007) Lipoxygenases in fruits and vegetables: a review. Enzyme and Microbial Technology, 40(4), 491-496.

Black EP, Setlow P, Hocking AD, Stewart CM, Kelly AL & Hoover DG (2007) Response of spores to high-pressure processing. Comprehensive Reviews in Food Science and Food Safety, 6(4), 103-119.

Bugg TDH (2003) Dioxygenase enzymes: catalytic mechanisms and chemical models. Tetrahedron. 59(36), 7075-7101.

Carrao-Panizzi MC, Beleia ADP, Prudencio-Ferreira SH, Oliveira MCN & Kitamura K (1999) Effects of isoflavones on beany flavor and astringency of soymilk. Pesquisa Agropecuária Brasileira, 34(6), 1045-1052.

Cavender GA (2011) Continuous high pressure processing of liquid foods: an analysis of physical, structural and microbial effects. PhD Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Cavender GA & Kerr WL (2011) Inactivation of vegetative cells by continuous high-pressure processing: New insights on the contribution of thermal effects and release device. Journal of Food Science, 76(7), E525-E529.

Chambers E, Jenkins A & McGuire BH (2006) Flavor properties of plain soymilk. Journal of Sensory Studies, 21(2), 165-179.

Chan W-M & Ma C-Y (1999) Modification of proteins from soymilk residue (okara) by trypsin. Journal of Food Science, 64(5),781-786.

Cheftel JC (1995) Review: High-pressure, microbial inactivation and food preservation. Food Science and Technology International 1(2-3), 75-90.

Cook EJ & Lagace AP (1985) Apparatus for forming emulsions. United States Patent No 4533254.

Corey M (2009) Developing functional food products through novel processing, ingredient, and shelf stability evaluation. PhD Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Cortes-Munoz M, Chevalier-Lucia D & Dumay E (2009) Characteristics of submicron emulsions prepared by ultra-high pressure homogenization: effect of chilled or frozen storage. Food Hydrocolloids, 23(3), 640-654.

Courregelongue S, Schlich P & Noble AC (1999) Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. Food Quality and Preference, 10(4), 273-279.

Cruz N, Capellas M, Hernandez M, Trujillo AJ, Guamis B & Ferragut V (2007) Ultra high pressure homogenization of soymilk: microbiological, physicochemical & microstructural characteristics. Food Research International, 40(6), 725-732.

Datta N, Hayes MG, Deeth HC & Kelly AL (2005) Significance of frictional heating for effects of high pressure homogenisation on milk. Journal of Dairy Research, 72(4), 393-399.

de Heij WBC, van Schepdael LJMM, Moezelaar R, Hoogland H, Master AM & van den Berg RW (2003) High-pressure sterilization: Maximizing the benefits of adiabatic heating. Food Technology, 57(3), 37-41.

Dickinson E & Stainsby G (ed) (1988) Advances in Food Emulsions and Foams. Elsevier Applied Science Publishers, London, UK.

Diels AMJ & Michiels CW (2006) High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Critical Reviews in Microbiology, 33(4), 201-216.

Donsi F, Ferrari G, Lenza E & Maresca P (2009) Main factors regulating microbial inactivation by high-pressure homogenization. Operating parameters and scale of operation. Chemical Engineering Science, 64(3), 520-532.

Draheim JE, Carroll RT, McNemar TB, Dunham WR, Sands RH & Funk MO (1989) Lipoxygenase isoenzymes: a spectroscopic and structural characterization of soybean seed enzymes. Archives of Biochemistry and Biophysics, 269(1), 208-218.

Dumay E, Chevalier-Lucia D, Picart-Palmade L, Benzaria A, Gracia-Julia A & Blayo C (2012) Technological aspects and potential applications of (ultra) high-pressure homogenization. Trends in Food Science & Technology, 31(1), 13-26.

Engler CR & Robinson CW (1981) Disruption of *Candida utilis* cells in high pressure flow devices. Biotechnology and Bioengineering, 23(4), 765-780.

Erdman Jr. JW & Fordyce EJ (1989) Soy products and the human diet. American Journal of Clinical Nutrition, 49(5), 725-737.

Farkas DF & Hoover DG (2000) High pressure processing. Journal of Food Science, 65(s8), 47-64.

FDA (1999) Health claims: Soy protein and risk of coronary heart disease (CHD). U.S. Food and Drug Administration, Silver Spring, MD, USA. Available at: http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=ec798ebc9e95c7e3a4552b798c0948aa&r=SECTION&n=21y2.0.1.1.2.5.1.13. Accessed 02 January 2013.

Floury J, Desrumaux A & Lardieres J (2000) Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Innovative Food Science & Emerging Technologies, 1(2), 127-134.

Floury J, Desrumaux A & Legrand J (2002) Effect of ultra-high-pressure homogenization on structure and on rheological properties of soy protein-stabilized emulsions. Journal of Food Science, 67(9), 3388-3395.

Floury J, Bellettre J, Legrand J & Desrumaux A (2004a) Analysis of a new type of high pressure homogeniser. A study of the flow pattern. Chemical Engineering Science, 59(4), 843-853.

Floury J, Legrand J & Desrumaux A (2004b) Analysis of a new type of high pressure homogeniser. Part B. study of droplet break-up and recoalescence phenomena. Chemical Engineering Science, 59(6), 1285-1294.

Gaffney BJ (1996) Lipoxygenases: structural principles and spectroscopy. Annual Review of Biophysics and Biomolecular Structure, 25(1), 431-459.

Galazka VB, Dickinson E & Ledward DA (1996) Effect of high pressure on the emulsifying behaviour of β-lactoglobulin. Food Hydrocolloids, 10(2), 213-219.

Galazka VB, Dickinson E & Ledward DA (2000) Influence of high pressure processing on protein solutions and emulsions. Current Opinion in Colloid & Interface Science, 5(3), 182-187.

Gaucheron F, Famelart MH, Mariette F, Raulot K, Michela F & Le Graeta Y (1997) Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. Food Chemistry, 59 (3), 439-447.

Geciova J, Bury D & Jelen P (2002) Methods for disruption of microbial cells for potential use in the dairy industry-a review. International Dairy Journal, 12(6), 541-553.

Gibson L & Benson G (2005) Origin, history, and uses of soybean (*Glycine max*). Department of Agronomy, Iowa State University, Iowa, USA. Available at: http://agron-

www.agron.iastate.edu/Courses/agron212/Readings/Soy_history.htm. Accessed 02 January 2013.

Gogate PR (2011) Hydrodynamic cavitation for food and water processing. Food and Bioprocess Technology, 4(6), 996-1011.

Guamis B, Lopez-Pedemonte T, Dumay E & Butz P (2003) Transfer of ultra high pressure homogenisation technology to food and cosmetic enterprises. Department of Food Science and Technology, Universitat Autonoma de Barcelona, Barcelona, Spain. Available at: http://www.cerpta.com/premsa/revistes%202010/projects.pdf. Accessed 02 January 2013.

Guerrero-Beltran JA, Estrada-Giron Y, Swanson BG & Barbosa-Canovas GV (2009a) Inactivation kinetics of lipoxygenase in pressurized raw soymilk and soymilk from high-pressure treated soybeans. Journal of Food Processing and Preservation, 33(2), 143-158.

Guerrero-Beltran JA, Estrada-Giron Y, Swanson BG & Barbosa-Canovas GV (2009b) Pressure and temperature combination for inactivation of soymilk trypsin inhibitors. Food Chemistry, 116(3), 676-679.

Guerzoni ME, Vannini L, Lopez CC, Lanciotti R, Suzzi G & Gianotti A (1999) Effect of high pressure homogenization on microbial and chemico-physical characteristics of goat cheeses. Journal of Dairy Science, 82(5), 851-62.

Guiavarc'h Y, Segovia O, Hendrickx M & Van Loey A (2005) Purification, characterization, thermal and high-pressure inactivation of a pectin methylesterase from white grapefruit (Citrus paradisi). Innovative Food Science & Emerging Technologies, 6 (4), 363-371.

Ha EYW, Morr CV & Seo A (1992) Isoflavone aglucones and volatile organic compounds in soybeans; effects of soaking treatments. Journal of Food Science, 57(2), 414-417.

Hand DB, Steinkraus KH, Van Euren JP, Hackler LR, El Rawi I, & Pallesen HR (1964) Pilot-plant studies on soymilk. Food Technology, 18(8), 139-142.

Hayes MG, Fox PF & Kelly AL (2005) Potential applications of high pressure homogenisation in processing of liquid milk. Journal of Dairy Research, 72(1), 25-33.

Hayes MG & Kelly AL (2003) High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. Journal of Dairy Research, 70(3), 279-305.

Hildebrand DF (1989) Lipoxygenases. Physiologia plantarum, 76(2), 249-253.

Hite BH (1899) The effect of pressure in the preservation of milk. West Virginia University Agricultural Experimental Station Bulletin, 58, 15-35.

Hite BH, Giddings NJ & Weakley CE (1914) The effect of pressure on certain micro-organisms encountered in the preservation of fruits and vegetables. West Virginia University Agricultural Experimental Station Bulletin, 146, 1-67.

Hoogland H, de Heij W & van Schepdael L (2001) High pressure sterilisation: novel technology, new products, new opportunities. New Food, 3, 21-26.

Horiba Scientific (2012) A Guidebook to Particle Size Analysis. Horiba Instruments, Inc., Irvine, CA, USA.

Howell RW and Collins FI (1957) Factors affecting linolenic and linoleic acid content of soybean oil. Agronomy Journal, 49(11),593-597.

Huang M & Gramoll K (2013) Thermodynamics – theory. OU Engineering Media Lab eCourses, The University of Oklahoma, Norman, OK, USA. Available at: http://www.ecourses.ou.edu/home.htm. Accessed 02 January 2013.

Hugas M, Garriga M & Monfort JM (2002) New mild technologies in meat processing: high pressure as a model technology. Meat Science, 62(3), 359-371.

Hymowitz T & Harlan JR (1983) Introduction of Soybeans to North America by Samuel Bowen in 1765. Economic Botany, 37(4), 371-379.

Iassonova DR, Johnson LA, Hammond EG & Beattie SE (2009) Evidence of an enzymatic source of off flavors in "lipoxygenase-null" soybeans. Journal of the American Oil Chemists' Society, 86(1), 59-64.

Indrawati, Van Loey AM, Ludikhuyze LR & Hendrickx ME (2000) Kinetics of pressure inactivation at subzero and elevated temperature of lipoxygenase in crude green bean. Biotechnology Progress, 16(1), 109-115.

Indrawati, Van Loey AM, Ludikhuyze LR & Hendrickx ME (2001) Pressure-temperature inactivation of lipoxygenase in green peas (*Pisum sativum)*: a kinetic study. Journal of Food Science, 66(5), 686-693.

Ireland PA, Dziedzic SZ & Kearlsey MW (1986) Saponin content of soya and some commercial soya products by means of high-performance liquid chromatography of the sapogenins. Journal of Science of Food and Agriculture, 37(7), 694-698.

Iwuoha CI & Umunnakwe KE (1997) Chemical, physical and sensory characteristics of soymilk as affected by processing method, temperature and duration of storage. Food Chemistry, 59(3), 373-379.

Johnson LA, Deyoe CW & Hoover WJ (1981) Yield and quality of soymilk processed by steam-infusion cooking. Journal of Food Science, 46(1), 239-248.

Kadam PS, Jadhav BA, Salve RV & Machewad GM (2012) Review on the high pressure technology (HPT) for food preservation. Journal of Food Processing and Technology 3(1), 135.

Kaneko S, Kumazawa K & Nishimura O (2011) Studies on the key aroma compounds in soy milk made from three different soybean cultivars. Journal of Agricultural and Food Chemistry, 59(22), 12204-12209.

Kobayashi A, Tsuda Y, Hirata N, Kubota K & Kitamura K (1995) Aroma constituents of soybean [*Glycine max* (L.) Merrill] milk lacking lipoxygenase isozymes. Journal of Agricultural and Food Chemistry, 43(9), 2449-2452.

Kon S, Wagner JR, Guadagni DG & Horvat RJ (1970) Ph adjustment control of oxidative off-flavors during grinding of raw legume seeds. Journal of Food Science, 35(4), 343-345.

Kumar V, Rani A, Tindwani C & Jain M (2003) Lipoxygenase isozymes and trypsin inhibitor activities in soybean as influenced by growing location. Food Chemistry, 83(1), 79-83.

Kuntz DA, Nelson AI, Steinberg MP & Wei LS (1978) Control of chalkiness in soymilk. Journal of Food Science, 43 (4), 1279-1283.

Kwok K-C, Basker D & Niranjan K (2000) Kinetics of sensory quality changes in soymilk during thermal processing, by parametric and non-parametric data analyses. Journal of the Science of Food and Agriculture, 80(5), 595-600.

Kwok K-C, Liang H-H & Niranjan K (2002) Optimizing conditions for thermal processes of soy milk. Journal of Agricultural and Food Chemistry, 50(17), 4834-4838.

Kwok KC & Niranjan K (1995) Review: Effect of thermal processing on soymilk. International Journal of Food Science and Technology, 30(3), 263-295.

Lakshmanan R, De Lamballerie M & Jung S (2006) Effect of soybean-to-water ratio and ph on pressurized soymilk properties. Journal of Food Science, 71(9), E384-E391.

Lander R, Manger W, Scouloudis M, Ku A, Davis C & Lee A (2000) Gaulin homogenization: a mechanistic study. Biotechnology Progress, 16(1), 80-85.

Laneuville SI, Paquin P & Turgeon SL (2000) Effect of preparation conditions on the characteristics of whey protein--xanthan gum complexes. Food Hydrocolloids, 14(4), 305-314.

Li R (2006) Soy products off-flavor generating, masking and flavor creating. In: Riaz (ed) Soy Applications in Food, pp 227-248. CRC Press, Boca Raton, FL, USA.

Li Y-Q, Chen Q, Liu X-U & Chen Z-X (2008) Inactivation of soybean lipoxygenase in soymilk by pulsed electric fields. Food Chemistry, 109(2), 408-414.

Li Y-Q, Tian W-L, Mo H-Z, Zhang Y-L & Zhao X-Z (2012) Effects of pulsed electric field processing on quality characteristics and microbial inactivation of soymilk. Food and Bioprocess Technology, (May), 1-10.

Liener IE (1986) Trypsin inhibitors: concern for human nutrition or not. The Journal of Nutrition, 116(5), 920-923.

Liu K (1997a) Nonfermented oriental soy foods. In: Liu (ed) Soybeans: Chemistry, Technology, and Utilization, pp 135-217. Chapman and Hall, New York, USA.

Liu K (1997b) Soybeans: Chemistry, Technology and Utilization (1st ed). Chapman and Hall, New York, USA.

Lo WY-L, Steinkraus KH, Hand DB, Hackler LR & Wilkens WF (1968) Soaking soybeans before extraction as it affects composition & yield of soymilk. Food Technology, 33(9), 1188-1190.

Lozano PR, Drake M, Benitex D & Cadwallader KR (2007) Instrumental and sensory characterization of heat-induced odorants in aseptically packaged soy milk. Journal of Agricultural and Food Chemistry, 55(8), 3018-3026.

Ludikhuyze LR, Indrawati, Van den Broeck I, Weemas CA & Hendrickx ME (1998a) High pressure and thermal denaturation kinetics of soybean lipoxygenase: a study based on gel electrophoresis. LWT-Food Science and Technology, 31(7), 680-686.

Ludikhuyze LR, Indrawati, Van den Broeck I, Weemas CA & Hendrickx ME (1998b) Effect of combined pressure and temperature on soybean lipoxygenase. 1. influence of extrinsic and intrinsic factors on isobaric-isothermal inactivation kinetics. Journal of Agricultural and Food Chemistry, 46(10), 4074-4080.

Luning PA, Carey AT, Roozen JP & Wichers HJ (1995) Characterization and occurrence of lipoxygenase in bell peppers at different ripening stages in relation to the formation of volatile flavor compounds. Journal of Agricultural and Food Chemistry, 43(6), 1493-1500.

Lv Y-C, Song H-L, Li X, Wu L & Guo S-T (2011) Influence of blanching and grinding process with hot water on beany and non-beany flavor in soymilk. Journal of Food Science, 76(1), S20-S25.

Malaki Nik AM, Tosh SM, Poysa V, Woodrow L & Corredig M (2008) Physicochemical characterization of soymilk after step-wise centrifugation. Food Research International, 41(3), 286-294.

Malaki Nik AM, Tosh SM, Woodrow L, Poysa V & Corredig M (2009) Effect of soy protein subunit composition and processing conditions on stability and particle size distribution of soymilk. LWT - Food Science and Technology, 42(7), 1245-1252.

Malvern Instruments (2012) A Basic Guide to Particle Characterization. MRK1806-01, Malvern Instruments Limited, Worcestershire, UK.

Matser AM, Krebbers B, van den Berg RW & Bartels PV (2004) Advantages of high pressure sterilisation on quality of food products. Trends in Food Science & Technology, 15(2), 79-85.

Matsuura M, Obata A & Fukushima D (1989) Objectionable flavor of soy milk developed during the soaking of soybeans and its control. Journal of Food Science, 54(3), 602-605.

McCrae C H (1994) Homogenization of milk emulsions: use of microfluidizer. International Journal of Dairy Technology 47(1), 28-31.

Messina MJ (1997) Soy food: their role in disease prevention and treatment. In: Keshun (ed), Soybean: Chemistry, Technology and Utilization, pp 443–447. Chapman and Hall Press, New York, USA.

Meyer RS (2000) Ultra high pressure, high temperature food preservation process. United States Patent No 6017572.

Meyer RS, Cooper KL, Knorr D & Lelieveld HLM (2000) High-pressure sterilization of foods. Food Technology, 54(11), 67-72.

Miles CP (1966) Process of producing milk from soy beans. United States Patent No 3288614.

Min S, Yu Y, Yoo S & Martin S St. (2005) Effect of soybean varieties and growing locations on the flavor of soymilk. Journal of Food Science, 70(1), C1-C11.

Minerich PL & Labuza TP (2003) Development of a pressure indicator for high hydrostatic pressure processing of foods. Innovative Food Science and Emerging Technologies, 4(3), 235-243.

Mizutani T & Hashimoto H (2004) Effect of grinding temperature on hydroperoxide & off-flavor contents during soymilk manufacturing process. Journal of Food Science, 69(3), SNQ112–SNQ116.

Moorman JE (1997) Microbicidal and rheological effects of high-pressure throttling (HPT). MS Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Morr C V & Ha EYW (1991) Processing to prevent formation of off-flavors in soy products. Comments on Agricultural and Food Chemistry, 2, 247-260.

Mustakas GC, Albrecht WJ & Bookwalter GN. (1972) Production of vegetable protein beverage base. United States Patent No 3639129.

Nelson AI, Steinberg MP & Wei LS (1976) Illinois process for preparation of soymilk. Journal of Food Science, 41(1), 57-61.

Nelson AI, Steinberg MP, Wei LS (1978) Whole Soybean Foods for Home and Village Use, INTSOY Series (no. 14). University of Illinois at Urbana-Champaign, Urbana, IL, USA.

N'Kouka KD, Klein BP & Lee S-Y (2004) Developing a lexicon for descriptive analysis of soymilks. Journal of Food Science, 69(7), S259-263.

North Carolina Soybean Producers Association (2013) The history of soybeans. North Carolina Soybean Producers Association Inc., Raleigh, NC, USA. Available at: http://www.ncsoy.org/ABOUT-SOYBEANS/History-of-Soybeans.aspx. Accessed 02 January 2013.

Norton T & Sun D-W (2008) Recent advances in the use of high pressure as an effective processing technique in the food industry. Food Bioprocess Technology, 1(1), 2-34.

Okubo K, Ijima M, Kobayashi Y, Yoshikoshi M, Uchida T, Kudou S (1992) Components responsible for the undesirable taste of soybean seeds. Bioscience Biotechnology, and Biochemistry, 56(1), 99-103.

Okubo K, Kobayashi Y & Tkahashi K (1983) Improvement of soymilk and tofu process on the behavior of undesirable taste component such as glucosides. Up-to-Date Food Processing (Japan), 18, 16-21.

Ortega-Rivas E (2007) Processing effects for safety and quality in some non-predominant food technologies. Critical Reviews in Food Science and Nutrition, 47(2), 161–173.

Pathomrungsiyounggul P, Grandison AS & Lewis MJ (2010) Effect of calcium carbonate, calcium citrate, tricalcium phosphate, calcium gluconate and calcium

lactate on some physicochemical properties of soymilk. International Journal of Food Science and Technology, 45(11), 2234-2240.

Patterson MF (2005) Microbiology of pressure-treated foods. Journal of Applied Microbiology, 98(6), 1400-1409.

Peck D (2004) The effects of high-pressure thottling versus thermal pasteurization on a blueberry-whey beverage. MS Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Pereda J, Ferragut V, Quevedo JM, Guamis B & Trujillo AJ (2007) Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. Journal of Dairy Science, 90(3), 1081-1093.

Poliseli-Scopel FH, Hernandez-Herrero M, Guamis B & Ferragut V (2012) Comparison of ultra high pressure homogenization and conventional thermal treatments on the microbiological, physical and chemical quality of soymilk. LWT – Food Science and Technology, 46(1), 42-48.

Popper L & Knorr D (1990) Applications of high-pressure homogenization for food preservation. Food Technology, 44(7), 84-89.

Poysa V & Woodrow L (2002) Stability of soybean seed composition & its effect on soymilk & tofu yield & quality. Food Research International, 35(4), 337-345.

Prawiradjaja S (2003) Process optimization for solid extraction, flavor improvement and fat removal in the production of soymilk from full fat soy flakes. MS Thesis. Department of Food Science and Technology, Iowa State University, Ames, Iowa, USA.

Rackis J J & Gumbmann M R (1981) Protease inhibitors: physiological properties and nutritional significance. In: Ory (ed) Antinutrients and Natural Toxicants in Foods, pp 203-237. Food and Nutrition Press, Westport, CT, USA.

Rackis JJ, Wolf WJ & Baker EC (1986) Protease inhibitors in plant foods. content and inactivation. In: Friedman (ed) Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods, pp 299-347. Plenum Publishing Corporation, New York, USA.

Rasanayagam V, Balasubramaniam VM, Ting E, Sizer CE, Bush C & Anderson C (2003) Compression heating of selected fatty food materials during high-pressure processing. Journal of Food Science, 68(1), 254-259.

Rastogi NK, Raghavarao KSMS, Balasubramaniam VM, Niranjan K & Knorr D (2007) Opportunities and challenges in high pressure processing of foods. Critical Reviews in Food Science and Nutrition, 47(1), 69-112.

Rawle A (2011) Basic Principles of Particle Size Analysis. PR2211, Malvern Instruments Limited, Worcestershire, UK.

Riener J, Noci F, Cronin DA, Morgan DJ & Lyng JG (2008) Combined effect of temperature and pulsed electric fields on soya milk lipoxygenase inactivation. European Food Research and Technology, 227(5), 1461-1465.

Robinson DS, Wu Z, Domoney C & Casey R (1995) Lipoxygenases and the quality of foods. Food Chemistry, 54(1), 33-43.

Rodrigo D, Jolie R, Van Loey A & Hendrickx M (2007) Thermal and high pressure stability of tomato lipoxygenase and hydroperoxide lyase. Journal of Food Engineering, 79(2), 423-429.

Rodrigo D, Sampedro F, Silva A, Palop A & Martinez A (2010) New food processing technologies as a paradigm of safety and quality. British Food Journal, 112(5), 467-475.

Roesch R (2002) Characterization of oil-in-water emulsions prepared with soy protein concentrate by high pressure homogenization. MS Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Roesch RR & Corredig M (2003) Texture and microstructure of emulsions prepared with soy protein concentrate by high-pressure homogenization. LWT-Food Science and Technology, 36(1), 113-124.

Rosenthal A, Deliza R, Cabral LMC, Cabral LC, Farias CAA & Dominugues AM (2003) Effect of enzymatic treatment and filtration on sensory characteristics and physical stability of soymilk. Food Control, 14(3), 187-192.

Rouhana A, Adler-Nissen J, Cogan U & Frokiaer H (1996) Heat inactivation kinetics of trypsin inhibitors during high temperature- short time processing of soymilk. Journal of Food Science, 61(2), 265-269.

San Martin MF, Barbosa-Canovas GV & Swanson BG (2002) Food processing by high hydrostatic pressure. Critical Reviews in Food Science and Nutrition, 42(6), 627–645.

Savitry A & Prakash M (2004) Sing a song of joy. Prepared Foods, 4, 25-25.

Sharma V (2008) Microbial inactivation kinetics in soymilk during continuous flow high pressure throttling system. MS Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Sharma V, Singh RK & Toledo RT (2009) Microbial inactivation kinetics in soymilk during continuous flow high-pressure throttling. Journal of Food Science, 74(6), M268-M275.

Shirgaonkar IZ, Lothe RR & Pandit AB (1998) Comments on the mechanism of microbial cell disruption in high-pressure and high-speed devices. Biotechnology Progress, 14(4), 657-660.

Shook CM, Shellhammer TH & Schwartz SJ (2001) Polygalacturonase, pectinesterase, and lipoxygenase activities in high-pressure-processed diced tomatoes. Journal of Agricultural and Food Chemistry, 49(2), 664-668.

Sivanandan L (2007) Characterization of soymilk produced by continuous flow high pressure throttling process. PhD Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA, USA.

Sivanandan L, Toledo RT & Singh RK (2008) Effect of continuous flow high-pressure throttling on rheological and ultrastructural properties of soymilk. Journal of Food Science, 73(6), E288-E296.

Sivanandan L, Toledo RT, Singh RK (2010) Rheological and ultrastructural properties and particle size distribution of soymilk as affected by processing methods. International Journal of Food Properties, 13(3), 580-598.

Sizer CE, Balasubramaniam VM & Ting E (2002) Validating high-pressure processes for low-acid foods. Food Technology, 56(2), 36-42.

Smelt JPPM (1998) Recent advances in the microbiology of high pressure processing. Trends in Food Science & Technology, 9(4), 152-158.

Smiddy MA, Martin J-E, Huppertz T & Kelly AL (2007) Microbial shelf-life of high-pressure-homogenised milk. International Dairy Journal, 17(1), 29-32.

Smith AK & Circle JJ (1972) Soybeans: Chemistry and Technology. AVI Publishing Company, Inc., Westport, Connecticut, USA.

Smith C, Van Megan W, Twaalfhoven L & Hitchcock C (1980) The determination of trypsin inhibitor levels in foodstuffs. Journal of the Science of Food and Agriculture, 31(4), 341-350.

Smith K, Mendonca A & Jung S (2009) Impact of high-pressure processing on microbial shelf-life and protein stability of refrigerated soymilk. Food Microbiology, 26(8), 794-800.

Soyfoods Association of North America (2013) Soy information: Sales and Trends. Soyfoods Association of North America, Washington DC, USA. Available at:

http://www.soyfoods.org/soy-information/sales-and-trends. Accessed 02 January 2013.

SPX (2008) APV homogenizers: Rannie and Gaulin. 3000-02-08-2008-US, SPX Corporation, Charlotte, NC, USA.

Stang M, Schuchmann H & Helmar Schubert (2001) Emulsification in high-pressure homogenizers. Engineering in Life Sciences, 1(4), 151-157.

Tewari G (2007) High-pressure processing of foods. In: Tewari & Juneja (ed) Advances in Thermal and Non-Thermal Food Preservation, pp 203-239. Blackwell Publishing Professional, Ames, Iowa, USA.

Thiebaud M, Dumay E, Picart L, Guiraud JP & Cheftel JC (2003) High-pressure homogenisation of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. International Dairy Journal, 13(6), 427-439.

ThinkQuest (2013) Protein digestion: a trip through the gut. Oracle Education Foundation, Redwood Shores, CA, USA. Available at: http://library.thinkquest.org/11226/main/c14txt.htm. Accessed 17 January 2013.

Toledo RT & Moorman JE (2000) Microbial inactivation by high-pressure throttling. United States Patent No 6120732.

Torres-Penaranda AV & Reitmeier CA (2001) Sensory descriptive analysis of soymilk. Journal of Food Science, 66(2), 352-356.

Torres-Penaranda AV, Reitmeier CA, Wilson LA, Fehr WR & Narvel JM (1998) Sensory characteristics of soymilk and tofu made from lipoxygenase-free and normal soybeans. Journal of Food Science, 63(6), 1084-1087.

Torrezan R, Tham WP, Bell AE, Frazier RA & Cristianini M (2007) Effects of high pressure on functional properties of soy protein. Food Chemistry, 104(1), 140-147.

Trujillo AJ, Capellas M, Saldo J, Gervilla R & Guamis B (2002) Applications of high-hydrostatic pressure on milk and dairy products: a review. Innovative Food Science and Emerging Technologies, 3(4), 295-307.

University of Kentucky (2009) Specialty soybeans. College of Agriculture Extension Service, University of Kentucky, Kentucky, USA. Available at: http://agron-www.agron.iastate.edu/Courses/agron212/Readings/Soy_history.htm. Accessed: Jan 04, 2013.

USDA (2013) Soybean (mature seeds, raw) Composition. USDA National Nutrient Database for Standard Reference, Washington DC, USA. Available at: http://ndb.nal.usda.gov. Accessed 04 June 2013.

van der Ven C, Master AM & van den Berg RW (2005) Inactivation of soybean trypsin inhibitors and lipoxygenase by high-pressure processing. Journal of Agricultural and Food Chemistry, 53(4), 1087-1092.

Wallace GM, Bannatyne WR & Khaleque A (1971) Studies on the processing and properties of soymilk II.– effect of processing conditions on the trypsin inhibitor activity and the digestibility *in vitro* of proteins in various soymilk preparations. Journal of the Science of Food and Agriculture, 22(10), 526-531.

Walstra P, Geurts TJ, Noomen A, Jellema A & Van Boekel MAJS (1999) Composition, structure, and properties. In: Walstra, Geurts, Noomen, Jellema and van Boekel (ed) Principles of Milk Properties and Processes, pp 3–26. Marcel Dekker AG, Inc., Basel, Switzerland.

Wang B, Xiong YL & Wang C ( 2001) Physicochemical And Sensory Characteristics Of Flavored Soymilk During Refrigeration Storage. Journal of Food Quality, 24(6), 513-526.

Wang R, Zhou X & Chen Z (2008) High pressure inactivation of lipoxygenase in soy milk and crude soybean extract. Food Chemistry, 106(2), 603-611.

Welti-Chanes J, Lopez-Malo A, Palou E, Bermudez D, Guerrero-Beltran JA & Barbosa-Canovas GV (2005) Fundamentals and applications of high pressure processing to foods. In: Barbosa-Canovas, Tapia Cano (ed) Novel Food Processing Technologies, pp 157–181. CRC Press LLC, Boca Raton, FL, USA.

Wicker L, Toledo RT, Moorman J & Pereyra R (2000) High pressure throttling of acidified pectin-casein dispersions from 310-MPa to atmospheric pressure. Milchwissenschaft, 55(1), 10-13.

Wilkens WF, Mattick LR & Hand DB (1967) Effect of processing method on oxidative off-flavors of soybean milk. Food Technology, 21(12), 86-89.

Wilson KA (1988) The proteolysis of trypsin inhibitors in legume seeds. CRC Critical Reviews in Biotechnology, 8(3), 197-216.

Wilson LA (1995) Soy foods. In: Erickson (ed) Practical Handbook of Soybean Processing and Utilization, pp 428-459. AOCS Press, Champaign, Illinois, USA.

Wilson MJ & Baker R (2000) High temperature/ultra-high pressure sterilization of foods. United States Patent No 6086936.

Xu B & Chang SKC (2009) Isoflavones, flavon-3-ols, phenolic acids, total phenolic profiles and antioxidant capacities of soy milk as affected by ultrahigh-temperature and traditional processing methods. Journal of Agricultural and Food Chemistry, 57(11), 4706-4717.

Yaldagard M, Mortazavi SA & Tabatabaie F (2008) The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. African Journal of Biotechnology, 7(16), 2739-2767.

Yuan S, Chang SKC, Liu Z & Xu B (2008) Elimination of trypsin inhibitor activity & beany flavor in soy milk by consecutive blanching & ultrahigh-temperature (UHT) processing. Journal of Agricultural and Food Chemistry, 56(17), 7957-7963.

Zhang H, Li L, Tatsumi E & Isobe S (2005) High-pressure treatment effects on proteins in soy milk. LWT-Food Science and Technology, 38(1), 7-14.

# APPENDIX A

## LIST OF ABBREVIATIONS

APC – Aerobic Plate Count

CFHPT – Continuous Flow High Pressure Throttling

CHP – Continuous High Pressure

CFU – Colony Forming Units

CFU/ mL – Colony Forming Units per mL

DW – Deionized Water

HDPE – High Density Polyethylene

HHP – High Hydrostatic Pressure

HPH – High Pressure Homogenization

HPP – High Pressure Processing

HPS – High Pressure Sterilization

HTST – High Temperature Short Time

LOX – Lipoxygenase

PEF – Pulsed Electric Field

PSD – Particle Size Distribution

PUFA – Poly Unsaturated Fatty Acid

RH – Relative Humidity

RI – Refractive Index

RPM – Rotations per Minute

SS – Stainless Steel

TI – Trypsin Inhibitor

TSA – Tryptic Soy Agar

UHPH – Ultra High Pressure Homogenization

UHT – Ultra High Temperature

# APPENDIX B

## SYMBOLS/ UNITS

° – degree

ρ – density

μm – micro meter

A - ampere

C – Celcius

$C_p$ – specific heat

g/L – gram per liter

MPa – mega Pascal

nm – nanometers