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Novel technologies for soy products, processing, and applications

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Novel technologies for soy products, processing, and applications

by

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Soybeans are an important source of protein and oil. On average, over one-third of the soybean mass is protein and about 20% is oil. One of the most common types of soy product for food applications sold in the Western marketplace is soymilk. Soymilk is the pasteurized extract of soaked ground soybeans, and soy protein isolates are purified fractions containing >90% protein (db). Soy protein isolate is extensively used as a food ingredient in many fabricated foods such as comminuted meat products. The recent increase in soy protein products consumption is the result of advances in achieving improved taste and recognition of health benefits. The U.S. Food and Drug Administration approved a health claim that soy protein positively impacts cardiovascular health in humans.

Novel technologies for producing and processing soy products are of great interest to the food industry. New technologies must assure food safety while maintaining nutritional, functional, and sensory characteristics that consumers demand. Thermal processing of soymilk leads to off-flavors, changes in color and reduced nutritional content. High-pressure processing (HPP) is a potential alternative for processing soymilk without exposure to elevated heat. Pressure treatment does not affect color and nutrients, and inactivates microorganisms. In the first study, our objective was to determine the impact of high-pressure processing conditions (i.e., pressure level, pressure hold time and temperature) and storage atmosphere (aerobic and anaerobic) on microbiological reduction and protein stability during refrigerated storage. We hypothesized that higher pressures, longer dwell time, and higher temperature (75°C) would yield greater microbial reduction immediately and over refrigerated anaerobic storage.

Commercial production of soy protein isolates traditionally uses defatted soybean meal that had the oil extracted with hexane. A new screw-pressing process using CO_2 as a displacement fluid and known as gas-supported screw pressing (GSSP) or Hyplex® offers an environmentally friendly way to produce soybean meal with little heat denaturation. Other traditional screw-pressing processes involve frictional heat that denatures protein decreasing protein solubility. High solubility, however, is required to obtain maximum soy protein isolate yields.

A simplified procedure to fractionate soy protein into glycinin- or β-conglycinin-rich fractions was developed by Deak and Johnson (2005, 2007). The Deak and Johnson method yields fractionated soy protein isolates with similar functional properties to commercial soy protein products. Surimi, also known as imitation crab meat, requires fillers and/or extenders to meet consumer demand. Soy protein isolate produced from hexane-extracted soybean meal is a common functional ingredient used in surimi processing. The objective of the second study was to determine the effects of fractionated GSSP soy protein isolates and moisture content on the physical properties of surimi produced from Alaskan pollock. We hypothesized that fractionated GSSP soy protein ingredients can be used effectively to extend surimi and the surimi can be further extended by adding water to above the normal level used in industry.

The research reported in this thesis strives to provide groundwork for future efforts to develop novel processes and techniques for producing and processing soy products for the food industry.

Thesis Organization

This thesis is organized into sections with a general introduction that covers the research problem and thesis organization. The literature review is in the following section and contains background information on the research problems. Chapters 3 and 4 are journal manuscripts, which is in press in Food Microbiology and has been submitted to Journal of Food Science, respectively. The format of the manuscripts follows that of the Journal of Food Science; including an abstract, introduction, materials and methods, results and discussion, references, and tables and figures following the text. This thesis concludes with a chapter offering comments on general conclusions and suggestions for future work.

CHAPTER 2. LITERATURE REVIEW

Introduction

Soybeans host a large population of microflora. Because the microorganisms found in soybeans get transferred and increase in numbers during the soaking and grinding process, soymilk must be treated to inactivate spoilage microorganisms and extend its shelf-life. Pasteurization is the most common practice used to extend soymilk shelf-life because it inactivates most known vegetative pathogens and spoilage bacteria (Kwok et al., 1995). Thermal treatment is also required to decrease content of anti-nutritional factors present in soy (Yuan et al., 2008). Thermal processing, however, affects nutritional and quality attributes of soymilk (Achouri et al., 2007). When soymilk is heat-treated it develops brown color and cooked flavor. Consumers are readily able to assess color and flavor so it is of great importance to the industry to remove any unacceptable characteristics. High-pressure processing of soymilk has been reported to be a potential alternative to thermal treatment (Lakshmanan et al., 2006; Kajiyama et al., 1995). High-pressure processing affects only non-covalent bonds so proteins, enzymes and DNA are damaged while color, taste and aroma compounds are not unaffected (Landau, 1967; Hayashi et al, 1989).

Soy protein is a common functional ingredient used in meat processing to increase gel strength, yield and lower production costs (Lin et al., 2000). Soy protein has two major storage proteins, glycinin and β-conglycinin. The two fractions make up about 70% of soy protein isolate (Dias et al., 2003). Glycinin and β-conglycinin each have their own unique functional qualities. Glycinin produces stronger, harder and tougher gels, while β-

conglycinin has higher water solubility, is a better emulsifier and affects elasticity of soy protein gels at high heating temperatures (Molina et al., 2001; Kang et al., 2005).

High-pressure Processing

Traditional methods in food processing rely on thermal treatments to extend the shelf-life and ensure microbiological safety of a food product. Nutritional and organoleptic changes to food can occur as side effects of the high temperatures used in processing. Most notably, vitamins and color and flavor compounds are adversely affected as well. The texture of the food can also be altered, for example vegetable and fruit tissues soften and become mushy (Smelt, 1997). To retain firmness, it is common to add chemical compounds to the food. Due to all of the changes that occur during processing, most processed foods are no longer similar to their original fresh forms, which consumers prefer.

Consumers today desire products with long shelf-lives that retain sensory characteristics of the original fresh product. This demand presents a challenge for the food industry, and justifies the need to develop and implement new processes. New technologies and processes (i.e. UV radiation, high-pressure processing and ohmic heating) are currently under extensive research. These technologies must strike a balance between improving the product's shelf-life and increasing food safety, while preserving the quality attributes of the food (McClements et al., 2001; Ortega-Rivas, 2007). Among them, high-pressure processing (HPP) has been adopted by the food industry for a few products including some condiments, meats, fruit, and vegetable juices (San Martin et al., 2002). Some of the advantages of the use of HPP for these products include better quality

and nutritional attributes of the products compared to the thermal-treated ones. For instance, pressure-treated strawberry jam was preferred over the traditional thermal-treated jam by a sensory panel, and the HPP-treated jam still retained 95% of its original ascorbic acid (Horie et al., 1991). However the main interest in HPP is due to its effects on pathogenic microorganisms and spoilage microorganisms and enzyme, therefore increasing the food safety and shelf-life of pressurized products without any need of chemical preservatives.

HPP was first attempted by Hite in 1899, who observed that the shelf-lives of bovine milk could be extended with the use of pressure. The process was largely ignored for nearly a century until consumer's demand for safe, additive-free, shelf-stable foods with maximum nutritional and sensory qualities drove the development of nontraditional food processing technologies (Zink, 1997).

An HPP system consists of a pressure vessel, a pressurization system, and a temperature control system. In batch systems, the foods are packed into flexible packaging and placed in a basket inside the pressure chamber. The foods are in a confined space containing a fluid that acts as the pressure-transmitting medium. The pressure is applied isostatically, spontaneously and uniformly throughout the product, which allows most food products to retain their original shapes (San Martin et al., 2002; Ortega Rivas, 2007). HPP is usually a batch process but the food industry has built semi-continuous lines with three or more vessels in a series. The pressure is held for the desired amount of time and then released. The level of pressure and holding time required are dependent on the food product and the purpose of the use of this technology. For example, if HPP is used to increase food safety, high pressure (i.e., ~ 600 MPa) is usually

applied. This technology could also be used to shuck oysters, and in this case lower pressure could be applied. There are two common pressurization methods, direct and indirect compression, used to generate pressure inside the vessel. Direct compression, although not used by the food industry, is the process where the volume of the chamber is reduced as pressure is applied. Indirect compression is used in the food industry. Indirect compression is the process of pumping a pressurizing medium, such as water, into the vessel without changing the volume of the chamber to reach the desired pressure.

Initial temperatures of HPP vessels can range from -20 to 100°C. During compression adiabatic heating occurs in the food system. This can be beneficial for maximizing effectiveness of HPP technology because the microorganisms would be undergoing two stresses (pressure and temperature) instead of just one stress (Ardia et al., 2004).

Impact of High-pressure Processing on Microorganisms

HPP induces changes in cell morphology, affects biochemical reactions, alters genetic mechanisms, and disrupts cell membranes, which all lead to microbial inactivation (Hoover et al., 1989; Smelt, 1998; Abee et al., 1999; Patterson et al., 1995). In the food industry, HPP has great potential for reducing the level of spoilage bacteria and pathogens in food (Lakshmanan et al., 2004; McClements et al., 2001; Ortegas-Rivas, 2007). Factors affecting HPP inactivation of microorganisms include treatment temperature, pressure level, duration of time that the pressure is held (dwell time), any antimicrobial substances present, the type of food matrix involved and the population of natural microflora affect the amount of microbes inactivated or killed (Cheftel, 1995;

Patterson et al., 1995; Smelt, 1998; Wuytack et al., 2002). Pressure, like temperature, can slow; stop or even accelerate microbial activity (Smelt, 1998).

Singer and Nichols proposed the fluid mosaic model to explain the basic structure of cellular membranes and pseudo-membranes of most organelles in microorganisms (Singer et al., 1972). In this model, a phospholipid bilayer is formed from the hydrophilic heads and hydrophobic tails of the lipids. Functional proteins penetrate the bilayer membrane to form an amphiphilic structure in which polar groups protrude from the bilayer into an aqueous phase, and nonpolar groups are buried in the hydrophobic interior of the bilayer. When pressure is increased, membrane fluidity decreases and a phase transition occurs. During pressure treatment, the phospholipid bilayer becomes agitated and the integral and peripheral membrane proteins begin to detach from the plasma membrane. As pressure increases, greater damaged is inflicted on the cells. Pressures of ~100 MPa can affect the nuclear membrane of yeasts while at higher pressures (400-600 MPa) there are pressure-induced changes in the mitochondria and the cytoplasm of the cell.

Under pressure, only non-covalent bonds are affected by the treatment. Proteins, enzymes and DNA could be damaged (Landau, 1967) while color, taste and aroma are not affected (Hayashi et al., 1989). The capability of HPP to inactivate microorganisms has been the subject of intensive research (O'Reilly et al., 2000; Smelt, 1998).

There is a considerable body of literature on the effect of HPP parameters and environmental conditions on microorganism inactivation. HPP inactivates many types of vegetative cells, while spores are resistant to pressures up to 1,000 MPa (Matser et al., 2004). Due to this resistance, the current trend is to implement the use of hurdle

technology which is the combination of HPP and some other treatment, such as a thermal treatment (Ross et al., 2003).

Mechanisms of inactivating *Listeria monocytogenes, Salmonella*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Salmonella typhimurium* have been extensively studied (Alpas et al., 2000; Bozoglu et al., 2004; Lakshmanan et al., 2004). There is evidence that pressure-injured cells will leak metal ions. This leakage becomes noticeable at pressures over 300 MPa (Nakatomi et al., 1993). It was observed that *Saccharomyces cerevisiae* cells underwent 25% volume reduction that corresponded to leakage of Na^+, Li^+ and Ca^{2+} ions after pressurization (Perrier-Cornet, 1998). The type of ions present in the food product, as well as the type of microorganisms present and temperature, can affect the level of baro-tolerance (Michiels et al., 1996).

DNA is believed to be cleaved during pressure treatment and could be another cause of inactivation. DNA cleavage is enzymatically controlled and can be reversible. High pressure interferes with DNA replication. In *Saccharomyces cerevisiae*, pressure induced tetraploidy (Hamada et al., 1992). DNA and RNA condense in *Listeria monocytogenes* and *Salmonella typhimurium* when under pressure (Mackey et al., 1994). Condensation has been seen in other cases and found to be reversible, since it is enzymatically controlled. It is hypothesized that under pressure endonucleases come into contact with DNA, which results in the DNA being cleaved.

Low water activity has a baroprotective effect on microorganisms and can increase resistance to pressure (Iwahashi et al., 1996). The shape of the bacterial cell can also affect sensitivity to pressure, with rods being more sensitive than cocci (Ludwig, 1996). Gram-positive microorganisms are more resistant to pressure than Gram-negative

(Hoover, 1989). Also, the sensitivity of microorganisms to pressure is dependent on the strain.

Temperature at which HPP occurs has a major effect on microbial inactivation; the optimum microbial lethality was observed at 50°C and 500 MPa (Gervilla et al., 2000). Under the same treatment the response can differ greatly between microorganisms. The effects of pressure between different species are believed to be dependent on pressure and are not the same for every organism.

Exponentially growing cells are more sensitive to pressure than cells in the stationary phase (Smelt, 1997; Mackey et al., 1995). *Lactobacillus plantarum*, a food spoilage organism, is more resistant to pressure when in the exponential phase growing at suboptimal temperature (Smelt et al., 1994).

Enzymes can be denatured under pressure. Enzymes have an optimum temperature where they are most resistant to pressure (Ludikhuyze et al., 2003). Similar results have been observed for microorganisms and bacteriophages, there has been some evidence showing that microbial enzymes could be the main target of pressure resistance in mesophilic microorganisms stabilized by pressure (Jaenicke, 1991). Other major targets of HPP inactivation could be protein denaturation, decrease in intracellular pH, and denaturation of enzymes associated with the efflux of protons. ATPase enzymes are involved in ion movement and can be denatured by pressure (Thom et al., 1984). When HPP inactivates the enzyme, the cell is no longer able to multiply. When a cell is damaged by HPP, it also becomes more sensitive to environmental stresses, which is a similar result with thermal treatment.

Microorganism Injury Recovery

It is now well established that the inactivation process occurs in two steps: a rapid linear decline in cells, followed by a slower decline, known as a tailing effect (Kalchayanand et al., 1998; Alpas et al., 2000). The tailing effect occurs at the pressure where almost all cells are inactivated and little to no recovery occurs after pressurization.

HPP can sub-lethally injure substantial amounts of cells (Wuytack et al., 2002; 2003). Injury can easily be measured by comparing the growth of pressure-treated cells on nonselective media to cells grown in selective media, or media containing substances that add stress to the cells, such as NaCl, sodium dodecyl sulfate or low pH (McClements et al., 2001; Patterson et al., 1995). Pressure-damaged *E. coli* cells were able to repair their outer and inner membrane after pressurization at 400 MPa for 2 min at 20˚C (Chilton et al., 1996). The sub-lethally injured *E. coli* was unable to repair its inner and outer membranes in the presence of bile salts, NaCl and different antibiotics. The injured cells were unable to repair the inner membrane, suggesting that many cellular processes are required to repair the inner membrane (Chilton et al., 2001)

The sub-lethally injured bacterium may not be detected because the injured cell cannot grow on selective media until it has recovered enough to multiply (Patterson et al., 1995). Injured cells can fail to grow on selective media immediately after treatment and, therefore, the food may be mistakenly considered void of microorganisms (Patterson et al., 1995; Wu, 2008).

Recovery during storage of cells injured during HPP treatment has been reported, and is a major food safety concern (Wu, 2008). Damaging the cell membrane is considered to be a critical step in the events leading to inactivation of pressure-treated

microorganisms (Smelt 1998; Wouters et al., 1998). With pressure treatment, however, some cells may survive after being damaged and repair any pressure-induced damage to their membranes. Pressure-induced injury in cells can be repaired within 1-15 days, which poses a problem for processors that cannot detect sub-lethally injured cells (Bozoglu et al., 2004). Injured cells that are unable to multiply and form colonies on nonselective media may survive, but may not be detectable immediately after pressurization. This could be misleading and the number of inactivated cells could be over-estimated because injury recovery is not taken into account. The detection level of the method will also play a role in estimating the efficacy of the pressure treatment. Inaccurately measuring inactivation may increase the risk of spoilage or even food poisoning and is a critical step in assuring the food safety of pressurized food. It has been suggested that cells can be sublethally injured with mild pressure and their growth could then be inhibited if the food is lightly preserved, it would be the same type of inhibition as injured cells grown on nonselective media with 4% NaCl added (Ulmer et al., 2000).

Certain strains of bacteria are able to develop baro-tolerance when subjected to cycles of pressurization, with the survivors being regrown after each cycle (Hauben et al., 1996). Injury recovery of pathogenic bacteria has been observed in various food matrices: nutrient broth, milk, phosphate buffer and ground pork after HPP treatment and being stored between 6 h and 4 wk at various temperatures (Bozoglu et al., 2004; Bull et al., 2005; Chilton et al., 2001; Ellenberg et al., 1999; Koseki et al., 2006).

Pressure Resistance in Microorganisms

It has not been discovered if pressure can induce cells to become more resistant to physical treatments, but it is known that cells subjected to stress other than pressure can become more resistant to pressure. One possible mechanism is that membrane-bound enzymes are stabilized during stress and can influence resistance (Smelt et al., 1997).

Bacterial spores are notoriously resistant to environmental stress, as well as thermal treatment and high pressure. It remains unknown what the mode of action is for pressure on bacterial spores. Certain pressure levels can induce spore germination and then the germinated spore is more sensitive to pressure and can be inactivated. Spore germination does not always need to be pressure-induced. Lowering the pH or heating can both induce activation. Activation is reversible, but germination usually quickly follows activation. The mechanism of how pressure induces activation remains unknown, it is possible that it could be similar to low pH or heating activation, which is reversible or could cause irreversible germination. Viruses vary genetically considerably and with that variance come different levels of pressure resistance. Bacteriophages, which are protein-DNA viruses, can be reduced significantly at pressures of 300-400 MPa (Brauch et al., 1990). The Sindbis virus, which has a lipid coating, can retain full virulence at pressures from 300 to 700 MPa at temperatures as low as -20°C (Butz et al., 1992).

<center>**Soymilk Processing**</center>

Soybeans are greatly valued by the food industry for its high oil and protein contents. Among all soy foods, soymilk is consumed in highest quantities (Savitry et al.,

2004). Soymilk is a liquid extract from soaked soybeans that have been ground and strained to remove all water-insoluble components (Guo et al., 1997; Munoz et al., 1998). The food industry has gone to great lengths to reduce and remove off-flavors and beany flavor that are considered unacceptable by most Western consumers. The resulting soymilk has mild flavor, which has led to wide acceptance of soymilk in the Western market (Wang et al., 1994; Huang et al., 2004).

Soymilk sales have rapidly increased in North America and even internationally. Soymilk sales have increased over 300% in the past decade. Sales in the United States alone went from $500 million in 2001 to $622 million in 2003. Annual growth in soymilk consumption has been 25% (Savitry et al., 2004). In addition to the improvement in taste, the increase in soy product consumption can be attributed to the U.S. Food and Drug Administration authorizing in 1999 the claim that soy proteins can help control heart disease in humans (Kennedy, 1995; Huang et al., 2004).

Soymilk can be sold in aseptic packaging to achieve a non-refrigerated shelf-life of at least a year (Yuan et al., 2008). Usually, soymilk is sold in the refrigerated section after ultra-high-temperature pasteurization that gives up to 12 wk of shelf-life (Kwok et al., 1995). Regardless whether the soymilk is refrigerated or shelf-stable, both products undergo heating processes, which alter the color, decreases the nutritive value, and can create cooked flavors (Guerra-Hernandez et al., 1999; Fernandez-Artigas et al., 1999). Thermal processing, therefore, affects nutritional and sensory attributes of soymilk. Production of strong off flavors is a challenge to developing soy foods that are appealing to consumers, and negatively impacts the use of heat-treated soymilk as an ingredient (Kwok et al., 2000; Achouri et al., 2007). More deterioration of color and flavor of soymilk during thermal

processing is associated with increased heating time (Kwok et al., 1995; 2000). Chemical changes in soymilk can continue during storage, but few studies have been conducted to evaluate the changes occurring in soymilk after treatment and during storage (Schroder et al., 1985; Erickson, 1997; Rysstad et al., 1998; Skibsted, 2000). Fresh soymilk, therefore, has a very short shelf-life, which limits consumption to the areas close to the production site.

HPP of soymilk has been reported in few studies to be a potential alternative to thermal treatment (Lakshmanan et al., 2006; Kajiyama et al., 1995); however, no investigations have focused on optimizing processing parameters on soymilk shelf-life extension.

Alaskan Pollock Surimi Production

Surimi is the Japanese term for mechanically deboned fish flesh that is minced and mixed with cryoprotectants to extend the frozen shelf-life, and cooked (Nagai et al., 2007). Ideal surimi is white in color, firm in texture, and moist. Surimi is commonly used as a base ingredient in the production of Kamaboko, a Japanese fish loaf, and shellfish substitutes commonly known in the United States as imitation crab meat (Park, 2005).

Surimi paste production begins immediately upon catching the fish, generally Alaskan pollock. The fish flesh is mechanically deboned and then minced into a paste. The paste is then washed several times. Depending on the species of fish, extensive washing can be necessary to remove fat and undesirable materials that may affect functionality or color of the surimi. The resulting surimi should be translucent and have a mild odor (Park, 1995).

After washing, cryoprotectants are added to prevent denaturation of actomyosin during frozen storage. It was discovered in the 1960's that low-molecular-weight

carbohydrates when added to surimi paste can stabilize the proteins and act as a cryoprotectant (Scott et al., 1988). Without a cryoprotectant the proteins in surimi undergo changes during frozen storage. The muscle proteins can become denatured and dehydrated which causes conformational changes to occur. Ice crystal formation, pH and ionic strength can also affect protein functionality (Park, 1994). Other changes during frozen storage include decreased in water-holding capacity and gel-forming ability of the surimi gels (Iwata et al., 1971). The decreased in functionality is due in part to the disintegration of myofibrillar proteins. The three-dimensional gel network cannot develop if the myofibrillar proteins are no longer structurally intact (Morrissey et al., 1993). After cryoprotectants are added, the commercial surimi paste is kept frozen for storage and shipping to processing plants.

During processing the surimi paste is thawed and chopped, and the protein content is adjusted to 78-85% moisture content by the adding of water. Salt is always added to surimi flesh to thicken the paste and extract the myofibrillar proteins. Solubilization of myofibrillar proteins is a prerequisite for the gel formation (Sano et al., 1988; Choi et al., 2000).

Protein gelation is fundamental to surimi production. One major concern in the production of surimi is the formation of modori. Modori is the weakening of a gel due to myosin degradation, and is a result of endogenous heat-activated proteases, which become active in the 50-70°C range (Jiang, 2000). The industry usually adds functional ingredients that contain protease inhibitors such as beef plasma protein, egg white and whey proteins to overcome this problem (Benjakul et al., 2004).

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The seafood analogs are prepared by adding ingredients such as starch, egg white proteins, salt and vegetable oils to the surimi paste (Campo et al., 2008). The paste is then heated to form an elastic gel (Numakura et al., 1990). The heating process is done in two steps to form a stronger gel than could be achieved by a single heating process (Park, 2005). The initial heating step is conducted at moderate temperature, around 40°C, which "sets" the gel. Setting is commonly used in the production of surimi (Lanier, 1986). A softer and more deformable gel is formed with the addition of sodium chloride when the surimi paste is held at low temperature without a second heating step but, a second heating step at higher temperatures yields a much stronger gel. Heat setting is not always necessary; surimi paste can be set at refrigerator temperatures if held overnight prior to further heating (Lanier et al., 1982). Industry prefers a short setting time to reduce cost and production time. The second heating step is conducted at high temperatures (>80°C) to form a rigid and irreversible gel (Montejano et al., 1984).

Alaskan Pollock

The ideal fish for surimi production is white fleshed and low in fat. Alaskan pollock is widely used in surimi production due to its white flesh, low fat, uniform size, ability to form strong gels and a large harvest size (Yoon et al., 2004). After the discovery that cryoprotectants prevent protein denaturation during frozen storage the Japanese could fish at sea for extended periods of time and could harvest, process and store the frozen surimi on the vessels. This increased surimi production and sales in Japan, and later globally. World-wide the harvest quantity and quality have steadily decreased over the last decade due to over-fishing and poor management practices. The

Russian Alaskan pollock industry collapsed and Japan's industry has steadily declined, as has America's industry. This trend has leveled off in recent years due to better technology and processing techniques that recover more meat and have less waste (Reed et al., 2008). Even with these advancements in processing only 50% of U.S. pollock captured is used for surimi production while the rest is utilized in fillet production.

Surimi Gelation

Alaskan pollock muscle is composed of striated muscle fibers that are in turn composed of myofibrils. Myofibrils are formed from contractile units called sarcomeres. The sarcomeres contain three types of filaments: thick, thin and connecting. Disassembly of the sarcomere is necessary to form a strong heat-induced gel. Myosin makes up 55-60% of the myofibrillar proteins. Myosin has both a globular domain, which is round in shape, and a fibrous domain, which is long and thin in shape. The globular domain is formed from two heavy amino acid chains, which are large polypeptides, and two pairs of light chains, which are small polypeptides. The N-terminal ends of the heavy chains fold into themselves to form an elongated pear shape to form the globular heads. The globular heads have ATPase activity, but postmortem, in the absence of ATP, the globular heads will bind to actin. The fibrous or "rod" domain has a C-terminal region and an N-terminal. The N-terminal of the rod domain connects the globular head to the C-terminal of the myosin (Park, 2005).

Actin constitutes 15-30% of the myofibrillar protein. Actin is the predominant protein found in the thin filaments of the sarcomere, and in the surimi itself. Actin is globular in shape, and will polymerize to form the actin filament which is referred to as

"fibrous actin". The fibrous actin is bound with myosin to form actomyosin. The heat-induced gel properties of actin are dependent on the concentration and properties of the actomyosin in the surimi.

There are other fractions of proteins associated with myosin or actin that are necessary for the structural integrity of the sarcomere. The fractions can be removed by solubilization or degradation. Disassembly of the sarcomere during processing is important for even distribution of protein in the heat-induced gel structure. The process of forming a gel involves denaturation, dissociation-association and aggregation of proteins (Hermansson, 1986).

Surimi gel is a three-dimensional network formed from hydrogen bonds, ionic linkages, hydrophobic interactions, and covalent bonds. Prior to heating, hydrogen bonds maintain the protein structures, during heating the hydrogen bonds are broken and the protein unfolds. The unfolded protein's peptide backbone becomes hydrated and the water in contact with the protein becomes structured or clustered. This hydration is important for water-holding capacity of the heat-induced gels formed from protein-protein aggregation (Park, 2005).

Salt bridges also known as ionic linkages, are abundant along the myosin rod domain. On the rod domain at neutral pH glutamic acid and aspartic acid are negatively charged, while lysine and arginine are positively charged. The attraction between charges forms salt bridges and the proteins form an aggregate which is insoluble in water. Salt bridges are considered to be the most important force in the assembly of myosin thick filaments (Miroshinichemnko et al., 2000). The addition of salt disrupts the attraction between charges and lead to disassembly of the thick filaments. This is why salt is added

to surimi paste; it breaks the salt bridges and disperses the proteins. Even dispersion of proteins is necessary for the development a flexible and elastic structure in the heat-induced gels (Niwa, 1992).

Hydrophobic interactions result from the unfolding of the proteins during heating. The interior of folded proteins is composed of hydrophobic amino acids, while the exterior of the proteins is composed of hydrophilic amino acids. The unfolded protein exposes its hydrophobic groups which promotes the formation of hydrophobic clusters, and protein-protein interactions (Park, 2005). Hydrophobic areas of a protein closely associate with other hydrophobic areas of proteins. This association results in protein aggregates formed from the binding of the proteins. Under certain conditions, hydrophobic interactions lead to a gel network. Disulfide bonds are formed by the oxidation of two cysteine residues on neighboring proteins. These covalent bonds are formed during heating surimi above 40°C, which occurs during the second heating step.

Texture and Color of Surimi

Texture and color are major quality characteristics of surimi. A surimi gel should be cohesive and elastic while being light in color. Assessing the texture of surimi after gelation is the primary method for determining its quality (Lanier, 1992).

High-grade surimi has a distinctive rubbery mouth feel that relates to a low value of the stiffness/cohesiveness ratio (Lanier, 1986). The rubbery mouth feel is due to the elastic behavior of the surimi gel (Niwa, 1992). Shear strain (gel cohesiveness) is also an indicator of protein quality, but is not affected by moisture content until the moisture level reaches or exceeds 81% (Hamann et al., 1992).

The hardness and strength of surimi gels can be affected by protein concentration, heating temperature, and heating time, as well as moisture content (Harper et al., 1978; Camou et al., 1989). At high moisture contents (>75%) surimi gels have rubber-like elastic characteristics with covalent bonding and hydrophobic interactions (Niwa, 1992; Lee et al., 1997; Benjakul et al., 2001). The mineral content of the water is also important. Calcium and magnesium can cause texture changes in surimi (Lee, 1990).

Water is added to maintain consumer accepted texture while decreasing the cost of production. The water molecules act as a protein stabilizer due to the proteins hydrophobic residues. In water, the protein structure can remain stable until heating when the hydrophobic sites are exposed. The addition of water disperses the proteins while allowing an expanded gel network to form during heating. The correct amount of water to add is important as too much water can decrease gel hardness (Lanier et al., 1985).

Whiteness of the surimi is also an important quality attribute (Choi et al., 2000). The whiteness value is calculated from the Lab values. The $L*$ value measures the lightness of the surimi gel, the $a*$ value is a measure of how red or green the gel is, and the $b*$ value is a measure of how yellow or blue the gel is.

$$Whiteness = 100 - [(100-L*)^2 + a*^2 + b*^2]^{1/2}$$

Water quality affects the color of surimi. Iron and manganese present in water can alter the color of the surimi gel. Increasing the moisture content of surimi gels creates a lighter and less yellow gel, which increases the whiteness values (Park, 1995). Concentration and properties of added ingredients also affect the color of the surimi gel. Vegetable oil creates a whiter gel. Increasing the oil concentration in the gel increases the lightness and yellowness of the gel. Concentration and type of starch added can alter surimi gel color.

The addition of incompletely swollen starch granules creates a more opaque and yellow gel as the concentration is increased while the addition of fully swollen starch granules create a more translucent and less yellow gel (Park, 1995).

Color and texture are affected by heating time and method (Bertak et al., 1995). The temperature in the second step of the heating process is important, excessively high heating temperature causes separation and reduced water-holding capacity in some gels due to the formation of large aggregates and pores. The large pores that are formed increase syneresis because the water is not held as firmly (Stanley et al., 1992). The greater the syneresis, the weaker and less stable the gel becomes.

Functional Additives in Surimi

The addition of filler ingredients has been used to extend surimi to meet the demand of the market. There are five proposed models for the spatial partitioning of a gelling protein and an additive (Ziegler et al., 1990). In single-phase gels, the additive remains soluble in the fluid of the gel matrix. In second-phase gels, separation occurs between the additive and the gel; this is commonly found when starch is added to the surimi paste and the paste is cooked with the final result having a distinct layer of starch separate from the surimi gel. The third model involves "complex" gels, the surimi and the additives form interactions which lead to a gel. Two or more proteins co-polymerizing to form a network; this is considered a fourth model. The fifth model is an interpenetrating network where there is an increase in shear stress without an increase in shear strain (Yongsawatdigul et al., 1996).

Ingredients used to extend surimi are usually considered to be natural and healthy, to suit consumer preferences. These ingredients also improve the texture of the surimi. The texture of surimi can be modified with the addition of starch, hydrocolloids and protein additives, which act as filler or extenders (Lee et al., 1992). Starch, dried egg white and soy protein isolate (SPI) are major functional ingredients in the surimi industry because of their abilities to form gels and retain water, while being light in color (Choi et al., 2000). Starch is mainly used to maintain gel strength while extending the surimi. Starch is commonly used as an extender because of its ability to swell, hold water, and maintain gel strength during refrigerated and frozen storage, while using less surimi (Park, 2000). Dried egg white has better gelling properties than liquid egg white and when added to surimi can inhibit gel softening, increase whiteness and act as a cryoprotectant. Heating temperature, water uptake, and the size of the swollen granule affect starch gelatinization in protein gel (Wu et al., 1985).

The functional advantages of protein additives are their abilities to improve gel firmness, elasticity, inhibition of heat-stable proteases, and anti-retrogradation of starch during refrigerated and frozen storage (Park, 2005). Replacing fish protein with SPI leaves less myofibrillar protein in the surimi and therefore less myofibrillar protein is available to be degraded. SPI is a common functional ingredient in meat processing added to enhance texture, yield and flavor and reduce production costs (Lin et al., 2000). Sugar, sorbitol and salt are commonly added in varying concentrations as cryoprotectants to stabilize myofibrillar protein, and maintain functionality of the fish proteins (Matsumoto, 1979; Park, 1988; Lanier, 1990).

Nutritional and Functional Properties of Soy Protein

Soybeans are a versatile commodity because of their unique functional properties, high protein content, nutritional value, and perceived health benefits (Deak et al., 2006). Soybean seeds contain between 35-46% protein content at maturity (Nagano et al., 1992). Soy protein is considered one of the most important ingredients used in the food industry for the production of gels (Salleh et al., 2004).

SPI is derived from defatted soy flakes by extracting with alkali and precipitating at pH 4.5. SPI contains >90% protein (dry basis) and is comprised mostly of glycinin and β-conglycinin which are the major storage proteins found in soy beans.. Glycinin (primarily 11S) and β-conglycinin (primarily 7S) make up about 70% of the protein in SPI (Dias et al., 2003). Glycinin is formed from acidic and basic polypeptides linked by disulfide bridges (Utsumi et al., 1987). β-Conglycinin is a trimeric glycoprotein consisting of three types of subunits, α′, α, and β, in seven different combinations (Thanh et al., 1976). At certain protein concentrations, the β-conglycinin subunits aggregate and become insoluble during heating (Utsumi et al., 1984).

Nutritional benefits and functionality are important properties to be considered when choosing a protein additive. Soy protein is a major source of vegetable protein due to its availability and low cost. In 1999, the U.S. Food and Drug Administration authorized the health claim that soy protein can help control heart disease in humans. Soy protein has the physiological function to lower cholesterol and triglycerides levels in human serum (Kito et al., 1993; Aoyama et al., 2001). It has been suggested that β-conglycinin fraction has greater health benefits than glycinin (Manzoni et al., 2003; Duranti et al., 2004).

Soy protein is largely utilized in the food industry because of its functional properties (Deak et al., 2006). The functional properties of soy proteins are due to their structure's surface hydrophobicity and sulfhydryl cross-linking. Glycinin and β-conglycinin each have their own unique functional qualities. Glycinin produces stronger, harder and tougher gels while β-conglycinin has higher solubility, is a better emulsifier and affects elasticity of soy protein gels at high heating temperatures (Molina et al., 2001; Kang et al., 2005). Processing, intrinsic and environmental factors affect the functional properties of protein ingredients (Kinsella, 1979). Commercial SPI can have different functional properties due to processing conditions that can cause differences in protein denaturation and aggregation (Hermansson, 1986). In meat applications, commercially produced SPIs are partially or fully denatured to enhance their functional properties (Hermansson, 1986; Chronakis et al., 1995).

New Processes for Making Soy Protein Isolate and Fractions

SPI is conventionally produced using hexane as the solvent to extract oil. Crown Iron Works (St. Paul, MN, U.S.A.) and Safe Soy Technologies (Ellsworth, IA, U.S.A.) have developed a new process using CO_2 as a displacement fluid to displace the oil from dehulled and flaked soybeans; the process has been termed gas-supported screw pressing (GSSP) or HYPLEX®. SPI and glycinin-rich and β-conglycinin-rich fractions were produced via the Deak and Johnson method at pilot-plant scale from GSSP soybean flour for this project (Deak and Johnson, 2005, 2007; Deak et al., 2006). Nazareth et al. (2009) reported similar functionality for SPI produced from GSSP meal as produced from

commercial white flakes (hexane extraction of dehulled, flaked soybeans and flash- or downdraft-desolventized to minimize protein denaturation).

To produce an enriched glycinin fraction, a reducing agent should be added before precipitating glycinin. The reducing agent is usually SO_2 in the form of sodium sulfite. Adding a reducing agent prevents co-precipitation of glycinin and β-conglycinin (Thiering et al., 2001). Glycinin preferentially binds calcium ions, which is surface charge dependent (Rao et al., 1976). The Deak and Johnson method was able to produce glycinin and β-conglycinin enriched fractions from GSSP defatted soybean meal using calcium chloride and sodium sulfite. The fractions had high yields of solids, protein and isoflavones, and similar protein purities compared to fractions produced by traditional methods. The GSSP fractions produced in the pilot plant had the same level of enrichment as soy protein fractions produced from white flakes in the laboratory. It has yet to be determined if GSSP soy protein ingredients can be used to extend surimi and if the functionality of these proteins would favor incorporation of additional water in surimi.

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CHAPTER 3. IMPACT OF HIGH-PRESSURE ON MICROBIAL SHELF-LIFE AND PROTEIN STABILITY OF REFRIGERATED SOYMILK

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Abstract

The effects of pressure (400, 500 and 600 MPa), dwell time (1 and 5 min) and temperature (25 and 75°C) on microbial quality and protein stability of soymilk during 28 days of storage (4°C) were evaluated under aerobic and anaerobic conditions. After processing and during storage, there were significant differences in total bacterial count (TBC), numbers of psychrotrophs (PSY) and Enterobacteriaceae (ENT), and protein stability between untreated (control) and pressurized samples ($P < 0.05$). Pressure applied at an initial temperature of 75°C resulted in a greater suppression in growth of PSY compared to TBC. No ENT was detected in pressurized samples throughout the storage period tested. Dwell time had no significant effect on log reduction of TBC at 25 or 75°C ($P > 0.05$). Pressure at 400 MPa (5 min), 500 and 600 MPa (1 and 5 min) produced 100%

sub-lethal injury in surviving bacterial populations irrespective of temperature. After 28

days of refrigerated storage, both aerobic and anaerobic pressurized samples had

better or similar stability as the control on day one of storage. Soymilk control samples

were spoiled after 7 days whereas pressurization increased soymilk shelf-life by at least 2

weeks. Pressure (600 MPa) at 75°C for 1 min not only significantly reduced initial

microbial populations and increased the microbial shelf-life but also extended the protein

stability of soymilk ($P < 0.05$).

Introduction

Consumer's demand for safe, additive-free, shelf-stable foods with optimum

nutritional and sensory qualities has driven the development of non-traditional food

processing technology (Zink, 1997). One must balance the improving product shelf-life

and increasing food safety while preserving sensory and nutritional quality attributes

(McClements et al., 2001; Ortega-Rivas, 2007). Among them, high-pressure processing

(HPP) has been adopted by the food industry for treating many foods including

condiments, meats, and fruits and vegetable (San Martin et al., 2002).

There is a considerable body of literature on the effects of HPP parameters and

environmental conditions on inactivating microorganisms. Treatment temperature, level

and duration of treatment, and amount of initial microflora affect the amount of microbes

inactivated (Cheftel, 1995). Recovery of sub-lethally injured cells during HPP has been

reported and is a major food safety concern (Wu, 2008). For example, pressure-injured

Escherichia

coli cells were able to repair their outer and inner membranes after pressurization (400 MPa) for 2 min at 20°C (Chilton et al., 2001). Injured cells may not grow on selective media immediately after treatment and there is the risk of mistakenly considering a food to be void of microorganisms (Patterson et al., 1995; Wu, 2008). The extent of recovery of sub-lethally injured cells depends on many parameters including type of food product, processing conditions, and storage conditions, which justify the need to determine sub-lethal recovery for a specific food product.

Soybean foods have become increasingly popular since the Food and Drug Administration approved the soy protein health claim in 1999 (FDA (1999)). According to that claim, 25 g of soy protein per day may reduce the risk of heart disease. Soymilk is a liquid extract from soaked and ground soybeans, which contains most of the soybean components including protein, lipid and saccharides (Guo et al., 1997). Consequently, fresh soymilk has a very short shelf-life, which limits consumption to areas close to the production site. Thermal processing is the most common practice used to improve the microbial safety and extend the shelf-life of soymilk because it inactivates vegetative pathogens and many spoilage bacteria (Kwok and Niranjan, 1995). The use of ultra high-temperature (UHT) is relatively new for soymilk production and the traditional processing involving temperature of 90–100°C, applied up to 30 min (Yuan et al., 2008). In some conditions, thermal processing, however, detrimentally affects nutritional and quality attributes of soymilk, and produces strong off flavors (Lozano et al., 2007). It limits the development of soy foods that are appealing to consumers and negatively impacts the use of heat-treated soymilk as an ingredient (Kwok et al., 2000; Achouri et al., 2007). Because of these detrimental effects of thermal treatment on soymilk

properties, other processing methods such as high-pressure homogenization, high-pressure throttling, and pulsed electric field have been applied to soymilk (Smiddy et al., 2007; Sivanandan et al., 2008; Li et al., 2008).

HPP of soymilk would be considered as a potential alternative to thermal treatment only if it could improve its microbial, sensorial, nutritional and quality attributes. Its effect on lipoxygenase activity, trypsin inhibitors, and protein properties and functionality, which are parameters that will affect soymilk attributes, has been previously reported (Tangwongchai et al., 2000; Van der Ven et al., 2005; Lakshmanan et al., 2006; Kajiyama et al., 1995). The benefits of applying high pressure to provide microbial safety and extend shelf-life have been reported for dairy and human milk, but not yet on soymilk (Viazis et al., 2008; Hayman et al., 2007; Garcia-Risco et al., 1998; Huppertz et al., 2006; McClements et al., 2001). The present study aims to evaluate the impact of HPP conditions (pressure, temperature and dwell time) on changes in bacterial populations and protein stability of refrigerated soymilk. Additionally, the extent of sub-lethal injury to populations of bacterial survivors in soymilk following HPP was determined. In the present study, processing conditions were chosen based on industry practices with dwell times of less than 5 min and maximum pressure of 600 MPa.

Materials and Methods

Soymilk production

Lipoxygenase-free cultivar soybeans (IA 1008) were washed with deionized water to remove dirt and soaked for 12 h at room temperature. After soaking, the beans were ground in a 4-L Waring heavy-duty laboratory blender (Waring, New Hartford, CT,

U.S.A.) at low speed for 1 min at a 1:8 soybean-to-water ratio. After blending, the slurry was pressed in a 100-mesh nylon filter sack and water was added to reach a final 1:10 soybean-to-water ratio. The pH of the soymilk was adjusted to pH 7.0 using 2 N NaOH.

High-pressure processing

Five- and 10-ml aliquots of soymilk were sealed with a tabletop vacuum chamber machine (Multivac Inc., Kansas City, MO, USA) in polyester bags (reference 404, KAPAK Corporation, Minneapolis, MN, USA) so that the headspace in the pouches was kept to a minimum. The pouches were 0.063 cm in thickness, an oxygen permeability of 118.65 $cc/m^2/day$ (ASTM D-3985) and a carbon dioxide permeability of 845 $cc/m^2/day$, at 23°C. Samples were pressurized in a FOOD-LAB 900 Plunger Press system (Stansted Fluid Power Ltd, Stansted, UK). A T-thermocouple was placed directly inside a polyester bag to record temperature of soymilk during HPP treatments. Soymilk samples were pressurized at 400, 500 and 600 MPa at initial temperatures of 25 and 75°C for dwell times of 1 and 5 min. For treatment at 75°C, soymilk was preheated for 5 min at 75°C prior to pressurization in a water bath, and were transferred into the HPP vessel and pressurized within 30 s after heat treatment. The initial temperature of the pressurization fluid inside the vessel was 25 or 75°C, and propylene glycol was circulated at these temperatures through the external jacket of the vessel. The average quasi-adiabatic temperature increases upon compression were 1 and 2°C/100 MPa at 25 and 75°C, respectively. The average rate of pressurization was 350 MPa/min and depressurization occurred within 5 sec.

Storage of soymilk

Both pressurized and non-treated (control) samples were stored at $4 \pm 0.4°C$ for up to 28 days. Designated samples were aseptically opened using a sterile scissors then closed, using metal paper clips, for aerobic storage conditions. This procedure simulated consumer use and storage of store-bought soymilk. The other treated and control samples remained sealed (anaerobic storage) to simulate unopened packages of soymilk.

Microbial analysis

Immediately after treatment, and every 4 days thereafter, duplicate samples of soymilk were analyzed for each treatment. One-ml aliquots of each sample were serially diluted (10-fold) in 0.1% peptone (Difco, Becton Dickinson, Sparks, MD). Aliquots (0.1-ml) of appropriate dilutions were plated onto tryptic soy agar (TSA; Difco). In instances when bacterial survivors were beyond detection in 0.1-ml samples, 1.0-ml aliquots of soymilk were surface-plated over five agar plates (0.2 ml per plate). Inoculated TSA plates were incubated at 30°C for 48 h and at 4°C for 7 – 10 days to determine total bacterial count (TBC) and numbers of psychrotrophs (PSY), respectively. The pour plate technique with TSA and violet red bile Agar (VRBA) overlay were used to enumerate *Enterobacteriaceae*. The inoculated TSA/VRBA plates were incubated at 35°C and bacterial colonies were counted at 24 h. TSA and TSA with 5% NaCl (TSAN) were used as non-selective agar and selective agar, respectively, for determining the percent injury among bacterial survivors just after HPP treatment. Percentage injury was calculated using the following equation:

Percent Injury = [(CFU/ml on TSA – CFU/ml on TSAN) ÷ CFU/ml on TSA] x

100

Stability and pH

Aliquots (1.5-ml) of soymilk were subjected to centrifugation (1,532 x *g*, 25°C, 30 min). The supernatant was decanted and the percentage of precipitate was calculated as the ratio of precipitate weight divided by the initial soymilk weight, multiplied by 100. On each day of analysis, soymilk pH was measured.

Statistical analysis

To eliminate the effect of time variations, the experiment was run in 2 replications, with each treatment combination represented in each replication. These balanced data were from a randomized complete block design. For each of the responses, the data were analyzed with a 5-way ANOVA blocking by replication. The procedure proc GLM in SAS 9.1 was used in the analysis. Fisher's LSD was calculated for the comparison between treatment combination means after the ANOVA null hypothesis of equal means was rejected using the ANOVA F-test.

Results and Discussion

Initial total bacterial count (TBC) and numbers of psychrotrophs (PSY) and *Enterobacteriaceae* (ENT) in untreated soymilk were 4.6, 3.7, and 3.7 log CFU ml^{-1}, respectively.

Effects of processing and storage on total microbial count

Log reductions in TBC of soymilk following HPP at 400, 500 or 600 MPa for 1 and 5 min are shown in Figure 1. Increasing pressure level (400 to 600 MPa) and dwell time duration (1–5 min) had no significant effect on log reductions in TBC at 25 or 75°C (P>0.05). For all pressures, increasing the initial treatment temperature from 25 to 75°C significantly improved the TBC log reductions from an average of 2.5 to 4.5. Based on the TBC, treating at 75°C and 400 MPa (5 min) resulted in reducing initial bacterial numbers by 4.5 log cycles indicating that numbers of viable cell were inactivated to levels less than our detection limit (1.0 CFU ml^{-1}). As expected, further increases in pressure to 500 and 600 MPa (1 and 5 min) gave similar results. In fact, the initial populations of natural microflora in soymilk were not sufficiently large to evaluate the extent of log reductions that resulted from pressures applied at 500 or 600 MPa at 75°C. This observation might explain the result that pressure did not seem to significantly affect TBC reduction at 75°C. At 25°C, HPP treatment from 400 to 600 MPa resulted in variable reductions in TBC that were not significantly different (P>0.05). This variability may be attributed to differences in the numbers and types of natural microflora of soymilk. Microbial sensitivity to pressure has been reported as being greatly dependent on species (Gervilla et al., 2000; Lopez-Pedemonte et al., 2007; Shao et al., 2007).

Overall, TBC reductions of soymilk under pressure were similar to those observed for raw bovine's milk. Nabhan et al. (2004) reported a 4.5 log reduction in TBC of raw bovine milk after pressurization (600 MPa) at 55°C for 5 min. Our results were consistent with observations of Huppertz et al. (2006). Those investigators concluded that a

significant reduction (>4.0 log units) in bacterial numbers by HPP treatment of bovine milk would require pressurization at 600 MPa.

In the present study, adiabatic heating during pressurization at 25°C increased soymilk temperature to 40, 45 and 48°C for 400, 500 and 600 MPa, respectively. For treatments at 75°C, the temperatures increased to 77, 84 and 85.5°C for 400, 500 and 600 MPa, respectively. The high level of reduction in TBC observed in soymilk pressurized at 75°C compared to 25°C is not surprising because the temperature combination reached after adiabatic heating and applied pressure is likely to exert more lethal effect on bacterial vegetative cells. Pressure-induced death of bacteria increases at higher temperatures and this effect is proportionately greater as temperature is increased above 35°C (Kalchayanand et al., 1998).

Refrigerated control soymilk reached the spoilage detection level (7.0 log CFU ml^{-1}) between 4 and 7 days (aerobic storage) and 7 and 11 days of anaerobic storage (data not shown). Soymilk pressurized at 400 MPa (25°C for 1 min) and stored aerobically reached 7.0 log CFU ml^{-1} between 14 and 18 days (Table 1). Extended shelf-lives of 18 and 21 days were observed after treating at 500 MPa (1 or 5 min) or 600 MPa for 1 min. For 600 MPa-treated samples (25°C), increasing the dwell time to 5 min extended the time to 25 days for soymilk to reach the spoilage detection level under aerobic conditions. All pressurized samples stored anaerobically were below the spoilage level for up to 21 days when pressures of 400–600 MPa were applied to soymilk at 25°C. TBC in those samples reached 7.0 log CFU ml^{-1} or greater at 25 or 28 days.

Applying pressure (400–600 MPa) to soymilk at 75°C markedly extended shelf-life irrespective of the aerobic or anaerobic storage or level of pressure. All samples had

viable counts of <7.0 log CFU ml^{-1} at 28 days; the TBC of 75°C-treated samples pressurized at 400, 500 and 600 MPa ranged from 6.14 to 6.81 log CFU ml^{-1} for aerobic storage and 5.29 to 6.07 log CFU ml^{-1} for anaerobic storage (Table 1).

The rationale for evaluating the TBC in food products is based on the inverse relationship between the level of initial microbial counts and shelf-lives of food products. The ability of a food processing method to substantially reduce numbers of food-borne microorganisms is important for extending the microbial shelf-lives of foods. In the present study, increased pressure (500 or 600 MPa) drastically reduced TBC to extend the microbial shelf-life of refrigerated soymilk. Although increased dwell time (5 min) did not significantly further reduce TBC, it caused much slower increase in bacterial populations during storage. This result is likely due to a greater severity of sub-lethal injury among bacterial survivors when pressure treating for 5 min. Depending on the severity of sub-lethal injury, bacterial cells may take a long time to repair their lesions before they can start growing to form visible colonies (Ray and Foegeding, 1992).

Compared to the shelf-life of aerobically stored non-treated soymilk (4–7 days) the refrigerated shelf-life of soymilk that was pressurized at 500 or 600 MPa (at 25°C) increased by about 14 days. Similarly, as a consequence of microbial inactivation, HPP (500 MPa, >55°C) of raw bovine milk increased the shelf-life to 21 days (Nabhan et al., 2004). The extended shelf-life of the anaerobically stored soymilk is not surprising considering that growth of the typical aerobic spoilage bacteria in milk is inhibited by reduced oxygen conditions (Jay et al., 2005). The largest extension in shelf-life observed for 75°C-treated pressurized soymilk reflects the drastic decrease in initial TBC due to the lethal combined effect of pasteurization temperature (75°C) and high pressure. The

use of relatively high temperatures in combination with high pressure has been shown to increase microbial inactivation, which is proportionately greater as temperature increases above 35°C (Kalchayanand et al., 1998). Although there are intrinsic compositional differences between soymilk and bovine milk, the results of the present study indicate that very similar extensions in shelf-life may be achieved by HPP.

Effects of processing and storage on psychrotrophic count

Viable counts of PSY in pressurized soymilk during aerobic storage at 4°C are shown in Table 2. HPP treatment at 25°C and 400 MPa for 1 and 5 min reduced initial numbers of PSY by approximately 3.4 and 3.7 log CFU ml^{-1}, respectively. When pressures were increased to 500 or 600 MPa, survivors were undetected (<1.0 CFU ml^{-1}). Similarly, no survivors were detected on the day of treatment when HPP was performed at 75°C, regardless of pressure and dwell time. PSY in 500- or 600 MPa-treated samples (25°C) were not detected until day 4 (Table 2). Viable counts steadily increased and reached greater than 6.0 log CFU mL^{-1} at day 14 (400 MPa, 25°C) and day 18 (500 MPa, 25 °C).

At 25°C soymilk pressurized at 600 MPa had the lowest psychrotrophic counts throughout storage. By day 28, viable counts reached 5.03 log CFU ml^{-1} (600 MPa, 1 min) and 3.32 log CFU ml^{-1} (600 MPa, 5 min). Numbers of PSY were beyond the detection limit up to days 4 and 7 in samples treated (400 MPa, 75°C) for 1 and 5 min, respectively. Also for the entire storage period, PSY were also not detected in samples treated with 500 or 600 MPa at 75°C and stored aerobically. A similar observation was

made for samples treated with 400, 500 or 600 MPa at 75°C and stored under anaerobic conditions. In contrast, numbers of PSY in untreated soymilk reached very high levels (9.0 log CFU ml^{-1}) after 11 days of anaerobic storage (data not shown).

Psychrotrophic bacteria are the main spoilage organisms of refrigerated food products. When numbers of PSY in milk reach 7 log CFU ml^{-1}, bacterial production of proteases and lipases cause detectable off-odors and off-flavors that render the milk spoiled (Stepaniak, 1991). For commercial soymilks, growth of PSY was reported after 14 days of refrigerated aerobic storage (Bai et al., 1998). For 20 h of refrigerated storage, no PSY were detected in aerobically packaged raw bovine milk treated at pressures >300 MPa at 25°C for 10–60 min. The long dwell time and short refrigerated storage period may explain the lack of cell enumeration in that study (Lopez-Fandino et al., 1996). Our observation that no PSY could be detected in pressurized soymilk (400–600 MPa, 75°C) during refrigerated storage for 28 days indicated that the PSY population was highly sensitive to HPP with thermal treatment. While obtained with high-pressure homogenization, similar observations were reported by Smiddy et al. (2007) and Thiebaud et al. (2003) for raw bovine milk pressurized at 100–300 MPa at 25–55°C. Garcia-Risco et al. (1998) also demonstrated significant extension in microbial shelf-life of raw bovine milk following HPP (400 MPa, 30 min, 25°C). The pressure-treated milk had a PSY count of <7 log CFU ml^{-1} after storage (7°C) for 45 days. In contrast, the non-treated milk had a PSY count of >7 log CFU ml^{-1} after 15 days (Garcia-Risco et al., 1998).

Effects of processing and storage on *Enterobacteriaceae*

After 7 days of refrigerated anaerobic storage numbers of *Enterobacteriaceae* (ENT) in control soymilk reached approximately 8.0 log CFU ml^{-1}. No ENT was detected in pressurized samples stored at 4°C for 28 days irrespective of pressure levels and temperature used in the present study (results not shown). Certain human enteric foodborne pathogens, such as *E. coli*, *Salmonella* and *Yersinia enterocolitica*, are members of the *Enterobacteriaceae* and are found in the intestinal tract of warm-blooded animals (Jay et al., 2005). The HPP effects on viability of *Enterobacteriaceae* in soymilk were evaluated in the present study because salmonellosis and yersiniosis in humans have been traced to soybeans and soy products (ICMSF, 1998). In a previous study involving HPP of bovine milk, *E. coli* was more pressure-sensitive than the indigenous microflora (Pandey et al., 2003). That finding supports our results indicating pressure-induced inactivation of *Enterobacteriaceae* and no growth of this family of bacteria in treated samples during 28 days of storage. Although initial viable numbers of ENT and PSY were similar in the present study, the survival of some PSY but no ENT suggests that a mixture of Gram-positive and Gram-negative bacteria were likely present in the PSY group. The elimination of *Enterobacteriaceae* by HPP conditions used in the present study indicates the potential of HPP for improving the microbial safety of soymilk.

Sub-lethally injured microorganisms

Populations of sub-lethally injured cells can be quantified by plate counts using a non-selective growth medium with and without added NaCl (Wu et al., 2008). Sub-lethally injured cells are sensitive to salt concentrations due to the membrane damage

sustained during pressure treatment (Chilton et al., 2001). As expected, the bacteria in untreated soymilk exhibited no injury; approximately the same numbers of bacterial colonies were enumerated on both TSA and TSAN. On the day of treatment, soymilk pressurized at 400 MPa, 25°C for 1 min resulted in 62% sub-lethally injured survivors (Fig. 2). At that same temperature, application of 400 MPa (5 min) and 500 or 600 MPa (1 or 5 min) resulted in 100% injury to the surviving bacterial population. Treatment at 75°C resulted in 100% injury regardless of pressure level and dwell time. These results are supported by studies indicating that with higher temperatures and pressures greater amounts of injury are induced (Lopez-Pedemonte et al., 2007; Bayındırlı et al., 2006).

In most instances, no bacterial colonies were detected on TSA or TSAN when samples of soymilk were plated following pressurization (400–600 MPa) at 75°C. These results precluded calculation of percentage of sub-lethal injury. On day 4 or 7 during refrigerated storage of HPP-treated soymilk, emergence of bacterial colonies indicated that viable bacteria were present in samples that were subjected to pressure treatments at 75°C (Table 1). It is likely that survivors were merely present in numbers <1.0 CFU ml^{-1}. Alternatively, survivors might have endured severe sub-lethal injury and were unable to grow even on the non-selective TSA. Bozoglu et al. (2004) demonstrated two types of sub-lethal injury (I1 and I2) in bacteria after pressurization of UHT 1% low fat milk. Type-I2 sub-lethal injury is a severe injury and only after repair (I2 to I1) are the cells able to grow on non-selective but not on selective agar. Formation of colonies on both non-selective and selective agars occurs only when sub-lethally injured bacteria fully repair their injury and convert from I1 to active cells. The present study indicates that although bacterial survivors might not be detected in pressurized soymilk on the day of

treatment, cells with I2 type injury could potentially be present in this food product. In this regard, it is important to conduct microbial analyses over a period of time during which repair of type-I2 injury could permit detection of type-I1 injured cells or active cells to better predict the microbial shelf-life of soymilk.

pH and particle stability

The natural pH of soymilk is approximately 6.7 and was adjusted to 7.0 before HPP treatments. A pH of 7.0 is recommended to maintain the viscosity of pressurized soymilk similar to the untreated soymilk (Lakshmanan et al., 2006). None of the treatment conditions changed the soymilk pH value on the day of treatment. During storage, the pH of the untreated soymilk decreased steadily to reach a value of 4.8 after 28 days (Table 3). Characteristics of untreated soymilk are difficult to find in the literature as thermal treatment is traditionally applied during soymilk production. Decreases in pH of only 0.6–0.7 units were reported during storage of soymilk prepared using a thermal treatment of 116°C (Achouri et al., 2007). Regardless of the pressure and atmospheric storage conditions, a similar pH decrease was observed during storage of pressurized soymilks; the pH of pressurized soymilk after 28 days of storage was >6.4.

Spoilage of foods is usually accompanied by chemical and physical changes in the matrix due to compounds produced during bacterial growth and acidification of the media is one of the consequences of this spoilage. The extent of acidification is related to many parameters including the matrix composition such as presence of fermentable sugars and proteins, and type and growth phase of microorganisms. Pascall et al. (2006) demonstrated that the pH of soymilk artificially inoculated with *Bacillus subtilis*

decreased by <1.0 unit in 60 days while the pH of soymilk with *Bacillus stearothermophilus* decreased from 6.2 to 4.4 after 24 h at 55°C. The presence of indigenous acid producing bacteria might explain the previously stated pH drop occurring in the control soymilk, while growth of bacterial survivors in the pressurized sample had a minor impact on the soymilk acidification. Liu and Chang (2008) attributed decreased soymilk pH prepared from soybeans stored under different conditions to the hydrolysis of neutral lipid to fatty acids and the oxidation of fatty acids. Factors influencing these changes might also have occurred in the samples analyzed in the present study.

The physical stability of the soymilk was assessed by measuring the percentage of total sedimentable particles after low-speed centrifugation. On the day of treatment, the untreated sample had a stability index of 5.7%, which was comparable to the value obtained for soymilk homogenized with 80°C water (Cruz et al., 2007). The stability index of the untreated soymilk increased to 24.2% after 28 days of storage and confirmed the visual observation of the increased precipitation of particles throughout storage. Solubility of soy proteins is pH-dependent and acidification of soymilk probably contributed to the changes in the protein stability of untreated soymilk. Soy proteins solubility exhibits a U-shape pattern, with a lowest solubility at the isoelectric point of pH 4–5 (Jung et al., 2005). Overall, the pressurized soymilk showed an improved stability factor of at least 3.0 after 28 days of storage.

Soymilk is a colloidal suspension containing approximately 5% protein and 10% solids (Lakshmanan et al., 2006), and suspended particle stability depends on many parameters including its composition and processing methods applied (Cruz et al., 2007; Nik et al., 2008; Ono et al., 1991). Large aggregates containing lipid, cell wall debris and

proteins were identified in unheated soymilk (Bodenstab et al., 2003; Nik et al., 2008) and increased soymilk stabilities of heated and heated/homogenized soymilk were related to rupture of these large aggregates. On the other hand, Cruz et al. (2007) explained increased stability of homogenized soymilk by formation of protein and fat globule aggregates.

Conclusions

Under the test conditions used in the present study, the shelf-life of refrigerated pressurized soymilk can be extended for at least 2 wk longer than that of untreated soymilk based on the spoilage level of 10^7 log CFU ml^{-1}. In addition, the stability of pressurized soymilk can be maintained for 28 days at 4°C. HPP treatment is capable of improving shelf-life without negatively impacting the stability of the soymilk. Results of the present study indicate that HPP has the potential of being an alternative commercial process to traditional thermal treatments for extending the shelf-life of refrigerated soymilk.

Acknowledgements

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Table 1.

Total bacterial count (log CFU/ml) in pressurized soymilk during storage at 4°C[a].

Storage	Temperature (°C)	Pressure (MPa)	Dwell Time (min)	Time (days)							
				4	7	11	14	18	21	25	28
Aerobic	25	400	1	3.71	4.31	6.88	6.68	7.23	7.73	8.66	8.80
			5	3.89	3.94	6.33	6.79	6.91	8.12	8.49	8.93
		500	1	3.35	4.71	6.51	6.09	6.62	8.03	8.15	8.16
			5	2.34	4.69	6.03	5.35	6.90	7.93	7.91	8.00
		600	1	2.14	4.17	5.67	5.56	6.15	7.51	7.47	7.70
			5	2.21	4.67	5.69	4.87	5.59	6.80	7.37	7.59
	75	400	1	1.57	2.96	4.50	5.01	5.71	6.52	6.59	6.81
			5	0.58	1.91	4.06	4.62	5.15	6.02	6.20	6.40
		500	1	0.42	2.58	4.28	4.13	5.66	6.24	6.33	6.50
			5	0.38	2.30	3.42	4.47	5.46	6.05	6.15	6.41
		600	1	0.00	0.56	3.08	3.88	5.34	6.01	6.14	6.37
			5	0.00	0.46	3.09	3.56	5.21	5.70	5.82	6.14
Anaerobic	25	400	1	3.74	3.55	5.69	6.66	6.35	6.74	8.06	8.76
			5	2.95	3.39	4.19	4.85	5.58	6.50	7.31	8.02
		500	1	2.79	3.68	5.72	6.29	5.84	6.94	6.67	7.82
			5	2.61	3.18	4.35	5.48	5.61	6.49	6.70	7.46
		600	1	2.41	3.91	4.89	7.38	6.52	6.94	7.39	8.11
			5	2.30	3.07	4.60	6.92	5.90	6.21	7.22	7.95
	75	400	1	0.86	3.18	4.63	5.29	5.65	5.89	5.96	6.07
			5	0.53	3.05	2.66	4.68	5.25	5.44	5.61	5.69
		500	1	0.74	2.86	4.20	4.40	5.36	5.39	5.55	5.59
			5	0.50	2.83	4.06	4.71	5.43	5.54	5.60	5.59
		600	1	0.00	2.02	3.03	4.55	5.23	5.25	5.31	5.40
			5	0.00	1.78	2.60	4.16	5.03	5.13	5.21	5.29

Overall aerobic LSD was 1.62; aerobic LSD values for treatment at 25 and 75°C were 1.48 and 1.13, respectively. Overall anaerobic LSD was 2.58; anaerobic LSD values for treatment at 25 and 75°C were 2.14 and 1.74, respectively.
[a]The experiments were repeated twice and the data are expressed as mean log CFU/ml.

Table 2.

Psychrotroph count (log CFU/ml) in pressurized soymilk during aerobic storage at 4°C[a].

Temperature (˚C)	Pressure (MPa)	Dwell Time (min)	Time (days)								
			0	4	7	11	14	18	21	25	28
25	400	1	0.26	2.70	5.54	5.82	6.23	6.43	6.48	6.6	6.66
		5	0.26	2.10	2.03	5.27	6.27	6.32	6.35	6.4	6.38
	500	1	–	1.39	2.10	4.81	5.57	6.21	6.21	6.22	5.79
		5	–	1.44	1.50	4.66	5.35	6.11	6.11	6.12	6.12
	600	1	–	0.68	0.92	3.61	2.61	4.79	5.00	5.03	5.03
		5	–	0.61	1.04	2.11	2.82	2.99	3.22	3.40	3.32
75	400	1	–	–	0.74	1.75	2.39	2.83	2.83	2.95	3.22
		5	–	–	–	0.87	1.73	1.92	2.33	2.53	2.85
	500	1	–	–	–	–	–	–	–	–	–
		5	–	–	–	–	–	–	–	–	–
	600	1	–	–	–	–	–	–	–	–	–
		5	–	–	–	–	–	–	–	–	–

The sign (–) indicates that the values were below the detection limit of 1 CFU/ml. Overall aerobic LSD was 2.36; aerobic LSD value for treatment at 25°C was 2.16.

[a]The experiments were repeated twice, and the data are expressed as mean log CFU/ml.

Table 3.

pH of pressurized soymilk during storage at 4°C under aerobic and anaerobic conditions[a].

Storage Condition	Temperature (°C)	Pressure (MPa)	Time (days)								
			0	4	7	11	14	18	21	25	28
Control		0.1	6.7	6.5	6.0	6.4	5.8	5.5	5.3	5.1	4.8
Aerobic	25	400	7.0	7.1	7.0	7.1	7.1	6.6	6.7	7.0	7.1
		500	6.9	7.0	7.1	6.9	7.0	6.5	6.8	6.6	6.4
		600	6.8	7.0	6.9	6.8	6.7	6.4	6.7	6.9	6.9
	75	400	7.0	7.0	7.1	7.1	7.1	6.9	6.6	7.2	6.7
		500	6.9	7.0	7.0	7.2	7.1	7.1	6.8	7.1	6.8
		600	6.8	6.5	6.8	7.0	7.0	6.9	7.1	6.6	7.1
Anaerobic	25	400	–	7.1	7.0	6.6	7.1	7.0	6.7	6.9	6.5
		500	–	7.1	7.1	6.5	7.1	7.0	6.8	6.8	6.5
		600	–	7.0	6.9	6.4	6.6	6.6	6.5	6.8	7.0
	75	400	–	7.0	7.0	7.0	7.1	6.8	7.2	7.1	7.0
		500	–	7.0	6.9	7.1	7.0	7.1	7.3	7.0	7.0
		600	–	6.9	6.7	7.0	6.8	7.0	7.0	7.0	6.6

Untreated soymilk was stored anaerobically. The sign (–) indicates that the pH was not determined as the day of treatment; there were no difference between anaerobic and aerobic samples. Overall aerobic LSD was 0.4; aerobic LSD value was 0.4 for treatment at 25 and 75°C. Overall anaerobic LSD was 0.44; anaerobic LSD values for treatment at 25 and 75°C were 0.5 and 0.4, respectively.

[a]The experiments were repeated twice, and the data are expressed as mean log CFU/ml.

Table 4.

Stability of pressurized soymilk during storage at 4°C under aerobic and anaerobic conditions[a].

Storage	Temperature (°C)	Pressure (MPa)	Time (days)								
			0	4	7	11	14	18	21	25	28
Control		0.1	5.71	8.89	12.56	14.72	16.54	18.71	20.40	21.46	24.20
Aerobic	25	400	0.73	2.57	2.94	2.64	4.75	4.81	5.03	6.63	7.17
		500	1.54	2.30	3.74	2.67	4.04	4.26	7.10	7.00	7.06
		600	4.29	3.56	3.82	4.37	5.47	5.37	7.47	5.94	6.74
	75	400	2.95	1.55	2.27	3.24	2.71	2.97	3.89	4.96	4.61
		500	1.93	1.97	2.10	2.67	2.51	5.31	4.93	5.45	4.70
		600	3.16	1.64	2.00	3.69	3.92	7.21	4.73	3.22	3.74
Anaerobic	25	400	–	1.59	2.52	1.97	2.78	4.47	5.71	3.33	2.49
		500	–	1.46	2.75	3.64	2.78	2.99	2.98	3.62	2.73
		600	–	2.82	3.00	3.09	2.90	3.79	3.63	5.03	3.94
	75	400	–	2.49	3.40	3.86	3.64	4.42	3.94	4.38	5.63
		500	–	4.13	2.46	1.93	4.11	4.59	4.23	5.06	5.32
		600	–	2.81	4.14	4.12	4.15	5.56	5.48	5.62	5.52

The sign (–) indicated that the stability was not determined as the day of treatment, there was no difference between anaerobic and aerobic samples. Overall aerobic LSD was 1.45;aerobic LSD values for treatment at 25 and 75°C were 1.63 and 1.26, respectively. Overall anaerobic LSD was1.38; anaerobic LSD values for treatment at 25 and 75°C were 1.34 and 1.46, respectively.
[a]The experiments were repeated twice and the data are expressed as mean log CFU/ml.
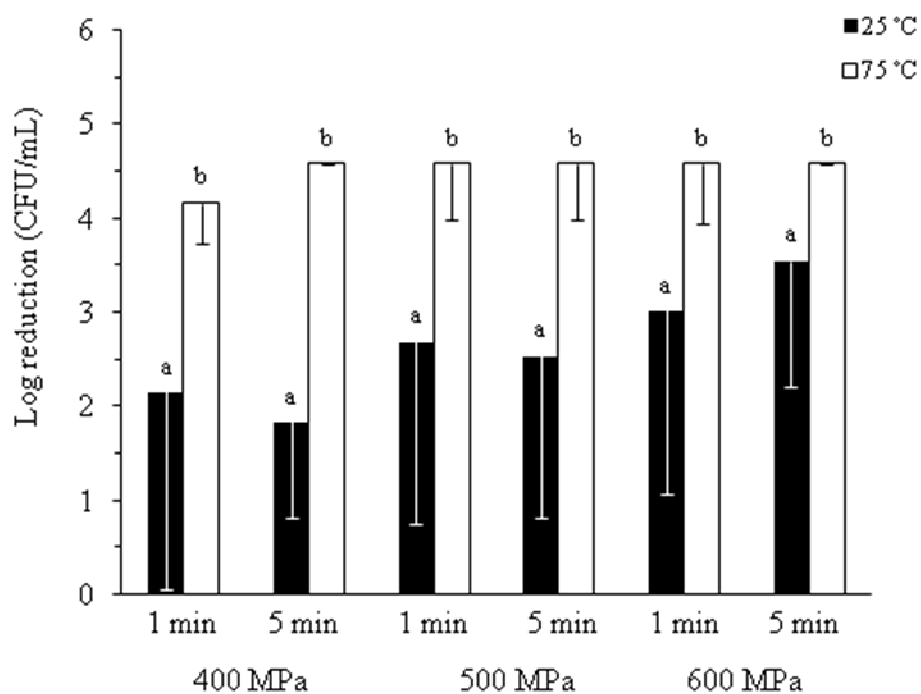
Fig. 1. Log reduction in initial total bacterial count of soymilk following pressurization. The data are expressed as mean log CFU/ml. LSD value between 25 and 75°C was 2.83. LSD value between 400, 500 and 600 MPa was 2.56. The bars represent the standard deviation.
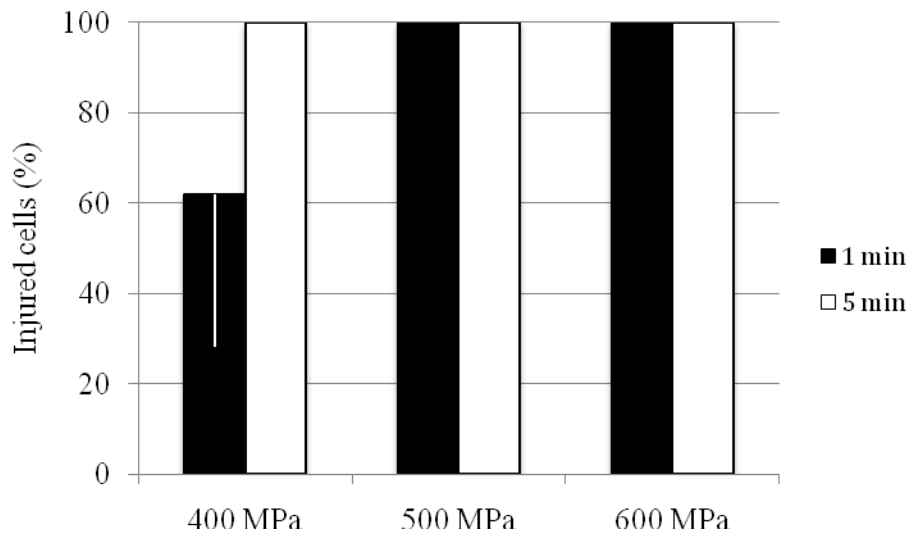
Fig. 2. Percentage of sub-lethally injured bacteria in soymilk following high-pressure treatment at 25°C. The bar represents one standard deviation.

CHAPTER 4. EFFECTS OF SOY PROTEIN INGREDIENTS, MOISTURE CONTENT AND SECOND-STAGE COOKING TEMPERATURE ON PHYSICAL PROPERTIES OF ALASKAN POLLOCK SURIMI

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ABSTRACT: The effects of added soy protein ingredients, methods of protein preservation, added moisture content, and second-stage cooking temperature on the texture and color of surimi gels made from Alaskan pollock were determined. Surimi samples were tested at various protein replacement levels (1, 3 and 5%), means of preservation (H_2O_2 and jet cooking), moisture contents (80, 81, 83 and 85%), and second-stage cooking temperatures (90 and 95°C). The type of soy protein used to extend surimi did not significantly affect gel texture and color. Preservation method of the soy protein ingredients significantly affected gel hardness at 95°C with jet-cooked soy protein producing harder gels. The level of protein replacement did not significantly affect surimi gel textural properties; fish protein could be replaced up to 5% with soy protein without adversely affecting texture. Gel hardness was

significantly increased by using 95°C second-stage heating temperature. Moisture level had no effect on surimi gel hardness or deformation but increased expressible water and decreased whiteness.

Introduction

Surimi is the Japanese term for mechanically deboned fish flesh that has been minced and mixed with cryoprotectants to extend frozen shelf-life (Nagai and others 2007). Surimi is commonly used as the base ingredient in the production of crab-flavored seafood (crab sticks). Seafood analogs are gelled protein products prepared by adding starch, egg white proteins, salt and vegetable oils to washed, minced fish muscle (Campo and others 2008). Surimi gel is a three-dimensional network and the texture of surimi after gelation primarily determines surimi gel quality (Lanier 1992). The hardness and strength of surimi gels are affected by added soy protein concentration and second-stage cooking temperature and duration as well as moisture content (Harper and others 1978, Camou and others 1989). Whiteness of surimi is also an important quality attribute (Choi and others 2000) with whiter gels being preferred.

Alaskan pollock is one of the most widely used fish species to produce surimi (Yoon and others 2004). In the United States, the demand for Alaskan pollock has increased while the harvest has decreased (Reed and others 2008). Filler ingredients have been used to extend surimi to meet market demand. The ingredients added to extend surimi should be considered to be healthy to suit consumer preferences, while improving the texture of the surimi (Lee and others 1992). Protein additives are widely used to increase gel strength in surimi. Sugar, sorbitol and salt are commonly added as

cryoprotectants to stabilize myofibrillar protein and maintain the functionality of the fish proteins (Matsumoto 1979, Park 1988, Lanier 1990). Starch, dried egg white and soy protein isolate (SPI) are preferred functional ingredients used by the surimi industry because of their abilities to form gels and retain water while being light in color (Choi and others 2000). SPI decreases proteolysis because it replaces fish protein so there is less myofibrillar protein to be degraded. The disintegration of myofibrillar proteins decreases gel-forming properties of surimi (An and others 1996) because it inhibits the development of a three-dimensional gel network (Morrissey and others 1993).

The major soy storage proteins, glycinin (Gly) and β-conglycinin (Bcon), have unique functional properties. Gly produces hard and tough gels while Bcon increases elasticity of soy protein gels at high heating temperatures (Kang and others 2005). Soy protein lowers cholesterol and triglycerides levels in human blood serum (Kito and others 1993, Aoyama and others 2001). Bcon has been associated more with these health benefits.

SPI is conventionally produced by using hexane as the solvent to extract the lipid and flash desolventizing the meal to prevent heat denaturation of soy proteins during solvent removal. Crown Iron Works (St. Paul, MN, U.S.A.) and SafeSoy Technologies (Ellsworth, IA, U.S.A.) have developed a new oil-extraction process using CO_2 as a displacement fluid to displace the oil from dehulled flaked soybeans when subjected to the high pressures of screw pressing. The process has been termed gas-supported screw pressing (GSSP) or HYPLEX®. GSSP is not supercritical but rather liquid CO_2 displaces the oil enhancing oil removal and the flashing of CO_2 cools the press to substantially reduce protein denaturation that is common to alternative screw-pressing methods. Nazareth and others (2008) reported that the

functionality of the SPI prepared from GSSP meal (GSSP SPI) is similar to the functionality of SPI produced from commercial hexane-extracted, flash-desolventized white flakes (WF SPI).

The most effective method of fractionating soy protein into Gly-rich and Bcon-rich fractions (SPF, soy protein fractions) is the method of Deak and Johnson (2005, 2007) and Deak and others (2006). Another process has been developed (Fig. 1) that removes the Bcon fraction while leaving the fiber and the glycinin in one fraction (Gly+Fiber-rich) (Deak and Johnson 2005).

The manufacture of soy protein ingredients involves producing aqueous solutions of protein, which are prone to microbial growth. Industry typically jet cooks SPI by direct steam injection and holding at 100-110°C for 10-20 sec before spray drying. The high temperatures used in jet cooking denature the protein but the high-shear tends to preserve many native functional properties by producing very small protein aggregates. Alternatively, H_2O_2 can be used as an antimicrobial agent and reduce protein denaturation (Deak and others 2007).

It is unknown whether GSSP soy protein ingredients (SPI or fractionated soy protein) can be advantageously used to extend surimi and whether these soy protein ingredients enable increasing the water content above the normal industry level. The objective of the present study was to determine the effects of GSSP SPI and fractionated soy protein ingredients, method of preservation, second-stage cooking temperature and moisture content on the physical properties of surimi produced from Alaskan pollock.

Materials and Methods

Chemicals

Sucrose, sodium chloride and sorbitol were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Spray-dried egg white was purchased from MP Biomedicals (Solon, OH, U.S.A.). Sodium tripolyphosphate was supplied by Acros Organics (Morris Plains, NJ, U.S.A.).Cornstarch was obtained from Cerestar (Hammond, IN, U.S.A.).

Soy protein preparation

SPI and soy protein fractions (SPF) glycinin-rich (Gly-rich), β-conglycinin-rich (Bcon-rich) and the glycinin- and fiber-rich (Gly+Fiber-rich) were produced by using the procedures of Deak and Johnson (2007) (Fig. 1) using GSSP soy flour. SPI was produced by alkali extraction and precipitation to remove the insoluble fiber and the adjusting the pH to 4.5 to precipitate the SPI. The Gly-rich and Bcon-rich fractions were produced by using a three-step process. The fiber was removed as in preparing SPI and removed and then adding $NaHSO_3$ and $CaCl_2$. The pH was adjusted to 6.4 to precipitate a Gly-rich fraction. The supernatant was adjusted to pH 4.8 to precipitate a Bcon-rich fraction. Preparation of the Gly+Fiber-rich fraction involved extracting a Bcon-rich fraction at pH 6.8 leaving behind a Gly-rich and fiber-rich fraction. All fractions were neutralized and either jet cooked at 105°C for 17 sec or treated with 0.1% hydrogen peroxide (H_2O_2) to reduce microbial load and assure food safety. All samples were spray-dried and stored in airtight containers at 4°C until used.

Protein content and profile analysis

Nitrogen contents were measured using the macro-Kjeldahl method (AOAC 1980). Protein content was calculated at N x 6.25.

Urea-sodium-dodecylsulfate polyacrylamide gel electrophoresis (urea-SDS-PAGE) was performed by using the methods of Rickert and others (2004) to determine the protein composition profiles of all fractions. Electrophoretic bands were identified by using a pre-stained SDS-PAGE low-range molecular-weight standard (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Gly and Bcon subunit bands were confirmed by using purified standards produced according to methods of O'Keefe and others (1991). Densitometry was carried out by using the Kodak 1D Image Analysis version 3.5 (Kodak, Rochester, NY, U.S.A.) on images scanned with a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ, U.S.A.). SDS-PAGE results were calculated as % composition: total storage protein in a given fraction = [(sum of storage protein subunit bands)/(sum of all bands)] x 100, fraction purity/composition = [(sum of subunit bands)/(sum of storage protein bands)] and subunit composition of a specific protein = [(subunit band)/(sum of subunits for the specific protein)]. All measurements were replicated at least four times and means reported.

Gelling properties of soy protein ingredients

To determine gelling properties of the soy proteins alone, the soy protein ingredients were brought to room temperature and then mixed with deionized water at room temperature at a 1:5 protein-to-water ratio. The mixture was stirred until the protein was completely dissolved (~1 h). The mixture was poured into stainless-steel cylinders

(25.4 mm inner diameter x 25.4 mm length) and sealed with lids on both ends and secured with a clamp (Fig. 2). The gels were set by heating them in a mineral oil bath at 40°C for 30 min. The gels underwent irreversible gelling during second-stage cooking(gel-setting) at 95°C for 20 min. The heating curve of the control surimi gel is shown in Fig. 3. The gels were cooled in an ice water bath for 3 min and stored in the refrigerator for 16 h.

Surimi preparation

Frozen Alaska pollock fillets were obtained from a local supermarket. Fillets were partially thawed and minced to uniformity in a KitchenAid mixer with a food grinder attachment (St. Joseph, MI, U.S.A.). Surimi was prepared according to the method of Benjakul and others (2004) with modifications. The minced fish was washed with cold water (5°C) at 1:3 (w/w) minced fish-to-water ratio. The mixture was gently stirred for 3 min and centrifuged (Sorvall RC 5B Plus, Thermo Scientific, Ashville, NC, U.S.A.) at 8000xg and 4°C for 30 min. Washing and centrifuging were repeated twice (once with deionized water and then with 0.2% NaCl in deionized water to aid dewatering). After the second centrifugation, the minced fish was squeezed between two layers of cheesecloth to remove excess water. The washed and dewatered mince was mixed with 4% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate, and kept frozen until used.

Surimi gel with added soy protein

The surimi prepared as above was thawed overnight in the refrigerator. Then, 1% egg white, 4% starch and 2% NaCl based on surimi weight were mixed with the SPI or

SPF (0, 1, 3 or 5% w/w minced fish). The mixture was added to the surimi (75, 73, 71 or 68% w/w) and mixed to a thick paste. The moisture level was adjusted to 80% with cold deionized water and 1% vegetable oil was mixed into the paste. The pastes were placed in the stainless-steel cylinders and cooked in a mineral oil bath for 20 min at 90 or 95°C after setting for 30 min at 40°C. After the two-step cooking procedure, the gels were cooled in an ice water bath for 3 min. The cooled gels were placed in airtight plastic containers and stored in the refrigerator for 16 h.

Surimi gels with increased moisture contents

The surimi prepared as above was thawed overnight in a refrigerator. Then, 1% egg white, 4% starch and 2% sodium chloride were mixed together with the SPI or SPFs (5% w/w). The mixture was added to the surimi (68% w/w) and mixed to a thick paste. The moisture level was adjusted to 80, 81, 83 or 85% with cold deionized water and 1% vegetable oil was mixed into the paste. The paste was placed in the stainless-steel cylinders. The pastes were cooked in a mineral oil bath for 20 min at 95°C after setting for 30 min at 40°C. The gels were cooled in an ice water bath for 3 min. The cooled gels were placed in airtight plastic containers and stored in the refrigerator for 16 h.

Texture profile analysis

Texture profile analysis (TPA) of the gels was performed with a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) and a 5-kg load cell. The gels were equilibrated and analyzed at room temperature. Breaking force (gel hardness) and

deformation (elasticity/deformability) were measured by using a cylindrical plunger (25.4 mm diameter) with a two-bite compression speed of 1.2 mm/min and 75% compression.

Color analysis

Color analysis was conducted by using a HunterLab Scan XE (Reston, VA, U.S.A.) and analyzed with Universal Software V4.10. Samples from each treatment were subjected to Lab and whiteness measurements. White and black standard plates were used for calibration. Whiteness was calculated by using the following equation (Park 1995).

$$Whiteness = 100-[(100-L)^2 + a^2 + b^2]^{1/2}$$

Expressible water

Expressible water was measured according to the method of Ng (1987). Cylindrical gel samples were sliced to 5.0 mm thickness, weighed and placed between five pieces of Whatman No. 1 filter paper; three pieces on the bottom and two pieces on the top. The standard weight (5 kg) was placed on the top of the sample for 2.0 min. The weight was removed and the sample weighed again (Y). The expressible water was reported as the percentage of lost water weight relative to the original sample weight (X).

$$Expressible\ moisture\ (\%) = [(X-Y)/X] \times 100$$

Statistical analysis

In the first experiment to evaluate the gelling properties for soy proteins alone, the treatments were replicated three times. In the second experiment, the experimental design was a 5 x 3 x 2 x 2 factorial design with 5 soy protein ingredients, 3 levels, 2 protein

preservation methods, and 2 setting temperatures. Three replicates were made for each treatment. In the third experiment, all analyses were run in duplicate for triplicate surimi preparations (n = 2 x 3). Results are reported as mean values of six determinations ± standard deviation (SD). Data were analyzed by Analysis of Variance. Differences among the treatment means were determined by using the least significant difference (LSD) test with significance defined at $P<0.05$.

Results and Discussion

Compositional properties of soy protein ingredients

Protein contents and profiles of the GSSP soy protein ingredients are shown in Table 1. The SPI prepared from GSSP meal contained only 85% protein (db), which is less than the minimum industry protein level of 90% for SPI. GSSP meal typically has a protein dispersibility index of about 70 compared to 80 for WF. The slightly less extractable protein of GSSP meal resulted in lower protein contents in SPI made from GSSP meal. The SPI prepared from GSSP meal had 1:1 Bcon-to-Gly ratio as is typical of SPI prepared from WF. The Bcon-rich fraction contained 80% protein and 67% Bcon, and was enriched to 2.4:1 Bcon-to-Gly ratio, where as the Gly-rich fraction contained 92% protein and 78% Gly, and was enriched to 0.1:1Bcon-to-Glyratio. The Gly+Fiber-rich fraction contained 65.4% protein and 54% Gly, and had 0.4:1 Bcon-to-Gly ratio; the fiber greatly reduced the protein content making it more like a protein concentrate (min 65% protein) than an isolate. Therefore, the three fractionated protein ingredients were substantially enriched in the targeted proteins.

Gelling properties of fractionated soy protein ingredients

Each soy protein fraction had substantially different textures as indicated by gel hardness values (Fig. 4). The gels prepared from the Bcon-rich fraction were significantly softer than the gels prepared from SPI or the Gly-rich fraction. H_2O_2-treated and jet-cooked proteins produced gels that were not significantly different from each other. The gels prepared from jet-cooked SPI and jet-cooked Gly-rich fraction were also not significantly different from each other. Gels prepared from the H_2O_2-treated Gly-rich fraction were significantly harder than all other gels. Gels prepared from H_2O_2-treated Gly-rich fraction were >100% harder than its jet-cooked counterpart and exhibited the look and feel of a hardboiled egg (Fig. 5). This observation suggests that native Gly, but not denatured Gly, gives harder gels.

Effects of type of soy protein ingredient on surimi quality

In general, surimi gels with added soy protein were as hard or harder than the control (without added soy protein); adding soy protein made the surimi gels more firm, except for the Gly+Fiber-rich fraction(Tables 2 and 3). Probably the fiber interfered with protein strands coming together to form a gel. In general, the type of soy protein added to the surimi gels did not have a significant impact on the gel hardness (Table 4). The H_2O_2-treated Gly-rich fraction did not produce harder surimi gels than the Bcon-rich fractions as the gelling properties of the 20% soy protein gels suggested. Therefore, fractionating soy protein did not produce fractions with superior hardness in surimi.

Deformation is the distance in compression that is needed to break a gel. Increased distance indicates more flexibility and more flexible gels are considered by the

surimi industry to be a good quality attribute. Protein type did not significantly affect the deformation of soy added surimi gels. All protein types were statistically similar (Table 5) to each other as well as the controls at 90 (Table 2) and 95°C (Table 3). Therefore, fractionating soy protein did not produce fractions with superior deformation in surimi.

Low expressible water is a desirable attribute in surimi because the product has better water-holding capacity and does not easily loose water. Soy protein type did not significantly affect expressible water: all soy protein products gave similar results (Tables 2 and 3). With the addition of soy protein, expressible water was not significantly different from the control (Table 6). At 95°C, the H_2O_2-treated Bcon-rich fraction significantly improved the expressible water compared to controls at 90 and 95°C as well as all other protein types.

Consumers expect white-colored seafood analogs and, therefore, a high whiteness value is desirable in surimi gels. Type of protein did not significantly affect surimi gel whiteness compared to the control (Fig. 5). Therefore, fractionated soy proteins do not offer any advantages to surimi color.

Effects of soy protein level on surimi quality

The level of protein replacement did not significantly affect hardness (Table 4) and no consistent trends for level of soy protein addition on hardness were observed (Tables 2 and 3). Higher replacement of fish protein with soy protein did not always have the same effects on surimi gel hardness. Our hypothesis that H_2O_2-treated Gly-rich fraction would increase hardness as more fish was replaced proved incorrect as did our hypothesis that increased level of Bcon-rich fraction would decrease hardness. The gels

prepared with 5% H_2O_2-treated Gly-rich fraction were softer than the controls without added soy protein. Our results, however, are consistent with those of Chang-Lee and others (1990) who reported that adding 1% SPI had no significant effect on the gel strength of whiting surimi. Yang and others (1992) reported that replacing 11% Alaskan pollock protein with SPI produced a stronger gel than surimi alone after setting at 55°C for 30 min.

Protein level had a significant impact on surimi gel deformation (Tables 2, 3 and 5). The effect of replacement level was temperature dependent. Gels of soy protein extended surimi cooked at 90°C did not have significantly different deformation values compared to the control. Jet-cooked Gly and H_2O_2-treated Bcon at 5% protein replacement and cooked at 95°C were the only gels significantly more flexible than the control gels. H_2O_2-treated SPI at 1% replacement and cooked at 95°C produced gels that were significantly more rigid than the control gel; but, higher replacement levels were not significantly different from the control. These findings are consistent with those of Luo et al. (2004) who reported that the breaking force and distance were significantly increased at 10% protein replacement with SPI prepared from WFs in Alaskan pollock surimi.

Protein level did significantly affect expressible water of soy protein extended surimi gels (Table 6). H_2O_2-Bcon at 1, 3 and 5% replacement, however, significantly reduced expressible water compared to the control and all other surimi gels.

Level of soy protein replacing fish protein significantly affected whiteness of surimi gels (Table 7). The effect of protein level was dependent on the temperature at which the gels were cooked. Replacing fish protein with 3 and 5% soy protein significantly affected the whiteness of the surimi gels when cooked at 95°C (Table 3).

These gels were significantly less white than gels cooked at 90°C (Table 2). Surimi extended with 3 and 5% jet-cooked Gly+Fiber-rich fraction and cooked at 90°C were significantly whiter than surimi gels with 5% jet-cooked SPI and cooked at 95°C. Colors were significantly whiter at lower replacement levels (0 and 1%). As more fish protein was replaced with soy protein, whiteness decreased. Surimi gels with 3% jet-cooked Bcon-rich fraction and cooked at 90°C at 3% were significantly whiter than surimi gels with 5% H_2O_2-treated Bcon and jet-cooked SPI when the gels were cooked at 95°C. Our observations are consistent with those of Luo et al. (2004) who observed that Alaskan pollock surimi at 10% replacement with SPI prepared from WFs were significantly darker than the control (without added SPI).

Effects of soy protein preservation method on surimi quality

The method used to preserve soy protein significantly affected surimi hardness when the gels were cooked at 95°C (Table 4) but not at 90°C (Table 2). At 95°C second-stage cooking temperature, jet-cooked soy proteins produced gels that were significantly harder than those produced with H_2O_2-treated protein ingredients (Table 3). The jet-cooked soy protein ingredients were fully denatured but the H_2O_2-treated soy proteins were not (Nazareth 2009).

Preservation method did not significantly affect expressible water (Table 6) of the surimi gels cooked at 90°C (Table 2) and 95°C (Table 3). Protein denaturing that occurred with jet cooking did not affect expressible water of the soy protein-added surimi gels.

The method used to preserve soy protein did not significantly affect the whiteness of the surimi (Tables 2, 3 and 7, Figure 5). These results were surprising and rejected our hypothesizes that jet cooking would have caused Maillard reaction browning whereas hydrogen peroxide would have bleaching of Maillard reaction products.

Effects of second-stage cooking temperature on surimi quality

Second-stage cooking temperature significantly affected hardness (Table 4). Cooking at 95°C (Table 3) produced harder gels than cooking at 90°C (Table 2).Higher second-stage cooking temperatures denature more protein and, thereby, increase hardness. We hypothesized that higher second-stage cooking temperatures would be required when using Gly in surimi because Gly denatures at 93°C whereas Bcon denatures at 75°C, but this hypothesis was rejected. Surimi gels prepared with 3% jet-cooked and H_2O_2-treated Gly and cooked at 95°C were significantly harder than the control and the Gly+Fiber-rich fraction cooked at 90°C. Surimi gels prepared with 1% jet-cooked SPI and 5% jet-cooked and H_2O_2-treated Bcon and cooked at 95°C were significantly harder than the control cooked at 90°C.

Second-stage cooking temperature did not significantly affect deformation (Table 5). At 90°C second-stage cooking, all surimi gels were as flexible as the control (Table 2) while most of the surimi gels cooked at 95°C were as flexible as the control (Table 3).

Second-stage cooking temperature significantly affected expressible water (Table 6). Cooking surimi at 90°C produced more expressible water than surimi cooked at 95°C. Higher second-stage cooking temperatures may cause more extensive protein denaturation; denatured soy protein may bind more water than undenatured soy protein.

Whiteness was significantly affected by second-stage cooking temperature (Table 7 and Figure 5). The surimi gels cooked at 90°C (Table 2) were significantly whiter than the gels cooked at 95°C (Table 3). Surimi gels made with 5% soy protein and cooked at 95°C were significantly less white than all other treatments. Park (1995) reported that increasing second-stage cooking temperature increased whiteness. At 95°C, all samples were as white as or whiter than the control. Adding more soy protein reduced whiteness especially when cooking at 90°C.

Effects of water level on surimi quality.

Esturk et al. (2006) and Reppond et al. (1997) reported that increasing moisture content decreased the strain and shear stress of surimi gels. The hardness values for Alaskan pollock surimi gels with different moisture levels are shown in Table 8. Increased moisture content did not significantly affect surimi gel hardness (Table 9) compared to the control (81% moisture). All surimi gels extended with soy protein products were not statistically different from the control. Therefore, soy protein (including fractionated soy protein) can be added to surimi without adversely affecting texture. The hardness of gels prepared with SPI prepared from GSSP meal and white flakes, however, were significantly different from each other (Table 4). Surimi gels with 83% moisture content were less hard than the control with no added soy protein at 81% moisture content. At 85% moisture content the surimi gels were less hard than the control at 81% moisture content.

Increasing the moisture contents of all soy protein-extended gels did not significantly affect surimi gel deformation. These findings are contrary to the report of

Hsu et al. (2002) that increasing moisture content in surimi gels decreased deformation. It is possible that the partial protein denaturation caused by GSSP was responsible for the soy proteins maintaining flexibility in surimi gels with increasing moisture content.

Moisture content significantly affected expressible water of surimi gels (Tables 8 and 9). Increasing the moisture content significantly increased expressible water in almost all gels. The control at 80% moisture and soy protein-extended gels at 81% moisture was not significantly different. Surimi gels with 83 and 85% moisture content had significantly more expressible water compared to the control but were not different from gels with 81% moisture content. The surimi gels prepared with Gly-rich, Bcon-rich and SPI prepared from white flakes had significantly less expressible water than the control. Replacing fish protein with up to 5% GSSP SPI did not cause significant changes to the expressible water of surimi gels. Our observations do not agree with the observations of others that expressible water is highly dependent on cooking temperature but not on moisture content of surimi gels (Park et al., 2008).

Moisture content significantly affected the whiteness of surimi gels (Table 9). The effect of moisture level was dependent on the protein type added to the surimi gels. The control gels were significantly whiter than all surimi gels extended with soy protein, regardless of moisture content, and became whiter as moisture content increased. As moisture content increased from 80 to 85%, the whiteness of surimi gels extended with soy protein significantly decreased (Table 8). SPI prepared from white flakes and GSSP meal were the least white of all surimi gels extended with soy protein. The jet-cooked Gly-rich fraction was the whitest of all surimi gels extended with soy protein. Our findings differ from those of others (Reppond and others 1997, Park 1995, Yoon and

others 1997) who reported that increasing the moisture content increased the whiteness of surimi.

Conclusions

Adding soy protein increases surimi gel hardness and can extend surimi up to 5% replacement without adversely affecting gel hardness, deformation, expressible water or whiteness. The type of protein added did not affect texture or color; therefore, there is no advantage of fractionated soy protein over SPI. The Gly+Fiber-rich fraction produced gels as hard as or less hard than the control and, therefore, it would not be advantageous to use this fraction to extend surimi. When cooked at 95°C higher replacement levels produced greater flexibility in the surimi gels, which is advantageous. Whiteness was decreased with the addition of soy protein ingredients. At 95°C second-stage cooking temperature, jet-cooked soy protein produced significantly harder gels than H_2O_2-treated soy protein. Second-stage cooking temperature of 95°C produced harder gels with less expressible water and less white color compared to gels cooked at 90°C. Moisture level did not affect hardness or deformation; therefore, added water can be used to extend surimi without adversely affecting texture. At 5% replacement, soy protein-extended surimi gels had similar expressible water properties to the control at 80% moisture content. The optimum texture and color of surimi gels were obtained by replacing fish protein with 5% GSSP soy protein, except for the Gly+Fiber-rich fraction at 85% moisture content.

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Table 1 – Compositional properties of soy protein ingredients prepared from GSSP meal.

Soy Protein Ingredient	Protein Content (% db)	β-Conglycinin (% total protein)	Glycinin (% total protein)	Others (% total protein)	Bcon:Gly ratio
SPI	85.0	47	43	10	1.1:1
Bcon-rich	80.3	67	28	5	2.4:1
Gly-rich	91.9	9	78	13	0.1:1
Gly+Fiber-rich	65.4	21	54	25	0.4:1

Table 2 – Textures and colors of surimi gels as affected by extending with soy protein ingredients when cooked at 90℃

| Property | Control | SPI | | | | | | Glycinin-rich | | | | | | Glycinin + Fiber | | | β-Conglycinin-rich | | | | | | LSD |
| | | Jet-cooked | | | H₂O₂-treated | | | Jet-cooked | | | H₂O₂-treated | | | H₂O₂-treated | | | Jet-cooked | | | H₂O₂-treated | | | |
	0%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	
Hardness[a]	338	590	612	799	631	532	513	754	399	434	377	635	554	314	424	333	693	633	415	647	494	636	138
Deform[b]	6.5	6.9	6.3	6.3	5.9	5.7	6.6	6.8	7.1	6.8	6.8	6.4	5.6	6.8	7.2	7.2	6.0	7.2	6.5	5.9	5.9	6.2	0.9
Ex Water[c]	43.5	30.5	30.9	27.8	38.6	27.4	30.6	30.9	39.4	39.0	38.5	33.9	31.9	36.8	36.2	42.0	29.1	29.2	33.0	29.9	37.0	29.3	3.4
Whiteness[d]	38.4	41.2	40.2	38.8	42.1	41.9	39.1	38.6	39.5	40.9	45.6	40.2	38.4	44.7	41.9	42.0	41.0	42.2	40.3	39.5	40.4	35.3	2.8

[a]Force (g) required to break gel, [b]Distance (mm) travelled when gel breaks, [c]Water (%) pressed from gels,
[d]Whiteness$=100-[(100-L)^2 + a^2 + b^2]^{1/2}$

Table 3 – Textures and colors of surimi gels as affected by extending with soy protein ingredients when cooked 95°C

Property	Control	SPI Jet-cooked			SPI H₂O₂-treated			Glycinin Jet-cooked			Glycinin H₂O₂-treated			Glycinin + Fiber H₂O₂-treated			β-Conglycinin Jet-cooked			β-Conglycinin H₂O₂-treated			LSD
	0%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	1%	3%	5%	
Hardness[a]	728	945	816	869	873	702	696	742	977	966	748	667	561	652	642	529	711	762	1026	902	674	1076	138
Deform[b]	6.7	6.0	6.6	6.9	5.5	5.9	6.8	6.1	7.4	7.5	5.9	6.5	9.0	7.0	6.8	7.1	7.3	7.5	8.1	6.5	6.3	6.8	0.9
Ex water[c]	27.6	24.8	25.7	24.3	26.2	23.2	26.2	25.5	24.1	24.9	25.8	25.6	28.0	28.9	24.1	27.5	25.6	26.5	23.6	22.4	23.1	20.2	3.4
Whiteness[d]	41.0	36.7	36.9	30.9	35.7	36.3	32.3	39.8	37.8	35.6	40.5	36.7	36.3	40.7	39.7	38.4	40.5	35.5	34.0	39.7	34.7	31.5	2.8

[a]Force (g) required to break gel, [b]Distance (mm) travelled when gel breaks, [c]Water (%) pressed from gels,
[d]Whiteness=100-[(100-L)^2 + a^2 + b^2]$^{1/2}$

Table 4 – ANOVA of the hardness of surimi gels as affected by extending with soy protein ingredients

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep[a]	2	18428.304	9214.152	0.22	0.8010
temp[b]	1	1051826.178	1051826.178	25.42	<.0001
prt[c]	2	110208.904	55104.452	1.33	0.2707
temp*prt	2	225584.761	112792.381	2.73	0.0725
preservation[d]	1	124766.413	124766.413	3.01	0.0869
temp*preservation	1	167371.320	167371.320	4.04	0.0482
prt*preservation	2	133788.175	66894.088	1.62	0.2059
temp*prt*preservatio	2	133587.420	66793.710	1.61	0.2064
level[e]	2	76675.058	38337.529	0.93	0.4008
temp*level	2	78948.661	39474.331	0.95	0.3902
prt*level	4	122258.693	30564.673	0.74	0.5688
temp*prt*level	4	259652.438	64913.109	1.57	0.1923
preservation*level	2	8680.643	4340.321	0.10	0.9006
temp*preservat*level	2	165005.481	82502.740	1.99	0.1439
prt*preservati*level	4	197237.756	49309.439	1.19	0.3222
temp*prt*prese*level	4	363184.275	90796.069	2.19	0.0785

[a]Replicate,[b]Cooking temperature,[c]Protein type,[d]Preservation method,[e]Level of protein replacement

Table 5 – ANOVA of the deformation of surimi gels as affected by extending with soy protein ingredients

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep[a]	2	0.92249725	0.46124863	0.42	0.6580
temp[b]	1	5.96782552	5.96782552	5.45	0.0225
prt[c]	2	5.93932889	2.96966445	2.71	0.0735
temp*prt	2	2.24712293	1.12356147	1.03	0.3639
preservation[d]	1	6.71280139	6.71280139	6.13	0.0157
temp*preservation	1	0.28198002	0.28198002	0.26	0.6135
prt*preservation	2	1.78739000	0.89369500	0.82	0.4464
temp*prt*preservatio	2	1.66739754	0.83369877	0.76	0.4710
level[e]	2	7.64398446	3.82199223	3.49	0.0359
temp*level	2	10.51182339	5.25591169	4.80	0.0111
prt*level	4	0.86263315	0.21565829	0.20	0.9392
temp*prt*level	4	6.97660214	1.74415053	1.59	0.1860
preservation*level	2	2.75353580	1.37676790	1.26	0.2909
temp*preservat*level	2	0.70682939	0.35341469	0.32	0.7253
prt*preservati*level	4	1.42459079	0.35614770	0.33	0.8602
temp*prt*prese*level	4	3.97044728	0.99261182	0.91	0.4652

[a]Replicate,[b]Cooking temperature,[c]Protein type,[d]Preservation method,[e]Level of protein replacement

Table 6 – ANOVA of the expressible water of surimi gels as affected by extending with soy protein ingredients

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep[a]	2	653.859346	326.929673	5.63	0.0054
temp[b]	1	1198.521959	1198.521959	20.64	<.0001
prt[c]	2	220.651907	110.325953	1.90	0.1572
temp*prt	2	293.128222	146.564111	2.52	0.0874
preservation[d]	1	0.372653	0.372653	0.01	0.9364
temp*preservation	1	10.302053	10.302053	0.18	0.6749
prt*preservation	2	23.407663	11.703831	0.20	0.8179
temp*prt*preservatio	2	132.298341	66.149170	1.14	0.3259
level[e]	2	12.814134	6.407067	0.11	0.8957
temp*level	2	28.369802	14.184901	0.24	0.7839
prt*level	4	106.388358	26.597089	0.46	0.7662
temp*prt*level	4	15.932700	3.983175	0.07	0.9912
preservation*level	2	69.679785	34.839893	0.60	0.5516
temp*preservat*level	2	31.787201	15.893601	0.27	0.7613
prt*preservati*level	4	141.123320	35.280830	0.61	0.6585
temp*prt*prese*level	4	157.190081	39.297520	0.68	0.6103

[a]Replicate,[b]Cooking temperature,[c]Protein type,[d]Preservation method,[e]Level of protein replacement

Table 7 – ANOVA of the whiteness of surimi gels as affected by extending with soy protein ingredients

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep[a]	2	78.4208574	39.2104287	3.72	0.0292
temp[b]	1	351.8486502	351.8486502	33.35	<.0001
prt[c]	2	41.7883366	20.8941683	1.98	0.1456
temp*prt	2	15.3651977	7.6825988	0.73	0.4864
preservation[d]	1	1.0237521	1.0237521	0.10	0.7563
temp*preservation	1	0.9756502	0.9756502	0.09	0.7619
prt*preservation	2	48.8865181	24.4432590	2.32	0.1061
temp*prt*preservatio	2	18.2805421	9.1402711	0.87	0.4249
level[e]	2	278.7836310	139.3918155	13.21	<.0001
temp*level	2	85.4613310	42.7306655	4.05	0.0216
prt*level	4	35.4630093	8.8657523	0.84	0.5042
temp*prt*level	4	39.9787565	9.9946891	0.95	0.4419
preservation*level	2	23.2856264	11.6428132	1.10	0.3374
temp*preservat*level	2	8.4177227	4.2088613	0.40	0.6725
prt*preservati*level	4	36.6159389	9.1539847	0.87	0.4878
temp*prt*prese*level	4	43.6306287	10.9076572	1.03	0.3959

[a]Replicate,[b]Cooking temperature,[c]Protein type,[d]Preservation method,[e]Level of protein replacement

Table 8 – Texture and color properties of surimi gels as affected by moisture content when cooked at 95°C

| Property	Control				5% Jet-cooked WF SPI				5% Jet-cooked GSSP SPI				5% Jet-cooked Gly-rich				5% Jet-cooked β-Con-rich				LSD
	80%	81%	83%	85%	80%	81%	83%	85%	80%	81%	83%	85%	80%	81%	83%	85%	80%	81%	83%	85%	
Hardness[a]	762	731	546	485	695	720	683	680	636	562	555	512	599	638	599	578	809	698	628	535	89
Deform[b]	6.7	7.3	6.8	6.5	7.5	7.8	7.7	7.1	6.9	7.3	6.5	6.4	7.5	7.5	7.1	6.2	8.1	7.7	7.4	6.6	0.7
Ex Water[c]	23.9	24.1	26.4	27.0	20.7	21.5	24.2	23.1	21.7	23.2	23.6	24.9	20.9	23.2	23.6	24.3	20.9	22.6	23.6	24.9	1.3
Whiteness[d]	42.0	39.0	41.1	45.9	34.4	26.1	26.9	25.5	30.9	26.2	28.1	26.7	35.1	36.2	33.7	35.1	34.0	30.8	29.3	30.6	1.7

[a]Force (g) required to break gel, [b]Distance (mm) travelled when gel breaks, [c]Water (%) pressed from gels,
[d]Whiteness$=100-[(100-L)^2 + a^2 + b^2]^{1/2}$

Table 9 – ANOVA of the texture and color properties of surimi gels as affected by moisture content

Property	Source	DF	Type III SS	Mean Square	F Value	Pr > F
Hardness	rep[a]	2	45484.3423	22742.1712	2.45	0.0995
	prt[b]	4	113990.3311	28497.5828	3.07	0.0274
	level[c]	3	180024.0463	60008.0154	6.47	0.0012
	level*prt	12	137525.8966	11460.4914	1.24	0.2953
Deformation	rep	2	6.57757000	3.28878500	4.45	0.0184
	prt	4	4.24661667	1.06165417	1.44	0.2409
	level	3	4.77151167	1.59050389	2.15	0.1098
	level*prt	12	8.11526333	0.67627194	0.91	0.5418
Expressible Water	rep	2	73.46512333	36.73256167	9.44	0.0005
	prt	4	61.94281000	15.48570250	3.98	0.0086
	level	3	93.50743167	31.16914389	8.01	0.0003
	level*prt	12	8.28691000	0.69057583	0.18	0.9987
Whiteness	rep	2	36.119443	18.059722	4.61	0.0161
	prt	4	1638.166693	409.541673	104.52	<.0001
	level	3	119.748965	39.916322	10.19	<.0001
	level*prt	12	204.512293	17.042691	4.35	0.0002

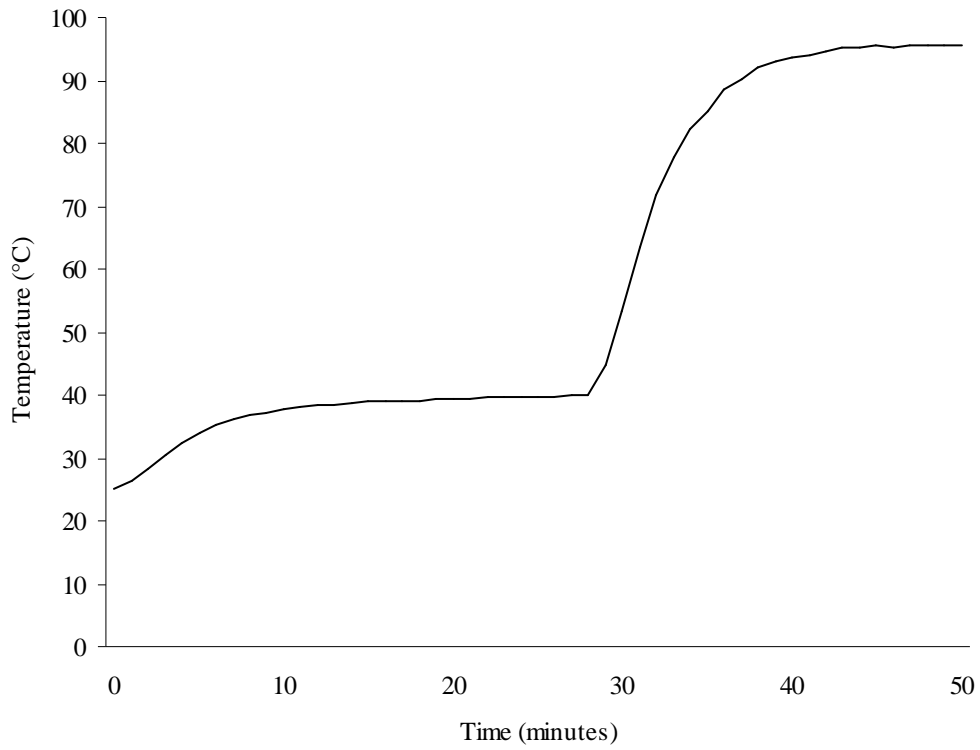[a]Replicate,[b]Protein type,[c]Level of protein replacement
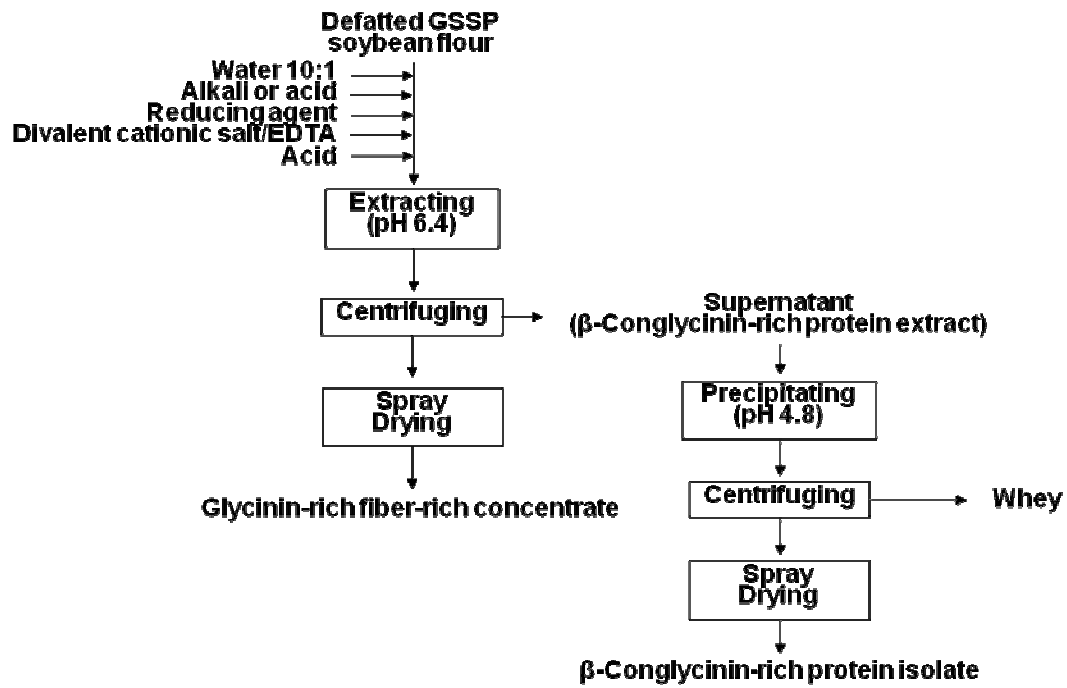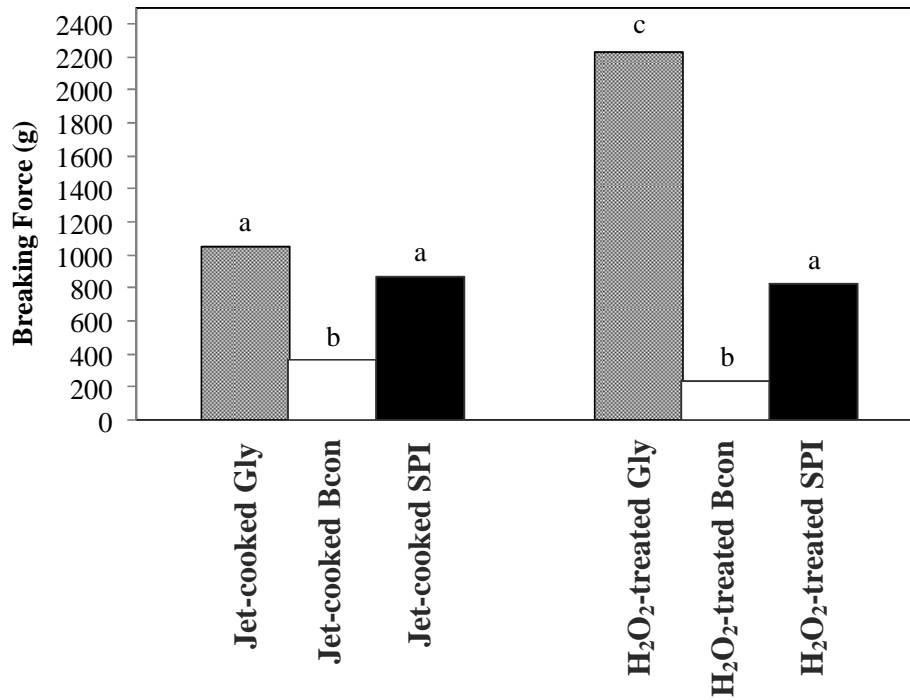
Deak and Johnson, 2007

Figure 1 – Preparation of a Glycinin+Fiber-rich fraction from GSSP soybean flour.

Figure 2 – Gel-forming device with clamp

Figure 3 – Heating curve of surimi gel prepared without soy protein ingredients. The gel was cooked at 40 and 95°C.

Figure 4 – Hardness of gels prepared with different soy protein ingredients. Gels were made at 20% protein and cooked at 95°C. Soy protein isolate (SPI) and glycinin-rich (Gly) and β-conglycinin-rich (Bcon) fractions were either jet cooked or hydrogen peroxide treated (H₂O₂).

H₂O₂ Gly **JC Bcon** **H₂O₂ SPI**

Figure 5 – Visual properties of 20% soy protein gels. Soy protein isolate (SPI) and Glycinin-rich (Gly) and β-conglycinin-rich (Bcon) fractions were either jet cooked (JC) or hydrogen peroxide treated (H₂O₂).

90°C

| Control | JC Gly | H₂O₂Gly | Gly+Fiber | JC Bcon | H₂O₂Bcon | JC SPI | H₂O₂SPI |

95°C

| Control | JC Gly | H₂O₂Gly | Gly+Fiber | JC Bcon | H₂O₂Bcon | JC SPI | H₂O₂SPI |

Figure 6 – Visual properties of surimi gels cooked at 90 and 95°C at 5% replacement level. Soy protein isolate (SPI) and Glycin-rich (Gly) and β-conglycinin-rich (Bcon) fractions were either jet cooked (JC) or hydrogen peroxide treated (H₂O₂). Gly+Fiber-rich fraction was treated with hydrogen peroxide only.

CHAPTER 5. GENERAL CONCLUSIONS

Chapter 2 of this dissertation is a study of the effects of high-pressure processing and storage conditions on the natural microflora of fresh unprocessed soymilk. The most effective treatment was the combination of HPP at 75°C followed by anaerobic storage, which reduced the microbial population the greatest while extending the protein stability of the soymilk. Pressures >400 MPa significantly reduced microbial counts. The total microbial load of soymilk was significantly affected by temperature. Dwell times of 1 and 5 min were not significantly different and, therefore, a dwell time of 1 min would shorten processing time and reduce processing costs. *Enterobacteriaceae* did not grow during storage, while only 400 MPa at 25°C aerobic storage allowed any psychrotroph growth. All HPP treatments induced injury. The shelf-life of treated soymilk was extended by at least 2 wk longer than untreated soymilk based on the spoilage level of 10^6 log CFU mL^{-1}.

The soymilk stability and pH were maintained throughout the storage study. HPP treatment enhanced shelf-life and shortened production time without affecting the quality characteristics of the soymilk. The present work shows that HPP has the potential to be an alternative commercial process to traditional thermal treatments for extending the shelf-life of refrigerated soymilk.

Chapter 3 involved studying the use of GSSP soy protein ingredients as an extender in Alaskan pollock surimi. The GSSP soy protein ingredients were preserved with two methods (jet cooking and treating with H_2O_2, the surimi gels were cooked at two different temperatures with different moisture contents. The type of GSSP soy protein did not affect gel texture or color, except for the Gly+Fiber-rich fraction. At 95°C

second-stage cooking temperature, jet-cooked soy protein produced significantly harder gels compared to H_2O_2-treated soy protein. Second-stage cooking temperature significantly affected gel hardness and deformation of surimi gels; the gels were harder and more flexible when cooked at 95 than at 90°C. Deformation was not affected by protein type, level, preservation method, cooking temperature or moisture content. Moisture content had no significant impact on gel hardness, or deformation. Whiteness significantly decreased with increased second-stage cooking temperature and moisture content. Our study shows that surimi can be extended by replacing fish protein with GSSP soy protein ingredients up to 5% and further extending the surimi by increasing the moisture content of the surimi gels to 85% without affecting gel hardness, deformation, or expressible water.

Recommendations for Future Research

Additional work is needed to analyze color, viscosity, flavor profile changes in pressure-treated soymilk during storage. Sensory attributes are important to consumers, so research is needed to analyze if HPP impacts color, texture and flavor in HPP-treated samples during storage.

Future work involving food applications with soy protein isolate is needed. Work is needed to formulate and produce a soymilk from GSSP soy protein isolate and fractions. A sensory analysis needs to be done as well to show that these products will be accepted by consumers.

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