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Effects of blanching on color, texture and sodium chloride content during storage time of frozen vegetable soybean modeling for commercial scale

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EFFECTS OF BLANCHING ON COLOR, TEXTURE AND SODIUM CHLORIDE CONTENT DURING STORAGE TIME OF FROZEN VEGETABLE SOYBEAN MODELING FOR COMMERCIAL SCALE

by

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Vegetable soybeans [*Glycine max* (L.) Merrill] are a green stage of soybeans, which have become increasingly popular among health-conscious Americans as an alternative low-fat and heart-healthy food. Vegetable soybeans (VSB) are also an excellent source of protein and fiber. However, the vast majority of the VSB consumed are imported, as they are not extensively grown and processed in the United States. The situation results in short supply and limited processing information.

The purpose of this study was to investigate the effects of water blanching (at 86, 92, 98 °C for 1m30s, 2m, 2m30s) and steam blanching (at 86, 92, 98 °C for 1m30s, 2m, 2m30s, 2m50s) on color, texture and sodium chloride content of frozen VSB during six-month storage time. It was hypothesized in this study that decreasing in blanching time and temperature from the conventional commercial process (98 °C for 2m30s – water blanching and 98 °C for 2m50s – steam blanching) would not affect the quality attributes of frozen VSB.

The results showed that blanching at temperatures lower than 98 °C for both methods did not completely inactive the peroxidase in VSB, which may cause quality losses during storage. Water blanching at shorter time than the control (2m30s) in

commercial processing experiment did not effectively tenderize the texture of VSB. On the other hand, blanching time of all experiments can be reduced to 1m30s with comparable quality to the conventional processes.

Blanching apparently affected quality of VSB while freezing and frozen storage had no significant effects on the final product. Optimal processing results in the improvement of production efficiency, increasing production yield and profits. Knowledge from this study is anticipated to be, more or less, supportive and informative for VSB producers in the United States and everyone who interested in this valuable commodity, vegetable soybeans.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Vegetable soybeans [*Glycine max* (L.) Merrill], aka green soybeans and edamame, are vegetable-type soybeans harvested at fresh green stage by handpicking when seeds reach the full pod physiological stage (R6), slightly before they are mature and dry (Fehr *et al*. 1971). Vegetable soybeans are perishable and have a short harvest time because of their critical weather requirements for growth. Vegetable soybeans are a functional food crop as they offer several benefits over yellow mature soybeans. For instance, they have less bitter and beany flavors (Rackis *et al*. 1972) and contain higher levels of ascorbic acid and beta-carotene (Bates and Matthews 1975; Simonne *et al*. 2000). Vegetable soybeans are a rich source of fiber, isoflavones, omega-3 fatty acid, and folic acid, which are known for many potential health benefits (Masuda 1991; Miles *et al.* 2000). Vegetable soybeans are one among a few green vegetables that contain all nine essential amino acids making them an excellent source of protein (American Culinary Federation 2010). However, raw vegetable soybeans are not digested easily by humans because of the presence of trypsin inhibitor (Rackis and Gumbmann 1981) and saponins (Tsukamoto *et al.* 1993). Trypsin inhibitor prevents protein digestion and interferes with protein absorption, while saponins cause bitterness and have in vitro hemolytic activity (Gesterner *et al.* 1968).

While vegetable soybeans have been consumed in Asian countries for centuries, their popularity has increased in United States markets in the past few decades with the “vegetarian fever” and “healthy food” trends. Vegetable soybeans are served as snacks, salad-mixes, and stir-fried. It has been estimated that 25,000 to 30,000 tons of vegetable soybeans are consumed each year in the United States (Chung 2013). The majority of vegetable soybeans consumed in the United States are imported from China and Taiwan even though the United States is the world largest soybean producer. Most of the domestic soybeans grown are for commercial field-type, i.e., oil production, livestock feed, and biofuel (Soyatech 2014; North Carolina Soybean Producers Association 2014). Data and information are therefore limited on commercial vegetable soybean production as well as production facilities in the United States. Although vegetable soybean seeds are available to United States suppliers, most vegetable soybeans are locally grown for household consumption. A study on commercial processing is thus necessary in order to facilitate improved preservation of vegetable soybeans from one harvest season to the next, and to supply the ever growing domestic demands. Commercial processing also increases the value of the commodity and allows for greater distribution and longer storage periods.

A combination of blanching and freezing has been established as the most suitable preservation method for vegetables, including vegetable soybeans (Reyes de Corcuera *et al.* 2004). Vegetable soybeans are marketed fresh or frozen, in the form of a pre-cooked product. Most of frozen vegetable soybeans on the market are salted for flavoring purposes. In commerce, the quality of frozen vegetable soybeans directly affects consumer purchasing decisions. Song *et al.* (2003) stated that vegetable soybeans should have balanced nutritional values and acceptable appearance quality. However, consumers preferred aesthetic appearance to nutritional quality. Masuda (1991) reported that quality requirements of vegetable soybeans were grouped into five major categories, which consisted of appearance, taste, flavor, texture, and lastly nutritional value. In terms of vegetable soybean palatability, four major quality attributes were required by the consumer, including appearance, flavor, taste, and texture (properties of structure).

In this research, industrial-scale processing of frozen vegetable soybeans was studied to determine the optimal processing conditions. This study focused on the blanching step, which is considered the critical point as it directly affects the quality of vegetable soybeans. It is expected that the results from this work will promote the industrial-scale processing of vegetable soybeans and support the growing demands of vegetable soybeans in the United States. In collaboration with Union Frost Co., Ltd., i.e., a frozen fruits and vegetables company in Thailand, the experiments in this research were conducted on both an industrial and laboratory scale. The commercial processing experiments, following the conventional process, and the laboratory experiments were conducted at the facility in Thailand and at the University of Nebraska-Lincoln (UNL), respectively. The latter study was completed with the modified methods as similar as possible to the commercial processing experiment. The experiments were designed to evaluate the effects of blanching time and temperature, as independent factors, and storage time on the key commercial quality attributes (color–greenness and brightness, texture–hardness, and flavor–sodium chloride content). The conventional processes conducted at the Union Frost Co., Ltd. facility were used as the controls for the experiment.

1.2 OBJECTIVES AND HYPOTHESES OF THE STUDY

The objectives of the study were (1.) to determine the optimal processing conditions (combination of blanching and freezing) for commercial frozen vegetable soybean preservation and (2.) to evaluate the effects of blanching, as a pre-freezing treatment, on the commercial quality of frozen vegetable soybeans (color, texture, and sodium chloride content) during six-month storage time.

The central hypotheses for this work are:

1. Decreased blanching time and blanching temperature will not affect the quality of vegetable soybeans compared to the conventional control process. Blanching with shorter time and lower temperature than conventional processes will not cause significant quality deterioration on focused attributes of vegetable soybeans during storage.
2. Frozen storage will have no or minimal effects on quality of vegetable soybeans. Additionally, frozen storage at -18 °C, or lower, will maintain the best quality of frozen vegetable soybeans for at least six months.

1.3 CONTENTS OF DISSERTATION

A background of vegetable soybeans, including their first introduction to the United States, classifications, and physical characteristics are described in Chapter 2. Additionally, chemical compositions and their health benefits as a functional food, as well as the current market situation of vegetable soybeans in the United States are also provided in this chapter.

In Chapter 3, commercial processing for frozen vegetable soybeans is described, including blanching, cooling, and freezing. Measurement approaches and general review of the processing effects on vegetable soybeans physical and chemical characteristics are discussed in this chapter.

Materials and methods used in both the commercial processing experiment and the laboratory experiment are listed in Chapter 4 as well as statistical analyses and measurements of the three focused quality attributes. Results from this study are discussed in two parts, (1) the effects of experiment conditions on commercial quality and (2) the effects of blanching factors, which were blanching methods, blanching time and temperature, and storage time, on focused commercial attributes.

Lastly, general conclusions drawn from this study are discussed in Chapter 5. Application of the study results in both academic and industry sectors, including potential and limitation for vegetable soybeans market in the United States, and recommendation for future studies also are provided.

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CHAPTER 2

VEGETABLE SOYBEANS (*GLYCINE MAX* (L.) MERRILL): BACKGROUND, CHARACTERISTICS, AND REVIEW OF CURRENT COMMERCIAL STATUS

2.1 ABSTRACT

Vegetable soybeans [*Glycine max* (L.) Merrill] are a green stage of soybeans that have become increasingly popular among health-conscious Americans as an alternative low-fat and heart-healthy food, as well as a good source of protein and fiber. However, the vast majority of the vegetable soybeans consumed are imported, as they are not extensively grown and processed in the United States. This situation results in short supply and limited processing information. The purpose of this chapter is to address the background, classification, and characteristics of vegetable soybeans. Additionally, the health benefits of vegetable soybeans as a functional food crop, and market trends in the United States are discussed.

Keywords: Vegetable soybeans, edamame, green soybeans

2.2 BACKGROUND

Vegetable soybeans in the United States were officially recorded in April 1855 by Peticolas of Mount Carmel, Ohio, in an article published in “Country Gentleman”. In December 1890, the first large-seeded vegetable-type soybean variety was introduced in America by Charles C. Georgeson, a professor of agriculture in Japan (William and Akiko 2009). Since then, vegetable soybeans have been used widely among the United States public through the boom of traditional Japanese culture. In July 1936, Green Shelled Soy Beans (canned) were the earliest known commercial green vegetable soybean product in the United States sold by Dr. John Harvey Kellogg’s Battle Creek Food Co., Michigan (William and Akiko 2009). Although the first wave of interest in green vegetable soybeans occurred during 1935-1947, interest almost disappeared after World War II until the late 1960s when the demand started to grow again. The first frozen green vegetable soybeans were imported from Japan in July 1966, and US demand has increased ever since. Today, vegetable soybeans can be purchased in supermarkets and are served in many restaurants across the United States.

2.3 CLASSIFICATION

Soybeans grown in the United States are classified as indeterminate, semi-determinate or determinate (McWilliams *et al*. 1999). Many southern varieties are determinate as they cease vegetative growth when the main stem terminates in a cluster of mature pods. Most northern varieties are indeterminate in growth habit. Indeterminate varieties develop leaves and flowers simultaneously throughout a portion of their reproductive period, with one to three pods at the terminal apex. Considering that soybean development is being driven by photoperiod, northern varieties vegetative growth is limited by season length. Semi-dwarfs, determinate varieties that are usually only 40-50% as tall as indeterminate varieties, are commonly grown in the Midwest (McWilliams *et al*. 1999). Soybean maturity groups are based on their adaptability at certain latitudes. These maturity belts run east to west in the United States with only about 100 to 150 miles from the north to the south for each belt. Maturity groups range from 000 in the extreme northern United States to VIII in the southern Gulf Coast states and most of Florida (Figure 1). Short day length and warm temperatures control soybean flowering. For instance, planting a specific variety further north than its adapted maturity range will extend the period of vegetative growth, delay flowering and maturity due to the extended summer day length and cooler temperatures, while planting further south causes earlier flowering and results in an earlier maturity (McWilliams *et al*. 1999).

Growth of soybeans consists of six vegetative stages and eight reproductive stages (McWilliams *et al*. 1999). Vegetative stages are VE (emergence), VC (cotyledon stage), V1 (first trifoliolate), V2 (second trifoliolate), V3 (third trifoliolate), V(n) (nth trifoliolate), and V6 (flowering will soon start). Reproductive stages are R1 (beginning bloom, first flower), R2 (full bloom, flower in top 2 nodes), R3 (beginning pod, 3/16" pod in top 4 nodes), R4 (full pod, 3/4" pod in top 4 nodes), R5 (1/8" seed in top 4 nodes), R6 (full size seed in top 4 nodes), R7 (beginning maturity, one mature pod), R8 (full maturity, 95% of pods on the plant are mature) (McWilliams *et al*. 1999). In particular, vegetable soybeans are harvested during their R6 stage while the seeds are developing. R6 stage is used to describe seed development, which also is known as the "green bean" stage or beginning full seed stage, and total pod weight will peak during this stage. This stage initiates with a

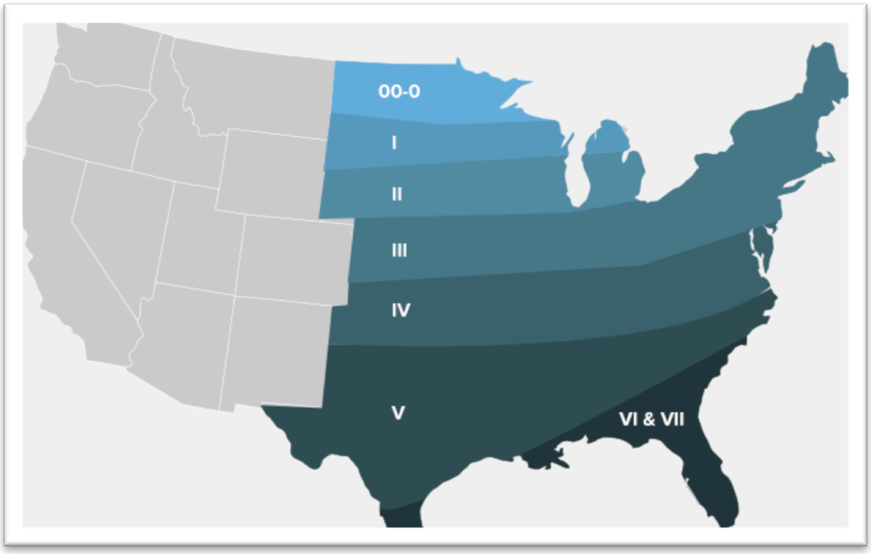

Figure 1. Soybean growing regions in the United States
Source: Monsanto Company 2014
(http://www.aganytime.com/asgrow/plant/Pages/Plant-Inner.aspx)

pod containing a green seed that fills the pod cavity on at least one of the four top nodes of the main stem. Growth, development and yield of soybeans are dependent on a variety's genetic potential interacting with environment and farming practices (McWilliams *et al.* 1999). Vegetable soybeans have a very short harvest window of only a few days. Therefore, immediate post-harvest cooling is essential to maintain product freshness. Early morning is the best time for harvesting as pods are cool and less field heat after harvest has to be reduced.

Physiologically, soybeans can be classified into two large groups, i.e. small-seeded field-types and large-seeded vegetable-types (William and Akiko 2009). Besides the seed size, the bean types do not differ in genetic or biochemical traits. From a qualitative perspective, however, the vegetable type has been selectively bred for large seed size, good flavor, and short cooking time to tenderize. In general, vegetable-type soybeans have slightly higher protein and slightly lower oil content compared to typical field-type soybeans. Vegetable-type soybeans also are preferable for consumption in fresh green state as they have better flavor and texture. Nonetheless, the majority of soybeans, more than 98%, in the world are small-seeded field-types, typically referred to as commercial types (William and Akiko 2009).

Physically, vegetable soybeans are classified into two main categories by their usages, i.e., perfect pods (3-seed pod and 2-big seed pod) and imperfect pods (2-small seed pod, 1-seed pod, atrophied pod, twisted pod, and defected pod). The characteristics used to classify physical properties include width, length, thickness, pod weight, pod projected area, and seed firmness (Sirisomboon *et al.* 2007).

2.4 CHARACTERISTICS AND CHEMICAL COMPOSITION

2.4.1 Physical characteristics

Vegetable soybeans are bright green in color with large seed sizes and soft textures. The pod color is important as bright green is the most desirable because surface color indicates the amounts of chemical components in the seed. Yellowing of the pods reflects declining freshness and degrading sugar, free amino acids, and ascorbic acid (Masuda 1991). Optimal pods have a good shape with spotless surfaces (Masuda 1991). Large grain size vegetable soybeans weigh 20-30 g/100 grains (Da Silva *et al.* 2009). Perfect pods, or export grade, consist of 3-seed pods, and big 2-seed pods, when the seeds are fully developed. Atrophied pods, twisted pods, and otherwise defected pods are considered imperfect pods and are not processed. The apparent density of vegetable soybeans is between 0.99-1.00, which is comparable to the density of water. Hence, the pods will float in water. In weighing, 500 g of vegetable soybean must contain at least 175-180 pods of 2-seed or 3-seed pods (Sitatani and Vasi 1992).

2.4.2 Chemical composition

Analysis of the nutrient content has shown that vegetable soybeans are an excellent source of numerous nutrients, including thiamine, protein, riboflavin and iron (Shanmugasundaram *et al.* 1989). Moreover, vegetable soybeans contain essential amino acids, but no cholesterol and little or no saturated fat. Additionally, vegetable soybeans are composed of nutritive compounds, such as isoflavones, omega-3 fatty acids, ascorbic acid, folic acid, and other essential vitamins and minerals. A study by Da Silva *et al.*

(2009) reported that food-type cultivar soybeans contained higher levels of aglycones, i.e., the more biological active form of isoflavones, and oleic acid than conventional soybeans.

Vegetable soybeans can be classified as a protein seed rather than oil seed. Soybean varieties most commonly grown contain on average 40% protein and 20% oil, depending on variety and strain (Rackis and Gumbmann 1981). Nutritive compounds in vegetable soybeans compared to yellow soybeans and soy nuts are shown in Table 1. Despite the nutritional compounds in vegetable soybeans, antinutritional factors, including trypsin inhibitors and phytates, are also present. However, a study showed that vegetable soybeans contained lower amounts of these antinutritional factors than conventional soybeans (Liu 1997).

Chemical components present in vegetable soybeans are related to their commercial qualities, including sweetness, flavor, and taste, as reported by Tsou and Hong (1991). This study further showed that the carbohydrate patterns of vegetable soybeans are different from those of grain soybeans as well as quantity. Vegetable soybeans contained a relatively high amount of starch and low amount of oligosaccharide compared to grain soybeans, which resulted in a milder and less bitter taste (Table 2). Tsou and Hong (1990) indicated that sucrose, which was the predominant sugar in vegetable soybeans, was responsible for their sweetness. Da Silva *et al*. (2009) also reported that food-typed soybeans contained high levels of fructose, glutamic acid, and alanine that correlated soybean mild flavor. These results supported studies by Masuda (1991) who also showed that vegetable-type soybean cultivars were milder and sweeter than conventional cultivars due to higher levels of sucrose, glutamic acid, and alanine amino acids. Analysis of amino acid patterns showed significant variation in glutamic

Table 1. Nutrition fact of vegetable soybeans compared to yellow soybeans and soy

Nutrition Fact	Green soybeans 1/2 cup cooked	%Daily Value	Mature/ yellow soybeans 1/2 cup cooked	%Daily Value	Soy nuts 1/4 cup plain	%Daily Value
Calories	127		148		194	
Total Fat	6g	9%	8g	12%	9g	14%
Saturated Fat	0.5g	3%	0g	0%	1g	5%
Total Carbohydrates	10g	3%	8g	2%	14g	5%
Protein	11g	22%	14g	28%	17g	34%
Cholesterol	0mg	0%	0g	0%	0mg	0%
Sodium	13mg	1%	0g	0%	1mg	0%
Dietary Fiber	4g	16%	6g	24%	4g	16%
Calcium	130mg	13%	88mg	8%	60mg	6%
Potassium	485mg	14%	442mg	12%	587mg	17%
Phosphorus	142mg	14%	210mg	22%	279mg	28%
Folate	100µg	25%	46µg	12%	88µg	22%
Source: USDA National Nutrient Database for Standard Reference Release 17						
Average Total Isoflavones	49mg/100g		24mg/100g		55mg/100g	
Source: USDA-Iowa State University Database on the Isoflavones Content of Foods-1999, USDA Nutrient Data Laboratory, Agricultural Research Service, 2008						

Table 2. The carbohydrate patterns of vegetable soybean and grain soybean (Tsou and Hong 1991)

Carbohydrate	**Vegetable soybeans (mg/g dry wt)**	**Grain soybeans (mg/g dry wt)**
Starch	83.2	0.66
Total sugar	110.2	102.4
Sucrose	99.14	62.05
Glucose	13.4	11.18
Fructose	8.95	0.73
Raffinose	0.16	14.85
Stachyose	0.95	25.38
Crude fiber	44.9	52.7

*Mean of three varieties: Kaohsiung No. 1, Tzurunoko, and Ryokkoh acid and alanine levels among the varieties tested, which clearly explained the differences in taste among vegetable soybean varieties (Tsou and Hong 1990). Undesirable features, including bitter, astringent, and metallic off-flavors were also detected in soybean seeds, as well as the so-called dry-mouth feeling (Okubo 1988). Sensory properties of undesirable components of soybeans are presented in Table 3.

As a functional food, vegetable soybeans are famous for their nutritional compounds, which provide numerous health benefits for humans. Major nutrients presented in vegetable soybeans, including isoflavones, omega-3 fatty acids, folic acid, and ascorbic acid, are described below.

Isoflavones – A hundred grams of raw green soybean contain a relatively high amount of isoflavones (48.95 mg) that can be further categorized as 20.34 mg of daidzein, 22.57 mg of genistein, and 7.57 mg of glycitein (US Department of Agriculture 2008). Isoflavones have garnered intense interest because numerous scientific reports support their potential properties as a phytoprotectant against many hormone-dependent diseases, including cancer, menopausal symptoms, cardiovascular disease and osteoporosis (Setchell and Cassidy 1999). Isoflavones are found exclusively in legumes, particularly in soybeans, but also in beans, sprouts, clover, and alfalfa (Rufer and Kulling 2006). Isoflavones are the excellent antioxidants that act by eliminating reactive oxygen species (ROS), the major cause of many diseases (Setchell and Cassidy 1999; Rufer and Kulling 2006). Sarkar and Li (2003) reported that the incidence of certain cancers were much higher in Americans compared to Asians, such as Japanese and Chinese, whose low risks of breast, colon, corpus uterine and prostate cancers have been thought to be related to

Table 3. Sensory properties of undesirable components of soybean (Okubo 1988)

Components	**Properties**	**Sources**
Phenolic acids	sour, bitter, astringent flavor	defatted seed
Oxidized phosphatidylcholine	bitter	defatted seed
Oxidized fatty acid	bitter	oxidized oil
Hydrophobic peptide	bitter	fermented products
Isoflavin	Objectionable taste, bitter, astringent, weak phenol-like taste	defatted seed
Daidzin	bitter, astringent	whole seed
Genistin	bitter, astringent	whole seed
Saponin	bitter	whole seed
A group saponin	bitter, astringent	hypocotyl
B group saponin	bitter, astringent	whole seed
Soyasaponin I	bitter, astringent	dried pea

their low fat diets and high intakes of foods high in isoflavones. In addition, incidence rates of cancers, such as prostatic, hypospadia, testicular, and other conditions linked to estrogen exposure have been reported to be lower in countries that have higher phytoestrogen intake (Setchell and Cassidy 1999). Several experimental studies showed that soy isoflavones exert anticarcinogenic effects on hormone-related cancers, which may be related to their estrogenic, antiestrogenic activities (Adlercreutz *et al*. 1995). Researchers have proposed that Asian women may experience milder symptoms during menopause than Western women because the soy foods in their diets provide phytoestrogens to supplement their declining estrogen levels (Schardt 2000).

Omega-3 fatty acids – Another essential compound in vegetable soybeans is omega-3 fatty acids. According to USDA data, the balance of fatty acids in 100 g of vegetable soybean is 361 mg of omega-3 fatty acids to 1794 mg of omega-6 fatty acids (US Food and Drug Administration 2012). Omega-3 fatty acids are considered essential fatty acids because they are needed to maintain health while they cannot be made by the human body. Omega-3 fatty acids are beneficial in prevention of various diseases, such as rheumatoid arthritis, depression, prenatal health, asthma, especially the properties of lowering triglycerides and boost heart health (Stone 1996; WebMD 2013). In addition, the research to date has reported that omega-3 fatty acids decrease risk for arrhythmias, thrombosis as well as reduce inflammatory responses (Stone 1996).

Omega-3 fatty acids have also been shown to exert positive heart health properties. For example, the American Heart Association (AHA) Science Advisory published the benefits of omega-3 fatty acids on cardiovascular disease (CVD) that dates back to 1996. Large-scale epidemiologic studies have further indicated that people at risk for coronary heart disease (CHD) benefit from consuming omega-3 fatty acids (Stone 1996). Additionally, Harris (1997) reported that about 4 g per day of omega-3 fatty acids decreased serum triglyceride concentrations by 25% to 30%, with accompanying increases in LDL cholesterol of 5% to 10% and in HDL cholesterol of 1% to 3%. Even though omega-3 fatty acids provide numerous health benefits, modest level of consumption were recommended, as they are high in calories. In addition, high doses of omega-3 fatty acids (3 g and above) will increase the risk of bleeding in some people.

Folic acid – Folic acid; aka folate, folicin, or vitamin B9; is another essential compound present in vegetable soybeans. The natural form of folic acid is preferred as it provides better absorption. Folic acid is essential for the daily formation of new skin, hair, and nail cells. Although folic acid is an important vitamin for everyone, it is especially vital for pregnant women. The protective effect of folic acid supplementation against neural tube defects (NTDs) in new borns has led to mandatory folic acid fortification in both the United States and the United Kingdom (Susan 2013). Numerous studies have confirmed that certain birth defects can be prevented by ingesting folic acid at dosages of 400 μg or higher daily (Medical Research Council Vitamin Study Research Group 1991). According to USDA data (US Department of Agriculture 2008), a 100 g dose of vegetable soybeans contains 311 μg of folate, or 78% of the daily recommended requirement. Folic acid is required for normal growth, development and functioning of the fetus, nervous system and bone marrow (National Institute of Health 2013). Studies have also shown that that folic acid plays a role in the prevention of colon cancer, inflammatory bowel condition, ulcerative colitis, esophageal, stomach and pancreatic cancer (Young-In 2004; Figueiredo *et al.* 2009).

Ascorbic acid – Another vital compound in vegetable soybeans is ascorbic acid or vitamin C. A 100 g sample of cooked vegetable soybeans contain 6.1 mg of vitamin C (10% DV) (US Department of Agriculture 2008). Because of its various health benefits, ascorbic acid is one of the most important vitamins for humans. Ascorbic acid is a coenzyme involved with the synthesis of body collagen, which is the structural protein of skin, connective tissue, tendon cartilage, and bone (Michels 2011). Ascorbic acid maintains skin elasticity, helps in iron absorption, improves resistance to infection, and prevents minor capillary bleeding. It is also essential for synthesis of bile acids (Michels 2011). Moreover, ascorbic acid is a powerful antioxidant and anti-inflammatory caused by ROS due to its ability to terminate chain radical reactions via electron transfer mechanisms (US Department of Agriculture 2012). As such the risk factors are decreased for diseases associated with these cellular stresses, such as endothelial dysfunction, high blood pressure, heart disease, asthma and cancer (Liu *et al.* 2002; Guz *et al*. 2007). In order to provide antioxidant protection, the Recommended Dietary Allowance (RDA) of ascorbic acid is 90 mg/day for adult men and 75 mg/day for adult women (Institute of Medicine 2000).

Potent health benefits of the nutrients mentioned earlier clearly indicate that vegetable soybeans are an excellent functional food. Consumption of vegetable soybeans offers numerous benefits over other green vegetables and, not surprisingly, vegetable soybeans will most likely become even more popular in the near future.

2.5 CURRENT STATUS AND MARKET TRENDS IN THE UNITED STATES

Chang-Chi Lin, CEO of Asia Foods Group of Companies, one of the largest Taiwanese frozen fruits and vegetables trading companies, stated that, with the “vegetarian fever” and “healthy-food” trends in the United States, soy products became an instant hit by 1997. Many media outlets introduced soy products, including vegetable soybeans, to the public. Moreover, the FDA has emphasized the benefit of vegetable soybeans by announcing that soybeans could lower the risk of heart disease (US Food and Drug Administration 2014). Four out of 10 consumers said they were aware of the FDA claim that consuming 25 g of soy protein per day reduces the risk of coronary heart disease (Soyfoods Association of North America 2013). It has also been reported that approximately 26% of consumers are aware of specific health benefits of soy in their diet (Soyfoods Association of North America 2013). According to the United Soybean Board (2013), a strong demand for soyfoods coincides with survey results that show that more than 75% of consumers perceive soy products as healthy, which is an 8% increase over 15 years. For the past three years, vegetable soybeans are second among the top three most consumed soy foods in the United States (Soyfoods Association of North America 2013). (Soybean products sales during 2008-2011 are presented in Table 4.). Vegetable soybeans were categorized in all other products category.

In the United States, there are two main vegetable soybean growers, SunRich in Minnesota and Cascadian Farms in Washington. Smaller producers are located in Arkansas, California, Minnesota, and Ohio. In 2000, the United States produced 11.6% of the world demand for frozen vegetable soybean, which was expected to increase dramatically (Lin 2001). The Soyfoods Association of North America (2013) reported that demand for vegetable soybeans in the United States grew at a rate of 12 to 15 percent per year and frozen vegetable soybean sales grew 4.3% from 2010 to 2011. These

Table 4. Soybean products sales during 2008-2011

Source: Soyfoods: The U.S. Market 2013, published by Soytech, Inc.

Category	**2008**	**2009**	**2010**	**2011**	**Percent Change (from 2010–2011)**
Tofu	$258	$252	$247	$255	3.2%
Soymilk	$1,156	$1,081	$1,043	$1,033	-1.0%
Meat Alternatives	$607	$622	$649	$662	2.0%
Energy Bars	$792	$808	$952	$1,092	14.7%
Soy Cheese, Cultured Soy (Soy Yogurt) & Frozen Soy Desserts	$221	$203	$186	$174	-6.4%
All Other Products	$2,094	$2,053	$2,039	$1,956	-4.0%
Total Sales (Millions)	$5,128	$5,020	$5,116	$5,172	1.1%

increases in soybean sales could be due, in part, to Americans demanding more but less expensive and alternative proteins considering the rising prices of meat. Chung (2013) estimated that $175–$200 million market, with 25,000 to 30,000 tons of edamame are currently being consumed each year in the United States. The increased US demand coupled with the rising production costs in China has provided an economical incentive to launch the production of vegetable soybeans in the United States. In the summer of 2012, a Texas-based Asian foods importer built the nation's first plant for vegetable soybeans production, i.e., American Vegetable Soybean and Edamame Inc., in Arkansas. This company has a fully operational system for handling vegetable soybeans, including receiving, processing, packaging, and shipping. Arkansas is expected to become the United States capital of vegetable soybeans as the state ranks 10th nationally for conventional soybeans. Additionally, Arkansas was the first state to develop a vegetable variety licensed for commercial production. Furthermore, an increasing number of local farmers were willing to grow a non-genetically modified vegetable soybeans (Chung 2013). A new vegetable soybean variety has thus been developed by Division of Agriculture, University of Arkansas, that is well adapted for production to their growing conditions. Local press reported 800-900 acres of vegetable soybeans were harvested in western Arkansas in 2012. Other efforts are on-going to develop commercial-scale vegetable soybeans production in Pennsylvania, Ohio, and Virginia (Pfeiffer 2013). (Vegetable soybean varieties currently available in the United States are shown in Appendix A.2.)

2.6 CONCLUSIONS

Vegetable soybeans are an immature stage of regular soybeans that have been selectively bred for larger seeds and softer texture. Vegetable soybeans are considered a functional food because they contain numerous health benefitting nutrients compared to other vegetables. The interest and popularity of vegetable soybeans in the United States has grown rapidly in the past two decades because of the mild and sweet taste, health benefits, and the alternative for a less expensive protein source.

Nonetheless, the majority of vegetable soybeans consumed in the United States are imported resulting in a critical gap in knowledge concerning the whole production system. Therefore, the commercial processing process of vegetable soybeans must be studied and promoted in order to supply the growing domestic demands and to improve the quality of vegetable soybeans for competitive global market.

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CHAPTER 3

COMMERCIAL PROCESSING OF FROZEN VEGETABLE SOYBEANS

3.1 ABSTRACT

Vegetable soybeans are marketed as frozen whole pods and kernels. Commercial processing of frozen vegetable soybeans consists of several steps, including blanching, cooling and freezing. Although freezing has been established as the most appropriate method to extend vegetable shelf life, including vegetable soybeans, blanching is applied as a pre-freezing treatment in order to decrease deterioration and extend shelf life by destroying spoilage microorganisms and inactivating deteriorative enzymes.

Commercial processing steps of frozen vegetable soybeans are described as well as measurement approaches used to evaluate vegetable soybean quality attributes, including color, texture and sodium chloride content. In addition, the effects of processing parameters on chemical compositions and physical characteristics of vegetable soybeans are mentioned in this chapter.

Keywords: vegetable soybeans, steam blanching, water blanching, Individual Quick Freezing

3.2 INTRODUCTION

Preservation of vegetables by blanching in water combined with freezing has long been established as the most appropriate to inactive enzymes thereby decreasing the rate of enzymatic deterioration (Williams *et al*. 1986). However, to this author's knowledge, there is still no scientific study or research regarding industrial-scale processing of vegetable soybeans available in the United States. A research report (Martins and Silva 2002) has been published on home-cooking methods of vegetable soybeans, but such applications are not suitable to industrial-scale processing due to the differences in blanching equipment, processing time and temperature. While home-cooking methods may vary depending on individual's preference, industrial-scale processing requires uniform and consistent products. In the frozen vegetable industry, freezing of foods normally consists of pre-freezing treatments, freezing, frozen storage, and thawing steps, which must be properly conducted to obtain optimum results (Fennema 1977). Blanching is also a critical point as it directly affects quality of final products. Optimizing both blanching and freezing processes should retain product quality during storage.

Vegetable soybeans are grown and harvested over a short period of the year because of their critical weather requirements for growth. Post-harvested vegetable soybeans continue to undergo chemical changes caused by enzymatic reactions, especially by lipoxygenase and peroxidase, that facilitate spoilage, deterioration, changes in color, flavor, and loss of nutrients (Schafer 2014; Bahceci *et al.* 2005). Hence, fresh vegetable soybeans must be processed as soon as possible to inactivate these enzymatic activities, minimize physical and chemical deterioration, and maintain their freshness and quality.

Even though the United States is the world's largest soybean producer, most of its domestic soybeans are commercial field-types (US Department of Agriculture 2015). The majority of vegetable soybeans consumed by humans are imported from China and Taiwan (Sirisomboon *et al.* 2007). As a result, studies and information remain limited regarding vegetable soybean production in the United States. Furthermore, only a few vegetable soybeans processing facilities are located in the United States as vegetable soybeans are able to grow in select climates and regions, where local production cannot satisfy domestic demands. In addition, vegetable soybeans are unable to retain their freshness for during distribution to national markets. Hence, developing processes that preserve vegetable soybeans while retaining their quality traits are necessary for taking the next step into commercial processing.

3.3 BLANCHING

Blanching is a traditional and widely used method for vegetable preservation, including vegetable soybeans. The main purposes of blanching are to inactivate enzymes that cause quality loss in the product during frozen storage and to reduce microbial load on the surface of vegetable soybeans. Peroxidase (POD) and lipoxygenase (LOX) are the most heat stable enzymes present in vegetables and their deteriorations have been used widely as indicators of sufficient heat treatment. Generally, it has been accepted that if POD is destroyed then it is quite unlikely that other enzymes survived (Halpin and Lee 1987). Raw vegetable soybeans also contain trypsin inhibitors and saponins that are not easily digested in the human body. However, Ku *et al*. (1976) stated that soaking and cooking improved digestibility of soybean products and inactivated 80% of initial trypsin inhibitor. Similarly, Savage *et al*. (1995) reported that soaking and blanching soybeans inactivated 80% of initial trypsin inhibitor and 99% of initial LOX activity, which was sufficient to reduce off-flavors of soybeans.

Physiological attributes and chemical composition of vegetable soybeans are changed by cooking, as is texture. In some cases, blanching negatively affects the product. For instance, blanching degrades and leaches nutritive components, such as sugars, minerals and vitamins (Cumming *et al*. 1981; Rincon *et al*. 1993; Vidal-Valverde and Valverde 1993). Rockland (1978) reported that cooking lima beans caused leaching of calcium and magnesium into the cooking water as well as starch gelatinization and protein denaturation. Similarly, boiling resulted in reduction of vitamin B6 and folacin in soybeans (Soetrisno *et al*. 1982) and oligosaccharides in dry mature soybeans (Rackis 1978). However, blanching can improve the quality of vegetable soybeans, including color stability and in texture while decreasing microbial counts (Gökmen *et al*. 2005).

Blanching is considered a pre-treatment when applied to foods before entering the main processing steps. The application of blanching, which varies from domestic boiling pots to commercial-scale blanchers, depends on product characteristics and the intended use of the final product. Generally, one of four blanching systems are utilized and include steam blanching, water blanching, gas blanching, and microwave blanching. In the food industry, blanching is a process typically used to remove air in the product prior to canning, to prevent ice crystal formation during freezing, to destroy enzymatic activity and to prevent color changes of a food product (Reyes de Corcuera *et al*. 2004). Song *et al.* (2003) determined that blanching of vegetable soybeans at a high temperature and for a short period of time (100 °C for 10 min) prevented loss of greenness, reduced seed hardness, and lessened leaching of sugars and water-soluble vitamins compared with other temperature-time combinations. Longer blanching times caused more color and nutritive value losses. In agreement with Lo *et al.* (2011), carrots subjected to high-temperature short-time (HTST) blanching contained more total galacturonic acid and total sugars in pectins than carrots blanched for a long time at low temperatures (LTLT).

Two common commercial blanching methods studied in this project were (1) water blanching, where vegetable soybean samples were immersed into boiling water for designated times and temperatures. Water blanching usually results in a more uniform treatment, allowing for lower processing temperatures. Water usually is heated indirectly with steam in a heat exchanger. Therefore, water quality must be ‘‘food-grade’’ (Reyes de Corcuera *et al*. 2004), and (2) steam blanching, where the mixture of food-grade steam and water (100 °C) directly heat the product in a heating chamber. Blanching time is controlled by the speed of the conveyor carrying product through heating chamber. Steam blanching usually is used for cut and small products and it is more energy-efficient and produces lower biological oxygen demand (BOD) than water blanching. In addition, nutrient leaching is reduced compared to water blanching (Reyes de Corcuera *et al.* 2004).

Cooling is a freezing pre-treatment applied to foods in order to prevent products from overcooking and to reduce the heat in product before entering the freezing unit. After blanching, vegetable soybeans are cooled down immediately by immersing or spraying with cool water, or a saline solution in the case of commercial processing to enhance the flavor of the product.

3.4 FREEZING

Freezing is one of the best food preservation methods when based on nutrient retention (Fennema 1977). Freezing also extends the shelf life of a food product by removing heat and reducing food temperature below its freezing point. The temperature used for freezing generally is less than -18 °C. At temperatures below 0 °C, deterioration caused by microbial activity enzymatic / oxidative reactions are decreased. Moreover, formation of ice crystals within the product reduces water availability for deteriorative reactions (Singh and Heldman 2009). Furthermore, extra and intracellular ice crystal formation may cause damage to cell walls and cell membranes of microbial cells. The storage temperature of -18 °C reliably prevents the growth of microorganisms, but permits enzymatic processes to continue (Geiges 1996).

Frozen foods have advantages over other kinds of food as they are available all year round and can be transported to greater distances with minimal degradation to product quality. Nutrients, color, and physical properties in frozen product may also be protected by other storage methods, however; there could be some losses during storage and pre-consumption period (Fennema 1988). Still, freezing can cause negative effects as Martins and Silva (2002) reported ascorbic acid, total vitamin C, starch, reducing sugars, chlorophylls *a* and *b* content, texture, and color can be affected by frozen storage. A number of factors obviously influence the final quality of frozen vegetable soybeans, such as the blanching method, blanching temperature, blanching time, storage temperature, and storage time. These factors need to be considered in order to achieve the highest quality of final products.

3.5 MEASUREMENT APPROACHES

Flavor, texture, and color are quality parameters that are typically assessed for fresh products, immediately after blanching and after a given storage time (Reyes de

Corcuera *et al.* 2004). These attributes strongly affect consumer-purchasing decisions; hence, they were focused on in this study (Chapter 4).

Food color is a critical factor for acceptance of food items by consumers. For the food color measurement, the L^* a^* b^*, or CIELab system, is the most used approach due to the uniform distribution of colors and it is very close to human perception of color. L^* is the luminance or lightness component, which ranges from 0 (black) to 100 (white). Parameters a^* from green (-*a*) to red (+*a*) and b^* from blue (-*b*) to yellow (+*b*) are the two chromatic components which range from -120 to 120 (León *et al.* 2006).

The texture of vegetable soybean is complex in nature and there is no standard available on the desired texture for vegetable soybean. There are many factors that might contribute to the hardness of vegetable soybean seeds. The possible factors reported to affect seed hardness are varieties, harvest stage, and cooking time (Tsou and Hong 1991). Because texture is a property related to the sense of touch, it can be measured by mechanical methods in units such as force. Texture testing is a well-established technique for evaluating the mechanical and physical properties of food structure for pre- and post-quality control checks. Texture testing has applications across a wide range of food types, including baked goods, cereals, confectionaries, dairy, fruit, vegetables, meat, and poultry. In texture analysis, standard tests using compression and tension are used to measure hardness, crispiness springiness, and other properties of food. Comparing the results from mechanical texture analysis with trained human sensory panels has shown that the measurements have a high correlation with the various sensory attributes associated with textural quality. In processing, texture analysis can be used for measurement and control of process variations such as temperature, humidity, and cooking time. The textural properties can be used to optimize the process (Brown 2010).

Flavor is another attribute that plays an important role in commercial food product acceptance. For marketing purposes, vegetable soybeans are salted in order to enhance flavor of the product. Based on general specification for commercial frozen vegetable soybeans, saltiness of the product should range between 0.3-0.5%. For this study, NaCl content in frozen vegetable soybeans was analyzed using Mohr's direct titration method.

Peroxidase is an enzyme commonly used as an indicator for adequate blanching process before freezing because it is more resistant to heat than most enzymes, and there are simple rapid assays to measure its activity (Barrett and Theerakulkait 1995; Reyes de Corcuera *et al*. 2004). According to the POD test, guaiacol (colorless) is oxidized by POD in vegetable soybeans, in the presence of hydrogen peroxide, and becomes tetraguaiacol (brown).

The complete reaction is:

$$\underset{\text{Colorless}}{4\ H_2O_2 + 4\ C_7H_8O_2\ \text{(guaiacol)}} \xrightarrow{\text{Peroxidase}} \underset{\text{Brown}}{\text{tetraguaiacol} + 8\ H_2O}$$

The amount of peroxidase can be qualitatively measured by spectrophotometric absorbance at wavelength setting of 500 nm; however, it was unpractical for the continuous industrial processing conducted in Thailand. Therefore, the test was modified to be suitable for the on-site application.

3.6 EFFECTS OF PROCESSING ON VEGETABLE SOYBEANS QUALITY

3.6.1 Effects on chemical compositions

Blanching is a conventional thermal process applied in the food industry in order to inactivate enzymatic activities responsible for the Browning reaction, deteriorative spoilage, and color changes in vegetables. Xu *et al.* (2012) reported that enzyme activity in vegetable soybeans was reduced dramatically by blanching, with 98% loss in activity after 2.5 min. Similarly, Sheu and Chen (1991) reported that POD activity was reduced by 90% after blanching for 1.14 min at 100 °C. A study showed that LOX enzymes were inactivated when beans were soaked and cooked, resulting in similar outcomes for both conventional cultivars and the null-LOX cultivar (Da Silva *et al*. 2009). Besides deteriorative enzymes, blanching also inactivates trypsin inhibitors, the antinutritional factors usually found in soy products. Mozzoni *et al.* (2009) reported that blanching, using steam-jacketed kettle, for approximately 2 min rendered 80% inactivation of trypsin inhibitor activity (TIA). Blanching process involves heat and water, which are the key factors in loss of water-soluble nutrients and vitamins in foods. Tosun and Yücecan (2008) studied the impact of processing and storage on vitamin C content of various commercial frozen vegetables, i.e., okra, green beans, spinach, and peas. They found significant decreases in the initial vitamin C content in the range of 19.1-51.5%, depending on the vegetable type and pre-freezing operations. The results showed that freezing process and storage time did not influence the vitamin C levels (except in green beans and spinach) but pre-freezing operations, including blanching, had a major impact on vitamin C content. The study also showed that physical disruption (slicing, cutting, etc.) prior to blanching apparently affected the leaching of water-soluble nutrients like vitamin C. These studies confirmed that blanching, as well as other pre-freezing treatments, affect nutrient content in blanched foods as water-soluble vitamins and nutrients leach out and degrade in blanching water. Similar effects on water-soluble nutrients, i.e. isoflavones, folic acid, and ascorbic acid, were observed. Setchell *et al.* (1998) reported that processing caused intra-conversions of isoflavones between the different forms while cooking soy products in water and resulted in leaching of isoflavones into the cooking water. Wang and Murphy (1996) studied the mass balance of isoflavones during the manufacture of various soy-based products. Their results showed that the main losses of isoflavones were related to the manufacturing steps, such as 49% isoflavones loss in the heating process of tempeh production.

Different blanching conditions also affect loss of water-soluble vitamins. A study reported the retention of vitamin C in vegetable soybeans after blanching at different conditions that blanching vegetable soybean at 100 ºC for 10 min had the minimal loss of vitamin C compared to blanching at 80 ºC for 30 min and 90 ºC for 20 min (Song *et al*. 2003). The study suggested that blanching fruits and vegetables at high temperature and short periods of time are the best conditions to preserve the nutrients. Besides blanching conditions, blanching methods also affect nutritive compounds in vegetables. Muftugil (1986) reported that ascorbic acid contents of water-blanched green beans were higher than those of steam blanched. Convection oven blanched green beans were lowest in ascorbic acid, and microwave treated samples were highest. Another study showed that water blanching resulted in a loss of 100% folic acid while microwave blanching caused 25.7% loss of folic acid in turnip greens when compared to control (Osinboyejo *et al*. 2003).

Compared to steam and microwave blanching, water blanching provides a uniform heat treatment (Reyes de Corcuera *et al*. 2004). Omega-3 fatty acids, another major nutrient found in vegetable soybeans, are polyunsaturated oils; and therefore, not soluble in water. Even though they do not leach out and degrade in blanch water, they are extremely susceptible to damage from heat, light, and oxygen. Thermal cooking, such as blanching, frying and toasting destroys their nutritional value and health benefits. A lab analysis showed that toasting at 350 ºF for 8-10 min caused a 5% loss of omega-3 fatty acids and α-linolenic acid (ALA) in walnuts (Fabulousfoods 2008).

Blanching seems to have undesirable effects on water-soluble nutrients. However, a study reported that blanching process increased the half-life of ascorbic acid in green beans. The result showed that half-life of ascorbic acid in unblanched green beans was 1.89 months, but increased to 2.15 and 3.48 months when blanched at 70 ºC for 2 min and 90 ºC for 3 min, respectively (Bahceci *et al*. 2005). There are many factors in any blanching process affecting water-soluble nutrient contents in foods, including degree of heating, surface area exposed to water, oxygen, pH and presence of transition metals. Underblanching stimulates enzymatic activities, which result in worse outcomes than not being blanched, while over-blanching causes loss of flavor, color, vitamins, and minerals. Hence, an appropriate blanching condition must be chosen to match the physiological characteristic of specific commodity. High temperature and short time (HTST) conditions have been recommended as the best conditions for fruit and vegetable blanching as they preserve nutrients, vitamins and organoleptic properties (Song *et al*. 2003).

Besides blanching, storage conditions also affect the chemical compositions of vegetable soybeans. Inappropriate storage results in quality degradation. A study reported that vitamin C content decreased by 4.66-fold more at room temperature (25 °C) than at refrigeration temperature (5 °C). Additionally, it was reported that weight loss of 2.77-fold more occurred at room temperature than at refrigeration temperature. Czaikosk *et al.* (2012) concluded that, at room temperature, the minimally processed vegetable-type soybeans, when stored in trays and wrapped in plastic wrap, caused a greater decrease in vitamin C content, more weight loss and larger changes in color parameters (*L**, *a**, *b**) than at refrigeration temperature (5 °C). The lower the storage temperature the better the quality of vegetable soybeans preserved. In long-term storage, freezing is considered as the best preservation method for fruits and vegetables.

3.6.2 Effects on physical characteristics

Texture, appearance and flavor are the most important qualities for commercial vegetable soybeans that gain consumer attraction. Processing directly affects physical characteristics of vegetable soybeans. A study reported that blanching resulted in a significant reduction of vegetable soybean hardness by 17.28% for sample blanched at 100 °C for 2.5 min and continued to decrease with increased blanching time (Xu *et al.* 2012). The decrease in hardness of vegetable soybeans during blanching was attributed to starch gelatinization and pectin solubilization (Song *et al.* 2003) when starch and pectin are responsible for hardness of vegetable soybeans. Textural changes of vegetable soybeans were affected by blanching as related to inactivation of pectinesterase (Steinbuch 1976). However, textural changes during frozen storage were attributed to moisture migration and ice recrystallization as the major causes. Moisture migration and ice recrystallization were believed to cause the increased hardness of vegetable soybeans during frozen storage. However, they can be minimized by maintaining small temperature fluctuations, small internal temperature gradients, and by the inclusion of internal barriers within a product and within a packaging (Zaritzky 2006). Moreover, blanching can be applied to vegetables as a pre-treatment of the freezing process in order to prevent crystal formation during commercial freezing by removing trapped air and metabolic gases within vegetable cells and replacing them with water, forming a semicontinuous water phase that favors a more uniform crystal growth during freezing (Reyes de Corcuera *et al*. 2004). Most of textural changes due to freezing and frozen storage would only become apparent after thawing and more noticeable in fruits and vegetables that have high water content (James 2006).

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CHAPTER 4

EFFECTS OF PROCESSING CONDITIONS ON QUALITY OF FROZEN VEGETABLE SOYBEANS

4.1 ABSTRACT

Blanching is a traditional and widely used method for vegetable soybeans preservation. Although combining blanching and freezing may be an excellent approach to prolong shelf life and retain vegetable soybeans quality, a critical gap of knowledge exists on this method and its effects. The purpose of this study was to determine an optimal combination of blanching and freezing conditions to retain high quality characteristics of final product. Vegetable soybeans were subjected to various blanching methods (water blanching and steam blanching), blanching temperatures (86, 92, 98 °C), and blanching times (1m30s, 2m, 2m30s, 2m50s), which were completed in a commercial facility. Color, texture and sodium chloride content of frozen vegetable soybeans during a six-month storage time were evaluated in response to the preservation methods applied. For example, the effects of blanching conditions on major commercial quality (color, texture, and sodium chloride content) of vegetable soybeans were evaluated during storage times of 1, 2, 3, 4, 5, and 6 months.

Blanching at a temperature lower than 98 °C did not adequately inactivate deteriorative peroxidase. Water blanching at shorter time than the control (2m30s) in commercial processing experiment did not effectively tenderize vegetable soybeans. On the other hand, decreasing blanching time in other experiments to 1m30s resulted in a final product comparable to that produced by a conventional process in terms of it quality traits. Color and sodium chloride content of vegetable soybean samples were not affected by blanching process. Additionally, freezing and frozen storage did not affect quality of vegetable soybeans during six months of storage.

Keywords: Pre-freezing treatment, blanching, freezing, vegetable soybean

4.2 INTRODUCTION

Processing plays an important role on the physiological characteristics of vegetable products. In order to evaluate the effects of processing and determine the optimal processing conditions for commercial frozen vegetable soybeans, a combination of blanching and freezing conditions was studied. Although water blanching and steam blanching are the most common blanching methods used in industrial-scale processing, this process must be optimized as it plays an important role in the quality of a final product during storage time. Blanching is able to destroy deteriorative microorganisms and inactivate enzyme activities resulting in high quality final products. As far as commercial frozen vegetable soybeans are concerned, effects of processing conditions (blanching methods – water blanching and steam blanching, blanching temperatures – 86, 92, 98 °C and blanching times – 1m30s, 2m, 2m30s, 2m50s) on three key commercial attributes (color, texture, and sodium chloride content) were studied for this project.

4.3 MATERIALS AND METHODS

An experimental approach was designed for optimal processing of frozen vegetable soybeans. In this study, blanching time and blanching temperature were considered independent factors, whereas color (-*a* value; greenness and *L* value; brightness), texture (hardness in Newton), and salt content (%salinity) were the responses. Vegetable soybeans, harvested in the same area and on the same day, were used as experimental units to represent population of inference.

The effects of each treatment on focused attributes were evaluated and compared to the controls (98 °C for 2m30s – water blanching and 98 °C for 2m50s – steam blanching). Totally, 23 treatments including control treatments were studied for this experiment, 11 water-blanched treatments and 12 steam-blanched treatments. (The experimental design for frozen vegetable soybean processing is presented in Table 1.) Based on an existing commercial processing process at a facility located in Lampang, Thailand, vegetable soybeans were water blanched at 98 °C for 2m30s or steam blanched at 98 °C for 2m50s prior to freezing. Therefore, these two treatments were assigned as the control treatments for water and steam blanching methods, respectively. Each treatment was completed in triplicate. In both (1) a commercial processing facility, and (2) laboratory setting.

4.3.1 Commercial processing experiment

In cooperation with Union Frost Co., Ltd., a frozen fruit and vegetable company in Thailand, the commercial processing experiment was completed as a part of real-world production. Vegetable soybeans, variety Xiemen75, were planted in Chiangrai, Thailand during the summer of 2012. They were handpicked and transported to the facility within 8 h of harvest in order to minimize both physical and chemical deterioration. Upon arrival at the facility, fresh vegetable soybeans were inspected and graded following company quality control standards. Fresh vegetable soybeans were conveyed through air blowers to remove light foreign material, such as dust, straws, and leaves. They were then washed in bubble washing tanks and soaked twice to remove dirt, soil and surface microorganisms. Small and imperfect pods were removed through shaking screens. Defected pieces were manually sorted followed by an additional washing in bubble washing tanks and finally conveyed through blancher. The vegetable soybeans were then

Table 1. Experimental design for commercial production

Blanching Time	Water Blanching (°C)			Steam Blanching (°C)		
	86	92	98	86	92	98
1m30s	Trt 1	Trt 5	Trt 9	Trt 12	Trt 16	Trt 20
2m00s	Trt 2	Trt 6	Trt 10	Trt 13	Trt 17	Trt 21
2m30s	Trt 3	Trt 7	Trt 11	Trt 14	Trt 18	Trt 22
2m50s	Trt 4	Trt 8	-	Trt 15	Trt 19	Trt 23

*Trt stands for Treatment

packed in mesh bags and labeled with treatment numbers. Each treated sample consisted of 12 kg of vegetable soybean. For the water blanching method, a two-metric ton rotary blancher was used (Figure 1). The blancher was filled with sufficient volume of water for sample immersion. Samples of fresh vegetable soybean (12 kg/treatment) were blanched at designated blanching conditions of temperatures (86, 92 and 98 °C) and times (1m30s, 2m, and 2m30s). Blanching time was recorded from the moment samples were immersed in boiling water and controlled by the speed of conveyor. All vegetable soybean samples were tested during three consecutive production shifts.

In the steam blanching process, a four-metric ton capacity continuous steam blancher was used (Figure 2). Samples of fresh vegetable soybeans were conveyed through blanching chamber where a mixture of food-grade steam and water (86, 92 and 98 °C) was introduced to the samples by spraying from above. Blanching time was controlled by conveyor speed (1m30s, 2m, 2m30s, and 2m50s).

Vegetable soybeans from each treatment were sampled for a peroxidase test. Peroxidase, a heat-resistant enzyme used as an indicator enzyme on residual enzyme activities, was tested for presence immediately after blanching. In this study, the peroxidase test was adapted for on-site testing by depositing a few drops of 0.5% guaiacol and 0.08% hydrogen peroxide substrates on half-kernel beans. The samples were visually monitored for color change within 3 min. Color of the mixtures changing from colorless to brick red indicated incomplete enzyme inactivation (Figure 3). Treatments with incomplete enzyme activities were rejected at the end of blanching stage.

Figure 1. Rotary drum blancher
(Suwit Machinery, Lamphun, Thailand)

Figure 2. Continuous steam blancher
(JV industrial Co. Ltd., Bangkok, Thailand)

Figure 3. Brick-red solution of incomplete lipoxygenase enzyme inactivation

Treatment samples with complete enzyme inactivation were conveyed through a cooling chamber to stop overcooking and reduce heat in the product. The cooling process consisted of immersing (for water blanching method) or spraying (for steam blanching method) samples with a cold saline solution (4 °C) for 4m40s to obtain final products with a commercial standard of 0.3% to 0.5% salinity. Vegetable soybean samples were drained properly by conveying through shaking screen prior to freezing in order to reduce excess moisture and prevent block formation.

Individual Quick Freezing (IQF) was used freeze the blanched vegetable soybeans. Therefore, each piece of product was placed on a cooling conveyer and subjected to a very low temperatures (-30 °C to -40 °C) using a cold air fluidization technique. Residence time was controlled by the conveyer speed as specifically designed for vegetable soybeans, which ensured that product core temperature decreased to -18 °C or below. Frozen vegetable soybean samples were stored in HDPE bags and cardboard boxes and kept at -18 °C or below until further analysis.

Referring to the POD test conducted in the commercial processing experiment, the results showed that blanching at temperatures lower than 98 °C did not effectively inactive POD enzyme, which could result in off-flavor and off-color in frozen product during storage. Hence, all treatments blanched at temperatures lower than 98 °C were eliminated from the study and were not carried on to laboratory experiment.

4.3.2 Laboratory experiment

Due to the availability of the equipment in laboratory, some experiment steps were modified to be as similar as possible to the commercial processing experiment. Regarding commercial processing results, only seven treatments (treatments blanched at

98 °C from both water blanching and steam blanching) had complete POD inactivation and were carried on to laboratory experiment.

In the laboratory experiment, Midori Giant vegetable soybeans were chosen as they had the most similar characteristics to variety Xiemen75, i.e., containing 2-3 large, sweet, and buttery seeds per pod with a clear pubescence, and having similar range of maturity days. Vegetable soybean seeds were bought from a supplier and planted in a research plot on East Campus at the University of Nebraska-Lincoln, Lincoln, Nebraska, in May 2013. Standard cultural practices were followed. Vegetable soybeans were harvested at R6 stage in August 2013 (95 days) by handpicking in the early morning. They were gathered in bundles and transported to shaded area where the pods were stripped off the stems. Vegetable soybean pods were packed in mesh bags and transported to processing unit within 2 h. Approximately 12 kg of vegetable soybeans per treatment was processed within 8 h of harvest.

In the preparation stage, vegetable soybeans were washed and soaked to remove dirt, soil, leaves, and stems prior to blanching. In water blanching method, a steam-jacketed blancher was used. Blancher was filled with an adequate volume of water for sample immersion. Vegetable soybean samples were immersed into near-boiling water (98 °C) for 1 m30s, 2m, and 2m30s for each specific treatment. Blanching time was recorded from the moment samples were immersed in boiling water.

In the steam blanching method, a batch steam blancher was used. Vegetable soybean samples were placed in blanching chamber and blanched with food-grade steam (100 °C) for 1m30s, 2m, 2m30s, and 2m50s for each specific treatment. After blanching, vegetable soybeans were cooled down by immersing in a 15-17% saline solution (4 °C) for 4m40s in order to reduce heat in the product and enhance salty flavor. Subsequently, each vegetable soybean treatment was packed in plastic bag and flattened the bag into a thin sheet. Vegetable soybean were frozen by placing a thin-sheet bag in Styrofoam coolers filled with a layer of dry ice, layer by layer, and covered until their core temperature decreased to -18 °C or below. Frozen vegetable soybeans were collected in HDPE plastic bags and cardboard boxes and stored at -18 °C or below until further analysis.

4.4 MEASUREMENTS AND ANALYSIS

4.4.1 Color measurement

The frozen vegetable soybean samples were thawed to room temperature prior to color measurement. A Chroma meter was calibrated using a white tile with values of L = 97.10, a = +0.08 and b = +1.97, where L^* is the luminance or lightness component, which ranges from 0 (black) to 100 (white), a^* is from green ($-a$) to red ($+a$), and b^* from blue ($-b$) to yellow ($+b$) are the two chromatic components which range from -120 to 120 (León *et al.* 2006). The color of the vegetable soybean peel, L^* (brightness), $-a$ (greenness), were measured using CR300 Minolta Color Reader (Minolta Camera Co.,Ltd., Japan). Each treatment measurement was completed in triplicate and the results were collected for statistical analysis (refer to Section 4.4.4).

4.4.2 Texture Profile Analysis

In this study, the compression force necessary to attain certain deformation of a single intact vegetable soybean was measured and recorded. Frozen vegetable soybean pod samples were thawed to room temperature, shelled and subjected to texture analysis using a Texture Analyzer TA.XtPlus (Texture Technologies Corp and by Stable Micro Systems, Ltd., Hamilton, MA) with 2-inch cylindrical probe in the commercial processing experiment. Compression force needed to achieve 90% deformation, at test speed of 2mm/s, from each treatment sample was measured and recorded in the commercial processing experiment. A Brookfield TexturePro CT (Brookfield Engineering Labs, Inc., Middleborough, MA) with a 1500 g load cell and a TA25/100 cylindrical probe was used in the laboratory experiment. Compression force needed to achieve 40% deformation, at test speed of 0.5 mm/s, from each treatment sample was measured and recorded in the laboratory experiment. The peak force of the first compression cycle, in Newton, represented the hardness of treatment sample. Each treatment measurement was completed in triplicate. As mentioned earlier, since there is no standard available on the desired texture for vegetable soybeans, samples from the commercial processing were used as the standard and control in the research.

4.4.3 Sodium chloride content measurement

NaCl content in frozen vegetable soybeans was analyzed using Mohr's direct titration method (adapted from AOAC Official Method 937.09 section 35.1.18 salt – chlorine as sodium chloride in seafood) (Appendix B.1). NaCl was initially extracted by blending 25 g of vegetable soybeans with 250 ml distilled water, and filtrated. Potassium chromate 5% (K_2CrO_7) (3 ml) was added into 30 ml of sample solution as an indicator and, subsequently, titrated with 0.1 M silver nitrate standard solution. The volume of silver nitrate used to complete the reaction in each treatment was recorded for statistical analysis. Chloride content was measured in the absence of acid.

4.4.4 Statistical Analysis

Ten replicates were prepared and processed for each of the treatments and control. Collected data were analyzed using SAS software (version 9.3, SAS Institute, Inc., Cary, NC). General Linear Models were used to detect significant differences of color, texture, and sodium chloride content among samples under different blanching conditions and storage times if they existed. Dunnett adjustment for multiple comparisons was used to compare each of a number of treatments with a single control. Probability (P) < 0.05 indicated significance. Results and discussion, as well as conclusions, were discussed in the following section.

4.5 RESULTS AND DISCUSSION

The experiment was designed to evaluate the effects of various combinations of blanching time and blanching temperature on vegetable soybean attributes as they were considered the critical points of the whole experiment. Blanching temperature must be high enough to completely inactive deteriorative enzymes. On the other hand, blanching temperature must not destroy functional nutrients and cause physical damages. Blanching time was another factor that needed to be considered. Vegetable soybeans should be blanched for sufficient time to achieve the acceptable level of tenderization. Because this study was intended for use for industrial-scale processing, blanching time that depends on conveyor speed had to be carefully controlled because the speed of blanching conveyor directly affects the flow of whole process. Blanching time must be well matched with blanching temperature in order to provide optimal quality to final product. Each combination was designed to have lower temperature and shorter time than control treatment to identify the optimal production process that increases production capacity, reduces waste production, and decreases energy consumption.

As the typical frozen storage period for commercial frozen vegetable soybeans is approximately six months (Tosun and Yücecan 2008), all frozen vegetable soybean samples in this study were stored at -18 °C or below and analyzed every 30 days during six months (0, 1, 2, 3, 4, 5, and 6 months) of storage.

4.5.1 Effects of experiment conditions on commercial vegetable soybean quality

Experiments were conducted at two different sites (Union Frost Co., Ltd., Thailand and University of Nebraska-Lincoln, Lincoln, Nebraska). Initially, there were 23 treatments from the experimental design, including 11 water-blanching treatments and 12 steam-blanching treatments (see Table 1). According to the commercial processing experiment that was conducted earlier during the summer of 2012, all 23 treatment samples were blanched at the designated temperatures and times. Immediately after blanching, every vegetable soybean treatment was sampled for a POD test. According to the results, POD was detected in all treatment samples blanched at temperatures lower than the control temperature (98 °C) for both water-blanched and steam-blanched methods. Incomplete enzyme inactivation leads to poor color, texture and off-flavor development of frozen vegetable soybeans during storage. Hence, all treatments blanched at temperature lower than 98 °C were eliminated for further study. Finally, 7 treatments were carried on to the next step, including Treatment 9 (water-blanched at 98 °C for 1m30s), Treatment 10 (water-blanched at 98 °C for 2 min), Treatment 11 (water-blanched at 98 °C for 2m30s; Control), Treatment 20 (steam-blanched at 98 °C for 1m30s), Treatment 21 (steam-blanched at 98 °C for 2 min), Treatment 22 (steam-blanched at 98 °C for 2m30s), and Treatment 22 (steam-blanched at 98 °C for 2m50s; Control).

(a) Texture Profile Analysis

Water blanching treatments – As far as commercial processing experiment was concerned, there was an increase in hardness of vegetable soybeans for all water-blanched treatments, including the control, during the first four months of storage (Figure 4). Data from the 5th and the 6th month were considered as outliers (not shown in the figure) as they were abnormally distant from other values, which may be attributed to the measurement error. The results showed that the texture of all treatment samples (Treatment 9 and Treatment 10) were significantly harder than the control treatment ($p<0.05$). Shorter water-blanching time than the control time (2m30s) did not effectively tenderize the vegetable soybean texture to the level of the control. Additionally, storage time had significant effect on the texture of vegetable soybean samples as they became harder over time ($p<0.05$).

According to the results from the laboratory experiment, hardness of water-blanched treatments ranged between 7.8 N to 15.0 N and had insignificant changes from month to month during storage (Figure 5). At the end of storage period, all water-blanched samples were as hard as they were at the beginning of the experiment. Additionally, hardnesses of the treatment samples (Treatment 9 and Treatment 10) were not significantly different from the control.

Steam blanching treatments – According to the commercial processing experiment, hardnesses of all treatment samples increased slightly during the first four

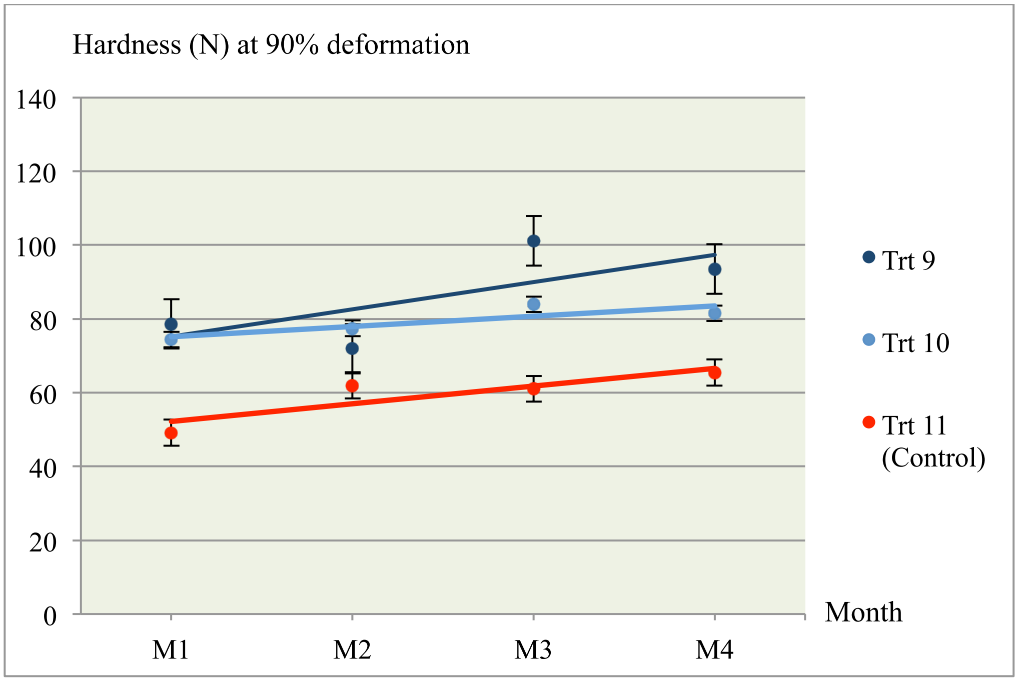

Figure 4. Texture profile analysis of commercial water-blanching samples at UNF

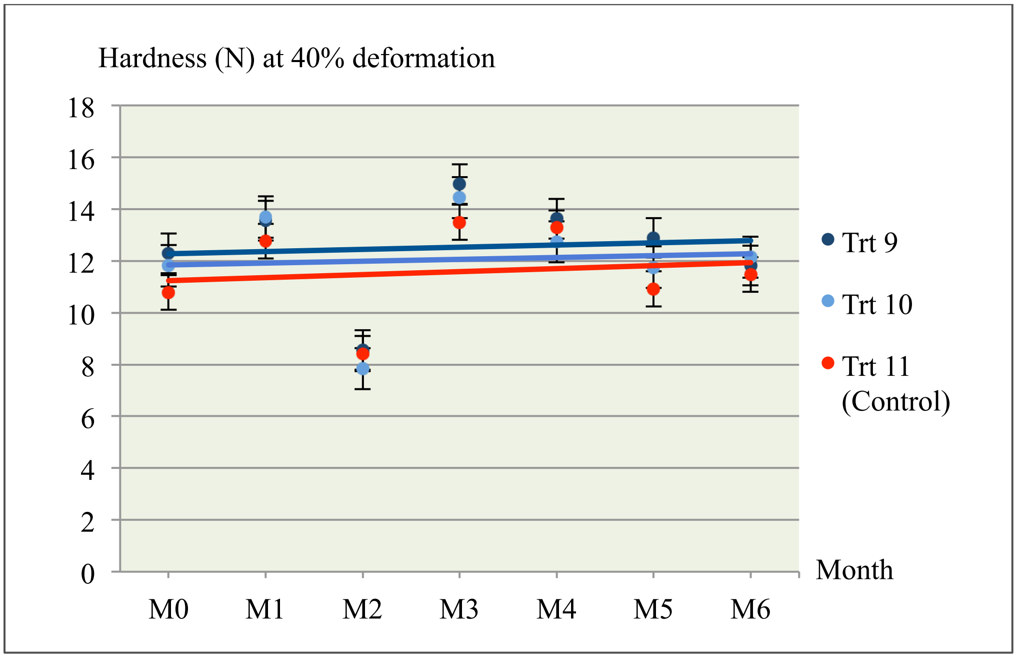

Figure 5. Texture profile analysis of laboratory water-blanching samples at UNL

months, except treatment 22 (Figure 6). However, they were not significantly different from that of the control and no significant changes during storage time were observed. Data from the 5th and the 6th month were considered as outliers and not shown in the figure. Hardnesses of all treatment samples from laboratory experiment ranged between 9.2 N to 14.5 N (Figure 7). Blanching treatments had no significant effects on the texture of all treatment samples compared to the control during storage time. According to the laboratory experiment, unexpected drops in all hardness values were observed in the 2nd month of storage for both water and steam blanching methods (Figure 5 and Figure 7). It was assumed to be a miscalibration error in the measurement procedure.

(b) Color measurement

L-value (Brightness) – According to water blanching method from both experiments, *L*-values of vegetable soybean samples from the commercial processing experiment ranged between 35.80 to 45.90, while those from the laboratory experiment ranged between 41.39 to 52.46. The brightness of treatments from both experiments was not significantly different from the controls and did not change significantly during storage. However, the brightness trends of both experiments were slightly different as the brightness of laboratory samples increased slightly, while that from commercial processing experiment decreased slightly over time. This may have been caused by the difference in varieties and the exposure to blanching media.

Likewise, the *L*-values of steam-blanched samples, from both experiments, had similar patterns to those of the water-blanched method. Brightness of steam-blanched samples from the commercial processing experiment ranged between 34.10 to 47.60,

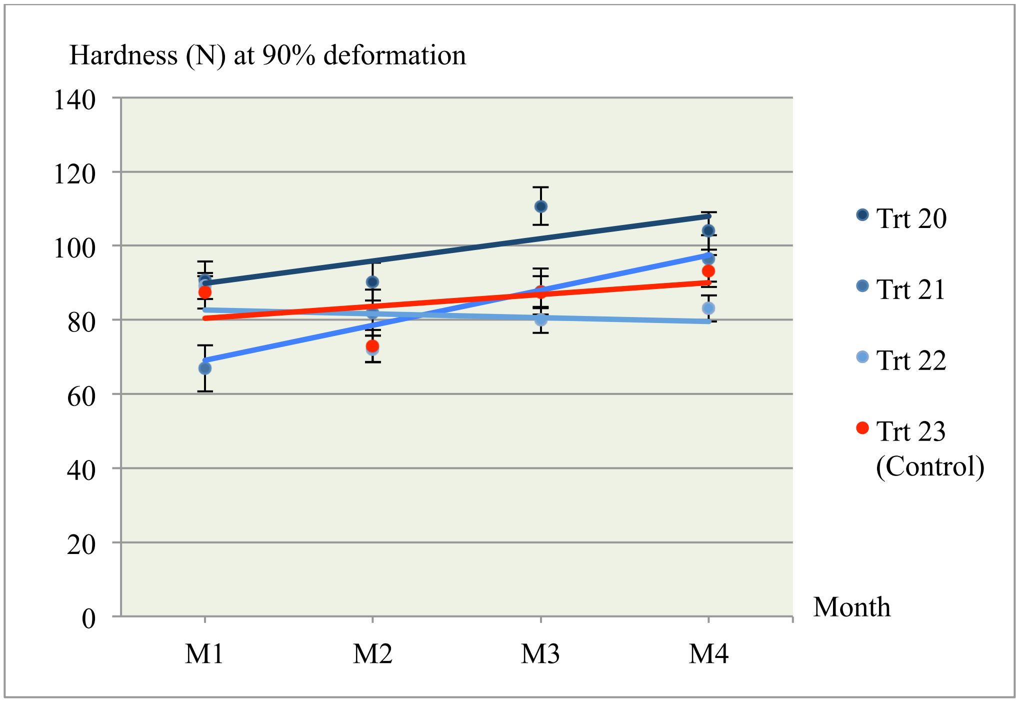

Figure 6. Texture profile analysis of commercial steam-blanching samples at UNF

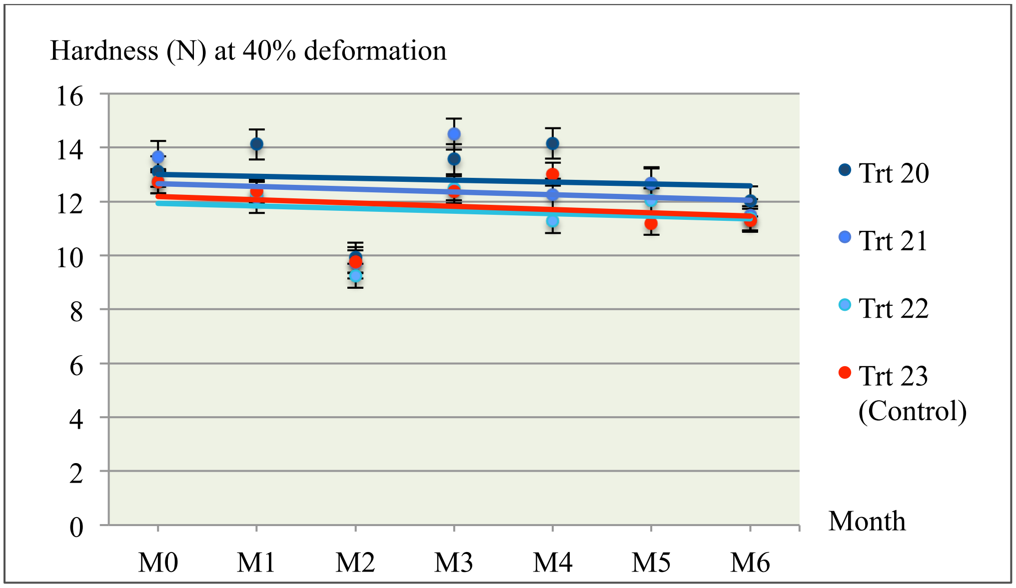

Figure 7. Texture profile analysis of laboratory steam-blanching samples at UNL

while those of the laboratory experiment ranged between 41.67 to 52.33. There were no significant differences in brightness among commercial processing treatments compared to the control and no significant changes were observed during storage. The brightnesses of laboratory treatments increased significantly during storage ($p<0.05$). Laboratory vegetable soybean samples became brighter after the 3rd month of storage.

-a value (Greenness) – The *-a* values of the water-blanched samples ranged between -14.62 to -9.80 for the commercial processing experiment and between -18.12 to -12.42 for the laboratory experiment. As far as the commercial water blanching experiment was concerned, there were no significant differences in greenness compared to the control and no significant changes during storage time. However, the water-blanched treatments from the laboratory experiment were significantly greener than the control ($p<0.05$). Moreover, the greenness of laboratory samples increased significantly over time ($p<0.05$) (Figure 8).

As far as steam-blanched method was concerned, the *-a* values of the commercial processing experiment ranged between -13.25 to -9.60 and between -18.53 to -11.47 in the laboratory experiment. There were no significant differences in greenness of vegetable soybean samples compared to the control in the commercial steam-blanching experiment during 6-month storage. In agreement with Martins and Silva (2003), color has been shown to be stable at low temperature ($T < -18$ °C). Color loss of green vegetables during frozen storage is attributed mainly to the fading of the vivid green to an olive brown color, due to chlorophyll's pheophytisation, the replacement of chlorophyll magnesium by hydrogen. At this low temperature, metal-chlorophyll

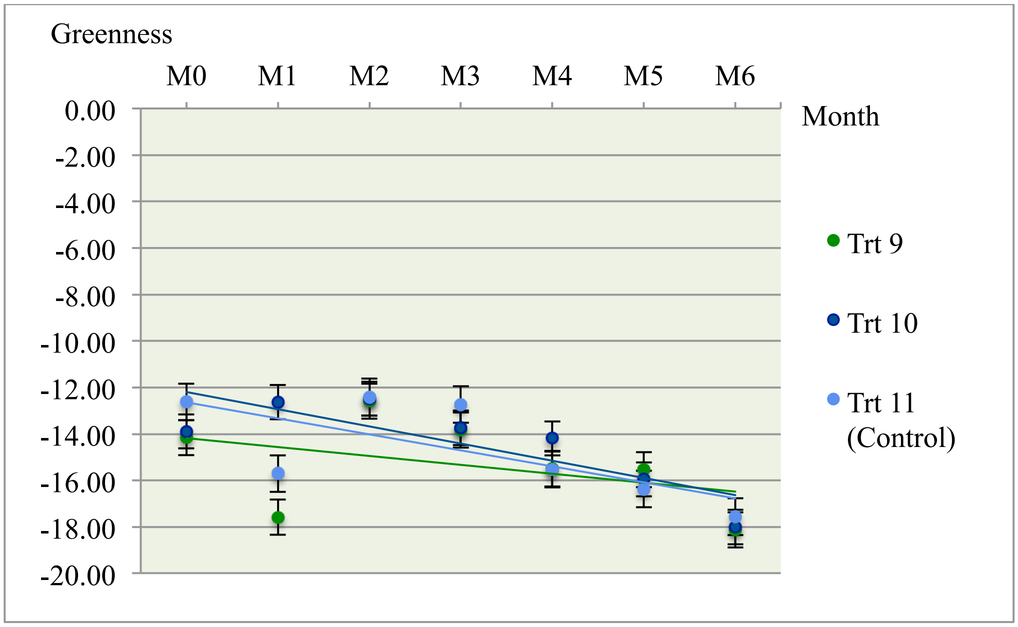

Figure 8. -*a* values of laboratory water-blanching samples at UNL

compounds, such as cupric-chlorophyll, retained a vivid green color (Martins and Silva 2003). However, the greenness of laboratory steam-blanched treatments was significantly greener than that of the control ($p<0.05$) (Figure 9).

Referring to the CIE color space diagram, the *L*-values and -*a* values of all vegetable soybean samples observed from the study fell into a very small area, approximately 0.2% of total area, of the diagram as shown in Figure 10. Even though there were significant differences between some treatments and the controls during storage time, the shade of brightness and greenness of vegetable soybean samples were all very similar and difficult to differentiate visually.

(c) Sodium chloride content

Vegetable soybean samples were cooled with 15-17% saline solution to provide the final product with commercial standard of 0.4±0.1% saltiness. During the commercial processing experiment, some data on sodium chloride analysis from Union Frost Co., Ltd. facility were missing. Hence, changes in sodium chloride content during storage time from the commercial processing section were not analyzed. However, the existing data showed that salt percentage of all samples were in acceptable range of 0.3-0.5% during the first three months of storage.

As far as the laboratory experiment was concerned, salt content of all treatment samples ranged between 0.19 to 0.64 (Figure 11). The results showed that the average saltiness of all treatments as within the acceptable standard range over storage time of six months.

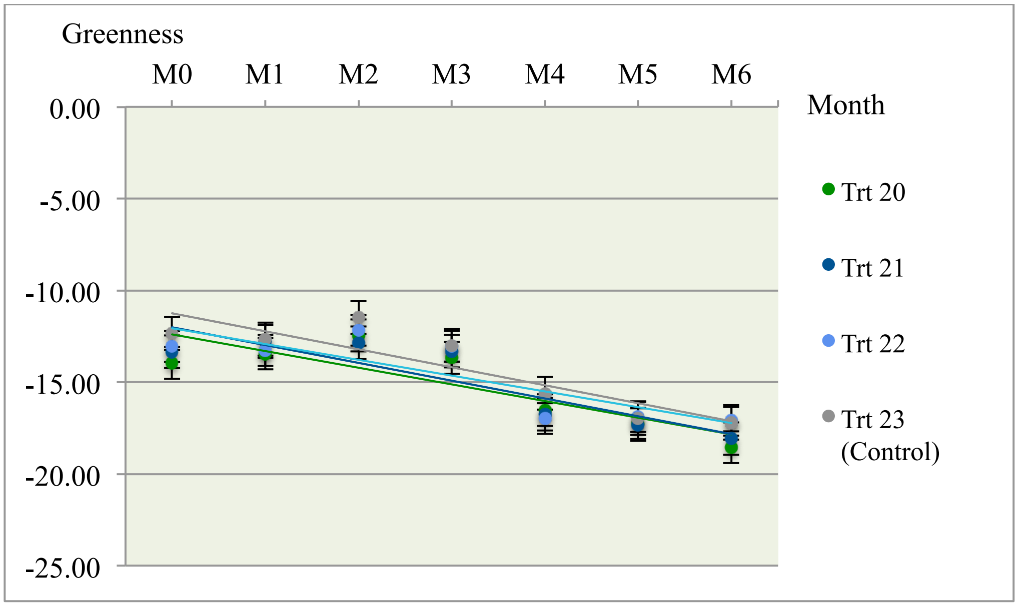

Figure 9. -*a* values of laboratory steam-blanching samples at UNL

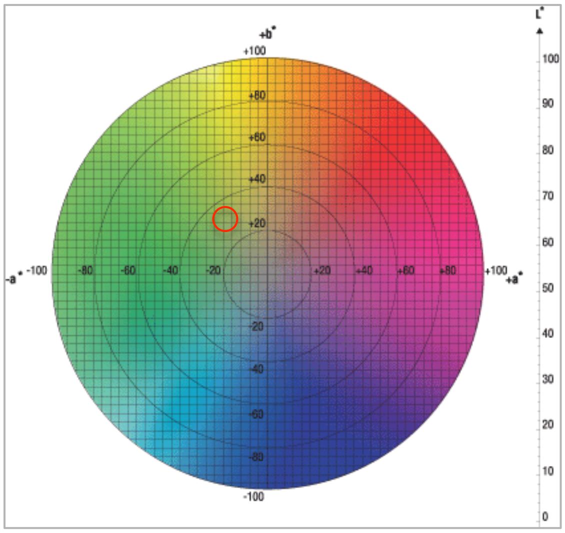

Figure 10. CIE Lab diagram showing area of all vegetable soybean color observed in the study

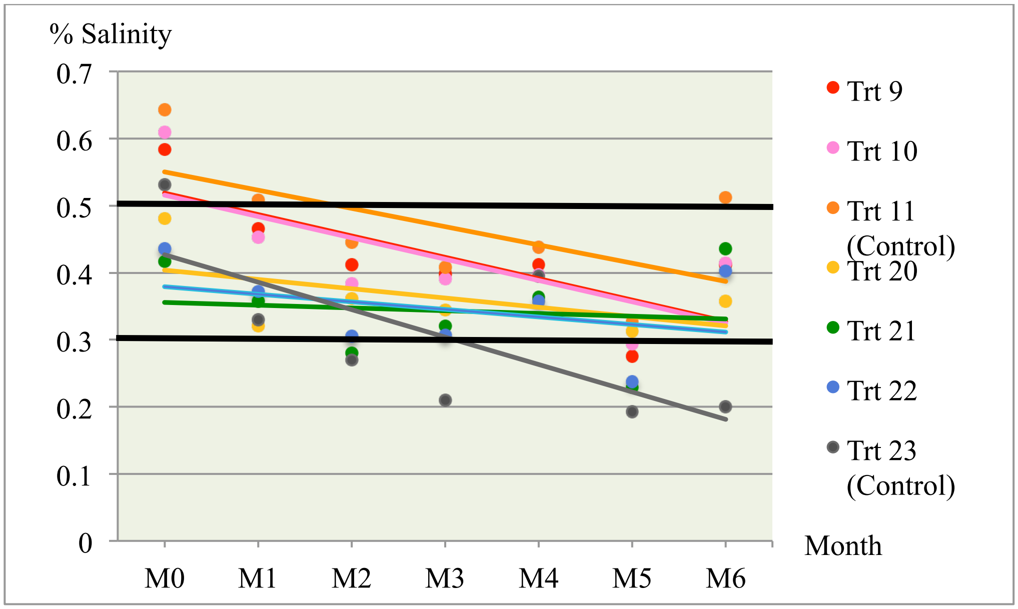

Figure 11. Salt content of all vegetable soybean samples

*Black lines indicate standard range of commercial saltiness (0.3-0.5%)

4.5.2 Effects of blanching factors and storage time on commercial quality attributes

(a) Effects of blanching method

Color – Regarding color measurement, the results from the commercial processing experiment showed that *L*-values (brightness) of water-blanched samples ranged between 35.80 to 45.90, while those of steam-blanched samples ranged between 34.10 to 47.60. *L*-value ranges of vegetable soybean samples from both blanching methods were very close to each other. Furthermore, vegetable soybean samples from both blanching methods shared similar patterns of change in brightness during storage time. According to the laboratory experiment, brightness of water-blanched samples ranged between 41.39 to 52.46, whereas those of steam-blanched samples ranged between 41.67 to 52.33. The results showed clearly that brightness pattern of samples from both blanching methods were similar and *L*-value ranges were very close.

Another color parameter is the -*a* value, or greenness of the bean shells, which is the very first quality parameter that attracts consumers. According to the commercial processing experiment, greenness of water-blanched vegetable soybean samples were in the range of -9.80 to -14.62, whereas those of steam-blanched were between -9.60 to -13.25. The results showed that -*a* values from both blanching methods were in the very close range. A similar trend of greenness was observed in the laboratory treatments. Greenness of all water-blanched treatments ranged between -12.42 to -18.12, while those of steam-blanched treatments ranged between -11.47 to -18.53. The results did not show any effects of blanching method on the greenness of vegetable soybean samples from the laboratory experiment.

Results from both the commercial processing experiment and the laboratory experiment showed clearly that blanching method did not have significant effects on brightness and greenness of vegetable soybeans. In this study, blanching temperature of the media was high enough to inactivate the enzyme responsible for color change in vegetable soybeans. Colors of vegetable soybeans from both blanching methods were well retained during storage time.

Texture Analysis – Results from texture profile analyses cannot be compared between water and steam blanching methods as blanching technique was different as well as the blanching media, which had different heat transfer coefficients (Fellow 2000).

Salt Content – Because the salting process was applied to vegetable soybean samples after blanching, it was not considered as a function of blanching method. Blanching method had no effect on salt content of vegetable soybean samples.

(b) Effects of blanching time and temperature

Results from the commercial processing experiment showed clearly that blanching temperature directly affected quality of vegetable soybeans. Blanching at temperatures lower than 98 °C, for both blanching methods, did not effectively inactive enzymatic activities of peroxidase which causes loss of quality during frozen storage. Therefore, 98 °C was the only blanching temperature factor studied in the further portions of the commercial processing experiment and the laboratory experiment.

Color – At blanching temperature of 98 °C, the results showed that water blanching at shorter time than the control (2m30s) did not cause significant differences in brightness of vegetable soybean samples compared to the control in both the commercial processing and the laboratory experiments. Similarly, steam blanching at shorter time than the control (2m50s) resulted in no significant differences in brightness of vegetable soybean samples compared to the controls from both experiments.

As far as the -*a* value results were concerned, decreasing blanching time in both blanching methods from the controls did not cause significant differences in the greenness of vegetable soybean samples from the commercial processing experiment. On the other hand, lowering blanching time significantly affected the laboratory processing samples. The greenness of laboratory treatments was significantly lower than that of the controls in both water blanching method ($p<0.05$) and steam blanching method ($p<0.05$). However, those differences showed the improvement, decrease in -*a* values, as vegetable soybean samples became greener.

Overall, the results showed clearly that blanching time, in the studied range (1m30s to 2m50s), had no obvious effects on brightness and greenness of vegetable soybean samples compared to the conventional processing time. In other words, decreased blanching time within the range of this study did not cause color degradation in vegetable soybeans. It can be concluded that, at blanching temperature of 98 °C, decreasing blanching time to 1m30s for both water and steam blanching methods had no negative effects on the appearance of vegetable soybeans.

Texture Analysis – According to water blanching method from the commercial processing experiment, the results showed that vegetable soybean samples blanched at a shorter time than the control (2m30s) had significantly harder texture than the control ($p<0.05$). Decreasing blanching time from the control time did not effectively tenderize the texture of treatment samples (Treatment 9 and Treatment 10) to the level of the control, which may cause unacceptable quality for frozen vegetable soybeans during storage time. On the other hand, decreased steam-blanching time to 1m30s had no significant effects on texture of all treatment samples compared to the control from the commercial processing experiment.

Referring to the results from the laboratory experiment, decreased blanching time to 1m30s in both water-blanching and steam-blanching methods had no significantly effects on texture of all treatment samples compared to the controls.

Based on the results from this study, at the same blanching temperature of 98 °C, water blanching time in the laboratory experiment can be reduced to 1m30s, while the same treatment could not be applied in the commercial processing experiment. It can be assumed that, besides blanching condition, characteristics of vegetable soybeans, i.e. variety, and chemical composition, also play an important role on the texture of final product. This concern should be taken into account when designing an appropriate processing condition.

Salt Content – Salting process was applied to vegetable soybean samples after blanching; therefore, blanching temperature and blanching time did not affect salt content of all vegetable soybean samples.

(c) Effects of storage time

All treatment samples were stored at -18 °C or lower, which was the optimal temperature to maintain for the best quality for frozen product. Hence, storage temperature was not considered as an independent factor in this study. A previous study reported that quality of commercial frozen vegetable was retained for six months.

Therefore, the vegetable soybean samples were sampled for analysis every 30 days for six months to evaluate changes among treatments during storage time.

Color – According to the commercial processing experiment, no significant differences in the *L*-values (brightness) and the -*a* values (greenness) of all vegetable soybean samples compared to the controls were observed during storage time. The colors of vegetable soybean samples were stable during six-month time. Similar results were observed in the laboratory experiment, except the brightness of steam blanching method and greenness of water blanching method. The brightness of steam-blanched samples did not change substantially during the first three months. However, it increased significantly (the beans became brighter) after the third month ($p<0.05$). Vegetable soybeans became brighter at the end of storage time compared to the beginning. In addition, there was a significant decrease in -*a* values of water-blanched samples ($p<0.05$), which showed that vegetable soybeans became greener during storage. The results were in agreement with Forni *et al*. (1991) that after six months of storage at -20 °C, only a small pheophytinisation of chlorophylls in peas were observed. The color was stable during storage time as evaluated by the objective measurements and by organoleptic evaluations.

The results confirmed that storage time had no negative effects on colors (brightness and greenness) of vegetable soybeans as they became brighter and greener compared to the beginning. Frozen storage at -18 °C and below can preserve the color of vegetable soybeans for at least six months as long as they were processed properly and the enzymes responsible for color degradation were destroyed effectively during blanching step.

Texture Analysis – According to the results from commercial processing experiment, storage time had significant effects on the texture of water-blanched samples. Vegetable soybeans from water-blanching treatments became significantly harder after the 3rd month of storage ($p<0.05$). Moisture migration and ice recrystallization were believed to cause the increased hardness of vegetable during frozen storage (Zaritzky 2006). During storage at the Union Frost Co., Ltd facility, temperature fluctuation from daily operation was possibly the major cause of moisture migration and ice recrystallization in vegetable soybean samples from the commercial processing experiment. These undesirable effects can be minimized by maintaining small temperature fluctuations, small internal temperature gradients, and by inclusion of internal barriers within a product and within a packaging (Zaritzky 2006). However, storage time had no significant effects on texture of steam-blanched vegetable soybean samples.

As far as the results from laboratory experiment were concerned, storage time had no significant effects on vegetable soybean texture from both blanching methods. Hardness of all vegetable soybean samples remained stable during storage period.

The different hardness patterns of vegetable soybeans between Xiemen75 (used in the commercial processing experiment) and Midori Giant (used in the laboratory experiment) were attributed to the differences in varieties, physiological characteristics, and chemical composition of vegetable soybeans. In addition, variation in commercial and laboratory practices may have affected the hardness of samples somehow, even though the laboratory set up was as similar to the commercial processing experiment as possible. For instance, the sample amount of vegetable soybean studied in the laboratory experiment was smaller than the commercial experiment, which possibly made experimental factors in laboratory more controllable. Measurement error was also possibly a cause of differences.

For further study, sensory evaluation is strongly recommended in order to determine if panelists can perceive the differences in texture between experimental samples and the control as well as changes in hardness of vegetable soybeans during storage.

Salt content – No significant changes in salt content were observed in all treatment samples during six months of storage time. Average salt content of all treatment samples was in the standard range of 0.4±0.1% (Figure 10). The results clearly confirmed that storage time had no effect on saltiness of vegetable soybeans.

4.6 CONCLUSIONS

Processing plays an important role in vegetable soybeans preservation, however; effects of processing are unavoidable. Blanching directly affected the quality of vegetable soybeans in various aspects, including color and texture.

Referring to the conventional processes at Union Frost Co., Ltd., blanching at temperatures lower than 98 °C did not effectively inactivate the deteriorative enzymes for both water and steam blanching. In the exception of water blanching method from commercial processing experiment, blanching time of all experiments can be reduced to 1m30s with comparable quality to the conventional processes. Freezing and frozen storage had no significant effects on the quality of vegetable soybeans. Vegetable soybeans can be stored up to six months at -18 °C and below without noticeable changes compared to the conventional processes. Besides maintaining quality of commercial frozen vegetable soybeans, appropriate processing conditions and storage also increase production capacity, efficiency, profits, and decrease energy consumption.

4.7 REFERENCES

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CHAPTER 5

GENERAL CONCLUSIONS, APPLICATION OF THE STUDY RESULTS AND RECOMMENDATION FOR FUTURE STUDY

5.1 CONCLUSIONS

According to the objectives stated earlier, this study was completed to identify the optimal processing conditions for commercial frozen vegetable soybeans, and to evaluate the effects of pre-freezing treatments on the commercial quality attributes (color, texture, and sodium chloride content) of vegetable soybeans during six-month storage time. The conclusions drawn from this study are:

1. The conventional process conducted at Union Frost Co., Ltd. facility was not the optimal process and could be improved;
2. Conventional processing process of frozen vegetable soybeans can be improved by decreasing steam blanching time to 1m30s without affecting the quality of the final product compared to current conventional process;
3. Blanching vegetable soybeans at temperatures lower than 98 °C was not adequate to inactivate enzymatic activities for both water blanching and steam blanching methods;
4. Blanching apparently affected quality of vegetable soybeans while freezing and frozen storage had no significant effects on final product;
5. Decreasing blanching time obviously would increase production capacity and decrease energy consumption, increasing profits, and reducing waste production; and
6. High Temperature Short Time (HTST) approach was applicable to identifying the optimal and specific blanching time for vegetable soybeans.

In industrial-scale processing, there are numerous factors that should be concerned carefully i.e. applicability of blanching approach and machinery in the facility, compatibility of blanching approach to product, operation cost, and production flow. A good production practice results in a good quality final product. Nevertheless, quality of vegetable soybeans also depends on other pre-processing factors i.e. genotype, harvest time, environment, etc. Therefore, the raw materials should be strictly controlled prior to entering the processing line.

5.2 APPLICATION OF THE STUDY RESULTS

Results and findings from this research are anticipated to be valuable and applicable for both academic and industrial sectors. Primarily, results from this research are beneficial and applicable to the frozen vegetable soybean industry in the United States. The outcomes can be used to improve the present process in industrial-scale processing in many states that have already launched growing vegetable soybeans. Moreover, the results can be used as reference and guideline for beginners in this business. Recently, there have been farmers in many states i.e. Arkansas, Iowa, Minnesota, and Colorado interested in growing and producing vegetable soybeans commercially. They have collaborated with state universities and government institutes in various aspects, i.e., cultivation, variety development. A numbers of vegetable soybean varieties have been developed for local farming. With this study, it is anticipated that local farmers, in the potential areas, will be encouraged to grow more vegetable soybeans in order to supply the fast growing demands in the United States. Knowledge from this project will add value to vegetable soybeans. This study also is expected to be supportive for vegetable soybean variety developers and farmers in the near future.

Potential for vegetable soybean market in the United States

- Meat-protein substitution

- Less expensive alternative protein source
- Supplying the fast growing domestic demands as Soybean Board (2010) estimated that vegetable soybeans would surpass all other soy-based products by 2020.
- Soybeans for livestock feed have faded
- Potential production for high value niche product

Limitation of vegetable soybean market in the United States

- Vegetable soybeans are perishable with short harvest and handling time. This limitation may require extra operational costs and efforts.
- Time and labor consumption during handpicking harvest. While mechanical harvest is still being developed, handpicking harvest is costly and time-consuming.
- Vegetable soybeans have specific growth condition requirements and they are sensitive to weather change. Inappropriate management may lead to crop failure.

5.3 RECOMMENDATIONS FOR FUTURE STUDY

As mentioned earlier, vegetable soybeans were introduced to American markets only in the past few decades. Hence, the information regarding vegetable soybeans, including breeding, planting, harvesting, processing, and marketing, in the United States is lacking. According to the US Department of Agriculture, it is not clear the amount of vegetable soybeans produced in the United States. The amount of vegetable soybeans produced is substantially less compared to field-type soybean. Most studies recently published are related to vegetable soybeans cultivation, variety development and domestic processing. To this author's knowledge, studies on commercial processing are not yet available. Research and studies on vegetable soybeans from farm-to-fork should be encouraged and supported by government, universities, and relevant organizations. The United States has the capability and potential for a comprehensive system of vegetable soybeans in aspects of lands, weather, harvest technology, and varieties development. Other criteria of interest are the desirability and marketable quality of selected varieties. For future studies, experiments on locally grown or U.S. commercial varieties are recommended in order to obtain more realistic and applicable outcomes for processing in the United States.

Limitation in this project was that only two varieties of vegetable soybeans were studied, Xiemen75 and Midori Giant. These two varieties may exhibit similar effects of processing on the concerned attributes but they may not entirely represent other commercial varieties of vegetable soybeans. In the United States, there are numerous vegetable soybean varieties available in the market. Each variety is different in physiological characteristics, i.e., genotype, height, yield, seed size, seed flavor, chemical compositions, and time to maturity, which may require specific processing conditions. Hence, specific characteristics of each vegetable soybean variety should be studied and selected appropriately to match the production region (latitude) for maximum production yield and to match the processing conditions.

With the limited number of panelist available and fresh vegetable soybean samples, sensory evaluation of frozen vegetable soybeans was not conducted in this study. Therefore, sensory evaluation is recommended for future study in order to evaluate consumer acceptability on commercial frozen vegetable soybeans and to observe if panelists can discriminate the differences between treatments if they exist. Sensory evaluation is needed along with scientific measuring instruments in order to ensure the product quality and the effectiveness of sensory panels.

Comparison between water blanching and steam blanching outcomes is another interesting topic for future study in aspects of energy consumption, waste management, and production yield. As to the academic aspect, the outcomes of this study can be used as reference for related studies and for further process development. For instance, a study on effects of processing on other vegetable soybean varieties and attributes, i.e., nutrition, sugar content, protein content is possible.

The outcomes of this study are anticipated to be, more or less, supportive and informative for everyone who is interested in this valuable commodity, vegetable soybeans.

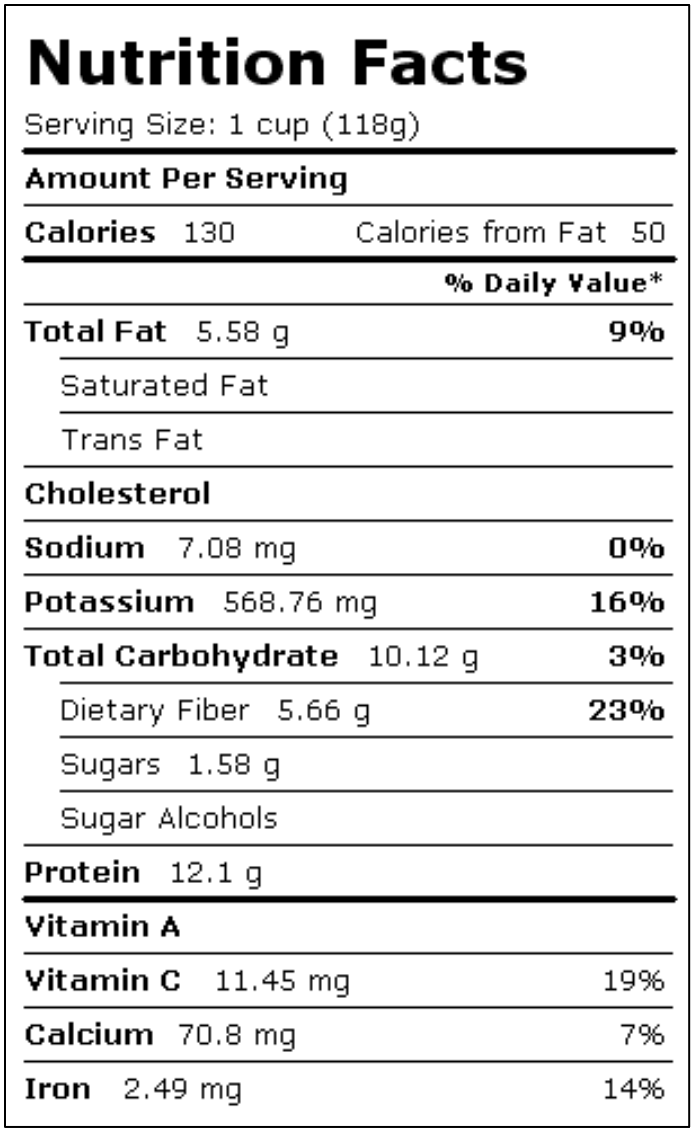
5.4 REFERENCES

Soybean Board. 2010. Food Use of Soy Protein Market Study.

APPENDICES

Appendix A. General information

Appendix A.1. Nutrition facts: 1 cup of unprepared frozen edamame



Nutrition Facts

Serving Size: 1 cup (118g)

Amount Per Serving

Calories 130 Calories from Fat 50

		% Daily Value*
Total Fat	5.58 g	**9%**
Saturated Fat		
Trans Fat		
Cholesterol		
Sodium	7.08 mg	**0%**
Potassium	568.76 mg	**16%**
Total Carbohydrate	10.12 g	**3%**
Dietary Fiber	5.66 g	**23%**
Sugars	1.58 g	
Sugar Alcohols		
Protein	12.1 g	
Vitamin A		
Vitamin C	11.45 mg	19%
Calcium	70.8 mg	7%
Iron	2.49 mg	14%

Appendix A.2. Vegetable Soybean varieties and days to maturity

Variety	Days to Maturity
Beer Friend	75-102
Bellesoy	N/A
Besweet 2020	75-87
Butterbeans (Green)	100-112
Early Hukucho	75
Envy (Green)	80-102
Gion	80-118
Green Legend	80
Green Lion	N/A
Haruno-Mai	102
Kenko	N/A
Kitanosuzu	108
Lucky Lion	80-112
Mana	117
Midori Giant	68-80
Miki	105
Misono Green	70-109
Mojo Green	80-90
Sapporo Midori	103
Sayakomachi	112
Sayamusume	75-108
Sayanishiki	N/A
Shirofumi	68-100
Shironomai	100-113
Taiwame	90
White Lion	90-110
Yukimusume	110

Appendix A.3. Seed supply companies

Seed Company	Website
American Takii, Inc.	www.takii.com
Evergreen Seeds	www.evergreenseeds.com
Fedco Seeds	www.fedcoseeds.com
Garden Guides	www.gardenguides.com
Johnny's Selected Seed	www.johnnyseeds.com
Kitazawa Seed Co.	www.kitazawaseed.com
Lockhart Seed	www.lockhartseeds.com
Nichols Garden Nursery	www.nicholsgardennursery.com
Osborn International Seed Co.	www.osbornseed.com
Pachamama Organic Farm	www.pachamamafarm.com
Rupp Seeds	www.ruppseeds.com
Sakata Seed America, Inc.	www.sakatavegetables.com
Seedex Inc.	www.seedexseed.com
Sow True Seed	www.sowtrueseed.com
Territorial Seeds	www.territorialseed.com
Vermont Bean Seed Company	www.vermontbean.com
Wannamaker Seed	www.edamameseed.com

Appendix A.4. Enzymes responsible for quality deterioration in unblanched vegetables (Barrett and Theerakulkait 1995)

Quality defect	Responsible enzymes
Off-flavor development	lipoxygenase lipase protease
Color changes	polyphenol oxidase chlorophyllase peroxidase (less extent) lipoxygenase
Nutritional changes	ascorbic acid oxidase thiaminase

Appendix B. Standard methods

Appendix B.1. AOAC Official Method 937.09 Salt (Chlorine as Sodium Chloride) in Seafood

Volumetric Method

First Action 1937

Final Action

A. Reagents

(a) Silver nitrate standard solution – 0.1M. Prepare as in 941.18A (see A.1.11) and standardize against 0.1M NaCl containing 5.844g of pure dry NaCl/L.

(b) Ammonium thiocyanate standard solution – 0.1M. Prepareas in 941.18D(b) (see A.1.11) and standardize against 0.1M $AgNO_3$.

(c) Ferric indicator – Saturated solution of $FeNH_4(SO_4)_2 \times 12H_2O$.

B. Determination

(a) Shellfish meats – Weigh 10 g meats, liquid, or mixed meats and liquid, into 250 mL Erlenmeyer or beaker.

(b) Other fish products – Use suitable size test sample, depending on NaCl content. Add known volume 0.1M $AgNO_3$ solution, more than enough to precipitate all Cl as AgCl, and then add 20 mL HNO_3. Boil gently on hot plate or sand bath until all solids except AgCl dissolve (usually 15 min). Cool, add 50 mL H_2O and 5 mL indicator, and titrate with 0.1N NH_4SCN solution until solution becomes permanent light brown. Subtract mL 0.1M H_4SCN used from mL 0.1M $AgNO_3$ added and calculate difference as NaCl. With 10g test sample each mL 0.1N $AgNO_3$ = 0.058% NaCl.

References: JAOAC 20, 410(1937); 23, 589(1940). CAS-7647-14-5 (sodium chloride)
http://files.foodmate.com/2013/files_2962.html

Appendix C. SAS codes for statistical analysis

General Linear Models and Dunnett adjustment of multiple comparisons

```
Data Water;
input Trt Month Hardness;
CARDS;

9     0     13.1
9     0     17.107
9     0     14.373
•
•
11    6     12.876
11    6     13.384
11    6     7.457
;


proc glm data=Water;
  class Trt;
  model Hardness = Trt Month Trt*Month;
  lsmeans Trt / pdiff=control ('11') adjust=dunnett;
run;

proc glm data=Water;
  class Month;
  model Hardness = Trt Month Trt*Month;
  lsmeans Month / pdiff=control ('0') adjust=dunnett;
run;
```

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