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Utilization of soybean components in foods

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Utilization of soybean components in foods

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ABSTRACT

On October 1999, the Food and Drug Administration approved the health claim of soy protein indicating that the food products high in soy protein may help lower coronary heart disease risk by consuming 25 grams of soy protein per day, as part of a diet low in saturated fat and cholesterol. Soybean has been traditionally a source of life in Japan, however, nowadays in Japan and the United States, a majority of portion of annual soybean production goes to animal feed but not for human consumption. Soybean is so beneficial for human consumption that more new applications to utilize soybeans are being studied and increased sales have resulted.

The objectives of this study were (1) to utilize the entire soybean components to develop, and commercialize soybean-based food products and (2) to improve human health, soy marketability, and reduce environmental wastes by utilizing entire soybean components though the development of those soy-based food products.

The first study achieved the objectives by using the commercial soybean cell powder. The new applications to utilize soy proteins, other than the bakery application, succeeded to develop soy-based tomato soups and pudding products that could met 50% and 100% FDA soy protein health claim. Oxidative stability of the commercial soy protein powder is the next issue to improve the quality of these developed products.

The second study used the commercial textured soy protein (TSP). The new formulation was easy to incorporate and produce a soy-based product. This study succeeded in the approach to utilize soy proteins by using TSP, soybean oil and non-meat derivative chicken-flavor. The developed product, a fried chicken-flavored TSP, had an enjoyable flavor and texture approved in the consumer test.

The third study finalized the objectives of this study by incorporating okara, a byproduct from soymilk production into a snack chip increasing its fiber and protein contents. The majority of trained panelists preferred the okara-based snack product developed in this study with no beany-flavor problem over the Japanese commercial product standard. The new okara-based snack food would provide high nutritional profile and improve human health, soy marketability, and reduce environmental wastes.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Soybean has been an increasingly important world commodity for a long time in our agriculture history. The reason that soybean cultivation has been world wide is because of its wide range of geographic adoption (Liu 1999). Therefore, the use of soybean into food products is very economical in many countries. Soybean is mainly used for food functionalities because of its unique chemical composition, and still it has good nutritional value for health benefits.

On October 1999, The Food and Drug Administration approved a front label health claim for products high in soy protein indicating that these foods may help lower coronary heart disease risk by consuming 25 grams of soy protein per day, as part of a diet low in saturated fat and cholesterol (Federal Register 1999). After the FDA approval of soy protein health claim, soyfoods sales have been increasing remarkably in food market. Total retail sales reached 4.0 billion dollars in 2003. The number of soy-based products increased 13.2 per year from 2001 to 2004 (The Soyfoods Association of North America, 2005). Although the increase of soyfoods sales in the market, only a small portion of annual soybean production goes for food consumption, and the majority is still used for animal feed (Liu 1999). The major reason that limits the use of soybeans for food consumption is 'beany-flavor or aroma' of soy-based food product. The flavor problem lowers consumer acceptance.

The overall objective of the study was to use soybean powder (soybean cells), textured soy protein (TSP), byproducts (okara) from soymilk production to develop, and commercialize soybean-based food products. Another goal was also to improve human health, soy marketability, and reduce environmental wastes by utilizing entire soybean components though the development of those soy-based food products for domestic and NASA mission.

The first study used commercial whole soybean cell powder. Soy protein powder is the top of usage of soybeans in food market. It eases the development of soy-based products for industries as seen in the soy-based energy bars which is the best seller in soyfoods market (The Soyfoods Association of North America, 2005). The objectives of the study was to evaluate the influence of the soybean cell powder on the color, texture and flavor of commercially available products, and to find the best formulation to develop new soy products that could have less undesirable beany-flavor/odor and enjoyable sensory perceptions, and still meet 100% FDA soy protein health claim for the marketing purpose.

The second study used commercial textured soy protein (TSP). Textured soy protein has been getting into the main stream of soyfood industries, because of the usage for meat alternatives. The soy-based meat alternative is third in sales in soyfood market next to soymilk (The Soyfoods Association of North America, 2005). The objectives of this study was to develop the best formulation to work TSP with flavors to develop new chicken- or shrimp-flavored TSP food products with nutritional benefits, and to improve soy marketability by utilizing soybean components.

The third study used dried okara powder. Okara was once consumed as a traditional food in Japan. However the gradual alteration from the traditional tofu processing method to the modern technology for mass production has been reducing the use of okara for human consumption in Japan. It is mainly used as fertilizers, animal feeds, and landfills, or incinerated as industrial wastes (Ohno and others 1996). The objectives of the third study were to use okara, which has high nutritional profile, from soymilk production to develop and commercialize soy-based snack foods, and to improve human health, soy marketability, and reduce environmental wastes.

Thesis Organization

This thesis is composed of a general introduction, a literature review, three papers, and a general conclusion. The literature review discussed topics related to soybean cell powder, textured soy protein, and okara which include the current data of soyfood market, nutritional benefits associated with soy protein powder, textured soy protein, and okara. Three manuscripts to be submitted for publication in the *Journal of Food Science* are included (chapter 2, 3, 4) in the thesis. This chapter includes an introduction, followed by materials and methods, results and discussion, conclusion, and figures and tables. Chapter 5 is the General Conclusions for the thesis. This thesis ends with appendices and references cited throughout the entire work under the Bibliography section (following the format of the *Journal of Food Science*).

LITERATURE REVIEW

Utilization of soybeans

Soybean has a wide range of geographic adoption and it is a most economical and valuable agricultural commodity (Liu 1999). It can be cultivated in most parts of the world. However, the soybeans for human consumption had not been focused on until early of 1990s in the United States (Messina M and Messina V 1991). Historically, soybeans have been used mainly for animal feed. There is a distinct feature of utilization of soybeans between Asian countries and the United States. Traditionally, the Asian countries have been used soybeans into various soyfoods for direct human consumption. Those traditional soy foods made from whole soybeans in Asian countries are tempeh, natto, miso, soy sauce, tofu and soymilk (Wilson 1992). In contrast, the majority of soybeans are manufactured into oil and meal in the United States. Soybean oil has been used as a major ingredient for almost every commercial edible oil and fat-content products (Liu 1999). Nowadays, new technologies introduce more utilization of soybeans in industries. The new soy-based foods have been developing and getting onto the main stream in the U.S. organic foods market (Soyatech, Inc. 2002). Those new soy-based foods are sometimes called “second-generation soyfoods” (Messina M and Messina V 1991). The “second-generation soyfoods” are the products which simulate familiar American food products such as energy-bas, bakery products, and meat alternatives. Therefore, it can ease to introduce soybean food products to American people. First of all, soybeans have to be processed into different forms for each purpose to cooperate into foods. Soybeans are processed and manufactured into soy flours, isolates, and concentrates for each functional property. The functional properties from those processed soybeans are emulsification, prevention of fat absorption, water absorption for uptake and retention, dough formation, cohesion, elasticity, color control for bleaching and browning, aeration, and texurization (Kinsella and others 1985; Kolar and others 1985). Soybean flours (powders), isolates and concentrates make easy to cooperate into food applications and they increase the utilization of soybeans. However, there are still several problems to use whole soybeans for direct food applications. Those problems are 1) undesirable beany-flavor of processed soyfoods (Kinsella and others 1985; Liu 1999), 2) oxidative instability of soy oil (Liu 1999; Ross and others 2000), 3) nutrition-related constraint (Liu 1999) lack of certain functional properties (Murphy and others 1997, Liu 1999), 5) low consumer acceptance as food (Murphy and others 1997; Liu 1999)

Nutritional benefits from soybeans

Raw whole soybeans are a good source of protein, fiber, calcium, iron, zinc, phosphorus, magnesium, and B-vitamins and vitamin K (Campbell and others 1985; Messia M and Messia V 1991; Schmidl and Labuza 2000). First of all, soy protein is the major component that provides health benefits for human. On October 26, 1999, the Food and Drug Administration approved a front label health claim for the products high in soy protein indication that these foods may help lower the risk of coronary heart disease (CHD) by consuming 25 grams of soy protein per day (Federal Register 1999). If the reference amount of food is 55 grams, it must contain at least 6.25 grams of soy protein, 3 grams or less total fat, 1 gram or less saturated fat or cholesterol, and 480 milligrams or less sodium to bear the health claim (21 CFR 101.12, 101.14, 101.62, 101.82. 2004. U.S. Food and Drug Administration).

The risk factor of coronary heart disease is the elevation of two types of cholesterols, low-density lipoproteins (LDL) and triglycerides, in plasma levels (Mitchell 1997; Friedmand and Brandon 2001). High-density lipoproteins (HDL), by contrast, can take up cholesterol from other lipoproteins (including LDL and triglycerides) in blood stream and body cells. Therefore, a low ratio of LDL to HDL helps lowering plasma cholesterol, and subsequently decreases the risk of cardiovascular diseases (Mitchell 1997; Friedmand and Brandon 2001). A possible mechanism of the cholesterol-lowering effect of soy protein is that soy protein decreases in the intestinal absorption of cholesterol, and modulate LDL receptor levels in liver (Friedman and Brandon 2001; Lin and others 2004).

Second, raw whole soybeans are rich in dietary fiber. Epidemiological studies suggested that consumption of dietary fiber could reduce the incidence of chronic disease, such as colon cancer (Schmidl and Labuza 2000). Unfortunately, scientific evidence of the effect of dietary fiber on colorectal cancer has not been clarified yet. However, several epidemiological studies showed the possible mechanisms of action of dietary fiber for the reduction of colon cancer (Emenaker 2003).

Third, the antinutritional components of soybeans are now studied as beneficial biological factors (Anderson and Wolf 1995). The antinutritional components are trypsin inhibitors, phytic acid, saponins and isoflavones. In recent years, the studies have been conducted to find the anticarcinogenic activity of isoflavones of soybeans on breast cancer of women. Isoflavones of soybeans, genistein and daidzein, are plant estrogen that present in high amounts in soybean. They are weak estrogens that can work for anticarcinogenic activity. The weak estrogens can bind to the estrogen receptor without a substantial estrogenic response ranging between 1/1000 and 1/1,000,000 of the activity of estradiol, which is natural human estrogen. Breast cancer is thought be estrogen dependent. Soybean isoflavones, particularly genistein, can compete with the potent estrogenic agonists from binding, and subsequently reduce breast cancer (Messina M and Messina V 1991; Hickman 2003). Soybean isoflavones have been also studied for the potential effect on the prevention of coronary heart diseases by lowering blood cholesterol (Ali and others 2004).

Whole soybeans

The cultivation history of soybeans goes back early of 2000 B.C. in China (Watanabe and Kishi 1984). Historically, soybeans have been provided an important source of nutrients in Asian countries. Today, soybeans have been planted all over the world with 73.0 million acres in 2002. The major usage of soybeans is as oilseeds in world production, which accounts for 55 percent of all oilseed production. From 2001 to 2002, soybean provided 83 percent of the domestic edible fats and oil in the United States (United Soybean Board 2002).

The composition of soybeans mainly consists of 35 to 40 percent of protein, 15 to 20 percent of oil, and from 20 to 28 percent of carbohydrates (Watanabe and Kishi 1984; Utsumi 1992; Liu 1999). Two predominant structural proteins of soybean are β-conglycinin (7 S) and glycinin (11 S). The composition and ratio of soy glycinin and β-conglycinin are varied in cultivars, but they influence tofu quality (Taira 1990; Murphy and others 1997).

Mature soybeans are very low in starch, less than 1% (Wilson and others 1978). They have other carbohydrates, cellulose, hemicellulose, and pectin (Watanabe and Kishi 1984). Cellulose is an indigestible dietary fiber that can promote a good bowel movement. It is an important component of soybean for human consumption to prevent rectal cancer (Watanabe and Kishi 1984; Emenaker 2003).

The storability of soybean is good with only about 13 percent of water content (Watanabe and Kishi 1984). It has a long term storage-life without deterioration, as long as it is stored under low temperature and humidity.

The off-flavor, or called 'bean-flavor' in this study has been a main problem to utilize soybeans into foods. The beany-flavor is formed by oxidation of the high proportion of unsaturated fatty acids, particularly linolenic acids in soybean. The enzyme, lipoxygenase, catalyzes the oxidation of polyunsaturated fatty acids of soy lipids, and leads the formation of volatile carbonyl compounds that release undesirable odor or flavor (Wilson 1995; Liu 1999). To control the undesirable 'beany-flavor', the recent genetic technology eliminates three isomers of lipoxygenase, L-1, L2, and L-3 from soybeans in Japan and the United States. Breeding soybean lacking lipoxygenase has been shown to succeed in controlling off-flavor developing in the processed soy-based food products (Wilson 1995; Torres-Penaranda and others 1998; Liu 1999).

Soy flours

The types of soy flours that have been commercially produced in the U.S. are raw or toasted defatted, low-fat, high-fat, full-fat, and lecithinated soy flours or flakes (De 1971; Berk 1992). The basic process of those soy flours are screening and grading the finished flour products after expelling or extracting most of the oil from clean and dehulled soybeans. Defatted soy flours contain less than 2% of residual oil. Low-fat, high-fat, and full-fat soy flours contain the oil in the rage of 5~6%, 15%, and 18~20% respectively. Lecithinated soy flour is made from defatted soy flour by adding lecithin in the rage up to 15% (De1971).

Other dried types of soybean products are isolated soy proteins, soy protein concentrates. Defatted soy flakes or flour which is treated with minimal heat treatment are the starting material for manufacturing process of isolates soy proteins. The low fat content of the flakes or flours is critical to keep good quality of manufactured isolated soy protein. Soy proteins are extracted and separated from defatted soy flakes or flours at neutral or mildly alkaline conditions by adjusting with food-grade alkali, such as sodium hydroxide. The relationship of protein solubility to pH is critical to extract soy protein from the starting materials. After a high-speed centrifuge treatment for the separation, insoluble materials are removed. Precipitated isolated soy protein is adjusted at pH 4.5 with food-grade acids (hydrochloric or phosphoric). The acidic protein curd is then collected by centrifugation or filtration. Soluble carbohydrates and other nonprotein residue are washed and removed. The final isolated soy protein is neutralized and it contains greater than 90% of soy protein. Additional thermal or enzymatic treatment is accomplished to obtain broad functional properties of isolated soy protein products (Kolar and others 1985).

Three common processes of soy protein concentrates are aqueous alcohol wash, acid wash, and hot water leaching. The basic manufacturing process of soy protein concentrates is removing soluble sugars from defatted soy flakes or flours. Proteins and insoluble polysaccharides are mainly remained after the process. As a result of the process, the protein content is increased, and the undesirable oligosaccharides (nondigestible carbohydrates) that cause flatulence are eliminated (Campbell and others 1985).

Soy flakes are made for edible products by flash-desolventized and carefully steam-heated to the desired NSI (nitrogen solubility index) value (Berk 1992). Full-fat soy flakes are alternative initial ingredients to whole soybeans for soymilk production (Wilson 1995).

Textured soy proteins

Protein texturization is an important process of soybean-based products to obtain a desirable fibrous structure for various meat alternatives. Thermoplastic extrusion is the most popular process to texturize soy proteins (Liu 1999). The raw materials, defatted soy beans or flours, or soy protein concentrates, is first steamed to give a water content of 15 – 40 %. The steamed material is then supplied to thermoplastic extrusion. The material is expanded from the various sizes of die of the extruder. The cause of expansion of material is due to a sudden lowering pressure after a great high pressure is applied (Watanabe and Kishi A 1984). There are two types of extruders to produce textured soy proteins (TSP), single-screw extruder, and twin-screw extruder. The study in chapter 3 used TSP produced by using a twin-screw extruder.

Okara

The fibrous residue remaining after soy milk production is called, okara or *unohama* in Japanese. Okara protein has low solubility but it has a good amino acid profile (Chan and Ma 1999). Chan and Ma (1999) analyzed the chemical composition of okara protein isolate. The dominant amino acids of their okara protein isolate were glutamic acid and aspartic acid. Okara protein isolate was also rich in leusine, arginine and lysine, but low in sulfur-containing amino acids, cyteine and methionine. The chemical analysis result from their study showed that the amino acid profile of the okara protein isolate was comparable to commercial soy isolates. The amino acid pattern of the okara protein isolate met or exceeded for FAO/WHO/UNU (1985) requirements.

Soybean oil

Soybean seeds are the most significant oilseed in the world and contain significant nutritional properties. They have been the major edible vegetable oil produced and consumed in the Unites States (Fehr and Hammond 1998). Regular soybean oil contains about 54% linoleic, 23% oleic, 11% palmitic acid, 8% linoleic, 4% stearic acid (Liu 1999). Saturated fatty acids tend to increase serum total cholesterol and they are significantly correlated with the incidence of cardiovascular diseases (Mitchell 1994). Palmitic acid is 70% of the total saturated fatty acid content of soybeans (Fehr and Hammond 1998). Palmitic acid increases serum total cholesterol, whereas, stearic acid appears to be less injurious to human health (Mitchell 1994; Fehr and Hammond 1998). The American Heart Association recommends a diet low in saturated fatty acids to prevent heart attack or stroke that is caused by elevating blood cholesterol (American

Heart Association 2005). Fehr and Hammond 1998 released the genetic engineered soybean lines which contained palmitic acid less than 6%, and stearic acid less than 3.0 % of the total fatty acid. The soybean lines are capable of forming a soybean or vegetable oil having a low saturated fatty acid content.

The unsaturated fatty acids of soybeans, oleic, linoleic, and linolenic fatty acids are the issue of oxidation of soybean oil. Particularly, because of a large portion of linolenic acid (polyunsaturated fatty acid), soybean oils lack oxidative stability during processing and storage conditions (Liu 1999). The oxidative instability of soybean oil develops off-flavor of soybean oil during food applications, too. Hydrogenation of soybean oil can increase oxidative stability of soybean oil by increasing the degree of saturation of double bonds of unsaturated fatty acids (Liu 1999). However the hydrogenation of soybean oil produces *trans* fatty acids that are the health issue for human consumption. The byproducts of hydrogenation of soybean oil, *trans* fatty acids, have a positive relationship between trans fatty acids and risk of coronary heart disease or myocardial infarction (Mitchell 1994). Alternatively, recent plant breeding technologies have been produced the soybean lines that are reduced linolenic acid content (Liu 1999; Roass and others 2000). Dr. Earl Hammond (Department of Food Science and Human Nutrition) and Dr. Walter Fehr (Department of Agronomy) collaborated to lead the development of the 1% linolenic acid soybean oil. The research team achieved the low expression for linolenic acid in soybean oil by combining three independent mutations (Fehr and Hammond 1998). The study in chapter 3 used the 1.2 % linolenic acid soybean oil. The oil that was refined from the low linolenic soybeans harvested in 2003 was donated by Dr. Walter Fehr (Department of Agronomy).

Soybean cells

U.S. Japan Cellfoods developed Unicell Soybean Powder, called USBP in chapter 2 of this study, to utilize the full nutritional benefits of soybeans without the "beany" flavor (Japan Cellfoods 2001). The Unicell Soybean Powder is the soybean flour produced by using an enzymatic treatment that can separate the individual soybean cells without breaking the cell walls. The basic advantage from the use of this soybean powder is that the nutrients (lecithin, saponin, isoflavones and fibers) can be consumed from the intact soybean cells and the soybean powder is still less 'beany-flavor' than the other commercial available soybean powders (Japan Cellfoods 2001).

The importance of the studies in this thesis was how we could utilize the entire soybean components into food applications. The studies consisted of three stages to achieve the utilization of entire soybean components. First stage was the utilization of soybean cells. The use of the Unicell Soybean Powder was the subject to utilize mainly soy proteins from the intact soybean cells for human health. The Unicell Soybean Powder is not a suitable ingredient for soymilk production. It has been used in the bakery industries in Japan and the United States. Our study expanded the usage of this soybean powder by approaching to the applications other than the bakery application. Second stage was the other way to utilize soy proteins and also to utilize soybean oils by developing a fried textured soy protein product. Third stage was the important subject to utilize the rest of components of soybeans by using okara, byproducts from soymilk production. When the okara is produced from soymilk products, this fiber- and moisture-enriched byproduct is hard to incorporate into modern food applications directly.

However, if the okara can be utilized entirely and directly into a food, it can contribute an enormous health benefits for human consumption. The last stage of the studies in this thesis achieved the contribution of health benefits from okara by developing a new soy-based food product. This new soy-based products could incorporate soybean components including fibers and minerals besides protein. Therefore it could contribute for reducing industrial waste for our environmental system. It also could overcome the 'beany-flavor' problem that has been seen in many commercial soy-based foods in the market. The last study of okara-based snack product could succeed to create a value-added product.

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Table 3 – Matched pairs t-test for the 1st chicken-flavored TSP sensory Analysis

Attributes	Matched pairs	P-value	t-test
Beany-flavor	4% - 3% (concentration)	0.1692	Not significant
	STRIP-N – STRIP-W (TSP)	0.5814	Not significant
Oily-flavor	4% - 3% (concentration)	0.3406	Not significant
	STRIP-N – STRIP-W (TSP)	0.0033	Significant
Chicken-flavor	4% - 3% (concentration)	0.0290	Significant
	STRIP-N – STRIP-W (TSP)	0.1963	Not significant
Color	4% - 3% (concentration)	0.4252	Not significant
	STRIP-N – STRIP-W (TSP)	<0.0001	Significant
Saltiness	4% - 3% (concentration)	0.0037	Significant
	STRIP-N – STRIP-W (TSP)	0.4799	Not significant
Chicken-aroma	4% - 3% (concentration)	0.1120	Not significant
	STRIP-N – STRIP-W (TSP)	0.8941	Not significant
Oily-aroma	4% - 3% (concentration)	0.1323	Not significant
	STRIP-N – STRIP-W (TSP)	0.5595	Not significant
Beany-aroma	4% - 3% (concentration)	0.3506	Not significant
	STRIP-N – STRIP-W (TSP)	0.7226	Not significant
Crispiness	4% - 3% (concentration)	0.0117	Not significant
	STRIP-N – STRIP-W (TSP)	0.0931	Not significant
Chewiness	4% - 3% (concentration)	N/A	
	STRIP-N – STRIP-W (TSP)	N/A	

STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

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CHAPTER 2. UTILIZATION OF SPRAYED-DRIED WHOLE SOYBEAN CELLS

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

Miki Katayama and Lester A. Wilson

Key words: soybean powder, soy protein, health claim, flavor, sensory

ABSTRACT

Unicell soybean powder (USBP) is a unique soybean powder containing spray-dried intact whole soybean cells. Two levels of USBP were used; 50% and 100% of the FDA soy protein health claim (6.25g protein per serving). Sensory tests were performed to evaluate the texture and flavor of the products with and without USBP. Instrumental and chemical methods were used to analyze the composition and physical appearance of all the products. Both levels of addition of the USBP increased whiteness and decreased the color of the products. The beverage products were treated with three different levels of homogenization and xanthan gum was added to stabilize the suspended solids and improve the viscosity of the beverages. The trained panelists could tell that the samples had the soybean powder due to an oxidized beany- flavor in batch 2. Chemical analysis revealed approximately ten times higher peroxide values in the second batch than in the initial batch of USBP. Utilizing high oxygen- and light-barrier packaging can prevent unsaturated fat oxidation. With a good drying and packaging system, USBP can be

utilized to develop new products that taste good and have health benefits for the consumers.

INTRODUCTION

Soy protein has various benefits for the food industry, because of its high quality protein, functionality (water-holding capacity, fat-holding, ability of emulsification, etc), and health benefits (Kinsella and others 1985; Schmidl and Labuza 2000). On October 26, 1999, the Food and Drug Administration approved a front label health claim for products high in soy protein indicating that these foods may help lower the risk of coronary heart disease (CHD) by consuming 25 grams of soy protein per day (Federal Register 1999). Those foods have to contain 6.25 grams of soy protein per serving and be low in saturated fat and cholesterol. Unicell soybean powder (USBP) is a unique soybean powder developed by using enzymatic treatment (pectinase) to separate soybean cells while leaving the soybean cell walls intact (Japan Cellfoods Co., Ltd. Hyogo, Japan). This soybean powder contains the same nutrients that are present in soybeans, including parts rich in protein, fiber and phytochemicals that are often lost in soymilk processing (Wilson 1992; Ikeda and Murakami 1995; Granata and Morr 1996). "Beany" or "grassy" odor associated with soy products is a major reason that limits the utilization of soybeans into foods. The major volatile compounds that are commonly found as contributors of those undesirable off-flavors or odors of soy products are hexanal, 2-pentyl furan, 2-pentyl pyridine, dimethyl trisulfide, 1-octen-3-ol, and *trans, trans*-2,4-nonadienal (Boatright and others 1997, 1999, 2001). The undesirable odor of soy products is formed by the oxidation of polyunsaturated fatty acids by lipoxygenases during processing

(Boatrigt and Crum 1997; Wilson 1992). Lee and others (2003) found that the singlet oxygen oxidation by light caused off-flavors in soy flour. Sensory panelists in their study could tell a significantly higher off-flavor in samples stored under light than in the dark.

Several studies had been conducted to overcome this undesirable off-flavor or odor of soybeans and tried to incorporate soybean ingredients into new foods. Friedeck and others (2003) studied the low-fat dairy-based ice cream by fortifying with soy protein isolates (SPI) at three different addition levels. Their sensory analysis revealed that the addition of SPI increased grassy and fatty flavors in intensity, and also increased thickness/viscosity and darkened the ice cream. This was in agreement with their instrumental analysis results. Drake and Gerard (200) studied consumer acceptance of soy-fortified yogurts. Soy protein concentrates (SPC) were added to yogurts (2.5% w/w) to meet the FDA labeling requirements for a "good source" of soy protein. The results showed that soy-fortified yogurts were less acceptable than yogurts without SPC at *p*-value less than 0.05, whereas consumer knowledge of soy health benefits increased acceptability scores of yogurts. Different from soymilk, which is made from soybean as a major ingredient, and the major sale of soy beverages in the U.S. market (The Soyfoods Association of North America 2005; United Soybean Board 2005), Goerlitz and Delwiche (2004) used soy-enhanced tomato juices and performed a consumer test with 100 judges to compare with two other commercial controls. The results showed that the nutritional label information did not significantly alter overall liking rating. The soy-enhanced tomato juice was significantly less preferred than the other commercially successful tomato juices.

The objectives of the study was to evaluate the influence of the USBP on the color, texture and flavor of commercially available products, and to find the best formulation to develop new soy products that could have less undesirable beany-flavor/odor, and still meet 100% FDA soy protein health claim for marketing purposes.

MATERIALS AND METHODS

Beverages, soups, and puddings

Two different batches of spray-dried USBP were shipped from Japan Cellfoods Co., Ltd. (Japan Cellfoods Co., Ltd. Hyogo, Japan). The first batch was shipped under temperature or humidity controlled conditions, whereas, the second batch was shipped commercially. The first study added USBP from the first batch to commercial or hand-squeezed fruit beverages. Commercial juices products (apple, grape, and tomato) were mixed with the USBP. Half of the treatments were homogenized at three different levels of pressure, 1000, 2000, and 3000 psi, with or without xanthan gum. The xanthan gum (TIC 1004T xanthan gum, TIC Gums, Inc. Maryland) addition levels were 0.15, 0.3, 1.0, 1.5 and 2.0 % (Tic Gums Inc. Maryland). The second study used USBP from the second shipped batch to incorporate in commercial canned tomato soups ("condensed" and "ready-to-serve", Campbell Soup Co., New Jersey). All the samples were treated under 2000 psi homogenization without gum addition. The third study used USBP in two different types ("instant" and "cook and serve") and two different flavors (banana and chocolate) of starch-based commercial pudding mixes (Kraft Foodservice, Illinois).

Sensory analysis

A triangle test was used as a discrimination test and performed on all of the pudding products with and without added USBP. Twenty-five untrained panelists evaluated the three digit random coded samples under red light to hinder the color differences among the samples. Three products were provided to the panelists, two being the same and the other a different one. The panelists chose the sample that they perceived to be the most different from the other two (Lawless and Heymann 1999).

Instrumental analysis

Microscopic pictures of the products were taken using a Zeiss Axioplan 2 bright-field microscope (Carl Zeiss MicroImaging, Inc., New York). The microscope was standardized with 1300 x 1030 scanned resolution, CES color balance, 100 neutro-density, and 63x oil immersion. The neutron density was adjusted between 1.5 to 2 when the pictures were taken. All images of samples were taken for 10 and 40 times magnifications.

Color was determined using a Hunterlab model 6100 Color Difference Meter, and standardized with black and white tiles (Hunter Associate Laboratory Inc., Reston, VA). The Hunter system was used to analyze the color of samples with 3 values, L (lightness/darkness), a (greenness/redness), and b (blueness/yellowness). A 10° Standard Observer, D65 light source, 1.7-inch port size and 1.0-inch area of view were used with three replications per treatment. The pH of all samples was measured with a Fisher Scientific AR 15 pH meter (Fisher Scientific International, Pennsylvania).

Chemical analysis

The initial and second USBP lots shipped from Japan were evaluated for composition (protein, nitrogen solubility index, trypsin inhibitor, crude fat by acid hydrolysis, and peroxide value) by using standard AOAC methods (AOAC International 2000; AOCS 1997).

Statistical analysis

All the sensory data were analyzed by using a mixed procedure with the SAS statistical program (SAS Institute Inc., Cary, N.C., U.S.A.).Binomial distribution was used to determine whether the panelists actually perceived a significant difference among three samples (control-0%, 50% and 100% of USBP addition to meet FDA health claim). The statistical differences were detected using the tables of correct judgments to establish significance at probability levels of 5% for the triangle tests (one-tailed) (Lawless and Heymann 1999).

RESULTS AND DISCUSSION

The first batch of USBP shipped from Japan had good color, low peroxide values, and less beany flavor than the second batch (Table 1). The peroxide content of the second batch was approximately ten times higher than the first batch. The first batch was used in the initial study, with commercial beverages and pudding mixes. These puddings (banana and chocolate flavored) products were found to be acceptable, with the 50% FDA Health Claim content being preferred. The second batch of USBP was used for the sensory analysis of pudding samples. The statistical data showed that the panelists in the triangle test could distinguish between the samples with the USBP from the second batch due to the oxidized off-beany flavor of these treatments. This problem could be solved by switching to a high barrier film bag (oxygen- and light-barrier packaging). By using USBP with a low peroxide value, the products would have better quality with less beany- or off-flavor problems that the previous studies also had in their soy-based research products.

The lowest homogenized pressure (1000 psi) used with the beverage products (apple, grape and tomato juice) maintained most of the USBP cell structure in the products, while the highest homogenized pressure (3000 psi) broke some of cells (Figure 1a and b). The gum addition to USBP beverage products did not prevent cell breakage under high homogenization pressure, however it reduced suspended solid separation in beverages and aided in product formulation. The beverage products also needed gum to stabilize (Figure 2a and b). The soybean cells remained intact in both "*cook and serve*" and "*instant*" pudding mixes (Figure 3a, b, c, and d). Overall, the addition of USBP to each of these foods increased thickness, with the highest level giving the thickest product. The texture and function of applications for health benefits could be gained from the USBP added pudding products, because the pudding research products were made from starch-based mixes that could mask the influence in thickness from the addition of USBP. The thickness problem was solved by adjusting the amount of liquid ingredient, such as milk or water, to the pudding mixes in this study. USBP also increased whiteness of these foods, which would need to be addressed in foods with high color intensity (Figure 4). Tomato soup with USBP would be the most acceptable product for consumers among the three different studies, because the strong tomato flavor masked the "beany flavor" from the USBP and the thickness of the mixture could be easily adjusted by adding water or milk into the products during preparation. The increase in whiteness (increased L value) of the tomato soup, by adding USBP, made the products creamier in appearance as in tomato soup made with milk (Figure 5).

CONCLUSION

The creamy appearance shown on pudding and tomato soup products can be desirable to consumers, because they are used to seeing tomato soups and puddings prepared with milk. The products can be a good source for vegetable protein, if the products are prepared only by adding water. If the products are prepared by adding milk, they become a good source of both of high soy protein and milk protein. Also, USBP fortified-tomato soup products will have the most health benefits among three research products, not only the most acceptable, because they contain isoflavones from soy and lycopene from tomato soup. Isoflavones and lycopene are natural antioxidants and they have been shown to reduce the risk of various cancers (Schmidl and Labuza 2000; Watson 2003; Campbell and others 2004; Nkondjock and others 2005). If USBP is dried properly and packaged in a high-barrier film bag to prevent oxidation, there should be no beany-/off-flavor problems when USBP is incorporated into products or used in new food products. This formulation and processing step are easily to scale-up and apply to the manufacture purpose. Therefore, it will be a relatively inexpensive way to produce the products for food industries. These advantages will make the products easier to reach the consumers and give the health benefits for them.

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Table 1- Composition of Unicell soybean powder

Composition	1st Batch	2nd Batch	Units
Protein	44.09	30.9	%
Crude fat by acid hydrolysis	19.4 (23.0 ± 2.0)	18.8	%
Free fatty acids	N/A	0.7	%
Peroxide value	10.0	107.9	meq/g
Trypsin inhibitor	<2,000	2,400	TIU/g
Nitrogen solubility	19.4	17.7	%

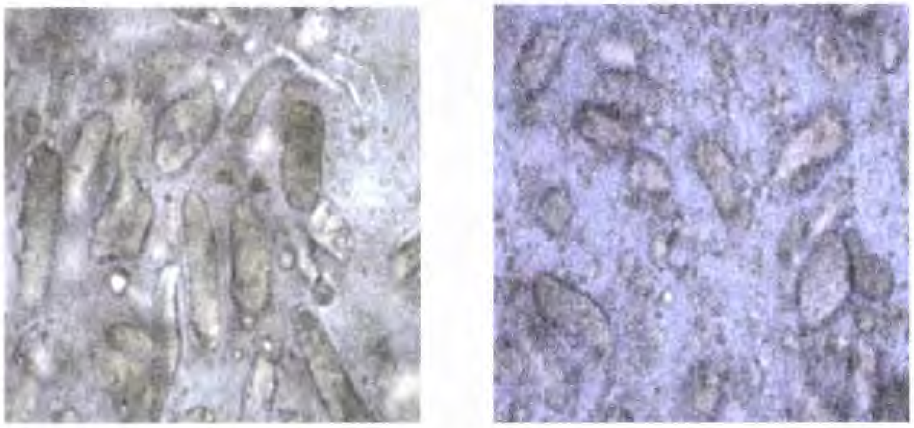

Figure 1-a **Figure 1-b**

Figure 1 – Effect of homogenizer pressure on USBP in grape juice samples: (a) 1000 psi, (b) 3000 psi, both was taken with 40X magnification.

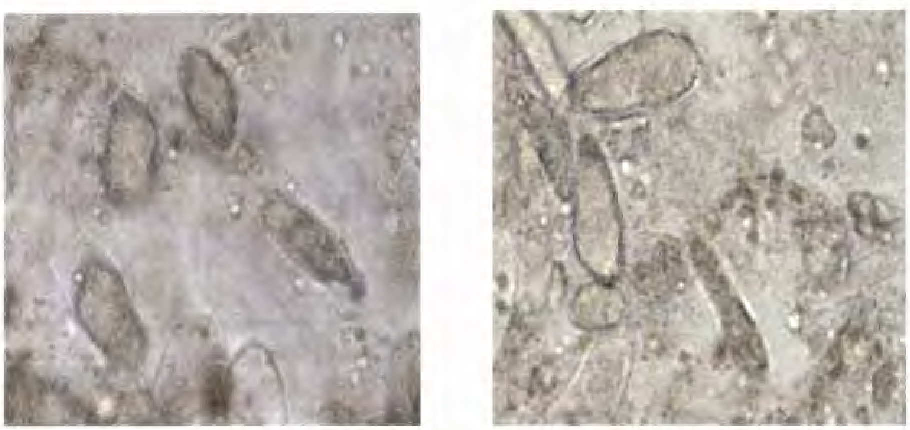

Figure 2-a **Figure 2-b**

Figure 2 – Effect of gum addition on USBP in tomato juice samples under 2000 psi homogenizer pressure. (a) treated without gum addition, (b) pudding sample was treated with gum addition. Both were taken with 10x magnification.

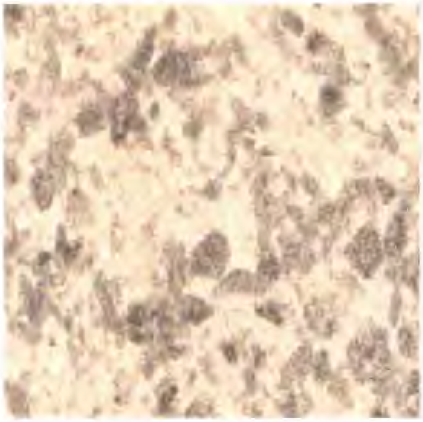

Figure 3-a

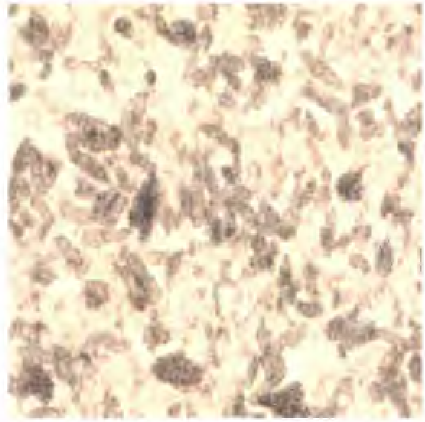

Figure 3-b

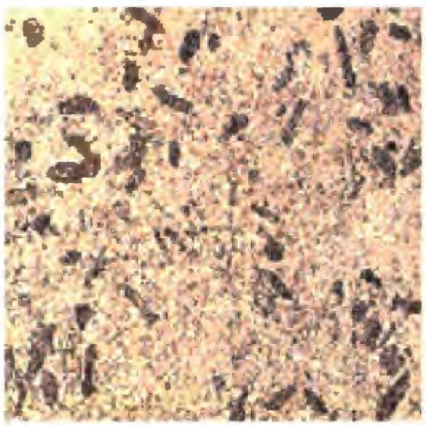

Figure 3-c

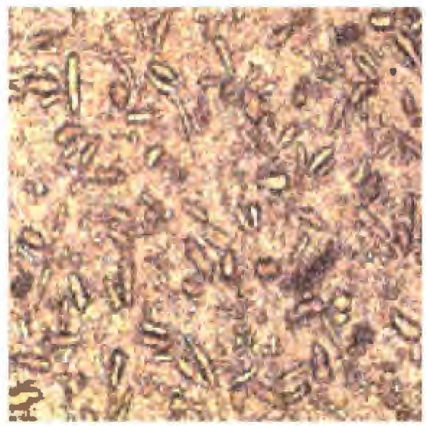

Figure 3-d

Figure 3 – USBP addition in flavored pudding samples ("cook and serve" and "instant") at two different levels. (a) "cook and serve" pudding sample contained soy protein to meet 50%, (b) "cook and serve" pudding sample contained soy protein to meet 100% FDA health claim. (c) "instant" pudding sample contained soy protein to meet 50%, (d) "instant" pudding sample contained soy protein to meet 100% FDA health claim. All pictures were taken with 10x magnification.

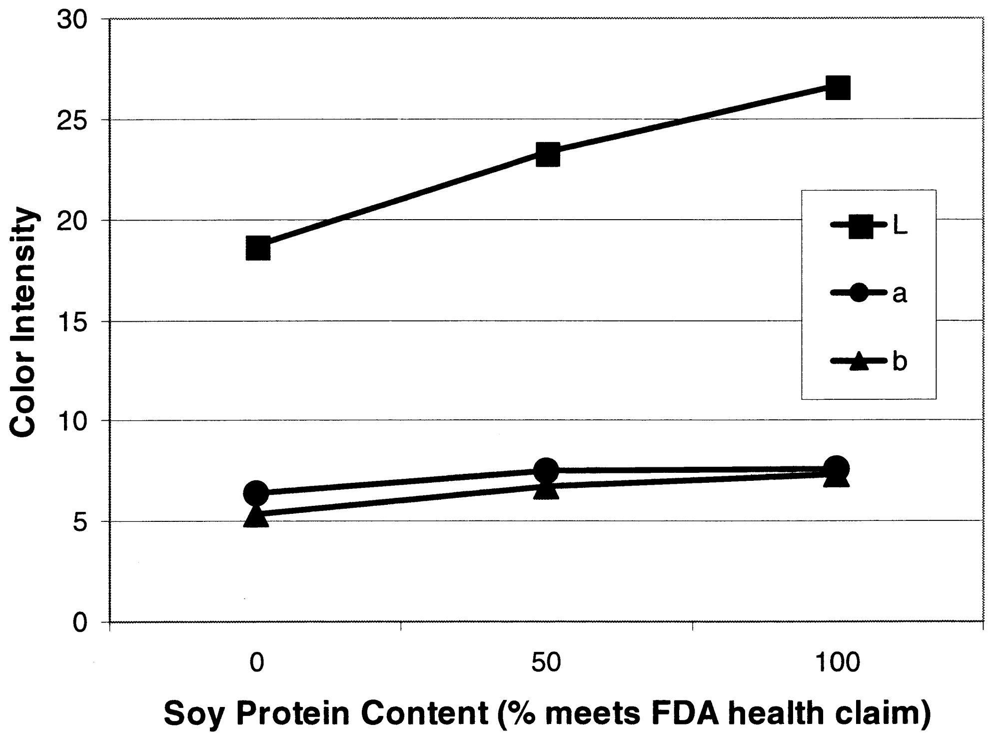

Figure 4 – Color measurement of chocolate pudding sample ("cook and serve") with two different addition levels of USBP (50% and 100% meet FDA soy protein health claim).

L: 0 = black; 100 = white

a: -a = green; +a = red

b: -b = blue; +b = yellow

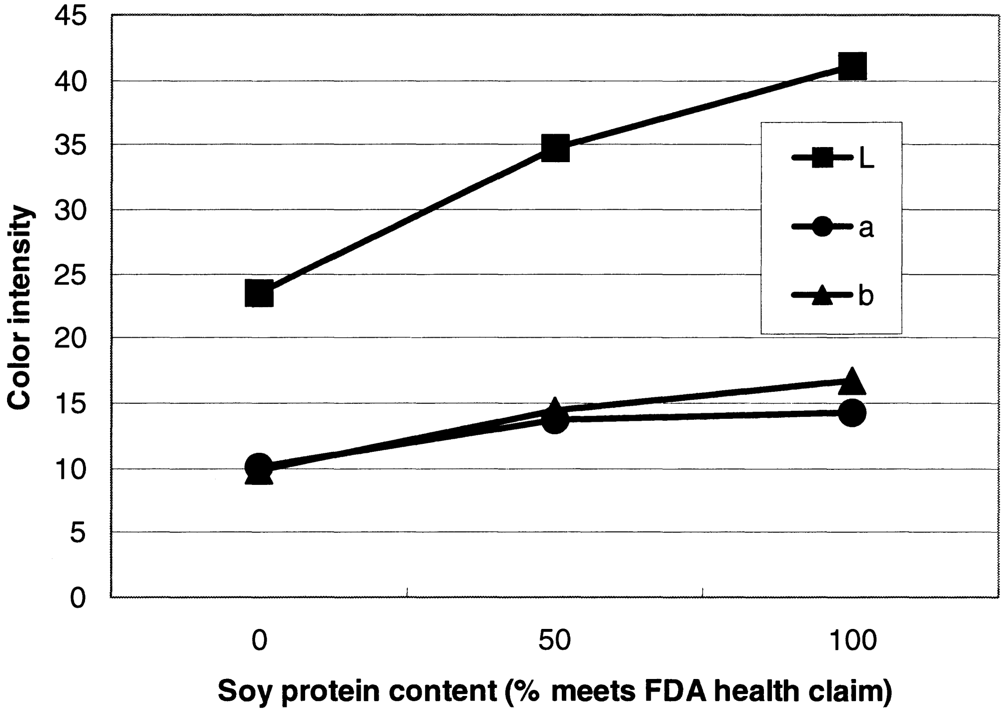

Figure 5 – Color measurement of condensed Tomato soup sample with two different addition levels of USBP (50% and 100% meet FDA soy protein health claim).

L: 0 = black; 100 = white

a: -a = green; +a = red

b: -b = blue; +b = yellow

CHAPTER 3. UTILIZATION OF SOYBEANS AND THEIR COMPONENTS THROUGH THE DEVELOPMENT OF TEXTURED SOY PROTEIN FOODS

A paper to be submitted for Journal of Food Science

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Key Words: textured soy protein, flavor, meat alternative, sensory study, consumer

ABSTRACT

Four different types of commercial textured soy protein (TSP) containing about 51% soy protein, and four different commercial flavors made from non-meat derivatives were studied to find an appropriate formulation to develop a new soy-based meat alternative food. The optimum condition that minimized hydration time and maximized flavor was chosen. Four descriptive analysis studies were conducted with minimum 14 of trained panelists. Baked and fried applications were tested during the sensory studies. The instrumental and chemical analyses were also performed to compare with the sensory results. All the results were statistically analyzed and used to prepare for a consumer preference test. A consumer preference test with 125 consumers was performed at a local grocery store. On a 9-point hedonic scale, the people who preferred the fried products gave an average 7.1 score. The people who preferred the baked products gave an average 6.7 score. As a result, 66% of the consumers preferred the fried products over the baked products, while 31% of consumers preferred the baked products.

INTRODUCTION

Soy protein foods have long been a major product in the organic foods market as health-promoting food products. Meat alternatives sales are the third in sale, next to soymilk sales, in soyfood products in the United States (The Soyfoods Association of North America, 2005). Textured soy protein (TSP), which is an extruded and fibrous-structured product, is mainly used as meat alternative food product. Textured soy proteins (TSP) are usually made from defatted soybeans or flour, and isolated soybean protein concentrates (Berk 1992). This study used textured soy protein made from defatted soy flour (approximately 8% fat dry basis) using a twin-screw extruder.

The primary problem that limits the incorporation of a large amounts of TSP into a meat-alternative product are undesirable flavors, called "beany" flavor or odor in this study. The undesirable "beany" flavor or odor is a result of the oxidation of unsaturated fatty acids by lipoxygenase enzymes during processing soy protein products (Wilson 1995). Chin (1999) studied the effects of different types of TSP in ground beef patties in a descriptive sensory study. Her study used isolates and ethanol-and acid-washed concentrates made from normal soybeans (lipoxygenase-present) and lipoxygenase-free soybeans to produce TSP using a single-screw extruder. There was no difference in juiciness, chewiness, and soy flavor between lipoxygenase-free and normal TSP-extended (20%) patties in her descriptive sensory study. Crowe (2000) performed the sensory analysis with 5 partially defatted soy flour TSP-extended ground beef samples, commercial TSP control, and 19% fat ground beef control. The trained panelists found no significant difference in hardness or chewiness between the TSP-extended ground beef sample and the 19% fat beef control. However, as a main attribute of TSP products, this study noted that soy flavor of the TSP-extended ground beef was significantly higher ($p<0.05$) than the 19% fat beef product. Taylor and Walsh (2001) performed a sensory study between their textured whey protein patties and a commercial TSP patty. The study concluded that there were no significant differences in appearance and texture among their treatments. There were significant differences in flavor, aftertaste and overall acceptance in their consumer panel test.

The unflavored TSP as raw materials used in our study were extruded products made from defatted lipoxygenase-present soybean flours. It had originally a strong soybean or oxidized bean flavor. The TSP as dried extruded products, have a long term shelf-life, at least one year, if they are stored in a dry and airtight container (The Soyfoods Association of North America 2005). However, the manufacturing plant where TSP is produced for this study uses crop bags during the storage. The latest stage of this study used TSP that was stored with crop bags for a relatively long time (more than 6 months), and the TSP had an additional oxidized bean flavor from this storage condition.

After the hydration process, the TSP had a sponge-like structure. This fibrous structure is the other challenge in this study, because it makes the products too chewy compared with real poultry or meat products.

The objectives of this study were to develop the best formulation to combine TSP with flavors to develop new chicken- or shrimp-flavored TSP food products with nutritional benefits, and to improve soy marketability by utilizing soybean components.

MATERIALS AND METHODS

Textured Soy Protein (TSP)

Four different shapes of commercial unflavored TSP (Heartland Fields LLC., West Des Moines, IA) were used to develop chicken-flavored or shrimp flavored TSP products. The composition data of these TSP from chemical analysis are shown Table 1.

Formulation of a new chicken-flavored TSP

1. Preparation

The amount of time to fully hydrate the textured soy protein was determined for water temperatures of 40°F, 140°F, 175°F, and 200°F before flavoring was added. The temperature matrix used still avoided the hazard range for food microorganisms. The optimum condition that minimizes hydration time and maximizes flavor was chosen.

Four different concentrated chicken flavors made from non-meat derivatives (Bell Flavors and Fragrances Inc., Northbrook, IL and Flavor & Food Ingredients, Inc., Middlesex, NJ) were diluted in hot water. Those 4 flavors were either a liquid, powder, or oil types. 30 g of unflavored TSP was hydrated with 100g of flavor solutions. Flavor concentrations of 0% (control), 2%, 3% and 4% were studied to find an appropriate concentration for each flavor. The flavored TSP samples were processed for a fried application. The flavored TSP samples were fried with 500g of a commercial low saturated soybean oil at two different temperatures, 356°F (180°C) and 375°F (191°C), using a household deep fryer.

2. Treatments for sensory evaluations

There were 3 descriptive sensory studies for chicken-flavored TSP products before a consumer preference test was performed. The first study used a powder type of chicken flavor and two types of strip shapes of TSP. Two different flavor concentrations were prepared, 3% as low concentration, and 4% as a high concentration of flavor solutions to hydrate the TSP before frying. Those two concentrations were applied to two types of strip shape of TSP (called STRIP-N and STRIP-W in this study). The difference between the two TSP was the opening size of die to produce a different shape of TSP. One was made with a narrow opening (called STRIP-N), and the other was made with a wider opening with a same die (called STRIP-W). A total of 4 treatments were provided to the first descriptive sensory study, 3% flavored STRIP-N (3%+STRIP-N), 4% flavored STRIP-N (4%+STRIP-N), 3% flavored STRIP-W (3%+STRIP-W) and 4% flavored STRIP-W (4%+STRIP-W).

The second sensory study for chicken-flavored TSP products used a liquid type of chicken flavor, and the same two types of TSP (STRIP-N and STRIP-W) used as the first sensory study. The flavor concentrations were also same as the first study. The application was same as the first study, a fried application. A total of 4 treatments were provided to the second descriptive sensory study, 3% flavored STRIP-N(3%+STRIP-N2), 4% flavored STRIP-N (4%+STRIP-N2), 3% flavored STRIP-W (3%+STRIP-W2), and 4% flavored STRIP-W (4%+STRIP-W2).

The third sensory study used the same powder type of chicken flavor as the first study used. The smallest size of TSP was used (called BIT). The shape of TSP was bits-like or crouton-like shape. As extruded products, the dimensions of BIT were varied. It was approximately 1-cm cubes. Two types of cooking processes were applied, baked and fried. Five grams (low concentration) or 6.7g (high concentration) of chicken powder flavor was weighted and dissolved in 30 g of hot water at 175 °F. Fifty grams of unflavored BIT was then marinated with this flavor solution, and stored in a freezer for 30 minutes. The frozen BIT was either baked in an oven at 320 °F, or fried with low saturated oil at 375 °F. Fifty grams of frozen BIT was baking for 12 minutes with heat, and resting for 2 minutes to take out extra moisture in an oven after the heat was turned off. The other 50g of frozen BIT was fried for 2 minutes and 30 seconds. Four treatments were provided to the third descriptive study, low and high concentration flavored baked samples, and the same for the fried samples. They were called, LB-BIT, HB-BIT, LF-BIT, and HF-BIT (L or H stands for low or high concentrations, B or F stands for baked or fried application).

Only the high concentration for flavor solution and the smallest size of TSP (BIT) used in the third study were chosen to use in the consumer preference test. Both baked and fried cooking processes were applied. Therefore, 2 treatments (HB-BIT and HF-BIT) were provided to the consumers in the preference test.

Formulation of a new shrimp-flavored TSP

Two powder types of shrimp flavors made from non-meat derivatives (Bell Flavors and Fragrances Inc., Northbrook, IL) were used in this shrimp flavor study. After the determination which TSP could work with these shrimp flavors best, the shreds shape of TSP (called SHRED) was chosen to use. A baked application was tried in this study to reduce the oiliness of the finished products. One gram of powder flavor was weighted from each shrimp flavor, and mixed. A total of 2.0g of mixed powder flavor was then dissolved in 18.0g of hot water at 175°F. 30g of unflavored SHRED was marinated with this flavor solution. This preparation was applied for all 4 treatments as the first preparation step. After the SHRED was flavored, 3g or 4g of a single flavor were weighted and sprinkled evenly over the flavored TSP. 1.5g, or 2.0g of this flavor and 1.5g or 2.0g of the other shrimp flavor were weighted and mixed. A total of either 3g or 4g of this mixed powder flavor were also sprinkled over the other two flavored SHRED. All 4 treatments were stored in a freezer for 30 minutes and baked in an oven at 320°F (160°C) for 14 minutes and 30 seconds with occasionally turning sides of TSP for even baking. The finished TSP products were warmed up at 200°F for 10 minutes before they were provided to the panelists in sensory evaluations. Those 4 treatments were called SL-SHRED, SH-SHRED, CL-SHRED, and CH-SHRED (S or C stands for single or combination flavor, L or H stands for low or high concentration).

Descriptive sensory analysis

After being approved by the Human Subjects Research Office at Iowa State University, sensory evaluations were performed at Iowa State University, the Center of Crops Utilization Research (CCUR) sensory facilities. The panelists were asked to sign a letter of informed consent. A descriptive analysis with 15- cm line scales (Lawless and Heymann 1999) was then performed with the trained panelists. The score on the 15-cm line scale was calculated raging from 0 as a minimum and 150 as a maximum intensity in each attribute. The panelists participated voluntarily and were instructed and screened during the training sessions. Subjects were trained in two 30-minutes sessions on two different days. The panelists were given a total of four pieces of flavored TSP as the reference standards for all attributes. Two pieces flavored with a lower, and the other two pieces flavored with a higher concentration than the chicken or shrimp flavor concentrations used during the real sensory sessions. The other reference standards used during the training sessions were the oil after fried samples for oily-aroma reference. The edible dried soybean flakes (Heartland Fields, LLC., West Des Moines, IA) from the same soy flour as the research TSP were used as the reference standards for soybean-flavor or aroma (called "beany-flavor or aroma" in this study). Each product had a 3-digit code and was presented to the panelists. It was also presented in a random order under controlled environmental conditions. The attributes to describe the samples were discussed and agreed upon by the subjects. The sensory attributes for all three chicken-flavored TSP studies were chicken-aroma and flavor, oily-aroma and flavor, beany-aroma and flavor (none/intense), exterior color(light/dark), saltiness(not salty/very salty), crispness (not at all/very crispy), and chewiness (not at all/very chewy). For the shrimp-flavored TSP study, shrimp-flavor and aroma were asked, instead of chicken-flavor and aroma, and the panelists agreed to have only crispness for texture attribute, because of the oiliness removed with baked products. Fourteen to twenty-one trained and screened panelists evaluated those attributes of samples on the score sheet and also left their comments at the last of each session.

Consumer preference test

Preference testing with a 9-point hedonic scale (Resurreccion 1998; Lawless and Heymann 1999) based upon the trained panelist results was performed in a local grocery store, Ames, Iowa. The Human subject form of this test was approved by the Human

Subjects Research Office at the Iowa State University. One hundred twenty-five consumers voluntarily joined in the test at the store. The panelists were provided a consent form, questionnaire sheet, and two pages of a 9-point hedonic scale. The questionnaire sheet asked the demographics of the panelists. Each of the two pages of a 9-point hedonic scale had a new scale with a new 3-digit code for the two research products, a baked and fried chicken-flavored products (HB-BIT and HF-BIT). The evaluation order of the samples was randomized when the samples were provided to the panelists. The panelists also randomly chose either baked or fried sample first to evaluate. The panelists indicated their preference on the hedonic scale that was balanced on a ballot with a ranging score from 9 (like extremely) to 1 (dislike extremely). They were also asked to choose the sample they preferred overall.

Instrumental Analysis

All the finished products were measured for color and texture. The instrumental results were compared to sensory panel data. A LabScan XE spectrophotometer with the Hunter system (Hunter Associate Laboratory Inc., Reston, VA) was used for the color analysis. The Hunter system had 3 values to explain the color of samples, L (lightness/darkness), a (greenness/redness), and b (blueness/yellowness) values. The parameters of the Hunter scale used in this study were D65 (daylight 65), 10° Standard Observer, 0.70 inch of port size, and 0.40 inch of area view. During four replications of sensory evaluation, the finished TSP samples from each treatment were randomly selected for the color analysis at every sensory session. The selected TSP samples were put in a plastic bug and crushed into small particles by using a roller to have an even color of samples. Three of 12g of the crushed TSP samples were weighted, placed in a plastic clear petri dish, and analyzed three spots from each dish for color. A total 9 of spots for each treatment were calculated to have an average at every session.

A TA.XT2i texture analyzer with TA-45 incisor knife blade (Texture Technologies Corp, Scarsdale, NY) was used for the texture analysis. The blade size was 1.5 mm wide x 11.0 mm long. During the first chicken-flavored TSP study, total 200 pieces of TSP were randomly selected and used to find appropriate parameters and methods for texture analysis. After the second chicken-flavored TSP study, the fixed parameters were used. Table 2 shows the parameters of this texture analysis. All the TSP used in this study varied in shape as extruded products. For texture analysis, the finished TSP with the same weight was selected. During the first and second chicken-flavored TSP studies, 5 pieces of TSP from each treatment with the same weight were selected. Three spots on each piece were chosen to analyze. After the first snap, the baked TSP in the shrimp-flavored TSP study became a breakable piece, and hard to have a third spot to analyze. Therefore, only 2 spots were selected for texture analysis for each piece. The bits-size of TSP was too small to have more than 2 spots for analysis. Ten pieces from each treatment with a same weight were selected from each treatment in the third chicken-flavored TSP study. Total 40 pieces for each treatment were analyzed and statistically calculated during 4 replications of sensory study.

Statistical Analysis

A random effects model was used to compare groups, while removing effect of correlation among the observations. For the sensory and instrumental data, the averages were used to summarize correlated data. The summarized data were then statistically analyzed using JMP 5.1.2, the Statistical Discovery Software (SAS Institute Inc, Cary , N.C., U.S.A.). A matched pairs t-test was set at a *P*-value of 0.05. Also a Tukey-Kramer HSD adjustment was used to minimize type I error inflation (Ramsey and Schafer 2002).

Chemical analysis

All the raw unflavored TSP and finished products were chemically analyzed by following the AOAC Official Methods (AOAC International 2000) procedures (934.01 for moisture by vacuum oven, 990.03 for crude protein by combustion method, and 954.02 for crude fat by acid hydrolysis) and the AOCS Official Methods (AOCS 1997 revised in 2004) procedures (Ba 12-75 for trypsin inhibitor activity, and Cd 8-53 for peroxide value).

RESULTS AND DISCUSSION

The trained panelists in the first chicken-flavored TSP study commented that STRIP-N had higher crispiness and lower chewiness than STRIP-W based on their internal senses. However, the statistical analysis resulted that there was no significant difference between the treatments in this sensory study, which disagreed with the panelist comments (Table 3 and 4). All the finished TSP samples that were produced at the same day as the sensory panel was performed were used to find appropriate parameters using A TA.XT2i texture analyzer. Low (3%) and high (4%) concentrations resulted in no significant difference in beany-flavor/aroma, oily-aroma/flavor, and chicken-aroma. The panelists found a significant difference only in chicken-flavor and saltiness in the concentration difference (Table 3). The color analysis showed that there was significant differences in all L, a, and b values in Hunter system between the two types of TSP, STRIP-N and STRIP-W (Table 5). The finished STRIP-W had a significantly higher L value (lighter color), lower a value(less red color), and higher b value(more yellow color) than the finished STRIP-N in Hunter system (numerical raw data are not shown). STRIP-W had a more fine-network structure than STRIP-N. STRIP-N had more rough air pockets in the structure after hydration. The structure difference made some difference between the two types of TSP in chemical analysis results (Table 6). STRIP-N had less moisture and more fat content than STRIP-W. Less moisture content of STRIP-N would give a crispy sensation to the panelists. The panelists noted in their comments that they felt the STRIP-N was crispier than the STRIP-W. However, a strong difference in texture was not seen in the statistical results (Table 3).

The second chicken-flavored TSP study used the same TSP, STRIP-N and STRIP-W as the previous study. The structure difference between the two TSP samples was obvious in the results from both sensory and instrumental analyses. The result from the sensory evaluations showed that there was a significant difference between the two TSP samples (Table 7). STRIP-N had a significantly higher crispiness and lower chewiness in sensory analysis (raw data are not shown). This result agreed with the results from the texture analysis (Table 8). The results from a TA.XT2i texture analyzer showed that TRIP-N had a significantly harder texture (more force required to break) than STRIP-W. This crispiness of STRIP-N could be related with less moisture content than STRIP-W (Table 9). The finished STRIP-W samples after being fried had almost two times higher moisture content than the STRIP-N samples. The internal color of both types of TSP was a significantly difference only in b value, which explained yellowness of the finished products (Table 10). The trained panelists perceived a significant difference in concentration difference only in flavor but not in aroma with the liquid-type of the chicken flavor (Table 7). According to their comments, the panelists preferred the powder type of chicken-flavor. Also the panelists who joined from the previous study noted that the liquid type of chicken-flavor had a weak chicken-flavor than the powder type. However, the statistical results did not show any significant difference between the liquid and the powder type of chicken-flavor from the overall sensory scores (Table11). The different types of flavor did not give any influence in flavor intensity.

To find another line extension for this new TSP product, shrimp-flavored TSP products were studied. A baked application was tested in this study based upon the panelist comments from the two previous chicken-flavor studies. Most of the panelists noted in their comments that oily-flavor and aroma could be the major problems for the fried TSP samples. Also, those finished products were not good for a long shelf-life. If the finished fried products were stored at room temperature one night, the texture of those products became soft and soggy finger-feeling. To solve this problem, the shrimp-flavored SHRED were baked in the oven and served to the panelists. There were significant differences in shrimp-flavor and saltiness between high and low concentrations of flavor (Table 12). The difference in flavor formulation (single or combination) did not make any significant difference in most of attributes, except crispiness in texture. The panelists perceived that the combination-flavored SHRED had a higher crispness than the single-flavored SHRED. The statistical results from texture analysis using a TA.XT2i texture analyzer also agreed with this human sensory result

(Table 13). The combination-flavored SHRED had a significantly higher hardness (more force required to break) than the single-flavored SHRED. Due to the proprietary rights to respect the flavor contributor company, the reason that made this significant difference in texture was unknown. However, it showed a difference in chemical composition in ingredients, or a difference in ratio of ingredients to make these two flavors. The color analysis using a LabScan XE spectrophotometer resulted in significant differences in lightness (L values) among the treatments (Table 14).The combination flavored SHRED had a higher L value. Higher concentration of flavor contributed a higher lightness (L value) to the finished SHRED products. The correlation coefficient, r was very high with 0.9777 between the lightness (L value) and the sensory results. However, the small sample size after averaging the results did not allow for statistical significance. In this study, the oiliness was not a problem for the trained panelists with baked samples, however, they noted that chewiness became a major problem to bite and eat the baked SHRED products. After hydration of the raw SHRED with flavor solutions, heating was applied to the flavored SHRED evenly in the oven, and excess moisture was taken out during resting time in oven. Because of irregular shapes of the raw extruded SHRED, some parts of SHRED were thick, and others were thin. The same amount of heat could go through the structure, take out moisture and give crispness to the thin parts, but not to the thick parts. The finished SHRED were left at room temperature, and warmed up to serve to the panelists. During this preparation time for the sensory panel, the finished SHRED which was most likely thicker than the others became chewy due to having more moisture content in its structure. The panelists were also not satisfied with either the single or combination shrimp flavors, because the flavors were too fishy-flavor for them.

The finished products did not have enough shrimp-notes. However, those shrimp flavors were only the flavors made from non-meat derivatives. The other shrimp flavors, which were tested before the sensory study was performed, were fishier flavored, or made from real shrimp. To solve two major problems, oiliness with the fried application and chewiness with the baked application, the smallest size of TSP, which was BIT (bits-like shape) was chosen in the third chicken-flavor TSP study, and both applications were used to test the small shape of TSP to see if those two problems could be solved.

The third chicken-flavored TSP sensory study used the same chicken flavor as the first chicken flavor study, which was the powder type. This chicken flavor was strongly preferred by all the panelists, and also it worked well with the fried application. The flavor was stable at the high frying temperature at 375 ^{o}F. It also could be held longer in the finished products even after several days. The trained panelists perceived that the fried products were significantly higher intensity in oily- and chicken-flavor/aroma, and saltiness in flavor attributes, and significantly higher crispiness in texture than the baked products (Table 15 and 16). Obviously, the fried samples had a significantly higher oiliness of flavor and aroma than the baked samples (Table 16).The chemical analysis showed that the fried BIT samples had more than three times higher fat content than the baked BIT samples (Table 17). The texture analysis resulted in significant differences (P<0.05) (Table 18). The application difference resulted in a significant difference both in human sensory and instrumental color analyses (Table 15 and 19). The sensory panelists scored a darker color for the fried BIT samples than the baked samples (Table 16). This result strongly agreed with the Hunter color analysis results. The fried samples had a lower L value (darker color), a higher a value (more redness), and a lower b value (less yellowness) than the baked samples. The correlations between the human and instrumental analyses were again not calculated statistically in this study, because of the small sample sizes after averaging the results in the instrumental analysis. However, the actual results from both sensory and instrumental analyses still could show a strong relationship in the application difference. The concentration difference also contributed significant differences in chicken-flavor/aroma, saltiness and color in sensory analysis. The major significant difference had been seen in saltiness ($P < 0.0001$).The trained panelist perceived a higher intensity in saltiness with the higher concentration of chicken-flavor. Most of the panelists noted in their comments that they liked tasting the products with higher concentration, and the saltiness were still not a problem for them. Based upon the comments from the first chicken-flavor sensory panelists, the crispiness between the fried STRIP-N (3% and 4%-STRIP-N) and fried BIT (L and HF-BIT) was compared, because the difference between two samples was only the shape of TSP. Other than the shape difference, all the conditions to produce both samples were same (flavor, application, ingredients, frying temperature, and etc), except the amount of water for flavor solution. Since all the finished products in the first chicken-flavored TSP study were sacrificed to find the appropriate parameters for texture analysis, the data for chewiness was not obtained. Therefore, the statistical calculation for chewiness to compare the two TSP was not performed. The texture of the fried BIT was significantly improved with a higher score than the fried STRIP-N in the first chicken-flavor study (Table 20). *P*-value for oily-aroma (oily smell) was not significantly difference, but it was close to 0.05. However, oily-flavor (oily taste) was significantly improved with the fried BIT, compared with the fried STRIP-N in the first study. At the end of the third preferred the fried products gave an average 7.1 score. The people who preferred the baked products gave an average 6.7 score. The research objectives of this study had achieved with the fried chicken-flavored bits-shape TSP (BIT) products after the results from the consumer preference test were obtained. The texture and oily flavor problems were further improved from the earlier stages of this study. Compared with the previous TSP studies that used TSP as a part of their ingredients, this study used whole raw unflavored TSP as the main ingredient. Also, all flavor ingredients were non-meat derivatives. Therefore, this research product was complete vegetarian menu.

CONCLUSION

The formulation of the flavored BIT was very simple with 3 steps, hydration with flavor solution, chilling in a short time, and frying. The ingredients were only TSP, chicken-flavor, and soybean oil. This simple formulation will allow the soyfood industry easier to scale-up and manufacture the products. Also utilizing soybean components will promote health benefits of soy protein. The oxidation flavor can be prevented with a high barrier packaging system. The high barrier package system will be required to block moisture, oxygen and light that can cause lipid oxidation flavor and textural changes. Those changes will increase the shelf-life of the raw and especially the finished product.

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Table 1 - Composition data of TSP from chemical analysis

	TSP			
Tests (units)	**STRIP-N**	**STRIP-W**	**SHRED**	**BIT**
Moisture by vacuum oven (%)	6.5	6.0	6.3	8.0
Protein – combustion (%)	49.4	51.4	50.8	53.4
Crude fat by acid hydrolysis (%)	12.2	12.9	9.8	8.3
Trypsin Inhibitor (TIU/g)	<2050	<2100	<2000	<2000
Peroxide value (meq/kg)	15.0	<5.4	8.5	7.1

STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die
SHRED: shred shape TSP
BIT: bit shape TSP

Table 2 – Parameters for texture analysis

Measurement	grams of force in compression return to start
Pretest speed	2.0 mm/s
Test speed	1.0 mm/s
Post test speed	10.0 mm/s
Distance	75% strain
Calibration distance	25 mm
Calibration	5 kg weight for 25 kilo load cell

Table 3 – Matched pairs t-test for the 1st chicken-flavored TSP sensory Analysis

Attributes	Matched pairs	P-value	t-test
Beany-flavor	4% - 3% (concentration)	0.1692	Not significant
	STRIP-N – STRIP-W (TSP)	0.5814	Not significant
Oily-flavor	4% - 3% (concentration)	0.3406	Not significant
	STRIP-N – STRIP-W (TSP)	0.0033	Significant
Chicken-flavor	4% - 3% (concentration)	0.0290	Significant
	STRIP-N – STRIP-W (TSP)	0.1963	Not significant
Color	4% - 3% (concentration)	0.4252	Not significant
	STRIP-N – STRIP-W (TSP)	<0.0001	Significant
Saltiness	4% - 3% (concentration)	0.0037	Significant
	STRIP-N – STRIP-W (TSP)	0.4799	Not significant
Chicken-aroma	4% - 3% (concentration)	0.1120	Not significant
	STRIP-N – STRIP-W (TSP)	0.8941	Not significant
Oily-aroma	4% - 3% (concentration)	0.1323	Not significant
	STRIP-N – STRIP-W (TSP)	0.5595	Not significant
Beany-aroma	4% - 3% (concentration)	0.3506	Not significant
	STRIP-N – STRIP-W (TSP)	0.7226	Not significant
Crispiness	4% - 3% (concentration)	0.0117	Not significant
	STRIP-N – STRIP-W (TSP)	0.0931	Not significant
Chewiness	4% - 3% (concentration)	N/A	
	STRIP-N – STRIP-W (TSP)	N/A	

STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

Table 4 – Difference in pairwise means by Turkey-Kramer HSD test for the 1st chicken-flavored TSP sensory study

Treatments	Beany-flavor	Oily-flavor	Chicken-flavor	Color	Saltiness	Beany-aroma	Oily-aroma	Chicken-aroma	Crispiness
	Attributes								
3%+STRIP-N	55.35^{A}	66.02^{A}	66.01^{A}	84.08^{A}	63.00^{A}	48.17^{A}	66.44^{A}	66.93^{A}	98.11^{A}
4%+STRIP-N	56.26^{A}	64.97^{A}	71.29^{A}	90.06^{A}	73.68^{A}	52.26^{A}	57.23^{A}	71.32^{A}	87.75^{A}
3%+STRIP-W	61.85^{A}	61.14^{A}	66.43^{A}	63.81^{B}	61.08^{A}	50.50^{A}	61.02^{A}	67.19^{A}	87.43^{A}
4%+STRIP-W	52.55^{A}	57.67^{A}	76.79^{A}	62.07^{B}	71.77^{A}	52.55^{A}	57.67^{A}	72.21^{A}	81.01^{A}

Means within a column with different letters are significantly different ($P < 0.05$)

3%+STRIP-N: flavored STRIP-N with 3% flavor concentration

4%+STRIP-N: flavored STRIP-N with 4% flavor concentration

3%+STRIP-W: flavored STRIP-W with 3% flavor concentration

4%+STRIP-W: flavored STRIP-W with 4% concentration

Table 5 – P-values from color analysis results in the 1st chicken-flavored TSP study

Matched Pairs	Hunter Color Values L	a	b
4% - 3% (concentration)	0.5799	0.4718	0.6050
STRIP-N – STRIP-W (TSP)	0.0057*	0.0115*	0.0031*

(*) indicates a statistically significant difference (p=0.05) between matched pairs.
STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

Table 6 – Chemical analysis results for the finished flavored TSP samples in the 1st chicken-flavor (powder) TSP study 1

	TSP treatments			
Tests (units)	3%+STRIP-N	4%+STRIP-N	3%+STRIP-W	4%+STRIP-W
Moisture by vacuum oven (%)	11.5	12.2	19.3	18.6
Protein – combustion (%)	30.8	29.8	29.1	28.3
Crude fat by acid hydrolysis (%)	40.3	42.7	38.9	38.8
Trypsin Inhibitor (TIU/g)	<2000	<2000	<2000	<2000
Peroxide value (meq/kg)	3.2	2.0	3.4	3.0

3%+STRIP-N: flavored STRIP-N with 3% flavor concentration
4%+STRIP-N: flavored STRIP-N with 4% flavor concentration
3%+STRIP-W: flavored STRIP-W with 3% flavor concentration
4%+STRIP-W: flavored STRIP-W with 4% concentration

Table 7 – Matched pairs t-test for the 2nd chicken-flavored (liquid) TSP sensory analysis

Attributes	Matched pairs	P-value	t-test
Beany-flavor	4% - 3% (concentration)	0.2119	Not significant
	STRIP-N – STRIP-W (TSP)	0.4921	Not significant
Oily-flavor	4% - 3% (concentration)	0.0483	Significant
	STRIP-N – STRIP-W (TSP)	0.0811	Not significant
Chicken-flavor	4% - 3% (concentration)	0.0427	Significant
	STRIP-N – STRIP-W (TSP)	0.9753	Not significant
Color	4% - 3% (concentration)	0.0016	Significant
	STRIP-N – STRIP-W (TSP)	<0.0001	Significant
Saltiness	4% - 3% (concentration)	0.0171	Significant
	STRIP-N – STRIP-W (TSP)	0.1216	Not significant
Beany-aroma	4% - 3% (concentration)	0.5019	Not significant
	STRIP-N – STRIP-W (TSP)	0.6874	Not significant
Oily-aroma	4% - 3% (concentration)	0.1299	Not significant
	STRIP-N – STRIP-W (TSP)	0.1446	Not significant
Chicken-aroma	4% - 3% (concentration)	0.5741	Not significant
	STRIP-N – STRIP-W (TSP)	0.2206	Not significant
Crispiness	4% - 3% (concentration)	0.5151	Not significant
	STRIP-N – STRIP-W (TSP)	0.0155	Significant
Chewiness	4% - 3% (concentration)	0.1292	Not significant
	STRIP-N – STRIP-W (TSP)	0.0344	Significant

STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

Table 8 – TA.XT2 results for the 2nd chicken-flavored TSP study

Matched Pairs	P-Values	t-test
4% - 3% (concentration)	0.0055	Significant
STRIP-N – STRIP-W (TSP)	0.0003	Significant

STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

Table 9 – Chemical analysis data for the finished flavored TSP samples in The 2nd chicken-flavor (liquid) TSP study

	TSP treatments			
Tests (units)	**3%+STRIP-N**	**4%+STRIP-N**	**3%+STRIP-W**	**4%+STRIP-W**
Moisture by vacuum oven (%)	4.1	5.0	8.1	7.9
Protein – combustion (%)	29.5	31.1	30.8	30.8
Crude fat by acid hydrolysis (%)	50.2	48.9	46.3	49.4
Trypsin Inhibitor (TIU/g)	<2000	<2000	<2000	<2000
Peroxide value (meq/kg)	2.8	2.7	1.8	1.9

3%+STRIP-N: flavored STRIP-N with 3% flavor concentration
4%+STRIP-N: flavored STRIP-N with 4% flavor concentration
3%+STRIP-W: flavored STRIP-W with 3% flavor concentration
4%+STRIP-W: flavored STRIP-W with 4% concentration

Table 10 – P-values from color analysis statistical results in the 2nd chicken-flavored TSP study

	Hunter Color Values		
Matched Pairs	**L**	**a**	**b**
4% - 3% (concentration)	0.2790	0.1942	0.0571
STRIP-N – STRIP-W (TSP)	0.1018	0.4985	0.0010*

(*) indicates a statistically significant difference (p=0.05) between matched pairs.
STRIP-N: strip shape TSP extruded with a narrow die
STRIP-W: strip shape TSP extruded with a wider die

Table 11 – Matched pairs t-test for powder and liquid types of chicken-flavor in sensory analyses

Attributes	Matched Pairs	P-Values	t-test
Chicken-flavor	3%+Liquid – 3%+Powder	0.2862	Not significant
	4%+Liquid – 4%+Powder	0.1555	Not significant
Chicken-aroma	3%+Liquid – 3%+Powder	0.5092	Not significant
	4%+Liquid – 4%+Powder	0.8728	Not significant
Beany-flavor	3%+Liquid – 3%+Powder	0.2433	Not significant
	4%+Liquid – 4%+Powder	0.4356	Not significant
Beany-aroma	3%+Liquid – 3%+Powder	0.3832	Not significant
	4%+Liquid – 4%+Powder	0.0451	Significant

3%+Liquid: low concentration of liquid type of chicken flavor
3%+Powder: low concentration of powder type of chicken flavor
4%+Liquid: high concentration of powder type of chicken flavor
4%+Powder: high concentration of powder type of chicken flavor

Table 12 – Matched pairs t-test for the shrimp-flavored TSP sensory analysis

Attributes	Matched pairs	P-value	t-test
Beany-flavor	H - L	0.0573	Not significant
	S - C	0.6253	Not significant
Fishy-flavor	H - L	0.0071	Not significant
	S - C	0.7492	Not significant
Shrimp-flavor	H - L	0.0087	Significant
	S - C	0.0984	Not significant
Color	H - L	0.2394	Not significant
	S - C	0.5343	Not significant
Saltiness	H - L	<0.0001	Significant
	S - C	0.7294	Not significant
Beany-aroma	H - L	0.2493	Not significant
	S - C	0.4590	Not significant
Fishy-aroma	H - L	0.0515	Not significant
	S - C	-2.0000	Not significant
Shrimp-aroma	H - L	0.4794	Not significant
	S - C	0.8191	Not significant
Crispiness	H - L	0.7533	Not significant
	S - C	0.0383	Significant

H: high concentration of flavor
L: low concentration of flavor
S: single flavor
C: combination flavor

Table 13 - Texture analysis results for the shrimp-flavored TSP study

Matched Pairs	P-Values	t-test
H - L	0.5124	Not significant
S - C	0.0077*	Significant

H: high concentration of flavor
L: low concentration of flavor
S: single flavor
C: combination flavor

Table 14 – P-values for color analysis statistical results in the shrimp-flavored TSP study

	Hunter Color Values		
Matched Pairs	L	a	b
H- L (concentration)	0.0010*	0.1436	0.0005*
S - C (flavor)	0.0006*	0.0086*	0.9651

(*) indicates a statistically significant difference (p=0.05) between matched pairs.
H: high concentration of flavor
L: low concentration of flavor
S: single flavor
C: combination flavor

Table 15 – Matched pairs t-test for the 3rd chicken-flavored TSP sensory analysis

Attributes	Matched pairs	P-value	t-test
Beany-flavor	H - L	0.1375	Not significant
	F – B	0.0531	Not significant
Oily-flavor	H - L	0.1452	Not significant
	F – B	0.0007	Significant
Chicken-flavor	H - L	0.0005	Significant
	F – B	0.0035	Significant
Color	H - L	0.0057	Significant
	F – B	<0.0001	Significant
Saltiness	H - L	<0.0001	Significant
	F – B	0.0012	Significant
Beany-aroma	H - L	0.9245	Not significant
	F – B	0.1207	Not significant
Oily-aroma	H - L	0.1678	Not significant
	F – B	0.0006	Significant
Chicken-aroma	H - L	0.0430	Significant
	F – B	0.0025	Significant
Crispiness	H - L	0.4023	Not significant
	F – B	<0.0001	Significant
Chewiness	H - L	0.2451	Not significant
	F – B	0.0063	Not significant

H: high concentration
L: low concentration
F: fried samples
B: baked samples

Table 16 – Difference in pairwise means by Turkey-Kramer HSD test for the 3rd chicken-flavored TSP sensory study

	Attributes									
Treatments	Beany-flavor	Oily-flavor	Chicken-flavor	Color	Saltiness	Beany-aroma	Oily-aroma	Chicken-aroma	Crispi-ness	Chewi-ness
LB-BIT	35.88^{A}	13.64^{B}	52.35^{B}	40.36^{B}	60.00^{B}	24.20^{A}	13.55^{B}	47.32^{B}	106.29^{AB}	50.63^{A}
HB-BIT	35.39^{A}	14.44^{B}	64.95^{AB}	44.46^{B}	79.41^{AB}	23.35^{A}	15.43^{B}	52.72^{AB}	101.74^{B}	52.08^{A}
LF-BIT	24.72^{A}	49.14^{A}	78.10^{AB}	105.17^{A}	79.35^{AB}	19.58^{A}	50.11^{A}	72.67^{AB}	121.40^{A}	26.98^{A}
HF-BIT	15.30^{A}	53.84^{A}	83.37^{A}	110.38^{A}	90.18^{A}	18.42^{A}	53.73^{A}	79.98^{A}	121.07^{A}	31.72^{A}

Means within a column with different letters are significantly different ($P < 0.05$)

Table 17 – Chemical analysis data for the finished flavored TSP samples in the 3rd chicken-flavor (powder) TSP study

	TSP treatments			
Tests (units)	**LB-BIT**	**HB-BIT**	**LF-BIT**	**HF-BIT**
Moisture by vacuum oven (%)	4.7	2.7	4.0	4.0
Protein – combustion (%)	53.9	54.5	41.6	41.3
Crude fat by acid hydrolysis (%)	7.8	8.0	28.6	26.4
Trypsin Inhibitor (TIU/g)	<2000	<2000	<2000	<2000
Peroxide value (meq/kg)	2.3	2.6	2.6	2.4

LB-BIT: low concentration flavored baked BIT
HB-BIT: high concentration flavored baked BIT
LF-BIT: low concentration flavored fried BIT
HF-BIT: high concentration flavored fried BIT

Table 18 - Texture analysis results for the 3rd chicken-flavored TSP study

Matched Pairs	P-Values	t-test
H - L	0.5866	Not significant
F – B	0.0509	Not significant

H: high concentration
L: low concentration
F: Fried samples
B: Baked samples

Table 19 – P-values for color analysis statistical results in the 3rd chicken-flavored TSP study

	Hunter Color Values		
Matched Pairs	**L**	**a**	**b**
H – L (concentration)	0.6582	0.0026*	0.2501
F – B (application)	<0.0001*	<0.0001*	<0.0001*

(*) indicates a statistically significant difference (p=0.05) between matched pairs.
H: high concentration
L: low concentration
F: Fried samples
B: Baked samples

Table 20 – Comparison with matched pairs t-test between fried STRIP-N and fried BIT in sensory analysis

Attributes	Treatments	Means	Mean difference	Std error	P-Values	t-test
Crispiness	BIT	121.60	27.19	4.81	<0.001	Significant
	STRIP-N	94.41				
Oily-flavor	BIT	49.77	-17.09	7.71	0.036	Significant
	STRIP-N	66.86				
Oily-aroma	BIT	50.67	-12.64	6.89	0.079	Not significant
	STRIP-N	63.33				

BIT: bits shape TSP
STRIP-N: strip shape TSP extruded with a narrow die

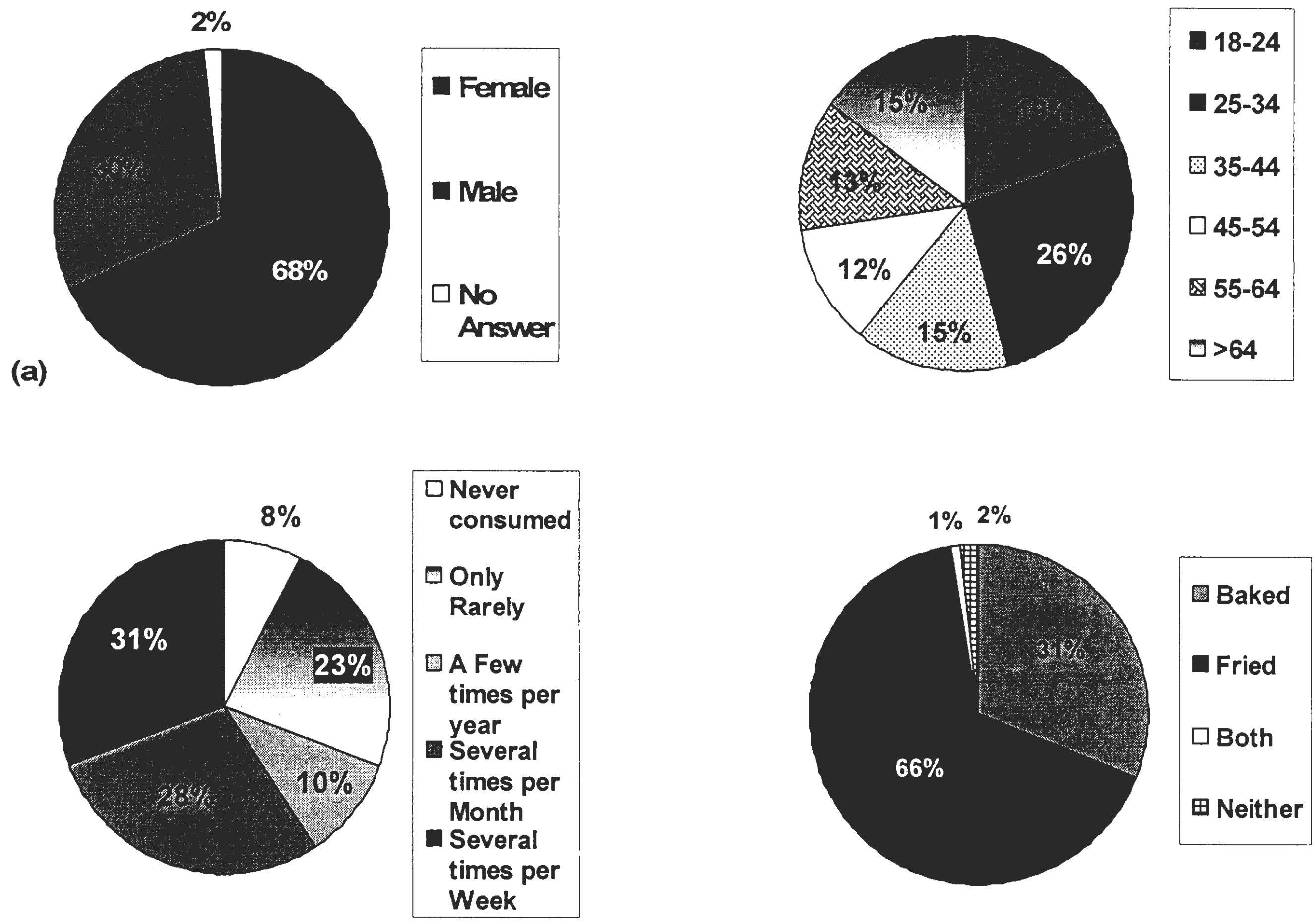

Figure 1 - Summary of consumer preference test in (a) gender, (b) age, (c) soy consumption, and (d) preference.

CHAPTER 4. UTILIZATION OF OKARA, BYPRODUCTS FROM SOYMILK PRODUCTION THROUGH THE DEVELOPMENT OF SOY-BASED SNACK FOODS

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Key words: okara, soybean oil, snack food, sensory

ABSTRACT

The study was conducted to develop a new soy-based food product which could utilize okara (a byproduct from soymilk production) and maximize health benefits from okara by consuming the product. A Japanese commercial fried okara snack product was used as a standard reference. Two types of dried okara powder, a commercially dried okara powder which had 7.7% moisture content made from regular (lipoxygenase-present) soybeans and a partially dried okara which had 44.3 % moisture content made from triple-null (lipoxygenase-free) soybeans, were used in this study. Also, two types of soybean oil, commercial low saturated soybean oil and commercial low linolenic acid soybean oil, were used in the same formulation to compare and find the best formulation to develop a soy-based food product.

Two descriptive analysis studies were performed with a minimum of 15 trained panelists. Baked and fried applications were tested during the early formulations before the first sensory study was performed. The instrumental and chemical analyses were also performed to compare with the sensory results. The results from the first sensory study

were statistically analyzed and used to reformulate the recipe for the second sensory study. The baked product made from commercial low saturated soybean oil and the partially-dried okara powder manufactured at the university facilities gave the closet flavor, texture, and appearance to the reference standard. The appearance of the final product may match the smooth surface of the standard by using a freeze-dried okara powder. However, the objectives of this study were achieved by developing the new product which was rich in protein and fiber with an enjoyable flavor and texture.

INTRODUCTION

The number of new soy-based products have been increasing 13.2% per year from 2001 to 2004, averaging 400 new products per year in the United States. The new soy-based products introduced in market were snacks (The Soyfoods Association of North America, 2005). Consumer awareness of health benefits from soy products was over 60 percent in 2004 (the United Soybean Board 2005). Soymilk sales were the second in sales to energy bars in soyfoods market (2004) (The Soyfoods Association of North America, 2005). Although the soymilk sale is selling well in the United States, a byproduct from soymilk production, okara, has not been utilized into soyfoods. There are mainly treated as industrial waste or animal feeds (Ohno and others1996; Chan and Ma 1999). Okara is fibrous residue after soymilk production, with good amino acid profiles. It contains about 20 – 27% protein (dry basis) (Watanabe and Kishi 1984; Chan and Ma, 1999).

Waliszewski and others (2002) studied the nixtamalized corn tortillas fortified with dried okara to determine amino acid composition and sensory changes at different levels of fortification. Higher levels of okara fortification were not acceptable for their trained panelists due to the undesirable flavor from okara. Up to 10% fortification of okara to the corn flour, there was no significant difference between regular corn tortillas and okara-fortified tortillas in sensory panel. The purpose of this study was achieved with the 10% okara-fortified corn tortillas which were rich in lysine and tryptophan meeting more than 90% of FAO requirement. The study concluded that the okara-fortified corn tortillas could be a good source of protein for Mexico and other countries.

Genta and others (2002) used okara to make a soy candy to increase the availability of soy proteins for human consumption and the production of soybean products. The study found that the lowest addition level, 18.3% (based on 100% formulation), of okara was the most acceptable and preferred by the judges. These two studies proved that okara was a good source of protein for human health. However, the undesirable flavor, called "beany" flavor in this study, of okara was a significant problem to develop an okara- or any kinds of soy-based food product. This undesirable "beany" flavor or odor is a result of the oxidation of unsaturated fatty acids by lipoxygenase enzymes during the process of soy protein products (Wilson 1995).

The objectives of this study were to use okara from soymilk production to develop and commercialize soy-based snack foods, and to improve human health, soy marketability, and reduce environmental wastes.

MATERILAS AND METHODS

Early formulation for a Gold Standard

Three different formulations were tried to find a Gold standard recipe based on the ingredients listed on the original Japanese product package. Main ingredients initially utilized in this study were all-purpose flour, commercially-dried okara powder, low saturated soybean oil, dark brown sugar, seasonings and salt. All the ingredients were commercially available. The first formulation was with baking powder and without water. The second formation was with baking powder and water. The third formulation was without baking powder. The dough was kneaded, rolled out into a sheet, sandwiched between two *sushi* bamboo mats, and pressed down with a roller to make a ridged surface. The thickness of rolled dough was 4 mm. The ridged shape dough was cut into 2.5cm-square. The squared dough were either fried at 350°F with low saturated soybean oil for 2 minutes in a deep-fryer (Pesto Frydaddy electric deep fryer, stock no. 05420. Eau Claire, WI), or baked in an oven (Caloric Prestige Series, Raytheon Company, Waltham, MA) at 395°F for 10 minutes (maximum baking time). The finished baked or fried products were tasted and compared with the original Japanese commercial products to see if they had similar texture, flavor and appearance.

Formulation of the first sensory evaluation

The first sensory study compared 2 formulations which differed in the type of oil from that of the original Japanese commercial product (rape seed oil). Low linolenic acid soybean oil which was developed by Dr. Walter Fehr (Department of Agronomy) and Dr. Earl Hammond (Department of Food Science and Human Nutrition) at Iowa State University was compared to low saturated soybean oil (Table 1). Both oils were commercially available.

Three different treatments, control (the Japanese commercial product as a standard reference), LinoB as the product made with low linolenic acid soybean oil, and SoyB as the product made with low saturated soybean oil. “B” stands for a baked application, whereas the Japanese products used a fried application. Table 2 shows the summary of the three treatments.

Sensory evaluation

After being approved by the Human Subjects Research Office at the Iowa State University, sensory evaluations were performed at Iowa State University, the Center of Crops Utilization Research (CCUR) sensory facilities. The panelists were asked to sign a letter of informed consent. A descriptive analysis with 15- cm line scales was then performed with the trained panelists. The score on the 15-cm line scale was calculated raging from 0 as a minimum and 150 as a maximum intensity for each attribute (Appendix A) (Lawless and Heymann 1999). The panelists participated voluntarily and were instructed and screened during the training sessions. During the training sessions, 16 out of 21 volunteers in the first sensory study, and 15 out of 17 volunteers in the second sensory study were selected to be panelists based on their ability to detect the important attributes of all food samples. The attributes to describe samples were discussed and agreed upon by the subjects. The sensory attributes for all samples were beany-flavor/aroma, oily-flavor/aroma, cinnamon-flavor/aroma, sweetness, saltiness, and crunchiness. Subjects were trained in two 30-minutes sessions on two different days. The reference standards used during the training sessions were commercial low saturated soybean oil as oily-aroma/flavor, commercial cooked okara as beany-flavor/aroma, and ground cinnamon powder as cinnamon-flavor/aroma. The other reference standards were two types of Asian commercial crackers, one was less and the other was very crunchy in texture. Each product had a 3-digit code and was presented to the panelists. The samples were also presented in random order under controlled environmental conditions. Four replications of a descriptive analysis were conducted. Each panelist evaluated all three treatments at each session. The sample size was two chips for each treatment. The panelists evaluated each treatment individually on the individual score sheet. For a future consumer preference test, the panelists were also provided a "your preference today" sheet (Appendix B) to indicate which treatment they preferred and to briefly discuss why. During the training sessions, the difference between a descriptive and a preference test and the purpose of including a preference test was discussed with the panelists.

Instrumental analyses

Color and texture of the samples were determined using instrumental methods for comparison to the results from the descriptive analysis panel. A LabScan XE spectrophotometer (Hunter Associate Laboratory Inc., VA) with the Hunter system was used for the color analysis. The parameters of the Hunter scale used in this study were D65 (daylight 65), 10°Standard Observer, 0.40 inch port size, and 0.25 inch area view. A TA.XT2i texture analyzer (Texture Technologies Corp, NY) with three point bend jig attachment and a TA-42 knife blade with sharp 45°chisel end were used for the texture analysis. The hardness of samples was measured on grams of force with the program set in compression return to start. The parameters of this program were 2.0 mm/s pretest speed, 1.0 mm/s test speed, 25 % strain distance, and 25 mm calibration distance. A 25 kilo load cell was calibrated with a 5 kg weight. Ten pieces were selected from each treatment, and used for texture or color analysis during the same time as sensory session was performed. Viscosity of oils was determined by using a DV-II+ PRO Digital Viscometer (Brookfield, Middleboro, MA). 600 ml of oil was transferred into a 600 ml beaker. A plate spindle No. 1 was placed into the oil. The viscosity of the oil sample was read from the viscometer dial reading on the percentage scale at 5, 10, 20, 50 and 100 rpm. The temperature of oil was maintained at 70.6 °F. The Herschel-Bulkley model was used for calculation of the consistency coefficient, K, called the viscosity with Newtonian fluid model (Steffe 1996). The measurement was duplicated.

Statistical analysis

A random effects model was used to compare groups, while removing effect of correlation among the observations. For the sensory and instrumental data, the averages were used to summarize correlated data. The summarized data were then statistically analyzed using JMP 5.1.2, the Statistical Discovery Software (SAS Institute Inc, Cary , N.C., U.S.A.). A matched pairs t-test was set at a *P*-value of 0.05. Also a Tukey-Kramer HSD adjustment was used in the first sensory study to minimize type I error inflation (Ramsey and Schafer 2002).

Chemical analysis

The raw okara powder, wet okara samples, and finished products were chemically analyzed by following the AOAC Official Methods (AOAC International 2000) procedures (934.01 for moisture by vacuum oven, 990.03 for crude protein by combustion method, 954.02 for crude fat by acid hydrolysis, 991.43 for total dietary fiber, and 984.27 for calcium, phosphorus, and potassium) and the AOCS Official Methods (AOCS 1997 revised in 2004) procedures (Ba 12-75 for trypsin inhibitor activity, Cd 8-53 for peroxide value).

Weight determination

The weights of 10 pieces (in grams) of the finished products for each treatment were determined. The measurement was repeated at each of the four sensory sessions. The average weight of each finished chip was determined based on the weight of the 40 pieces.

Reformulation for 2nd sensory study

Based on the results from the first sensory study, the recipe was reformulated to produce a product that was more similar to the original Japanese commercial product. The new main ingredients were a hand-made partially dried okara powder and light brown sugar (Table 3). The okara, which was manufactured from soymilk production in the CCUR pilot plant at Iowa State University, was frozen immediately following processing. The frozen wet okara was removed from the freezer, heated up for 7 minutes in a microwave (1.4 kilowatt, model # JE2810A 002, General Electric, Louisville, KY), and partially dried in an oven at 320 °F (Caloric Prestige Series, Raytheon Company, Waltham, MA) at the CCUR kitchen to an average moisture content of 44.3 %. The amounts and ingredients included in the seasonings were also modified from the previous formulation. Based on the results of the first sensory study, low saturated fatty acid soybean oil was used.

The new formulation was compared to the original Japanese product by the descriptive evaluation panel. The sensory evaluation method was the same method as for the first study. There were only 2 different treatments. One was the control, the original Japanese product standard, and the other was SoyBPO using commercial low saturated soybean oil as same as the first sensory study, but using the handmade partially-dried okara powder. The instrumental, chemical, statistical analyses and weight measurement were also performed as in the first study

RESULTS AND DISCUSSION

Early Formulation

Because of the different food laws in Japan, the Japanese nutrition information and ingredients list were different from the United States laws for food labeling. The Nutrition Labeling and Education Act, which amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Fair Packaging and Labeling Act (FD&C Act) requires all processed food products to bear nutrition labeling and nutrient claims (21CFR101.9, 4-1-03 edition, U.S. Food and Drug Administration). The Japanese Agricultural Standard (JAS) states the JAS Law (Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products, Law No. 175 in 1950) for food labeling regulation in Japan (Notification No. 513 of the Ministry of Agriculture, Forestry and Fisheries of March 31, 2000). The laws state that the manufacture or distributors need to provide all ingredients used to produce the processed products on the label.

However, currently in Japan not all the processed food products are required to bear all nutrition information and details of ingredients used in the products. As a result, although it is the exact same type of processed product as the other commercial products, it can have a different ingredients list and nutrition list (Report No. 5 from the Ministry of Agriculture, Forestry and Fisheries. December 2004). This case has an exemption for the ingredients that have high allergic risks (administrated in December 2004). Therefore, the early recipes involved ingredients and processing modifications to produce the proper flavor and texture from the ingredients information on the original label. When the dough was fried in low saturated soybean oil at 350 °F, the products broke apart into crumbs, and floated in the oil. When the water was added to the recipe, gluten formation developed in the dough during the kneading process. The addition of water allowed the products to be formulated with less oil amounts and resulted in less frying problems. The fried application could integrate flavors of the ingredients into the final products compared with the baked products. The fried products resulted in a milder flavor than the baked products. For example, the fried products had less spicy taste than the baked ones. This decrease in spiciness might result from the high temperature of oil (volatile loss and the penetration of oil into the dough during frying process might decompose or extract the components of spices and chemical reactions). The fried products were too dark in color and the texture was too soft. The texture of fried products was more like a doughnut product rather than a chip product, because of less crispness. The oiliness of the finished fried products was expected to be a major problem for future marketing purpose. Therefore, a baked application was chosen for the next application due to frying problems.

The baked application gave more crispness to the finished products. However, the texture of baked products resulted in more like a puffy cookie product. This problem was due to the addition of baking powder, which gave some proofing-expansion to the dough. Also the taste was spicier than the fried products. The appearance of the baked products was brighter in color than the fried products. The finished products also had strong of a cinnamon flavor that was away to be expected. The recipe was reformulated without baking powder and with less amount of cinnamon powder than the second recipe. When this reformulated recipe was tested, several improvements were seen in the baked products. The baked application with this recipe resulted in less oily problems and more crispiness than the fried application. Also, the finished products had a unique flavor from each of the flavoring ingredients. After all those results were discussed, the baked application was selected to evaluate in the first sensory evaluation.

First sensory evaluation

During the training sessions, two pieces of the original Japanese commercial products were provided to each panelist. The panelists were very interested in the flavor and texture of the original Japanese products in the initial evaluation. Some of the panelists were not satisfied with the thin texture of the LinoB and SoyB. The Japanese commercial product had a very thick and hard texture. It was so hard that the panelists took a longer time to finish one piece than consuming one piece of LinoB and SoyB. Compared with the hard texture of the Japanese product, the thin texture of LinoB and SoyB was easy to eat and swallow it. Therefore, during the training sessions, those panelists commented that it was too short a time for them to perceive details of the flavors of the LinoB and SoyB due to the too thin thickness. However, after the sensory evaluations started, some changes had been seen in their comment sheets. The Japanese commercial product was fried food products using rape seed oil. Some of panelists noted that the oily-aroma and flavor of the original Japanese fried products gave them a feeling of fullness quickly. Although the freshest package of the Japanese products was opened and provided to the panelists at every session, those panelists detected an oxidized, rancid oil flavor in the original commercial products. Also, most of panelists agreed that the texture of the original products was too hard and resulted in tiredness from extra force to allow jaw movement to bite and chew them. This comment was discussed with a few panelists during the training sessions, but it was noted by more panelists after one sensory session. Because of the baked products removed oiliness, the panelists also commented that LinoB and SoyB gave them a healthy feeling, too.

The matched pairs t-test showed that there were significant differences in oily-aroma/flavor, cinnamon-flavor, saltiness, sweetness, and crunchiness in sensory analysis (Table 4). This statistical result agreed with the result using the Turkey-Kramer HSD test in oily-aroma/flavor, sweetness, saltiness, and crunchiness (Table 5). Due to the large standard deviations, the results from Turkey-Kramer HSD test did not show a significant difference in cinnamon-flavor/aroma among the treatments.

The results from color analysis using a Labscan XE spectrophotometer revealed more numerical and statistical details in color difference (Table 6). The lightness in L value of LinoB was in a midpoint between the Japanese product (control) and SoyB. This result supported the sensory result in color attribute (Table 4). SoyB was significantly lighter than the control. The color of LinoB was also not significant different from control (Japanese product) in sensory panel. Redness (a positive a value) and yellowness (a positive b value) of control were significantly higher than LinoB and SoyB. Based on the results from both sensory and instrumental analyses, the lightness (L value in Hunter system) could be a major contributor to influence the visual perception of panelists.

The viscosities of low linolenic acid soybean oil and low saturated soybean oils resulted in the same viscosity. The consistency coefficient K of low linolenic acid soybean oil was 0.000535 (≈ 0.0005), and low saturated soybean oil was 0.000482 (≈ 0.0005). Therefore, the viscosity of oils was not a major influence on the hardness of finished products.

The results from the texture analyzer strongly agreed with the sensory results (Table 7). It showed that The Japanese product (control) required a significantly higher force to break the product than the two other samples.

However the weights of pieces between control and LinoB or SoyB were significantly different (Table 8). The piece weight of control was almost two times heavier than the piece weight of others. This problem was fixed in the next recipe before the second sensory study was performed.

Although the piece weight of the Japanese commercial product was approximately two times heavier than that of LinoB and SoyB, the protein, total dietary fiber, and mineral (calcium, phosphorus, and potassium) contents of LinoB and SoyB were much higher than those of the Japanese commercial product (Table 9). These results showed health benefits by consuming those products. One disadvantage of those products could be higher fat content if the piece weight of both products was adjusted to be same as the control, Japanese commercial product.

During the preparation of the second sensory study

There was no significant difference in beany-aroma/flavor between the low saturated soybean oil and the low linolenic acid soybean oil (Table 4 and 5). However, according to their mean scores, the low saturated soybean oil seemed to contribute a less beany-aroma/flavor than the low linolenic acid soybean oil. Beany-aroma or beany-flavor is the major problem to develop a soy-based food product. Therefore, the reformulated recipe for the second sensory study used only low saturated soybean oil (Table 3). This recipe that substituted the okara manufactured from soymilk production for the commercial dried okara powder gave a cruncher texture than the previous recipe. It also contributed a similar flavor with the Japanese commercial product. The addition of curry powder and white sesame powder instead of black sesame powder improved the flavor and appearance. The thickness and weight of finished product were adjusted to be the same as the Japanese commercial product. After the adjustments, the thickness was 3.0 mm, and the piece weight was 1.5 g for the new sample. The new sample made from this reformulated recipe was called SoyBPO. PO stands for the partially-dried okara powder manufactured in the CCUR pilot plant at the Iowa State University.

The chemical analysis revealed that the partially-dried okara powder had approximately five times higher moisture content than the commercial dried okara powder (Table 10). The total dietary fiber content of the partially-dried okara powder was very close to that of commercial dried okara powder. This result could explain if the partially-dried okara powder had equal moisture content to the commercial dried okara powder had, the total dietary fiber of this powder could be five times higher than that of the commercial dried okara powder. The protein content of this powder was also diluted with the higher moisture content compared to that of the commercial dried okara powder. Therefore, the protein content of the partially-dried okara powder could be higher than the result shown in Table 10. This theory also could support the mineral contents (calcium, phosphorus, and potassium). Another health benefits using this okara powder would be a low fat content in finished products.

Second sensory study

The results from the second sensory evaluation showed that there was no significant difference in beany-flavor/aroma, sweetness and saltiness between the SoyBPO (research product made from the reformulated recipe) and control (the Japanese commercial product) (Table 11). Those attributes were the main attributes needed to find a right flavor formulation. The panelists commented that they did not perceive any 'beany-flavor/aroma' from neither of the products. Instead, they preferred the entire flavor of SoyBPO over the Japanese commercial product. The comment agreed with the sensory results (Table 11). SoyBPO had less beany-flavor/aroma than the Japanese product. There were significant differences in oily-aroma and flavor, cinnamon-aroma and flavor, color, and crunchiness. The original Japanese commercial product had a more oily-aroma and flavor because they were fried products. According to the panelist comments, the Japanese commercial products had an oxidized aroma and flavor from rancidity (lipid oxidation). The crunchiness of the commercial products was too intense for most of the panelists. SoyBPO had a just right texture and flavor for them. They also noted a healthier feeling on the baked research products. At the end of the second sensory study, their overall preference score was 82.1% for SoyBPO, the research product, 12.8% for the Japanese commercial product, and 5.1% undecided. According to the comments from the undecided panelists, they perceived that two products had too close flavor profile. The difference in flavor seemed to be a major contributor for their preference, because they did not comment about any difference in texture.

There were many improvements seen in the results from instrumental analyses. There was no significant difference in lightness of surface color between the two treatments (Table 12). This result showed a major improvement in appearance.

When the weight measurement was performed, there was no significant difference in piece weight. The piece weight of both products was 1.55 grams in average (data not shown). The statistical results using a texture analyzer showed that there was a significant difference in hardness between the treatments (Table 13).

The finished research product, SoyBPO, from the second sensory study had more fiber and protein contents than control, the Japanese commercial products (Table 14). The mineral content was also higher than the Japanese commercial product. The fat content was also improved than the previous recipe used in the first sensory study.

CONCLUSION

The Japanese commercial product has a plain and smooth color appearance, whereas the SoyBPO has a textured surface (Figure 1). The appearance of SoyBPO will be able to match the Japanese commercial product using freeze-dried okara. The wet okara can be frozen right after it is manufactured from soymilk production. The frozen wet okara then will go to a freeze-drying system to make dried okara. The freeze-dried okara will be pulverized to make fine okara powder. By using this fine okara powder, the appearance of finished products will become a smoother surface.

The moisture content of partially-dried okara powder had approximately 5 times more moisture than the commercial dried powder, however, the fiber content was same. This means if the partially-dried okara could be manufactured to have same moisture content (~8.4% as dry basis) as the commercial dried okara, its fiber content would be much higher than the commercial one. Therefore, it is possible to produce a new fiber-rich okara snack with this okara powder. Also, the protein content will be higher than the current data in this study.

According to the comments from the majority of panelists, SoyBPO in the second sensory study had a better hardness for the panelists than the Japanese commercial product at the end of the study. The hardness will be enough to be an advantage for packaging and transportation systems. Less lipid oxidation of the baked research products is also an advantage for marketing purpose. It will have a longer shelf-life than a fried product, such as the Japanese commercial okara product.

Many advantages and improvements obtained from SoyBPO in this study will promote the use of okara, byproducts from soymilk production, in food industry. It will then contribute to health benefits for humans and the reduction of environmental waste.

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Table 1 - Formulation for the first sensory evaluation

Ingredients		grams*	Formulation (100% basis)
Wheat flour		70.0	31.0
Commercial dried okara powder		40.0	18.0
Water		60.0	27.0
Dark brown sugar		25.0	11.0
Low saturated soybean oil/ Low linolenic acid soybean oil		15.0	7.0
Seasonings		10.3	5.0
	(Mayonnaise)	(6.0)	
	(Black sesame)	(3.0)	
	(Caramel)	(1.0)	
	(Cinnamon)	(0.2)	
	(Red ground pepper)	(0.1)	
Salt		2.7	1.0

All ingredients were commercially available.
* grams were total 223g for 170 pieces in average.

Table 2 - Summary of three treatments for the first sensory evaluation

Treatments	Types of oil	Application
Control (Japanese product)	Rape seed	Fried
SoyB	Low saturated soybean	Baked
LinoB	Low linolenic acid soybean	Baked

Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.

Table 3 - Formulation for the second sensory evaluation

Ingredients	grams*	Formulation (100% basis)
Wheat flour	75.00	40.0
Partially-dried okara powder	25.00	13.0
Water	30.00	16.0
Light brown sugar	20.00	10.6
Low saturated soybean oil/	15.00	8.0
Seasonings	16.15	8.6
(Mayonnaise)	(6.00)	
(White ground sesame)	(6.00)	
(Caramel syrup)	(4.00)	
(Curry powder)	(0.07)	
(Red ground pepper)	(0.05)	
(Ground cinnamon)	(0.03)	
Salt	7.20	3.8

* grams were total 188.35g for 90 pieces in average.

Table 4 – Matched pairs t-test for the 1st sensory analysis

Attributes	Matched pairs	P-value	t-test
Beany-aroma	LinoB – Control	0.6620	Not significant
	SoyB – Control	0.7312	Not significant
Oily-aroma	LinoB – Control	0.0152	Significant
	SoyB – Control	0.0094	Significant
Cinnamon-aroma	LinoB – Control	0.0247	Significant
	SoyB – Control	0.0823	Not significant
Saltiness	LinoB – Control	<0.0001	Significant
	SoyB – Control	<0.0001	Significant
Sweetness	LinoB – Control	0.0005	Significant
	SoyB – Control	0.0006	Significant
Beany-flavor	LinoB – Control	0.0895	Not significant
	SoyB – Control	0.2022	Not significant
Oily-flavor	LinoB – Control	0.0026	Significant
	SoyB – Control	0.0012	Significant
Cinnamon-flavor	LinoB – Control	0.0087	Significant
	SoyB – Control	0.0085	Significant
Color	LinoB – Control	0.1764	Not significant
	SoyB – Control	0.0782	Not significant
Crunchiness	LinoB – Control	<0.0001	Significant
	SoyB – Control	<0.0001	Significant

Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.

Table 5 – Difference in pairwise means by Turkey-Kramer HSD test for the 1st sensory analysis

Attribute	Control	LinoB	SoyB
Beany-aroma	23.36^{A}	26.36^{A}	26.06^{A}
Oily-aroma	52.95^{A}	22.11^{B}	23.63^{B}
Cinnamon-aroma*	13.84^{A}	32.17^{A}	27.27^{A}
Beany-flavor	22.63^{A}	29.75^{A}	26.95^{A}
Oily-flavor	44.08^{A}	19.27^{B}	15.47^{B}
Cinnamon-flavor*	14.34^{A}	36.38^{A}	35.94^{A}
Sweetness	82.06^{A}	55.84^{B}	57.91^{B}
Saltiness	62.45^{A}	20.34^{B}	20.63^{B}
Color	60.27^{A}	68.88^{A}	69.94^{A}
Crunchiness	125.03^{A}	86.16^{B}	91.66^{B}

0 = minimum intensity; 150 = maximum intensity.
Control = Japanese commercial deep-fried product; LinoB = research baked product Made with low linolenic acid soybean oil; SoyB = research baked product made with low saturated soybean oil.
Means within a raw with different subscript letters are significantly different ($P < 0.05$)
* indicates there was no significant difference among the treatments due to the large standard deviations.

Table 6 – Difference of pairwise means for Hunter color values

Treatment	Hunter Color Values L	a	b
Control	50.98^{B}	9.78^{A}	19.15^{A}
LinoB	52.87^{AB}	8.61^{B}	17.34^{B}
SoyB	53.90^{A}	8.13^{B}	17.54^{B}

L: 0 = black; 100 = white
a: -a = green; +a = red
b: -b = blue; +b = yellow
Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.
Means within a raw with different subscript letters are significantly different (P < 0.05)

Table 7 – Matched pairs t-test for the results from TA.XT2i texture analyzer

	Control	LinoB	SoyB	Matched pairs: Control – LinoB	Matched pairs: Control - SoyB
Mean	5259.09	1379.09	1652.41		
MD				3880.00	3606.67
Std Err				283.39	331.17
P-value				<0.0001	<0.0001
t-test				Significant	Significant

Unit of mean values is grams of force.
Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.
MD = mean difference; Std Err = standard error: P-value (<0.05).

Table 8 – Matched pairs t-test for the results from weight determination

				Matched pairs	
	Control	**LinoB**	**SoyB**	**Control – LinoB**	**Control - SoyB**
Mean	1.53	0.74	0.80		
MD				0.79	0.73
Std Err				0.03	0.03
P-value				<0.0001	<0.0001
t-test				Significant	Significant

Unit of mean values is grams.
Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.
MD = mean difference; Std Err = standard error: P-value (<0.05).

Table 9 – Chemical analysis data of finished products in the 1^{st} sensory study

Tests (units)	**Control**	**LinoB**	**SoyB**
Moisture by vacuum oven (%)	2.4	3.6	2.9
Protein – combustion (%)	7.6	14.6	15.0
Crude fat by acid hydrolysis (%)	17.6	17.7	18.4
Total dietary fiber (%)	3.8	9.9	10.0
Calcium (%)	0.03	0.16	0.16
Phosphorus (%)	0.08	0.17	0.17
Potassium (%)	0.19	0.50	0.49
Trypsin Inhibitor (TIU/g)	<2,000	<2,000	<2,000
Peroxide value (meq/kg)	18.9	2.9	5.1

Control = Japanese commercial deep-fried product; LinoB = research product made with low linolenic acid soybean oil; SoyB = research product made with low saturated soybean oil.

Table 10 - Composition data of raw okara powder from chemical analysis

Tests (units)	Commercial dried okara powder	Partially-dried okara powder
Moisture by vacuum oven (%)	8.4	44.3
Protein – combustion (%)	31.7	17.1
Crude fat by acid hydrolysis (%)	14.7	7.6
Total dietary fiber (%)	33.6	33.0
Calcium (%)	0.36	0.23
Phosphorus (%)	0.37	0.21
Potassium (%)	1.35	0.65
Trypsin Inhibitor (TIU/g)	3,350	<2,000
Peroxide value (meq/kg)	30.7	17.3

Commercial dried okara powder = dried okara powder manufactured commercially; partially dried okara powder = dried okara powder manufactured in CCUR pilot plant at the Iowa State University.

Table 11 – Matched pairs t-test for the 2nd sensory analysis

	Mean of matched pair		MD	Std Err	P-value	t-test
Attribute	Control	SoyBPO				
Beany-aroma	27.20	20.07	7.13	5.21	0.1951	Not significant
Oily-aroma	52.39	35.65	16.73	4.83	0.0042	Significant
Cinnamon-aroma	28.83	52.51	-23.68	4.95	0.0004	Significant
Beany-flavor	27.70	17.14	10.55	7.34	0.1741	Not significant
Oily-flavor	56.32	20.14	36.18	6.13	<0.0001	Significant
Cinnamon-flavor	18.29	63.71	-45.42	8.24	<0.0001	Significant
Sweetness	69.97	61.89	8.08	3.78	0.0525	Not significant*
Saltiness	48.14	37.76	10.38	6.33	0.1250	Not significant
Color	57.05	76.96	-19.92	4.22	0.0004	Significant
Crunchiness	120.91	89.64	31.27	5.91	0.0001	Significant

0 = minimum intensity; 150 = maximum intensity.

Control = Japanese commercial deep-fried product; SoyBPO = research product made with low saturated soybean oil and partially-dried okara powder.

MD = mean difference; Std Err = standard error: P-value (<0.05).

* indicates that a significant difference might be seen between the treatments if the sample size became bigger.

Table 12 –Matched pairs t-test for Hunter color values

	Hunter Color Values L	a	b
Control	51.77	9.22	19.97
SoyBPO	53.03	8.06	17.40
MD	-1.26	1.17	2.56
Std Err	1.12	0.28	0.50
P-value	0.2909	0.0026	0.0006
t-test	Not significant	Significant	Significant

L: 0 = black; 100 = white
a: -a = greenness; +a = redness
b: -b = blueness; +b = yellowness
Control = Japanese commercial deep-fried product; SoyBPO= research product made with low saturated soybean oil and partially-dried okara powder.
MD = mean difference; Std Err = standard error: P-value (<0.05).

Table 13 – Matched pairs t-test for the results from TA.XT2i texture analyzer

			Matched pairs
	Control	**SoyBPO**	**Control – LinoB**
Mean	4119.03	3188.35	
MD			930.68
Std Err			389.90
P-value			0.0445
t-test			Significant

Control = Japanese commercial deep-fried product; SoyBPO = research product made with low saturated soybean oil and partially-dried okara powder
MD = mean difference; Std Err = standard error: P-value (<0.05).

Table 14 – Chemical analysis data of finished products in the 2nd sensory study

Tests (units)	Control	SoyBPO
Moisture by vacuum oven (%)	2.4	2.3
Protein – combustion (%)	7.6	11.4
Crude fat by acid hydrolysis (%)	17.6	18.8
Total dietary fiber (%)	3.8	7.4
Calcium (%)	0.03	0.13
Phosphorus (%)	0.08	0.14
Potassium (%)	0.19	0.28
Trypsin Inhibitor (TIU/g)	<2,000	<2,000
Peroxide value (meq/kg)	18.9	6.1

Control = Japanese commercial deep-fried product; SoyBPO = research product made with low saturated soybean oil and partially-dried okara powder

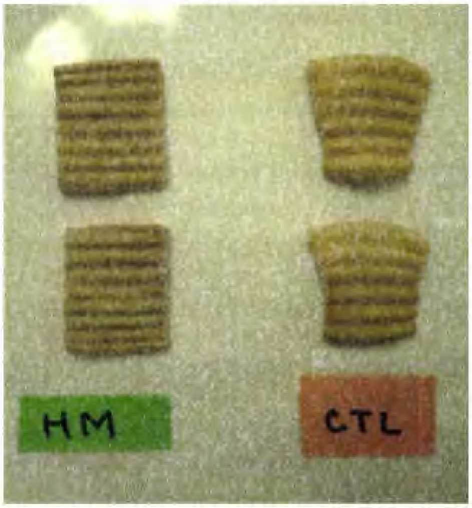

Figure 1 – Pictures for comparison of appearance between Japanese product and SoyBPO. HM = SoyBPO (left), CTL = Japanese product (right).

CHAPTER 5. GENERAL CONCLUSION

This study succeeded in the utilization of the entire soybean components through the development of new soy-protein fortified tomato soup and pudding products, chicken-flavored fried TSP product, and okara-based snack products. In recent years, many commercial soy-based products were produced by using cold applications and introduced into the organic foods market, such as soy-based smoothies, juices, and yogurts. The results from this study are hoped to contribute the application methods to utilize soy protein powders providing the scientific information. Based upon the results in this study, Unicell Soybean Powder needs better drying, better packaging procedures to improve and protect the flavor quality. Textured soy proteins have been used for the meat alternative products, and promoted the utilization of soybean for human consumption. The formulation developed in this study allows easy to incorporate TSP products into a diet as stand-alone products or ingredients. It can be marketed inside and outside of the United States, and then it will provide the nutritional benefits of soybeans to consumers. The utilization of okara was not a new concept, but a traditional custom in Japan, however, because of bulk production of soymilk in Japan and the United States, it has been become a minor usage for human consumption and a major waste problem. The development of new formulations to utilize okara for human consumption again will encourage more research into study of nutritional and functional benefits of okara, and eventually it will provide the maximum benefits of okara to bring back to our life. The disadvantage of okara which is high-moisture content can be beneficial, too. If the manufacturing plant where okara is produces is close to the food processing industry, the residual water from drying process of okara can be one of the ingredients for the formulation developed in this study.

The disadvantage of utilization of soybeans for human consumption had been overcome in this study, including beany-flavor of soybean products. Soybean is an amazing gift for human life. Future studies of soybean utilization will be seen as more economical, nutritional, functional, and provide benefits that can contribute to human life in the world as per the results of this study.

APPENDIX A

Sensory score sheet

Sensory Evaluation of Soy-based (Okara) Snack Food Products

Panel Code:
Date:

Please evaluate aroma and color first, and taste, flavor and texture second. Please also make sure to rinse your mouth with water, and/or have crackers between the samples to remove the aftertaste.

[AROMA]

1. Beany-aroma

None ———————————————— Intense

2. Oily -aroma

None ———————————————— Intense

3. Cinnamon-aroma

None ———————————————— Intense

[COLOR]

Light ———————————————— Dark

[TASTE]

Sweetness

Not sweet ———————————————— Very sweet

Saltiness

Not salty ———————————————— Very salty

[FLAVOR]

1. Beany-flavor

Less flavor ———————————————— More flavor

2. Oily-flavor

Less flavor ———————————————— More flavor

3. Cinnamon-flavor

Less flavor ———————————————— More flavor

[Texture]

Crunchiness

None ———————————————— Very crunchy

Comments:

Thank you for your participation!

APPENDIX B

"Your preference today" sheet

Your preference today
I like:
Reasons:

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As last, I would like to pray for the victims in the 1995 Kobe Earthquake (Great Hanshin Earthquake) in Japan as one of the survivors.