ABSTRACT

Title of Document: Texture optimization of soy protein isolate post high-moisture extrusion as an alternative dietary protein source

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Nutrition and Food Science

Soy protein isolate (SPI) has been used as an alternative protein source in texturized meat analogs due to its high protein content and health benefits. Twin-screw high-moisture extrusion was capable of texturing and shaping SPI into fibrous slabs similar to that of cooked skinless chicken breast yet harder and more rubbery due to significant post-extrusion moisture loss. The texture of extruded SPI was further optimized in the present study to reduce hardness and rubberiness. The combination of acetic acid treatment under pH 4.5 at 65°C for 50 min with addition of 0.1% (w/v) mixture of cornstarch and xanthan gum at a 3:2 (w/w) ratio yielded a tender SPI meat analog with desirable color closest to that of cooked skinless chicken breast. A novel vegetarian nugget based on the modified SPI meat analog was formulated and received consumer acceptance superior to commercial counterparts in its texture without detectable soy flavor.

Texture optimization of soy protein isolate post high-moisture extrusion as an alternative dietary protein source

By

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Chapter 1: Introduction

Vegetarian foods occupy a larger-than-ever shelf space in today's market due to the consumers' increasing health concerns (Craig, 2010; Istudor et al., 2010) and environment awareness (Gussow, 1994; Saxe, 2011). This has also led to an increase of vegetarian restaurants and has contributed to continuing market growth (Vegetarian Resource Group, 2009). Early research on soybean composition documented that soybeans can be an alternative protein source in the human diet (Nagata et al., 1998) with accumulating nutritional (Velasquez and Bhathena, 2007) and health benefits (Arjmandi et al., 1996; Azadbakht et al., 2007; Xiao, 2008). In fact, soy protein has long been used as the most popular plant protein source in products such as soy flour and cookies (Hoogenkamp, 2005; Wang et al., 2008; Mohsen et al., 2009). In particular, texturized soy protein was formerly used as a partial meat replacement in dry fermented sausage (Pereira et al., 2010) and co-extruded snack sticks (Hoogenkamp, 2005; Singh et al., 2008; Costa et al., 2010). According to Mintel™ Report (2008), meat and poultry substitutes were accounted for the second-largest market share in vegetarian foods (Fig.1.1).

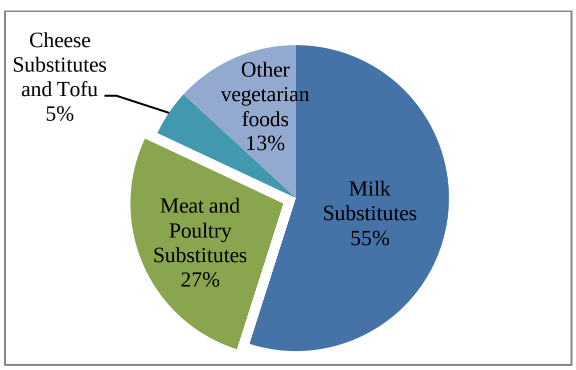


Fig.1.1 Types and U.S. market shares of vegetarian foods (adapted from Mintel, 2008)

Soy protein isolate (SPI), the most refined form of soybean protein extracted from defatted soy flour (DSF), with the highest protein content and good bioavailability compared to soy protein concentrate (SPC) (Terrel et al., 1975; Andrade et al., 2010). SPI has a cholesterol-lowering effect in humans partially due to its isoflavone contents, which also reduce the risk of cancer, cardiovascular diseases, osteoporosis, and menopausal symptoms (Erdman, 2000; Meyer et al., 2002; Hsu et al., 2010; Jiang et al., 2011). The protein structure of SPI can be modified by different factors such as temperature, pH, pressure, and addition of polysaccharides to achieve desirable physicochemical properties (Carp, 2001; Jiang et al., 2010).

Meat analogs made from SPI by twin-screw high moisture extrusions have been shown to exhibit textural attributes closest to cooked chicken breast (Hsieh et al., 2009) and other meat analogs (Chen et al., 2010). The nutritional value as well as protein digestibility of SPI was not significantly changed after extrusion (Hsieh et al., 2009), indicating a quality dietary alternative protein source. However, even after short storage or transportation under refrigeration, the texture of SPI meat analog deteriorates and becomes very chewy and rubbery, rendering the product unacceptable to consumers. Therefore, there is a dire need to further improve the texture of SPI meat analog post-extrusion that could translate to different formats of the product, while maintaining consumer acceptability regarding the texture and flavor.

Chapter 2: Literature Review

2.1 Soy Protein

Soybeans are a major agricultural crop worldwide. With the protein content ranging between 40-50%, which is considered the highest protein content among legumes (Soybean Board, 2011), soybeans represent the principal form of vegetable protein available in the human diet (Nagata et al., 1998). In addition to containing all three of the macronutrients (protein, carbohydrate, and lipid) that are required for good nutrition, soybeans have long been promoted as the only vegetable that contain complete protein (ASAIM, 2007), containing an adequate proportion of all nine of the essential amino acids necessary for the dietary needs of humans or other animals (Brandsch, 2006; Medline Plus Medical Encyclopedia, 2006). Moreover, soy protein is assigned the highest attainable protein score of 1.0, as determined by the internationally accepted Protein Digestibility Corrected Amino Acid Score (PDCAAS) method (Hoogenkamp, 2005). A score of 1.0 indicates that 100% of the essential amino acids required by a 2-5 year-old child can be digested from that protein score. Proteins with a PDCAAS of 1.0 are considered equally high in quality and meet all of the essential amino acid requirements for humans, especially children. Table 2.1 shows the PDCAAS score of some popular sources of protein.

As the most widely used soy, soy protein contains amino acid concentrations similar to those of meat protein, including beef, pork, and turkey, cow's milk, and egg whites (McDonald et al., 2009), and has long been used as an alternative protein source in vegetarian foods (Erdman, 2000). There are two main forms of soy protein that are commercially available: soy protein concentrates (SPC) and SPI. The former is made by removing part of the carbohydrates (soluble sugars) from defatted soy flour and could be in the forms of granules, or spray-dried (Daftary, 1976; Konwinski, 1992; Henk, 2005). The latter, commonly used as a component of various meat or meatless products, is obtained by solublizing and separating protein out of the flakes, followed by precipitation in the isoelectric point (pI) range for β-conglycinin and glycinin (pH 4 to 5) (Hoogenkamp, 2005; Jiang et al., 2010). Table 2.2 outlines the typical basic composition of deffatted soy flour, SPC, and SPI.

Table 2.1. Protein Digestibility Corrected Amino Acid Score (PDCAAS) scores for a variety of protein sources (adapted from Hoogenkamp, 2005)

Protein source	PDCAAS
Soy protein isolate	1.00
Casein	1.00
Egg white	1.00
Beef	0.92
Rolled oats	0.57
Ground nut meal	0.52
Whole wheat	0.40

Table 2.2. Typical composition of defatted soy flour (DSF), soy protein concentrate (SPC) and soy protein isolate (SPI) (adapted from ASAIM, 2007)

Component	DSF (%)	SPC (%)	SPI (%)
Protein	52	65	86
Fiber	16	22	-
Carbohydrates	16	-	-
Moisture, ash, other	16	13	14

A growing body of evidence reveals soy protein to be a highly nutritive material with the potential to promote health and mitigate certain human disease factors (Erdman, 2000; Horiuchi et al., 2000; Torre-Villalvazo et al., 2008; Moon et al., 2011). For instance, soy protein, along with the products derived from it, has been identified as a cholesterol-lowering food (Fukui et al., 1993; Pipe, 2009; ASAIM, 2007). Cholesterol, at elevated levels, has been conclusively linked with cardiovascular disease, a major cause of illness and death in the Western Hemisphere (NHF, 2001), and is often associated with the dietary intake of foods of animal origin such as meat, eggs, and milk (Anderson, 1995). While direct evidence of soy proteins' effects on cardiovascular heart disease remains debatable, however, some researchers are convinced that consumption of soy protein could reduce the risk of developing cardiovascular diseases (Kurowska et al., 1995; Azadbakht et al., 2008).

2.1.1 Soy Protein Isolate (SPI)

SPI has been used to enrich the protein content of food products, including those for the school lunch program, and can also be used as a replacement for milk protein (Hoogenkamp, 2005; Tuker et al., 2010). SPI, the most refined form of soy protein, is obtained by centrifugation after extraction from the defatted soy flour (Sorgentini, 1995) and retains all the aforementioned health benefits (Barbosa et al., 2006; MacDonald et al., 2009). In addition, research that involved feeding rats a diet containing SPI indicated higher bone mineral density than a regular diet (Chen et al., 2008; Evans et al., 2007). Nearly all of the fat, fiber and soluble carbohydrates have been removed and due to the extraction process and its high nutritive value, SPI is a premium ingredient (Hoogenkamp, 2005).

SPI contains two major functional components, glycinin (11S) and β-conglycinin (7S globulins) (Sorgentini et al., 1995). These two components are highly linked to the structure of SPI due to the subunits of 11S consisting of two polypeptide components linked via disulfide bonds (AB); one disulfide bond has acidic (A) and the other has basic (B) isoelectric points (Badley, 1975; Bainy et al., 2008; Jiang et al., 2011). Research has demonstrated that alkali will cause glycinin to disassociate, inducing subsequent unfolding as a result of disulfide bond cleavage (Kensella et al., 1979; Jiang et al., 2011). In addition, heat-denaturation of soy proteins greatly modifies the structure of glycinin (Jiang et al., 2010; Shi and Sun, 2011). Upon heating, the disulfide bonds linking the acidic-basic subunits of 11S globulin are cleaved, thus separating the polypeptides, hence, all of the subunits may dissociate and re-associate in different ways (Kensella et al., 1985; Nik et al., 2009). The 7S globulin is a trimetric glycoprotein, which consists of three types of subunits: α, α' and β (Carp, 2001). The rest of the soy protein isolate consists of γ-conglycinin, basic 7S globulins, lipoxygenase, agglutinins, and β-amylases (Petruccelli, 1995). Figure 2.1. gives a simplified flow chart of the processing scheme commonly used to obtain SPI.

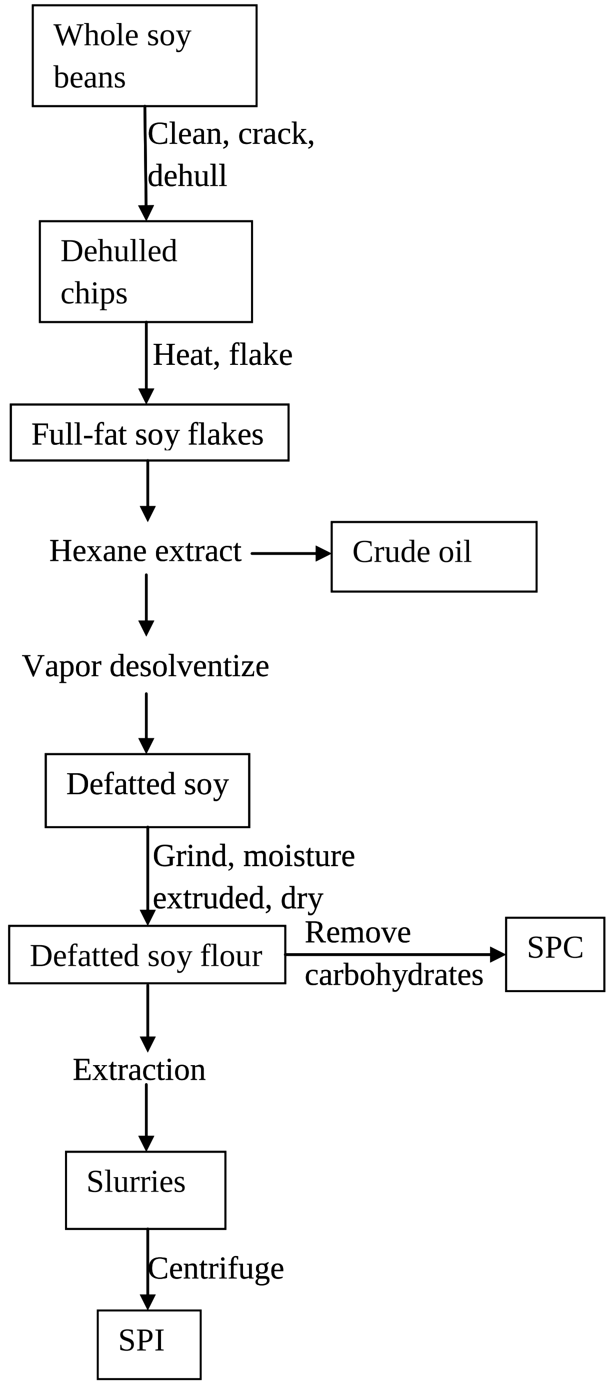


Fig. 2.1. Production procedure for making soy protein isolate (SPI) and soy protein concentrate (SPC) from whole soybeans (adapted from Hoogenkamp, 2005)

2.1.2 Factors affecting SPI properties

Numerous amounts of research has been conducted to evaluate the effects of various factors such as pressure, pH, temperature, polysaccharides, and salts that affect the properties of SPI (Table 2.3) (Hermansson,1986; Carp, 1998; Kim, 2004; Puppo, 2005; Jaramillo, 2011). The emulsifying capabilities of 7S and 11S could be significantly improved by pressure, both pH and polysaccharides can increase the solubility of the SPI (Carp, 1998; Kim, 2004), in addition, polysaccharides can also increase the stability of SPI (Ye, 2008), and finally, salt can cause the denaturation temperature to increase by approximately 8 ºC (Braga, 2006).

2.1.3 Texturized SPI

Texturized SPI, usually obtained by extrusion, has been used as a meat replacement in dry fermented sausage and co-extruded snack sticks (Hoogenkamp, 2005; Qammar et al., 2010); it is also a major component in fabricating the structure of meat analog (Rareunrom et al., 2007), and is considered to be the major type of texturized plant protein (TPP). The extrusion of texturization isolate does not appear to reduce the nutrient content of human diets, compared with that of non-extruded soy protein isolates (Hsieh et al., 2009). Research has demonstrated that the extruded soy protein isolate yields a similar growth rate in the weight of rats within a certain period of time to that obtained from commercial soy protein isolates, indicating that the process does not significantly alter the overall digestibility of the soy isolate (Hsieh et al., 2009).

Table 2.3. Various processing parameters affecting the physicochemical properties of soy protein isolate (SPI)

Parameters	**Effects**	**References**
Pressure	• Significantly improves the emulsifying activity of 7S and 11S at 400 and 200 MPa • In terms of structural properties, increased pressure levels decrease the α-helix content and increase the random coil content • Significantly increases aggregate formation (combined with insoluble and soluble aggregates) to a similar extent between pressure levels of 200-600 MPa • Extends the molecular structure of soluble aggregate formation above 400 MPa	Puppo, 2005 Tang, 2009
Temperature ↑	• Increases the dispersibility, corresponding to an increases in hydrophobicity • Increases storage modulus and hardness of glucono-δ-lactone induced gel with soy protein • Heat-denature helps modify the structure of glycinin	Hermansson, 1986 Kim, 2004
pH ↑	• Significantly increases solubility	Jaramillo, 2011
Polysaccharides ↑	• Improves solubility • Increases stability	Ye, 2008 Carp, 1998
Salt ↑	• Increases denaturation temperature by about 8ºC	Braga, 2006

Additionally, general sensory evaluation of foods indicated that the moisture content of the product is a significant factor in relation to "toughness," "chewiness," "springiness," and "mushiness" (Lin et al., 2002). According to this study, products with a more orderly directional structure possess a higher degree of hardness or chewiness (Lin et al., 2002). As chewiness and hardness remain the largest textural hurdles in consumer acceptance, particularly in Western-style diets, it is critical that the texture of the extruded SPI be further modified in order to expand the market for these products, enabling more consumers to enjoy the nutritional quality of extruded/texturized SPI.

2.2 Extrusion and Extruders

A thermo-mechanical operation providing continuous mixing, kneading, and shaping (Akdogan et al., 1999), extrusion cooking involves three key steps: (1) the raw material is fed into a hopper and gradually mixed (mixing); (2) the mixture is forced to flow through the passage between a rotating screw and a stationary barrel, usually steam-heated (kneading); and (3) the well-mixed ingredients are pressurized against the end of the barrel and exit via a small outlet called die (Sebastian et al., 1991; Riaz, 2000). It is the combination of all three steps that determines the physical attributes of the final product (Akdogan et al., 1996; Sun et al., 2011). Four types of commonly used extruders include: single-screw wet extruders, single-screw dry extruders, single-screw interrupted-flight extruders, and twin-screw extruders. Table 2.4 highlights the different characteristics with the pros and cons of each type of extruders currently available.

Among those extrusions mentioned before, regular and high moisture extrusion, different in the moisture content during the process, are widely used among industries (Akdogan et al., 1999). In fact, extrusion has long been used to fabricate meat-like texture and plexilamellar structure using plant protein (Burgess and Stanley, 1976).

Table 2.4. Comparison of the key characteristics of different extruders (adapted from Riaz, 2000)

Extruders	Features	Advantages	Disadvantages	Applications
Single-screw wet extruder	Live bin Feeding single screw Preconditioning cylinder Extruder barrel Die Knife	Easy operation Less training required Low cost Higher capital investment	Poor mixing ability Not self-cleaning enough Limitation on the size, species of raw ingredients	Precooked or thermally modified starches
Single-screw dry extruder	Live bin Feeding single screw Preconditioning cylinder Screw segments Steam-locks Extruder barrel Die Knife	Relatively low capital investment Can be adjusted to fit all types and sizes of installations Less training required	High power requirement Limitation on sizes of final products Functions poorly with ingredients with high fat content and highly viscous materials Initial moisture content is important	Recycling wet waste from food and animal by-products

Table 2.4. Characteristics of different extruders (Riaz, 2000) (cont.)

Single-screw interrupted-flight extruder	Feeding zone Rotating worm shaft Single screw extruder barrel Die Knife	Relatively less expensive Easy to operate Easily replaceable A wide variety of preconditioners can be adapted Lower power requirement High shear, turbulent mixing action can knead solid formulation	Limitation on heating in the barrel Limitation on maximum barrel temperature (150°C) Less versatile Difficult to control processing conditions	Oilseed preparation for solvent extraction
Twin-screw extruder	Live bin Feeding screw Preconditioning cylinder Extruder barrel Jacketed heads Rotating screw Die Knife	Uniformly-shaped products Higher internal fat content ingredients can be accepted (up to 18%) Variety in the range of raw materials that can be included: oily, sticky, or wet Wide range of sizes of the materials	More expensive and higher cost to maintain Relatively complicated to operate	Ravioli; meat analog; spaghetti

Nevertheless, high moisture extrusion is gaining popularity due to the fact that the products obtained often have a more tenderized texture compared to other types of extrusion and the moisture of the product is easy to control (Akdogan et al., 1999; Ranasinghesagara et al., 2006; Singh et al., 2007; Sun et al., 2011).

2.2.1 Effects of extrusion

During the extrusion process, the control of operating parameters, including prior processing history of feed materials, material feed rate, screw speed and configuration, barrel temperature, and die configuration, has a critical effect on the physical properties and chemical characteristics of the final product (Lin et al., 2000; Chevanan et al., 2008; Wei et al., 2009), which can directly or indirectly impact the final product's nutritional quality (Table 2.5). For instance, due to the high barrel temperature, most vitamins are destroyed, whereas the mineral content of final products may be increased as a result of the abrasion of the interior of the extruder barrel and screws by certain types of food materials.

Moreover, the texture of meat analog made from texturized SPI via extrusion is found to depend upon such processing parameters as moisture content and cooking temperature (Singh et al., 2007). The higher the moisture content, the lower the viscosity the product (Lin et al., 2002). On the other hand, reduction in the moisture content can cause the texturized soy product to become more directionally aligned, thus yielding a product showing similar texture to that of skinless chicken breast (Ranasinghesagara et al., 2006). However, increase in product hardness becomes

Table 2.5. Effects of extrusion process on nutrients contents

Nutrients	Nutrition Effects	Reference
Carbohydrates	Starch increases the rate of gelatinization at much lower moisture levels (12-22%) Branches on amylopectin molecules sheared off	Jin, 1994
Protein and amino acids	The digestibility of protein is improved from the enzyme-access sites	Camire, 2001
Lipids	Products will have lower lipid levels, and the recovery of lipids is improved	Camire, 2001
Dietary fiber	Total fiber values will remain balanced due to the increase in soluble fiber and the decrease in insoluble fiber	Camire, 2001
Vitamins	Most of the vitamins will be destroyed, aside from Vitamins D, K, and B_2	Camire, 2001 Andersson,1990
Minerals/Metals	The mineral/metal content (including possibly hazardous metal fragments) of the final products may increase	Camire, 1993

evident after frozen storage and consequently renders the product unpalatable. In order to enhance the quality of the product, it is critical that the hardness be reduced without giving off any soy (beany) flavor.

2.3 Polysaccharides

2.3.1 Xanthan Gum

Xanthan gum, a polysaccharide derived from the bacterial coat of *Xanthomonas campestris*, has served as an important commercial microbial polysaccharide (Katzbauer, 1997). This polymer consists of a linear (1-4)-β-D glucose backbone with a negatively charged trisaccharide side chain on each second glucose residue (Braga, 2006).

Xanthan gum is soluble in both cold and hot water, and its thermal stability against hydrolysis is generally superior to many other water-soluble polysaccharides or polymers (Stokke, 1996). It is also stable over a broad range of pH values, not only on account of its stability but also due to its shear-thinning behavior, also referred to as pseudoplasticity (Katzbauer, 1997). This stability can be explained by the fact that the conformational status of the polymer molecules contained in xanthan gum is stabilized by hydrogen bongs (Cuvelier, 1986). Pseudoplasiticy enhances certain sensory qualities (flavor release, mouth feel) in food products (Katzbauer, 1997).

Due to its stability, xanthan gum has been utilized in a variety of different industrial applications (Katzbauer, 1997). Table 2.6 summarizes its different applications, including usage as a food additive in order to stabilize and thicken food products (Jansson and Kenne, 1975). The Food and Drug Administration (FDA) has approved the use of xanthan gum without any specific quantity limitations, as xanthan is non-toxic and does not inhibit growth (Kennedy and Bradshaw, 1984). In addition, xanthan gum is non-sensitizing and does not cause skin or eye irritation (Garcia-Ochoa, 2000).

2.3.2 Starch

As a carbohydrate that occurs in granular form in the organs of plants, starch is derived from the Anglo-Saxon starch and connotes strength or stiffness (Swinkels, 1985). Microscopy reveals starch to be composed of tiny, white granules, ranging from about 2 to 100 μm in diameter. Starch is derived from many commercial sources, including cereal grains (corn, wheat, sorghum, and rice), tubers (potato), roots (tapioca, sweet potato, and arrowroot), and the pith of the sago palm (Swinkels, 1985), with major starches and their properties summarized in Tables 2.7 and 2.8 (Beyum et al., 1985). Starch can be considered to be a condensation of the glucose polymer, of which two types exist: a linear-chain molecule termed amylose and a branched polymer of glucose termed amylopectin (Beynum, 1985).

Cornstarch, which is obtained from the wet milling of corn, makes up the largest portion of the market for starch-based products in the U.S. (Long, 1985). Furthermore, it is proposed that it possesses various health benefits, such as reducing the glycemic index, preventing coronary heart disease and certain cancers, as well as functioning as a prebiotic (Zhu, 2010).

Table 2.6. Applications and functionalities of xanthan gum (adapted from Katzbauer, 1997)

Applications	**Examples**	**Concentrations (% w/w)**	**Functionalities**
Food	Salad dressing	0.1-0.5	Emulsion stabilizer Suspending agent Dispersant
	Bakery products	0.1-0.4	Improve the cohesion of starch granules Contribute to the structure Increase shelf-life Facilitate pumping Control the battery rheology
	Frozen foods	0.05-0.2	Increase the freeze-thaw cycles
	Syrup, toppings, relishes, sauces	0.05-0.2	Thickener Heat stability and uniform viscosity Combination with other hydrocolloids
Cosmetics and pharmaceutical	Toothpaste	N/A	Easy extrusion from the tube or pump dispenser Keep a stable stand on the brush Improve the dispersion on and rinsing from the teeth Improve the thickness Give a bright, shiny cord with short flow behavior
	Creams, eye couture gels	0.2-1	Thickener and stabilizer Feel gentle and soft
Agriculture	Feed and pesticide	0.03-0.4	Suspension stabilizer Improve spreadability Reduce drift Increase cling and permanence
Petroleum production	N/A	0.1-0.4	Reduce the lubricant or friction

Table 2.7. Physical properties of different starches (adapted from Beyum, 1985)

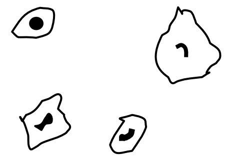
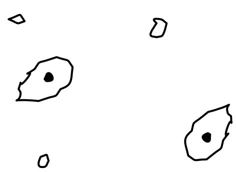
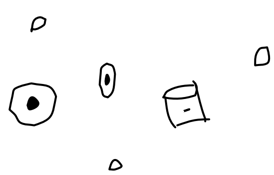
Name	Source	Microscope appearance (illustration of the shapes)	Raw material composition (% by weight)
Cornstarch	Corn		Starch: 60 Starch on dry substance: 71 Moisture: 16 Protein (N×6.25): 9 Fat: 4 Fiber: 2
Potato starch	Potato		Starch: 18 Starch on dry substance: 82 Moisture: 78 Protein (N×6.25): 2 Fat: 0.1 Fiber: 0.7
Wheat starch	Wheat		Starch: 64 Starch on dry substance: 74 Moisture: 14 Protein (N×6.25): 13 Fat: 2 Fiber: 3
Tapioca starch	Tapioca		Starch: 26 Starch on dry substance: 77 Moisture: 66 Protein (N×6.25): 1 Fat: 0.3 Fiber: 1

Table 2.8. Physical and chemical properties of variable starch granules (adapted from Beyum,1985)

Property		**Corn starch**	**Potato starch**	**Wheat starch**	**Tapioca starch**
Physical attributes	Type	Cereal	Tuber	Cereal	Root
	Size range (diameter, μm):	3-26	5-100	2-350	4-35
	Shape:	Round, polygonal	Oval, spherical	Round, lenticular	Oval, truncated
Chemical composition, dry basis* (%, w/w)	Moisture at 20°C	13	19	14	13
	Lipids	0.6	0.05	0.8	0.1
	Protein (N×6.25)	0.35	0.06	0.4	0.1
	Ash	0.1	0.4	0.15	0.2
	Phosphorus	0.015	0.08	0.06	0.01

*except for moisture content.

Chapter 3: Hypothesis and Objectives

3.1 Hypothesis

It was hypothesized that the physicochemical properties of high-moisture-extruded SPI meat analog can be modified by a combination of pH, temperature, and polysaccharides to achieve a product with desirable texture and flavor.

3.2 Research objectives

The ultimate goal of this project was to improve the texture of post-extruded soy protein isolate meat analog that could be further formulated into a novel product to expand its applicability. In order to fulfill this goal, there were three specific objectives:

1. To characterize the physical and textural attributes of the extruded SPI after frozen storage as the baseline for improvement.
2. To modify and optimize the texture of SPI meat analog against cooked skinless chicken breast.
3. To formulate a novel nugget product utilizing modified SPI meat analog and assess its acceptability organoleptically.

Chapter 4: Materials and Methods

4.1 Materials

SPI meat analog samples post high-moisture twin-screw extrusions were provided by j-Green Nature Foods Co. (Cumberland, MD) under an exclusive license agreement with Dr. Fu-hung Hsieh at the University of Missouri after two-week's storage in the freezer. For comparison purpose, 100% Nature Fresh Chicken (Giant Food, Landover, MD) skinless chicken breast was employed. The texture of the SPI meat analog was optimized using acetic acid, xanthan gum, and cornstarch. Commercial vinegar (Richfood, Spokane, WA) was used as a source of acetic acid to rehydrate the SPI meat analog; it was chosen not only because of its acidity but also because of its ability to mask soy flavor. Ticaxan® xanthan gum and food grade cornstarch were supplied respectively by TIC gums (Belcamp, MD) and Hodgson Mill (Effingham, IL). Both xanthan gum and cornstarch were added to act as stabilizers for SPI. Cornstarch was chosen not only because of its stability but also because of its sheer lack of fat and sodium. The novel vegetarian chicken nugget product, M nuggets, was formulated using the modified SPI meat analog. Potato starch flour (ENTERG), white pepper (McCormick), onion salt (McCormick) and corn flake crumbs (Kellogg's) were used to complete the recipe.

4.2 Methods

4.2.1 Analysis of SPI meat analog

Moisture content

The moisture content of the soy protein isolate meat analog was determined by the AOAC oven method at 105 ℃ (AOAC 984.25).

Crude protein content

The crude protein content of the SPI meat analog was determined using the Nitrogen Analyzer Series II (PerkinElmer Precisely, Waltham, MA). A 120 mg sample was ground and prepared for each run. The crude protein content was calculated by the equation below utilizing the proper conversion factor for soy protein (Mosse, 1990).

$$Crude\ protein\ content\% = 5.65 \times nitrogen\ content \times 100\%$$

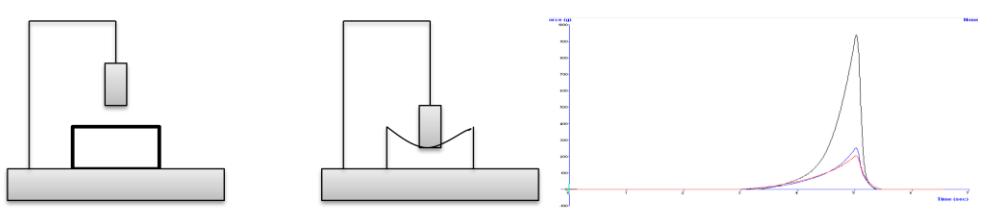
The nitrogen content was read directly from the nitrogen analyzer.

Color evaluation

The color of the SPI meat analog was measured using a ColorFlex colorimeter (Hunter Lab). The spectral data L^* (lightness), a^* (redness or greenness), and b^* (yellowness or blueness) were recorded. The digital images in the research were recorded onto a SmartMedia memory card in a Canon camera (G11). This method was also applied to the modified SPI meat analog during the formulation when determining the color.

Texture analysis

The hardness of the SPI meat analog was determined using a TA-XT2i (TA Instrument, Robbinsville, NJ) equipped with a 25-kg load cell and a 38mm diameter test probe. The samples were cut into 4 cm × 4 cm × 2 cm (L×W×H) blocks averaging 5 grams in weight and compressed to half of the original height at a test speed of 3 mm/s. Data was recorded at the max force, in grams, and the peak occurred when the probe depressed the sample by 10 mm, reached the middle of the meat analog and went back to the original stage. Figure 4.1 illustrates the process in which the data is calculated and the output returned. This method was also applied to determine the hardness of cooked chicken breast and modified SPI meat analog in order to compare cooked chicken breast meat with SPI meat analogs.



a. b. c.

Fig. 4.1. Illustration of the measurements of product hardness using a texture analyzer: a). Side view of an analyzer with probe; b). The probe start to press the sample to the middle of the block and returns to get the maximum force (hardness); c). Texture profile captured after the analysis

4.2.2 Formulation for modified meat analog

Temperature optimization

The SPI meat analog was prepared into blocks with a size 4 cm ×4 cm × 2 cm (L×W×H) averaging 5 gram and rehydrated in warm water with controlled temperature (55°C, 65°C, 75°C, and 85°C), and the mass was recorded every 10 min until it became constant to obtain the maximum moisture content. Afterwards, the water absorption capacity (WAC) was recorded as gram of water retained per gram of dried meat analog using the following equation:

$$WAC = [\frac{W_2 - W_1}{W_1}] \times 100\%$$

where W_1 and W_2 are the weights of the sample before and after rehydration, respectively.

As mentioned before, the texture of the samples was analyzed using the texture analyzer to assess the hardness of the meat analog.

pH optimization

The SPI meat analog blocks 4 cm × 4 cm × 2 cm (L×W×H) were rehydrated in water at different pH values, 3.5, 4.5, 5.5, and 7.0, at 65°C for 50 min. The pH values were carefully adjusted by gradual addition of vinegar into water. The temperature and time were chosen according to the temperature and time optimization study, which showed that the SPI meat analog yielded the least max force in texture analysis, corresponding to the hardness of the meat analog. The texture was analyzed afterward using the texture analyzer.

Effects of polysaccharides

The SPI meat analog blocks 4 cm × 4 cm × 2 cm (L×W×H) were rehydrated in a cornstarch and xanthan gum solution comprising of 0.1% of the total volume of the solution. The cornstarch to xanthan gum ratio (w/w) was optimized at 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0. In order to fine tune the effect of pH, the texture of the samples was measured with pH value controlled on a 0.5 interval from 3.0 to 7.0. Samples of 5 gram SPI meat analog were ground and placed on an aluminum foil plate, and the moisture loss was determined by measuring the mass every 10 min at both refrigerator temperature (4℃) and room temperature (22℃), respectively, until the mass became constant. The temperatures were chosen to mimic the way in which consumers handle the product.

Texture stability

To evaluate the texture stability against the optimized storage temperature and treatments, SPI meat analog blocks 4 cm × 4 cm × 2 cm (L×W×H) were treated for 50 min in a solution of pH 4.5. The total of 0.1% (w/v) polysaccharide mixture was then added at the cornstarch to xanthan gum ratio (w/w) of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0. The texture was measured after 24 hrs in storage at 4℃.

Scanning electron microscope (SEM)

SEM imaging was performed using a Hitachi SU-70 scanning electron microscope (Hitachi high technologies America Inc, Roslyn Heights, NY) to provide visual confirmation of the microstructures of the samples. Rehydrated samples (treated in acetic solution with polysaccharides) were cut into small pieces (0.2 cm thick, 0.4 cm wide and 0.6 cm high). The samples were then freeze-dried at -110°C for 72 hrs in a freeze drier (Thermo Savant). Each sample was fixed on an aluminum stage of 1 in. diameter with the cut side facing up. All samples were coated in gold. The SEM was operated at 3 keV of back scatter electron (BSE)/secondary electron (SE).

4.2.3 Formulation of novel product (M nuggets)

The SPI meat analogs were treated under the optimal condition determined in section 4.2.2 and ground in a food processor (Cuisinart, East Windsor, NJ). The ground product was incorporated into the recipe outlined in Table 4.1.

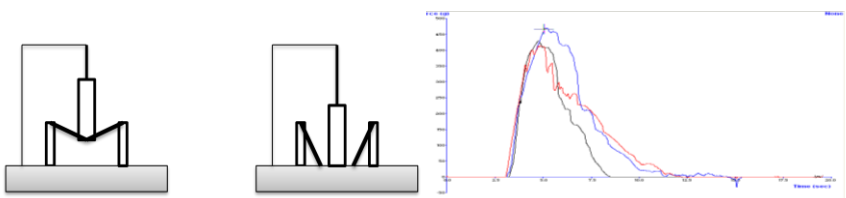
Table 4.1. Formulation of the novel product M nugget

Ingredients	**Quantity**
Ground meat analog	100 g
Potato starch flour	1 tablespoon
White pepper	1/6 teaspoon
Onion salt	½ teaspoon

4.2.4 Analysis of M nuggets

Texture analysis

Each chicken nugget (25 g at 3 cm L × 10 cm W × 2 cm H) made of modified SPI meat analog was measured for firmness and toughness using the TA-TX2i texture analyzer equipped with a 25-kg load cell and a cutting board probe. Data was recorded as max force in grams that occurred when the cutting board probe broke down the nuggets. Fig.4.2 illustrates the process during the evaluation. Commercial chicken nuggets (Banquet®) and vegan chicken-free nuggets (Wealth & health®) were chosen for comparison.



a. b. c.

Fig. 4.2. Illustration of the measurements of product hardness using a texture analyzer: a). Side view of the texture analyzer with the probe; b). The probe breaking the sample; c). Texture profile captured throughout the analysis

Sensory evaluation

The organoleptic quality of the nugget products, including off (soy) flavor, chicken flavor, chewiness, and toughness, along with consumer acceptance were measured by a panel of 29 self-reported consumers recruited from the university population through email announcements. Additional to the M nuggets developed in the present study, commercial chicken nuggets (Banquet®), which won the Chefbest award for the best taste (Chefbest.com, 2009), and vegetarian chicken nuggets (Wealth & health vegan nuggets chicken free) were employed for comparison. The sensory analysis protocols received approval by the Institutional Review Board (IRB) at the University of Maryland (College Park, MD). The approved form can be found in Appendix A.

Prior to tasting, participants were asked a series of questions to gather demographic data including gender and age. All samples were labeled with random 3-digit codes. An ordinal-type rating scale, where 5 = extremely and 1 = none, was employed for off flavor, chicken flavor, chewiness, and toughness evaluations. Consumers scored all products for overall acceptability on a 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely. Consumers were provided with saltine crackers and ambient temperature drinking water for palate cleansing between samples. The IRB approved sensory evaluation sheet could be found in Appendix B.

<u>Nutrition label</u>

The nutrition label of M nuggets was obtained using Genesis R&D SQL (Genesis 9.7, esha Research, Salem, OR).

4.2.5 Statistical analysis

The data was analyzed using analysis of variance (ANOVA) by SAS (9.2.2 version, SAS, Cary, NC), followed by the Dunnett's test when comparing with the control or the Tukey's test when comparing amongst samples. The complete statistical analysis can be found in Appendix C.

Chapter 5: Results and Discussion

5.1 Properties of the SPI meat analog

The high-moisture extruded SPI meat analog without further modification was found to contain 70.75% moisture, slightly less than the 78.55% moisture content reported in cooked chicken breast (Murphy, 1998). The 25.61% protein in the sample was also higher than the 20.40% protein in cooked chicken breast, indicating that the meat analog could be considered a high-protein source (CFR). The dark, yellowish color with a red tone (L*55.38; a*3.62; b*17.42) of SPI meat analog was shades darker than cooked chicken breast (L* 64.87; a* 0.37; b* 14.37), hence could not be considered a desirable color when mimicking cooked chicken breast. The hardness of the meat analog, which was measured by a texture analyzer and reported as the max force registered when pressing the sample till it reached half the distance of the sample thickness, was found to be 1834.9 g, which was significantly different ($P>0.05$) from that of cooked chicken breast (432.6 g).

5.2 Formulation of modified SPI meat analog

5.2.1 Temperature

While cooking SPI meat analog under 75 and 85°C gave a plateaued moisture content after 40 min (Figure 5.1), the WAC was also found to increase with increasing temperature (Table 5.1), which means the higher the cooking temperature, the juicier the SPI meat analog, since WAC is critical in determining product texture after rehydration (Ning and Vilota 1994). However, both 75 and 85°C were considered unacceptable because most proteins got denatured at temperature above 70°C (Braga, 2006). At 65°C, the meat analog reached its maximum moisture content after 50 min, which was shorter than the 60 min under 55°C (Figure 5.1). Interestingly, the lowest hardness (500.9 g) of the SPI meat analog was found at 65°C (Table 5.1), which yielded the most tender meat analog among all temperatures studied. Ning (1994) reported that the WAC of a product is mainly dependent upon the size of the air cells in the samples, and higher cooking temperatures expanded those cells and created more open spaces in the product's structure, consequently lowering the hardness. However, the increased hardness at temperatures above 70°C could be attributed to protein denaturation, most likely the changes of glycinin structure due to overheating (Hermansson, 1986; Kim, 2004).

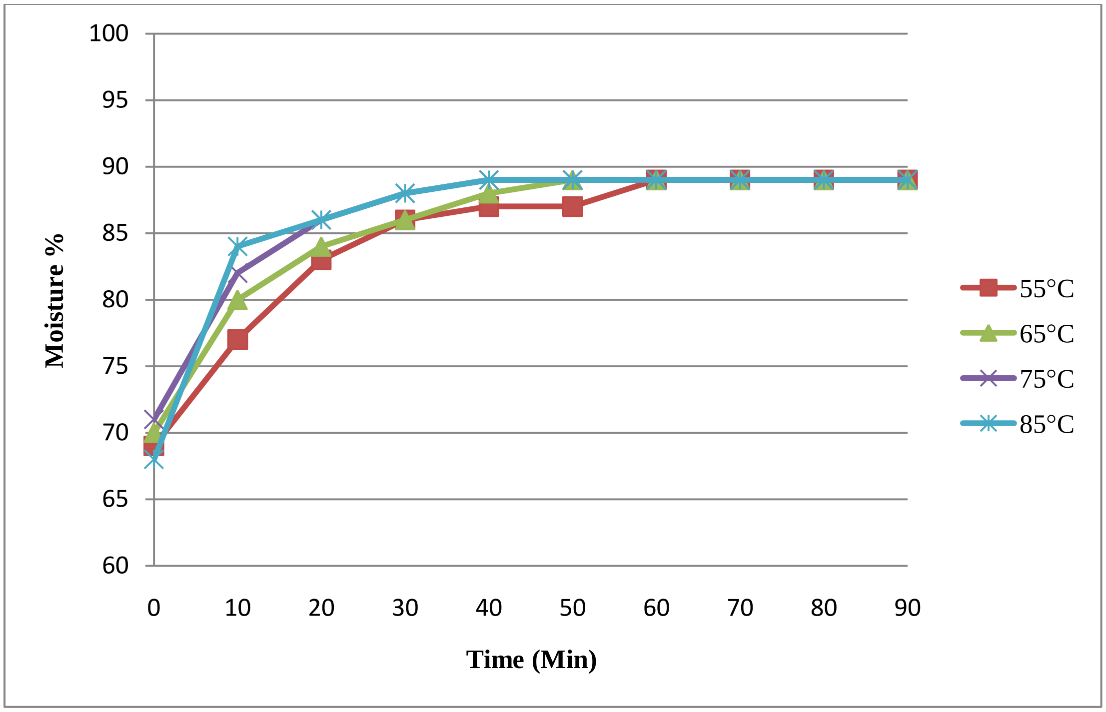


Fig.5.1. Moisture absorption time profile of soy protein isolate (SPI) meat analog treated at different temperatures; n=3

Table 5.1. The water absorption capacity (WAC) of soy protein isolates (SPI) meat analog and the resulting hardness of the product treated with different temperatures

Temperature (°C)	WAC[1] (%)	Hardness[2] (g)
55	165[a]	782.9±8.5
65	171[a]	500.9±6.5
75	179[b]	669.8±12.4
85	188[b]	716.4±26.9

[1] Values in the same column followed by the same superscript were not significantly different by Tukey's test ($P<0.05$)

[2] Values are means ± SD, n=3

5.2.2 pH

The hardness of the SPI meat analog was found to be dependent on pH. At pH 4.5 the lightest maximal resistance force (364.0 g) of the sample was received compared with those treated by other pH conditions (Table 5.2), indicating that, in order to produce the desirable texture, pH 4.5 should be employed to reduce the hardness of SPI meat analog.

Table 5.2. The effect of pH on the hardness of soy protein isolate (SPI) meat analog; Specific pH value was achieved by varying the vinegar-to-water ratio

pH value	Vinegar : water	Hardness[1] (g)
3.5	1 : 92	465.8±14.4
4.5	1 : 455	364.0±8.5
5.5	1 : 1897	452.6±16.4
7.0	Water only	532.1±23.6

[1] Values are means ± SD, n=3

It is known that a certain pH value could raise the solubility of SPI. Since the isoelectric point (pI) of soy protein is between pH 4 and 5 (Jaramillo, 2011; Henk, 2005), it is postulated that at pH 4.5 more SPI became dissolved, hence reduced the hardness of the product. A pH value away from the pI could thus increase the hardness of the product.

5.2.3 Polysaccharides

The effect of polysaccharides, namely cornstarch and xanthan, on the hardness of SPI meat analog was investigated under different pH conditions, which were adjusted by mixing vinegar with water following the ratios indicated in Table 5.3. In general, polysaccharides increased the hardness of the SPI meat analogs compared with the control (Fig. 5.2). However, a pH of 4.5 still yielded the least hardness of the product, even with the polysaccharides mixture. This could be attributed to the fact that pH 4.5 is the closest to the pI of SPI. Addition of cornstarch and xanthan in general could help the formation of gel-like structure (Beynum, 1985), which in turn increased the hardness of the SPI meat analog. The ratio between cornstarch and xanthan, however, did not exhibit specific trend towards product hardness. Nevertheless, the ratio of 2:3 (cornstarch:xanthan) offered the least hardness, whereas 3:2 produced similar product hardness when pH was controlled at 4.5.

Table 5.3. Specific pH values correspondent to the vinegar-to-water ratios in the solution

pH value	Vinegar : water
3.0	1 : 20
3.5	1 : 92
4.0	1 : 235
4.5	1 : 455
5.0	1 : 1056
5.5	1 : 1897
6.0	1 : 2032
6.5	1 : 2647
7.0	Water only

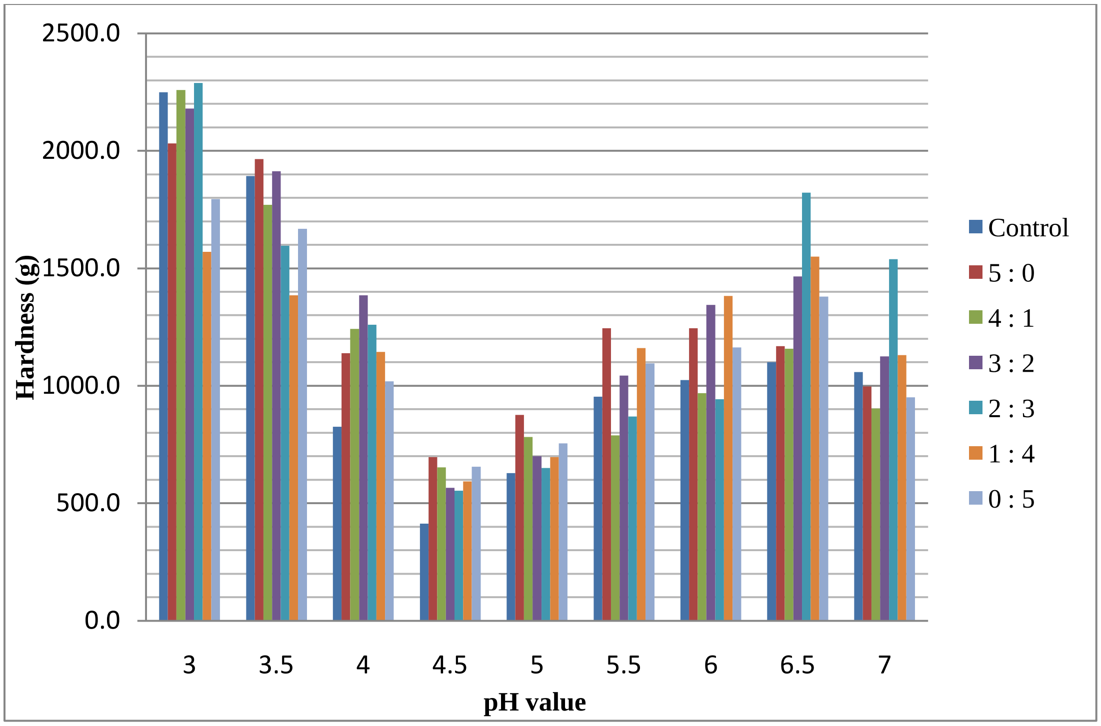


Fig.5.2. The hardness of soy protein isolate (SPI) meat analog treated under different pH values with a different cornstarch-to-xanthan ratio (w/w). Ratio 5 to 0 indicates cornstarch only, and the ratio shifted by continuingly decreasing cornstarch while increasing xanthan until 0 to 5 ratio was reached, which indicates xanthan gum only. The control represents the samples received neither cornstarch nor xanthan gum treatment; n=3

To simulate the temperatures the SPI meat analog could be stored at, the product moisture loss under two temperatures (4 and 22°C) were studied. It was found that samples stored at 4°C retained moisture better than at 22°C (Fig. 5.3). Additionally, the cornstarch: xanthan ratio of 3:2 appeared to assist in moisture retention as seen by the least moisture loss at both temperatures. This finding was promising, as moisture retention is the key to a tender and juicy product that consumers desire for.

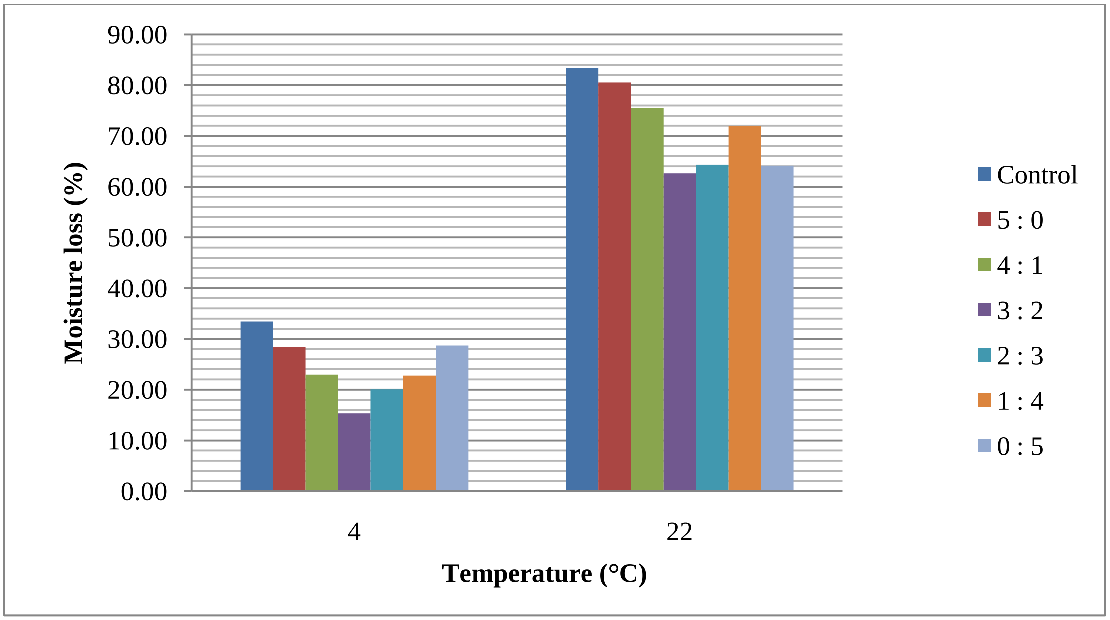


Fig.5.3. Moisture loss of soy protein isolate (SPI) meat analog under two different temperatures up to 24 hrs. Ratio 5 to 0 indicates cornstarch only, and the ratio shifted by continuingly decreasing cornstarch while increasing xanthan until 0 to 5 ratio was reached, which indicates xanthan gum only. The control represents the samples received neither cornstarch nor xanthan gum treatment; n=3

5.2.4 Texture stability

Since 4°C was found the ideal storage temperature for SPI meat analog regarding minimal moisture loss, the stability of the product under 4°C was further studied. As seen in Fig. 5.4, addition of cornstarch and xanthan gum slightly raised the hardness of the meat analog upon treatment; however, they did slow down the rate that SPI meat analog turned rubbery, indicating an extension of texture stability. In fact, the control, which was SPI meat analog treated with acetic acid but without polysaccharides, reached the hardness level similar to that of meat analog after 24 hrs. The retardation of texture hardening in this case could be attributed to the possible formation of a gel layer outside the meat analog, which served as a moisture barrier that prevented the moisture from escaping the meat analog during storage.

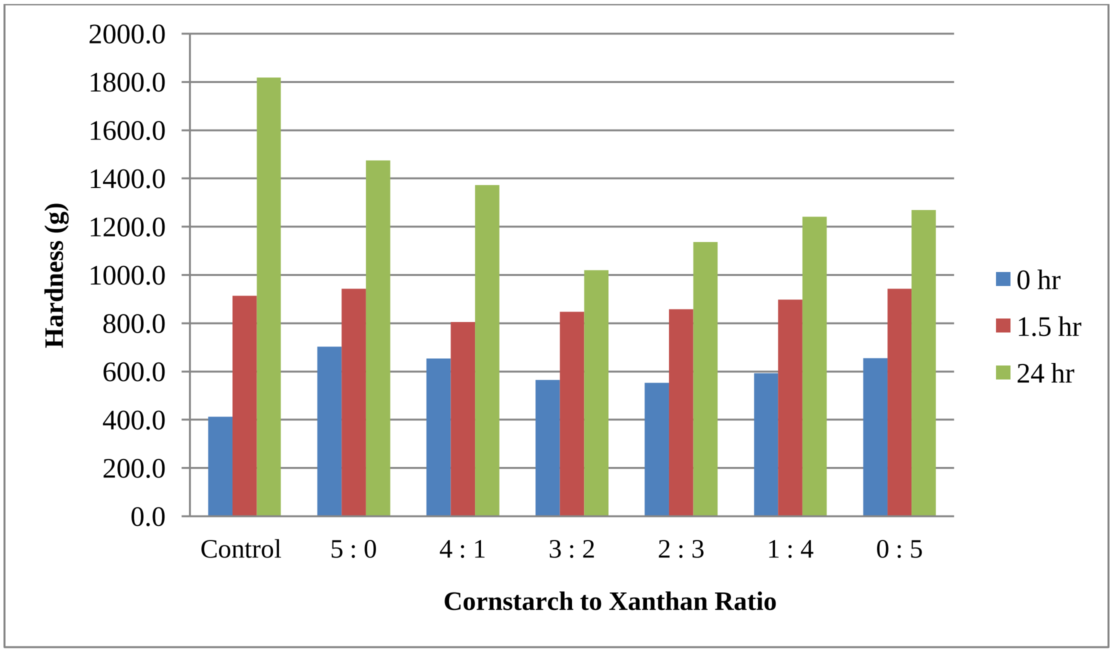


Fig.5.4. The effect of storage time (up to 24 hrs at 4°C) on the hardness of soy protein isolate (SPI) meat analog treated with polysaccharide solutions at different cornstarch-to-xanthan ratios (w/w). Ratio 5 to 0 indicates cornstarch only, and the ratio shifted by continuingly decreasing cornstarch while increasing xanthan until 0 to 5 ratio was reached, which indicates xanthan gum only. The control represents the samples received neither cornstarch nor xanthan gum treatment; 0hr means newly made modified SPI analog; n=3

5.2.5 Color evaluation

Just as different temperatures changed the rates of moisture loss, they also changed the color (Fig. 5.5). Significant changes in the color of the treated SPI meat analog occurred at different temperatures (P>0.05, Table 5.4). Moreover, addition of the cornstarch/xanthan mixture decreased the a* value significantly (P>0.05) compared to the control.

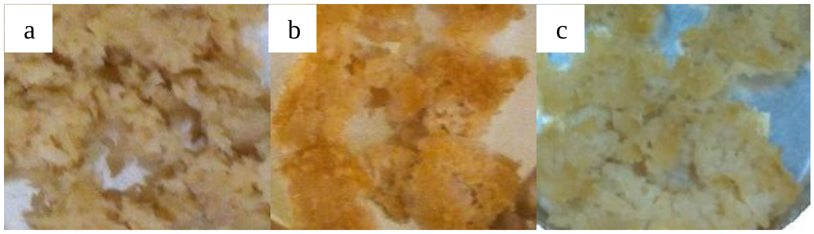


Fig.5.5. Comparison of the color of SPI meat analog undergone different treatments. a) Ground soy protein isolate (SPI) meat analog immediately after acid and polysaccharide treatments. b) Stored at 22°C for 24 hrs. c) Stored at 4°C for 24 hrs

As the product moisture decreased, the meat analog grew darker (Table 5.4). Moreover, instead of the white-yellow color, the meat analog turned yellowish according to the b* measurements.

The color change of meat analog could be due to the sugar-amino browning reactions. In general, the browning rate increases as the water content decreases (Eichner, 1972), which could cause the meat analog color to turn yellow-brownish. There was no significant difference ($P<0.05$) on each of the values within the same temperature. However, it was found that significant changes on L*, a*, and b* values existed across different temperatures. Moreover, after 24 hrs, all of the values were significantly changed compared with the newly treated product at both storage temperatures. It was intriguing to note that both L* and a* values were significantly decreased while the b* value was increased because of the browning reactions. Thus a large amount of moisture loss during storage could cause the browning reaction that rendered the color of the SPI meat analog unacceptable.

Table 5.4. Comparison of the color of ground modified SPI meat analog after 24 h storage at different temperature[1,2,3]

Treatment C:X[4]	Before			After					
				22°C***			4°C***		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control	64.20±0.13[c]	2.38±0.18[b]	14.69±2.18[b]	37.23±0.13[a]	4.93±0.43[c]	18.44±1.90[a]	52.05±0.63[a]	3.22±0.37[b]	18.49±0.73[a]
5to0	64.24±0.04[c]	1.38±0.05[a]	14.56±0.05[b]	38.59±0.35[b]	5.36±0.19[b,c]	20.47±1.06[a]	52.60±0.30[a]	2.83±0.55[a,b]	17.38±1.33[a]
4to1	62.60±0.54[b]	1.28±0.05[a]	14.34±0.33[b]	39.75±0.11[b,d]	5.54±0.08[a,b,c]	19.79±0.90[a]	52.52±0.34[a]	4.40±0.44[a,b]	19.43±0.60[a]
3to2	60.13±0.18[a]	1.60±0.34[a]	13.81±0.10[b]	40.73±0.30[d]	4.53±0.05[a,b,c]	19.10±0.75[a]	51.37±0.51[a]	3.00±0.60[a,b]	18.25±1.49[a]
2to3	61.36±0.52[a]	1.78±0.05[a]	14.76±0.07[b,c]	40.37±0.34[d]	3.77±0.31[a,b]	17.82±0.60[a]	50.33±0.28[a]	2.67±0.60[a]	19.06±1.05[a]
1to4	61.55±0.17[a]	1.31±0.04[a]	12.55±0.25[a,c]	39.57±0.47[c]	3.46±0.40[a]	14.07±1.52[a]	52.16±0.70[a,b]	3.65±0.67[a]	19.35±1.93[a]
0to5	61.36±0.15[b]	1.24±0.03[a]	12.06±0.03[a]	43.79±0.19[e]	3.21±1.11[a]	18.41±0.24[a]	52.33±0.68[b]	2.48±0.17[a]	19.65±0.98[a]

[1] Values in the same column followed by the same superscript were not significantly different by Tukey's test ($P<0.05$)
[2] *** indicates significant different when compared with the untreated (before) samples by Dunnett's test ($P>0.05$)
[3] Values shown are means ± SD, n=3
[4] C:X means the ratio of cornstarch to xanthan gum; 5 to 0 means cornstarch only, and the amount of cornstarch decreases until it reaches the last, 0 to 5, which means xanthan gum only; Control represents that samples treated without cornstarch and xanthan gum

5.2.6 Scanning electron microscope (SEM)

To visualize the microstructure of the meat analog under different conditions, the samples were examined under SEM (Fig. 5.6). It was found that addition of acid and heat helped to create the air cells between the layers (Fig. 5.7) compared with the SPI meat analog, which had almost no air cells or layers (Fig. 5.6). Furthermore, introducing cornstarch and xanthan gum into the treatment formed the bond to connect the two layers (Fig.5.8).

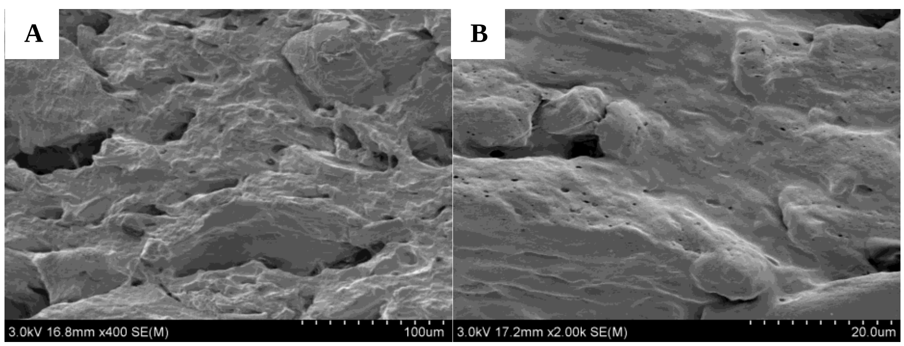


Fig. 5.6. Scanning electron micrographs of untreated soy protein isolate (SPI) meat analog after frozen storage; A) 400× magnification. B) 2000× magnification

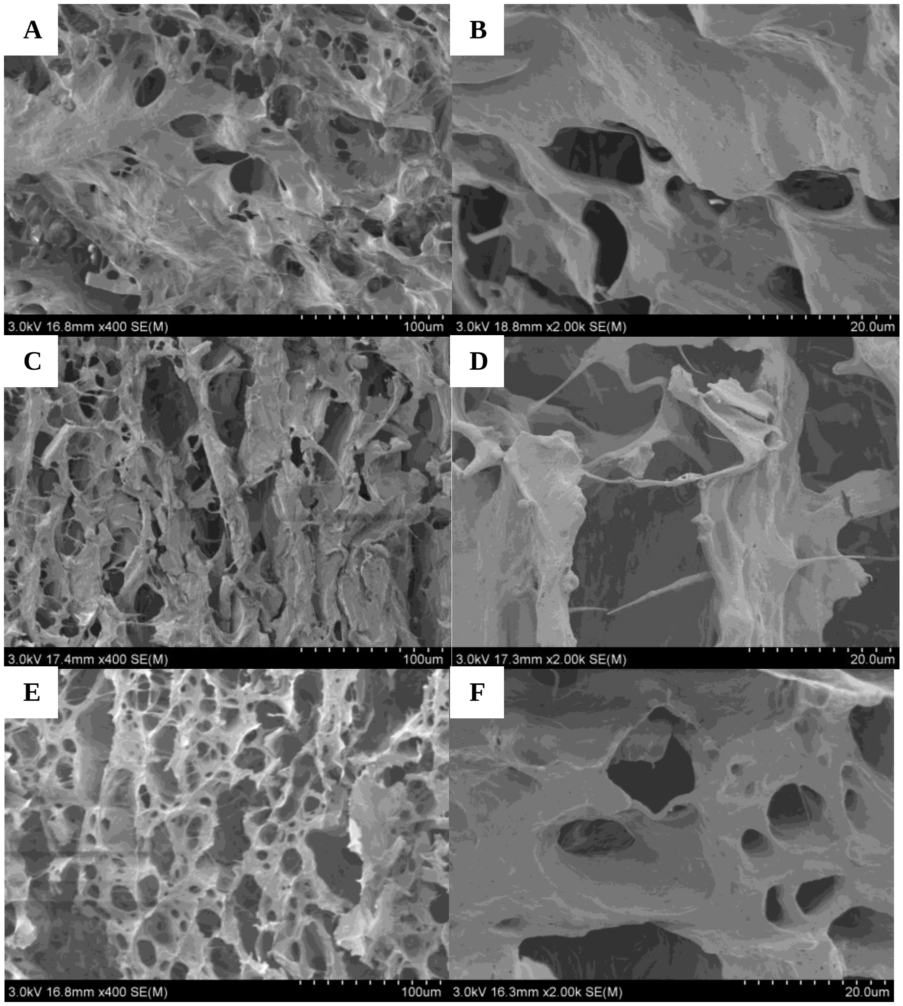


Fig.5.7. Scanning electron micrographs of untreated soy protein isolate (SPI) meat analog when treated in acetic acid solutions for 50 min at 65°C; A) pH 3.5 (400×). B) pH 3.5 (2000×). C) pH 4.5 (400×). D) pH 4.5 (2000×). E) 5.5<pH<7.0 (400×). F) 5.5<pH<7.0 (2000×)

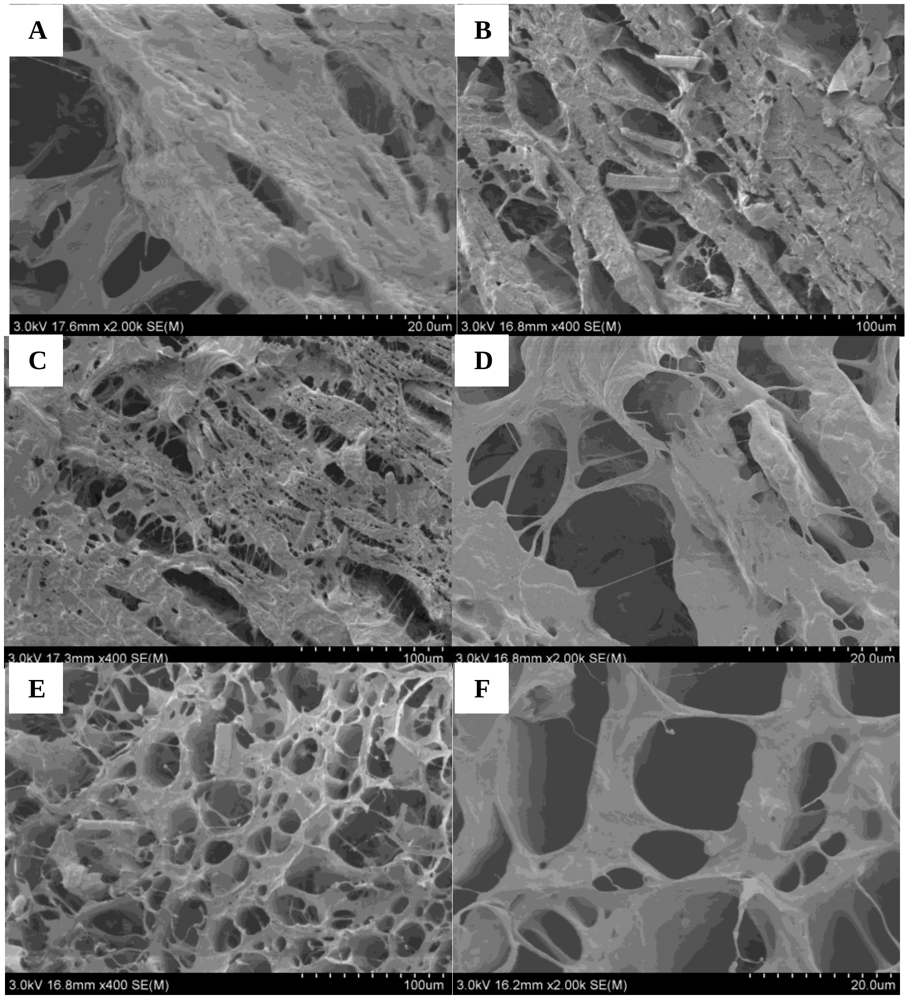


Fig. 5.8. Scanning electron micrographs of soy protein isolate (SPI) meat analog when treated not only in the acetic acid solution but also with cornstarch and xanthan mixture (C:X=3:2 w/w); A) pH 3.5 (400×). B) pH 3.5 (2000×). C) pH 4.5 (400×). D) pH 4.5 (2000×). E) 5.5<pH<7.0 (400×). F) 5.5<pH<7.0 (2000×)

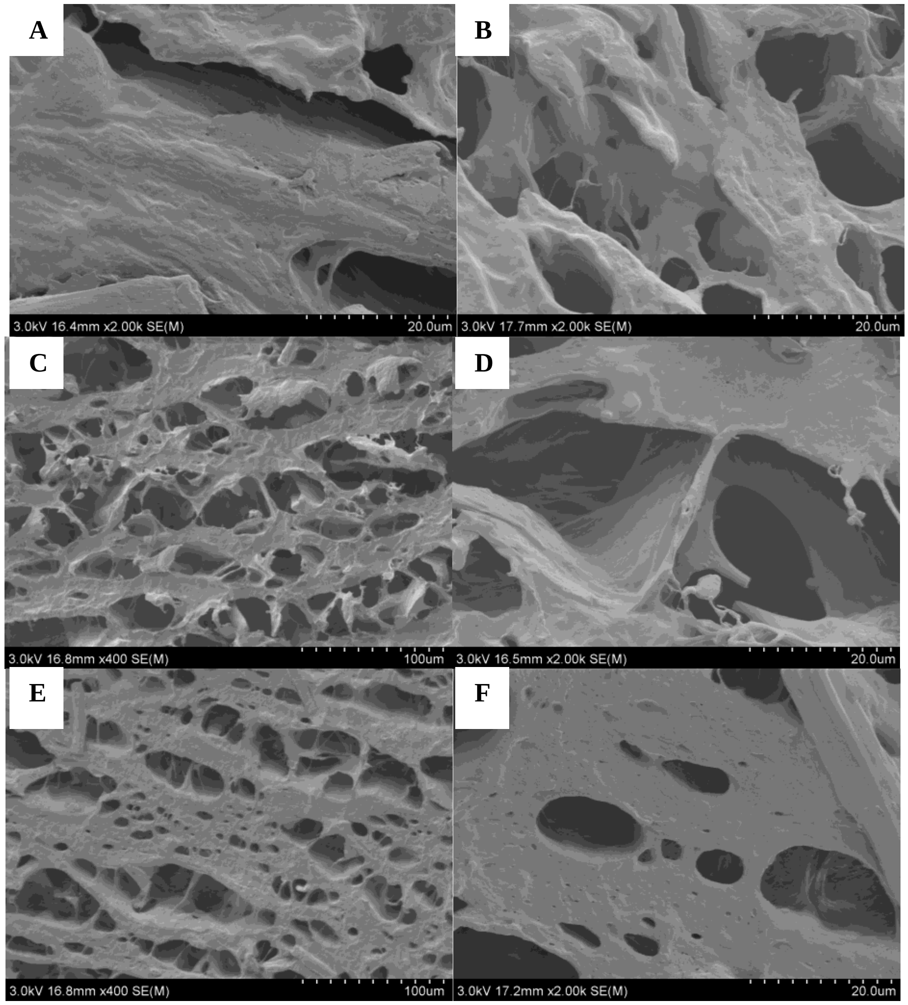


Fig. 5.9. Scanning electron micrographs of soy protein isolate (SPI) meat analog when treated in the acetic acid solution only after 24 hrs stored at 4ºC; A) pH 3.5 (400×). B) pH 3.5 (2000×). C) pH 4.5 (400×). D) pH 4.5 (2000×). E) 5.5<pH<7.0 (400×). F) 5.5<pH<7.0 (2000×)

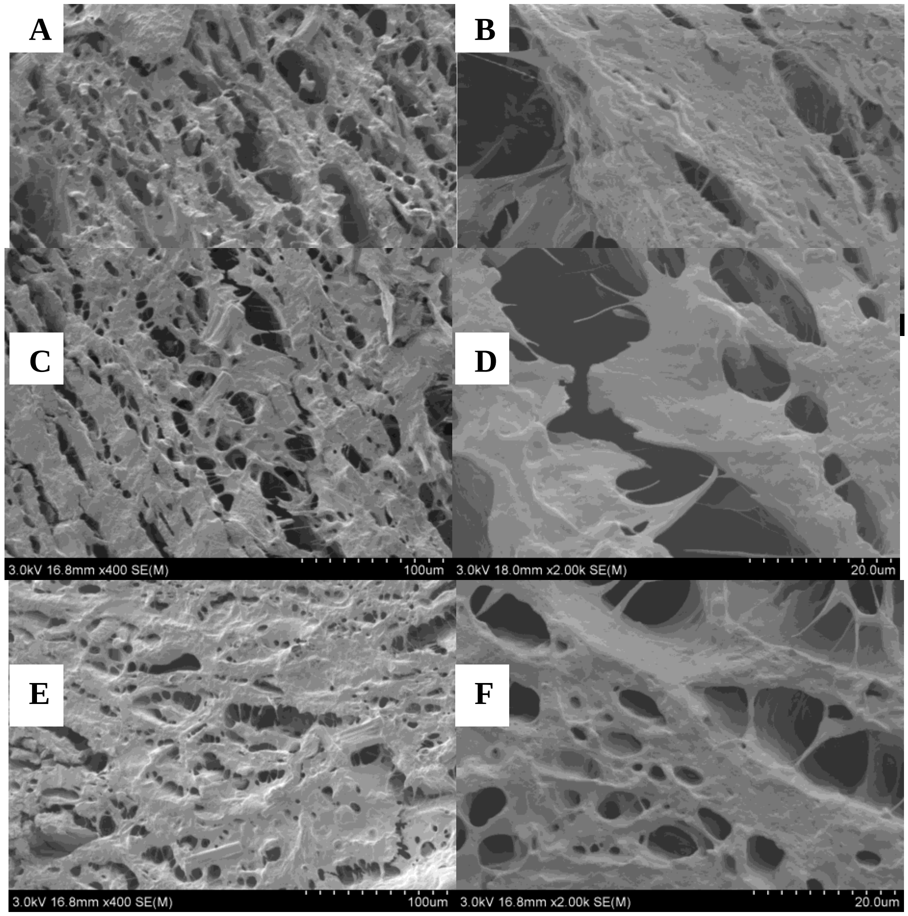


Fig. 5.10. Scanning electron micrographs of soy protein isolate (SPI) meat analog when treated not only in the acetic acid solution but also with cornstarch and xanthan mixture (C:X=3:2 w/w) after 24 hrs stored at 4°C; A) pH 3.5 (400×). B) pH 3.5 (2000×). C) pH 4.5 (400×). D) pH 4.5 (2000×). E) 5.5<pH<7.0 (400×). F) 5.5<pH<7.0 (2000×)

In general, it was reasonable to postulate from all of the SEM pictures that air cells entered the structure, and the product formed layers instead of collapsing together (Fig. 5.6) (Ranasinghesagara et al., 2006), hence helped the structure of the SPI meat analog to be tender. Unlike other pH conditions (Fig. 5.7. B; Fig.5.7. F), at pH 4.5 the least hardness of the SPI meat analog could be evidenced with the giant gaps between the layers, leading to the tenderizing of the meat analog (Fig.5.7. D). However, Fig. 5.9 showed that the structure of the samples treated by acetic acid only started to collapse after 24 hrs. Nevertheless, by adding cornstarch and xanthan gum into the treatment, some connecting bonds were formed to join the layers (Fig 5.8), and this helped extend its texture stability. Fig.5.8. (E, F) showed that the structure of the SPI meat analog at pH greater than 5.5 was very similar to that at pH 4.5. However, after 24 hours of storage, the air cells disappeared, collapsing the structure again regardless whether the analog was treated with the polysaccharide mixture (Fig. 5.10. F) or not (Fig. 5.9. F). Overall, storage at 4°C after 24 hrs appeared to shrink the air cells and the layers stuck back together (Fig. 5.8, Fig.5.9); however, with the help of cornstarch and xanthan gum, most of the air cells remained, especially when the pH equaled 4.5 (Fig. 5.9. D; Fig.5.10. D).

5.3 Modification of SPI meat analog

The SPI meat analog was modified according to the results gathered in section 5.2. The results suggested that, in order to achieve the desired texture, the SPI meat analog should be rehydrated in the solution made by vinegar and water with the ratio of 1 to

455 (pH of 4.5), heated up to 65°C, and then added with 0.1% (w/v) total amount of cornstarch and xanthan gum with the ratio of 3 to 2 (w/w). In the end, the product was heated for 50 min to finish the modification.

The modified SPI meat analog possessed different properties from the counterpart. The moisture content of the modified SPI reached 80.52%, which was a 10% increase from the sample; the protein content was slightly reduced to 17.25%, which could still be considered as a high-protein dietary source. In addition, the color of the modified product was lighter, namely light yellow-white (Fig. 5.11), compared with the SPI meat analog (L*60.13; a*1.60; b*17.42) (Table 5.5).

Table 5.5. Comparison of key properties among cooked skinless chicken breast, soy protein isolate (SPI) meat analog (after frozen storage), and modified SPI meat analog

	Cooked skinless chicken breast	**SPI meat analog**	**Modified SPI meat analog**
Moisture content	78.55%[1]	70.75%±0.12	80.52%±0.09
Protein	20.40%[1]	25.61%	17.25%
Hardness[3] (g)	432.6[a]±34.3	1834.6[b]±82.1	485.9[a]±36.6
Color[2,3]	L* 64.87±0.34[c] a* 0.37±0.34[a] b* 14.37±0.52[a]	L*: 55.38±0.34[a] a*: 3.62±0.01[c] b*: 17.42±0.14[b]	L*: 60.13±0.18[b] a*: 1.60±0.34[b] b*: 13.81±0.09[a]

[1] Data were adapted from Murphy, 1998
[2] Values in the same column followed by the same superscript were not significantly different by Tukey's test (P<0.05)
[3] Values are means± SD, n=3

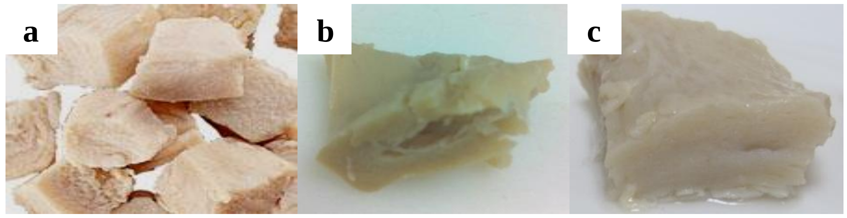


Fig.5.11. Photographic presentation of: a) cooked skinless chicken breast, b) soy protein isolate (SPI) meat analog (after frozen storage), and c) modified SPI meat analog

It was found that the modification process successfully decreased the hardness of the SPI meat analog (Table 5.5), reaching the level that was not significantly different from that of the cooked chicken breast (Table 5.5, $P<0.05$). Moreover, the color of the modified SPI meat analog was visually undifferentiatable from that of the cooked chicken breast, although considerable discrepancy still exists in the L* and a* measurements; nevertheless, the yellowness, represented by the b* value, of the SPI meat analog was reduced during the modification to the level that was not significantly different from the cooked chicken breast ($P<0.05$) (Table 5.6). Thus, it could be summarized that the modification process not only improved the texture but also the appearance of the SPI meat analog, drawing the product closer to the properties of the cooked chicken breast.

5.4 Novel product (M nuggets)

5.4.1 Formulation of M nuggets

A novel vegetarian product, i.e., the M nugget (Fig. 5.13) was developed using the modified SPI meat analog following the recipe described in section 4.2.3. Fig. 5.12 illustrated the procedure of making M nuggets.

Potato starch flour was chosen to take the place of the regular wheat flour in the original recipe due to the fact that it is gluten- and wheat-free, has no saturated fat and sodium, which could leverage the nutritional quality of the SPI meat analog with improved taste without introducing any negative nutritional factors. Moreover, the potato starch flour could also help form and shape the modified SPI meat analog before further processing. Both onion salt and white pepper served as the flavoring agents. Not only did they give the product tasteful flavor, but they also helped mask any undesirable flavor.

The M nuggets were shaped into square blocks not only considering the convenience during storage and shipping but also how they could provide an even and stable heat transfer during pan frying. The ball shaped version was created before the square shape; however, it was found that deep fat frying must be applied to thoroughly cook the ball product, which brought up another unhealthy concern associated with fat.

Additionally, products in the ball shape left a very strong oily smell after cooking due to the deep fry, which was unpleasant and could lead to rancidity for the final product. Thus, a square shape was selected after the comparison.

In the M nugget recipe, corn flake crumbs acted as a shield to protect moisture during frying, and it gave the final product a crispy coating besides its protein content with low sodium. Corn oil was chosen to act as a medium in which to pan fry the shaped and coated nuggets.

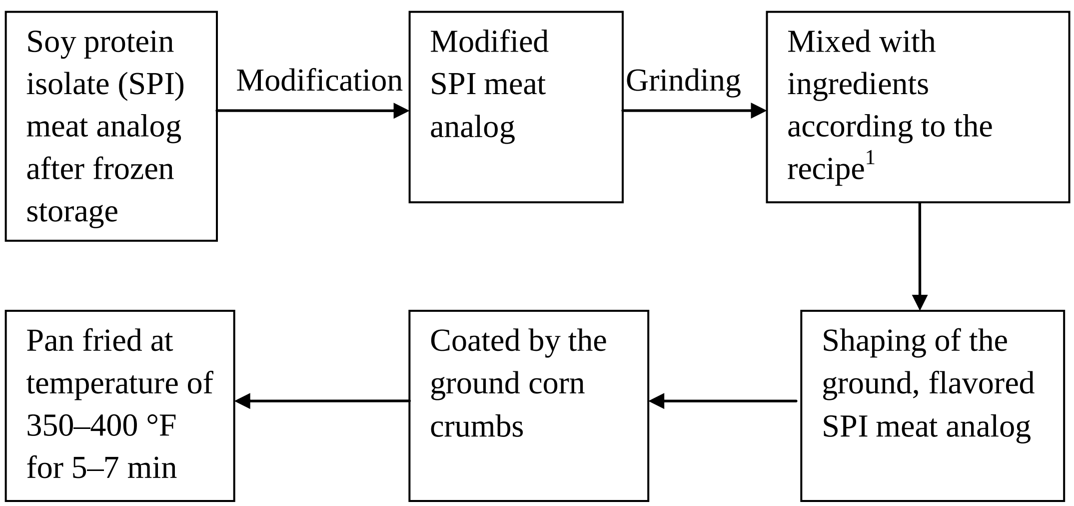


Fig. 5.12. Procedure for making the M nuggets; [1]recipe was illustrated in Table 4.1.

Fig. 5.13. Photographic presents the M nugget

5.4.2 Analysis of M nuggets

The toughness of M nuggets, as reported by the max force exerted by the M nuggets upon breakage, was found to be ca. 483.8g, which was in line with the values of commercial chicken nuggets and vegan chicken-free nuggets (Table 5.6).

Table 5.6. Comparison of product hardness among commercial chicken nuggets, M nuggets, and commercial vegan chicken-free nuggets

Product	**Hardness (g)[1,2]**
Commercial chicken nuggets	438.8±56.6[a]
Commercial vegan chicken-free nuggets	440.4±25.5[a]
M nuggets	483.8±31.9[a]

[1] Values in the same column followed by the same superscript were not significantly different by Tukey's test ($P<0.05$)
[2] Values are means ± SD, n=3

The basic nutrition facts of all three products were summarized in Table 5.7. The actual labels of each of the products can be found in Appendix D.

Table 5.7. Comparison of the nutrition facts among commercial chicken nugget, M nuggets, and commercial vegan chicken-free nuggets for a serving size of 85 g

	Commercial chicken nuggets	**Commercial vegan chicken-free nuggets**	**M nuggets**
Calories	210	120	120
Total fat (g)	11	2	1
Saturated fat (g)	2	0	0
Trans fat (g)	0	0	0
Cholesterol (mg)	15	0	0
Total carbohydrates (g)	17	14	9
Dietary fiber (g)	3	2	0
Sugar (g)	1	0	0
Sodium (mg)	400	450	400
Protein (g)	11	14	20

The M nuggets were found to have better nutrition facts than the commercial chicken nuggets and the vegan chicken-free nuggets. In terms of calories, the M nuggets contained only 120 calories per serving, which was the same as the vegan chicken-free nuggets but much lower than the commercial chicken nuggets. Besides, a 2% DV of total fat (1 g) was also good for human consumption, as well as the zero saturated fat, trans fat, and cholesterol, which can help prevent cardiovascular disease and control cholesterol levels. The lower sodium content also made this product more competitive on the market due to people's concern about Hypernatremia. The protein content of M nuggets was the highest among all three products, which provides a good source of protein for vegetarians that suffer inevitably from shortage of protein in traditional vegetarian diets.

5.4.3 Sensory evaluation of M nuggets

The toughness and chewiness of the M nuggets were not significantly different from the commercial chicken nuggets, whereas the off flavor, mainly the soy flavor, was not detectable in the M nuggets based on the sensory evaluation (Table 5.8), giving a score similar to that of the commercial chicken nuggets. On the other hand, all panelists could detect at different levels soy flavor in the commercial vegan chicken-free nuggets. However, the chicken flavor in vegan chicken-free nuggets and the M nuggets were found to be significantly lower compared to commercial chicken nuggets. Overall, non-vegetarian consumers liked M nuggets as much as they liked the commercial vegan chicken-free nuggets in terms of both the product in general and the texture; although the average scores below 5 indicated that they slightly dislike both products. Moreover, significant difference was found in the preference for the product in general between M nuggets and commercial chicken nuggets; this could be attributed to that the non-vegetarian consumers still preferred the real meat. According to the comments gathered from the sensory evaluation, most non-vegetarians complained about the fake meat and expressed a keen desire for the real meat, so further comparison between vegetarian consumers and non-vegetarian consumers was analyzed (Table 5.9).

Table 5.8. Sensory evaluation on the texture, flavor, and consumer acceptances of commercial chicken nuggets, commercial vegan chicken-free nuggets, and the M nuggets; data shown were analyzed based on the non-vegetarian consumers [3]

		Commercial chicken nuggets	Commercial vegan chicken-free nuggets[1]	M nuggets[1]
Texture	Toughness	1.8±0.8	2.0±0.9	1.4±0.7
	Chewiness	2.4±1.0	2.7±1.0	2.0±1.1
Flavor	Off flavor (Soy)	1.8±0.8	2.7±1.2**	2.5±1.5
	Chicken	2.9±1.5	1.9±1.8**	1.3±1.5**
Like product[2]		5.6±1.5[a]	4.4±1.7[a,b]	3.6 ±2.1[b]
Like texture[2]		5.1±1.6[c]	3.8±1.4[d]	3.4±1.9[d]

[1] ** represented that the data were significant different from the control (Commercial chicken nuggets) by Dunnett's test (P>0.05)
[2] Values in the same row followed by the same superscript were not significantly different by Tukey's test (P<0.05)
[3] Values are means ±SD, n=29; 1 point stands for none and 5 point stands for extreme

Table 5.9. Comparison of consumer acceptance of commercial vegan chicken-free nuggets and the M nuggets between vegetarian and non-vegetarian consumers[3]

		Commercial vegan chicken-free nuggets[1]	M nuggets[1]
Like product	Vegetarian[2]	5.1±1.2[a,A]	6.0±0.7[a,A]
	Non-vegetarian[2]	4.4±1.7[a,B]	3.6±2.1[a,B]
Like texture	Vegetarian[2]	4.9±1.2[a,C]	6.8±0.4[b,C]
	Non-vegetarian[2]	3.8±1.4[a,D]	3.4±1.9[a,D]

[1] Values in the same column followed by the same capitalized superscript were not significantly different by Tukey's test (P<0.05)
[2] Values in the same row followed by the same superscript were not significantly different by Tukey's test (P<0.05)
[3] Values are means ± SD, n=29; 1 point stands for dislike extremely and 9 points stand for like extremely

There was a significant difference between vegetarians and non-vegetarians in the preference of the commercial vegan chicken-free nuggets and M nuggets. While vegetarians liked M nuggets as much as they liked the commercial vegan chicken-free nuggets (Table 5.9, $P<0.05$), it is important to note that vegetarians preferred the flavor and texture of M nuggets to that of the commercial vegan chicken-free nuggets. Specifically, the M nuggets were not as mushy as the commercial vegan counterparts. Contrary to the significant soy flavor detected by the panelists, significant soy flavor reduction in the M nuggets was found to contribute to the acceptance of the product (Table 5.8). Not only will the M nuggets offer vegetarians with a nutritionally sound product without compromising taste, further improvement in the nuggets' chicken flavor will broaden its market share into non-vegetarian sectors.

Chapter 6: Conclusions

Post-processing frozen storage was found to adversely affect the quality of high-moisture-extruded SPI, yielding products with unsatisfactory texture (hard and rubbery) and undesirable color (dark and yellowish-brown). Such shortcomings were improved significantly by employing a series of optimized treatment combining pH adjustment, temperature modification, and polysaccharides. The modified SPI meat analog was softer than the original with a lighter color after rehydrating the SPI meat analog in an acetic acid solution at pH 4.5 and 65 °C for 50 min alongside the treatment with a cornstarch/xanthan mixture at a ratio of 3:2 totaling 0.1% (v/w).

Additionally, a novel product “M nugget” utilizing the modified SPI meat analog was successfully developed to mimic chicken nuggets, as evidenced by its desirable texture while eliminating soy flavor. These characteristics were confirmed by organoleptic evaluation against commercially available conventional chicken nuggets and vegan nuggets. The M nugget was shown to have significantly higher consumer acceptance especially among vegetarians for its texture; moreover, soy flavor was not detected. Not only are the texture and flavor of the M nugget desirable, its low fat, low sodium, and high protein content also makes the M nugget one competitive dietary protein source on the market.

Appendix A: Approval from IRB of University of Maryland, College Park

IRB Protocol Approval

University of Maryland IRB <no-reply@umresearch.umd.edu> **Fri, Apr 8, 2011 at 2:58 PM**
To: "Y. Martin Lo" <ymlo@umd.edu>, Haiqin Ge <melodyguh@gmail.com>

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

Initial Application Approval

DO NOT REPLY TO THIS EMAIL ADDRESS AS IT IS UNMONITORED

To:	Principal Investigator, Y. Martin Lo, NFSC Student, Haiqin Ge, NFSC
From:	James M. Hagberg IRB Co-Chair University of Maryland College Park
Re:	IRB Protocol: 11-0189 - Modifying the texture of post-extruded meat analog and a novel product development
Approval Date:	April 08, 2011
Expiration Date:	April 08, 2014
Application:	Initial
Review Path:	Exempt

The University of Maryland, College Park Institutional Review Board (IRB) Office approved your Initial IRB Application. This transaction was approved in accordance with the University's IRB policies and procedures and 45 CFR 46, the Federal Policy for the Protection of Human Subjects. Please reference the above-cited IRB Protocol number in any future communications with our office regarding this research.

Recruitment/Consent: For research requiring written informed consent, the IRB-approved and stamped informed consent document will be sent via mail. The IRB approval expiration date has been stamped on the informed consent document. Please note that research participants must sign a stamped version of the informed consent form and receive a copy.

Continuing Review: If you intend to continue to collect data from human subjects or to analyze private, identifiable data collected from human subjects, beyond the expiration date of this protocol, you must submit a Renewal Application to the IRB Office 45 days prior to the expiration date. If IRB Approval of your protocol expires, all human subject research activities including enrollment of new subjects, data collection and analysis of identifiable, private information must cease until the Renewal Application is approved. If work on the human subject portion of your project is complete and you wish to close the protocol, please submit a Closure Report to irb@umd.edu.

Modifications: Any changes to the approved protocol must be approved by the IRB before the change is implemented, except when a change is necessary to eliminate an apparent immediate hazard to the subjects. If you would like to modify an approved protocol, please submit an Addendum request to the IRB Office.

https://mail.google.com/mail/?ui=2&ik... 1/2

Unanticipated Problems Involving Risks: You must promptly report any unanticipated problems involving risks to subjects or others to the IRB Manager at 301-405-0678 or jsmith@umresearch.umd.edu

Additional Information: Please contact the IRB Office at 301-405-4212 if you have any IRB-related questions or concerns. Email: irb@umd.edu

The UMCP IRB is organized and operated according to guidelines of the United States Office for Human Research Protections and the United States Code of Federal Regulations and operates under Federal Wide Assurance No. FWA00005856.

1204 Marie Mount Hall
College Park, MD 20742-5125
TEL 301.405.4212
FAX 301.314.1475
irb@umd.edu
http://www.umresearch.umd.edu/IRB

Appendix B:

Evaluation sheet:

Name (Optional): ___________ Product Code___________

Date______________

Please check one of the boxes that represent your opinion about the taste intensity of the product you are evaluating:

Intensity of taste		Texture		Flavor	
		Toughness	Chewiness	Soy	Chicken
None	1				
Slight	2				
Moderate	3				
Strong	4				
Extreme	5				

Please check the term that best reflects your attitude about the product whose code matches the code on this scored:

How much do you like this product (product code)?

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

How much do you like this product for its texture?

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Appendix C: Statistic Analysis

ANOVA comparison of hardness between cooked chicken and SPI meat analog

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'TEXTURE COMPARISON BETWEEN COOKED CHICKEN AND   SPI
MEAT ANALO';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA TEXTURE;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT HARDNESS @;
  OUTPUT;
  END;
CARDS;
CHICKEN 655.6 649.1 593.2
UNTREATEDSPI 1865.2 1849.9 1789.6
PROC PRINT DATA=TEXTURE;
RUN;
PROC ANOVA DATA=TEXTURE;
CLASS SAMPLES;
MODEL HARDNESS=SAMPLES;
RUN;
```

Tukey comparison of WAC

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'WAC COMPARISON AMONG DIFFERENT TEMPERATURES';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA WACCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT WAC @;
  OUTPUT;
  END;
CARDS;
55DEGREE 162 179 166
65DEGREE 169 182 175
75DEGREE 181 186 175
85DEGREE 185 192 195
PROC PRINT DATA=WACCOMPARISON1;
RUN;
PROC ANOVA DATA=WACCOMPARISON1;
CLASS SAMPLES;
MODEL WAC=SAMPLES;
```

```
MEANS SAMPLES/TUKEY;
RUN;
```

Dunnett comparison of L value at different temperatures

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON L VALUE AMONG DIFFERENT
TEMPERATURES';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
  DO i=1 TO 7;
   INPUT L @;
   OUTPUT;
   END;
CARDS;
BEFORE    64.20 64.24 62.60 60.13 61.36 61.55 61.36
22DEGREE  37.23 38.59 39.75 40.73 40.37 39.57 43.79
4DEGREE   52.05 52.60 52.52 51.37 50.33 52.16 52.33
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL L=SAMPLES;
MEANS SAMPLES/DUNNETT ('BEOFRE');
RUN;
```

Dunnett comparison of a* value at different temperatures

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON A VALUE AMONG DIFFERENT
TEMPERATURES';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
  DO i=1 TO 7;
   INPUT A @;
   OUTPUT;
   END;
CARDS;
BEFORE    2.38 1.38 1.28 1.60 1.78 1.31 1.24
22DEGREE  4.93 5.36 5.54 4.53 3.77 3.46 3.21
4DEGREE   3.22 2.83 4.40 3.00 2.67 3.65 2.48
PROC PRINT DATA=COLORCOMPARISON1;
```

```
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL A=SAMPLES;
MEANS SAMPLES/DUNNETT('BEFORE');
RUN;
```

Dunnett comparison of b* value at different temperatures

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON B VALUE AMONG DIFFERENT
TEMPERATURES';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 7;
  INPUT B @;
  OUTPUT;
  END;
CARDS;
BEFORE    14.69 14.56 14.34 13.81 14.76 12.55 12.06
22DEGREE  18.44 20.47 19.79 19.10 17.82 14.07 18.41
4DEGREE   18.49 17.38 19.43 18.25 19.06 19.35 19.65
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL B=SAMPLES;
MEANS SAMPLES/DUNNETT('BEFORE');
RUN;
```

Tukey comparison of L* value at 4 degree Celsius among different ratio

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON L VALUE AMONG DIFFERENT RATIO AT
4 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT L @;
  OUTPUT;
  END;
```

```
CARDS;
CONTROL 51.48 52.73 51.94
5TO0    52.27 52.84 52.68
4TO1    52.89 52.46 52.22
3TO2    51.96 51.05 51.09
2TO3    50.61 50.05 50.34
1TO4    52.81 51.42 52.26
0TO5    51.56 52.86 52.58
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL L=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of a* value at 4 degree Celsius among different ratio

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON A VALUE AMONG DIFFERENT RATIO
AT 4 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT A @;
  OUTPUT;
  END;
CARDS;
CONTROL 2.90 3.14 3.62
5TO0    2.30 3.39 2.80
4TO1    4.27 4.89 4.03
3TO2    3.70 2.72 2.60
2TO3    2.73 2.05 3.24
1TO4    2.92 2.88 4.14
0TO5    2.28 2.54 2.61
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL A=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of b* value at 4 degree Celsius among different ratio

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON B VALUE AMONG DIFFERENT RATIO AT
4 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT B @;
  OUTPUT;
  END;
CARDS;
CONTROL 17.84 18.35 19.28
5TO0    15.54 19.07 17.54
4TO1    20.12 19.03 19.15
3TO2    19.93 17.69 17.12
2TO3    17.90 19.94 19.33
1TO4    20.48 17.13 20.45
0TO5    20.77 18.92 19.25
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL B=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of L* value at 22 degree Celsius among different ratio

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON L VALUE AMONG DIFFERENT RATIO AT
22 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT L @;
  OUTPUT;
  END;
CARDS;
```

```
CONTROL 37.16 37.15 37.38
5TO0    38.70 38.20 38.88
4TO1    39.77 39.85 39.64
3TO2    40.94 40.87 40.39
2TO3    40.36 40.04 40.71
1TO4    39.99 39.07 39.66
0TO5    43.82 43.97 43.58
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL L=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of a* value at 22 degree Celsius among different ratio

```
M 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON A VALUE AMONG DIFFERENT RATIO
AT 22 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT A @;
   OUTPUT;
   END;
CARDS;
CONTROL 4.86 5.39 4.54
5TO0    5.58 5.25 5.26
4TO1    5.47 5.52 5.63
3TO2    4.57 4.55 4.47
2TO3    3.98 3.42 3.92
1TO4    3.90 3.15 3.32
0TO5    2.95 2.25 4.92
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL A=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of b* value at 22 degree Celsius among different ratio

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'COLOR COMAPRISON ON B VALUE AMONG DIFFERENT RATIO AT
22 DEGREE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLORCOMPARISON1;
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT B @;
   OUTPUT;
   END;
CARDS;
CONTROL 19.75 19.30 16.27
5TO0    21.28 20.86 19.26
4TO1    20.79 19.54 19.05
3TO2    19.86 19.06 18.36
2TO3    17.83 18.41 17.22
1TO4    15.65 13.92 12.63
0TO5    18.17 18.65 18.42
PROC PRINT DATA=COLORCOMPARISON1;
RUN;
PROC ANOVA DATA=COLORCOMPARISON1;
CLASS SAMPLES;
MODEL B=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of the texture among untreated SPI meat analog, pretreated SPI analog and cooked chicken breast

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2' TEXTURE COMPARISON AMONG UNTREATED MEAT ANALOG,
ACIDIC MEAT ANALOG AND COOKED CHICKEN BREAST MEAT';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA TEXTURECOMPARISON1;
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT MAXFORCE @;
   OUTPUT;
   END;
CARDS;
UNTREATEDMEATANALOG  1802.9 1851.7 1850.1
```

```
ACIDICMEATANALOG   462.9 490.9 503.8
CHICKENBREASTMEAT  384.4 412.0 431.1
PROC PRINT DATA=TEXTURECOMPARISON1;
RUN;
PROC ANOVA DATA=TEXTURECOMPARISON1;
CLASS SAMPLES;
MODEL MAXFORCE=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of L* value among untreated meat analog, treated meat analog and cooked chicken breast

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'L VALUE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLOR;
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT L @;
   OUTPUT;
   END;
CARDS;
CHICKEN   64.85 64.54 65.22
UNTREATED  55.72 55.38 55.04
PRETREATED 60.00 60.05 60.34
PROC PRINT DATA=COLOR;
RUN;
PROC ANOVA DATA=COLOR;
CLASS SAMPLES;
MODEL L=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of a* value among untreated meat analog, treated meat analog and cooked chicken breast

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'A VALUE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLOR;
```

```
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT A @;
   OUTPUT;
   END;
CARDS;
CHICKEN   0.20 0.16 0.76
UNTREATED  3.63 3.60 3.63
PRETREATED 1.22 1.74 1.85
PROC PRINT DATA=COLOR;
RUN;
PROC ANOVA DATA=COLOR;
CLASS SAMPLES;
MODEL A=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of b* value among untreated meat analog, treated meat analog and cooked chicken breast

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'B VALUE';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA COLOR;
INPUT SAMPLES $ @;
  DO i=1 TO 3;
   INPUT B @;
   OUTPUT;
   END;
CARDS;
CHICKEN    13.79 14.54 14.78
UNTREATED  17.52 17.47 17.26
PRETREATED 13.71 13.90 13.82
PROC PRINT DATA=COLOR;
RUN;
PROC ANOVA DATA=COLOR;
CLASS SAMPLES;
MODEL B=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison of toughness among commercial chicken nuggets, M nuggets and commercial vegan chicken-free nuggets

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'TEXTURE COMPARISON AMONG COMMERCIAL CHICKEN
NUGGETS, SX NUGGETS AND VEGAN NUGGETS';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA TEXTURECOMPARISON1;
INPUT SAMPLES $ @;
 DO i=1 TO 3;
  INPUT MAXFORCE @;
  OUTPUT;
  END;
CARDS;
CHICKENNUGGETS 405.4 406.8 504.2
SXNUGGETS      501.7 447.0 502.8
VEGANNUGGETS   428.0 469.8 423.5
PROC PRINT DATA=TEXTURECOMPARISON1;
RUN;
PROC ANOVA DATA=TEXTURECOMPARISON1;
CLASS SAMPLES;
MODEL MAXFORCE=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Dunnett comparison on toughness of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL TOUGHNESS SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA TOUGHNESS;
INPUT SAMPLES $ @;
 DO i=1 TO 19;
  INPUT TOUGHNESS @;
  OUTPUT;
  END;
CARDS;
CHICKEN 2 2 1 1 1 2 2 1 1 1 2 2 2 1 3 3 2 3 3
VEGAN   3 2 2 2 2 1 1 2 2 1 1 1 1 1 2 3 2 3 3
MNUGGETS 1 1 1 2 1 1 1 1 4 1 1 1 1 1 1 3 1 2 1
PROC PRINT DATA=TOUGHNESS;
RUN;
PROC ANOVA DATA=TOUGHNESS;
```

```
CLASS SAMPLES;
MODEL TOUGHNESS=SAMPLES;
MEANS SAMPLES/DUNNETT('CHICKEN');
RUN;
```

Dunnett comparison on chewiness of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL CHEWINESS SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA CHEWINESS;
INPUT SAMPLES $ @;
  DO i=1 TO 19;
   INPUT CHEWINESS @;
   OUTPUT;
   END;
CARDS;
CHICKEN 4 4 2 1 4 2 3 2 2 2 3 1 2 3 1 4 3 3 2
VEGAN   2 2 3 2 3 2 4 2 1 2 2 3 3 3 2 3 2 3 2
MNUGGETS 3 5 1 3 1 2 2 2 1 1 5 2 1 1 1 2 3 1 2
PROC PRINT DATA=CHEWINESS;
RUN;
PROC ANOVA DATA=CHEWINESS;
CLASS SAMPLES;
MODEL CHEWINESS=SAMPLES;
MEANS SAMPLES/DUNNETT('CHICKEN');
RUN;
```

Dunnett comparison on off flavor of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL SOY FLAVOR SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA SOYFLAVOR;
INPUT SAMPLES $ @;
  DO i=1 TO 19;
   INPUT SOY @;
   OUTPUT;
   END;
CARDS;
CHICKEN 1 3 2 1 1 2 1 2 2 3 2 3 1 1 3 2 2 1 1
VEGAN   1 3 1 4 2 3 1 3 4 4 4 1 1 4 3 3 2 3 4
```

```
MNUGGETS 2 5 4 5 4 5 1 1 1 3 2 4 1 1 3 2 1 2 2
PROC PRINT DATA=SOYFLAVOR;
RUN;
PROC ANOVA DATA=SOYFLAVOR;
CLASS SAMPLES;
MODEL SOY=SAMPLES;
MEANS SAMPLES/DUNNETT ('CHICKEN');
RUN;
```

Dunnett comparison on chicken flavor of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL CHICKEN FLAVOR SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA CHICKENFLAVOR;
INPUT SAMPLES $ @;
  DO i=1 TO 19;
   INPUT FC @;
   OUTPUT;
   END;
CARDS;
CHICKEN 5 3 3 2 4 3 2 3 2 2 1 2 4 4 2 3 3 5 4
VEGAN   4 3 2 1 1 2 2 1 2 1 1 3 1 2 1 3 3 2 2
MNUGGETS 2 1 1 1 1 2 1 1 1 1 1 1 1 1 1 3 2 1 1
PROC PRINT DATA=CHICKENFLAVOR;
RUN;
PROC ANOVA DATA=CHICKENFLAVOR;
CLASS SAMPLES;
MODEL FC=SAMPLES;
MEANS SAMPLES/DUNNETT('CHICKEN');
RUN;
```

Tukey comparison on consumers' like product of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL LIKE PRODUCT SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA ALLLIKEPRODUCT;
INPUT SAMPLES $ @;
  DO i=1 TO 19;
   INPUT LIKEPRODUCT @;
   OUTPUT;
   END;
```

```
CARDS;
CHICKEN  8 7 5 7 5 3 6 5 6 8 6 4 6 7 6 7 3 4 6
VEGAN    6 5 2 3 7 5 3 4 2 6 3 3 6 6 4 3 7 3 6
MNUGGETS 3 4 4 4 2 1 7 8 7 1 1 3 2 3 6 3 2 4 4
PROC PRINT DATA=ALLLIKEPRODUCT;
RUN;
PROC ANOVA DATA=ALLLIKEPRODUCT;
CLASS SAMPLES;
MODEL LIKEPRODUCT=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison on consumers' like texture of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'ALL LIKE TEXTURE SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA ALLLIKETEXTURE;
INPUT SAMPLES $ @;
  DO i=1 TO 19;
   INPUT LIKETEXTURE @;
   OUTPUT;
   END;
CARDS;
CHICKEN 9 7 5 4 7 3 7 5 5 5 3 4 5 7 4 3 6 6 5
VEGAN   4 4 2 3 3 3 4 3 2 3 5 2 6 6 4 4 7 3 4
MNUGGETS 3 4 3 3 3 1 6 4 8 1 1 2 2 2 5 3 3 3 7
PROC PRINT DATA=ALLLIKETEXTURE;
RUN;
PROC ANOVA DATA=ALLLIKETEXTURE;
CLASS SAMPLES;
MODEL LIKETEXTURE=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison on vegetarians' like product of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'V LIKE PRODUCT SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA LIKEPRODUCT;
```

```
INPUT SAMPLES $ @;
  DO i=1 TO 10;
   INPUT LIKEPRODUCT @;
   OUTPUT;
   END;
CARDS;
VEGAN   3 7 4 5 4 6 5 6 5 6
MNUGGETS 6 5 6 7 6 6 6 5 6 7
PROC PRINT DATA=LIKEPRODUCT;
RUN;
PROC ANOVA DATA=LIKEPRODUCT;
CLASS SAMPLES;
MODEL LIKEPRODUCT=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison on vegetarians' like texture of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'V LIKE TEXTURE SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA LIKETEXTURE;
INPUT SAMPLES $ @;
  DO i=1 TO 10;
   INPUT LIKETEXTURE @;
   OUTPUT;
   END;
CARDS;
VEGAN  3 7 5 4 4 6 5 6 4 5
MNUGGETS 7 7 7 7 7 7 6 6 7 7
PROC PRINT DATA=LIKETEXTURE;
RUN;
PROC ANOVA DATA=LIKETEXTURE;
CLASS SAMPLES;
MODEL LIKETEXTURE=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison on non-vegetarians' like product of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
```

```
TITLE2'NV LIKE PRODUCT SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA LIKEPRODUCT;
INPUT SAMPLES $ @;
 DO i=1 TO 19;
  INPUT LIKEPRODUCT @;
  OUTPUT;
  END;
CARDS;
VEGAN   7 5 6 3 6 6 3 2 3 4 3 7 3 2 3 5 6 6 4
MNUGGETS 3 4 4 2 2 3 6 3 1 2 1 4 4 1 3 7 8 4 7
PROC PRINT DATA=LIKEPRODUCT;
RUN;
PROC ANOVA DATA=LIKEPRODUCT;
CLASS SAMPLES;
MODEL LIKEPRODUCT=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Tukey comparison on non-vegetarians' like texture of sensory evaluation

```
DM 'LOG; CLEAR; OUT; CLEAR;';
TITLE 'HAIQIN (MELODY) GE';
TITLE2'NV LIKE TEXTURE SENSORY';
OPTIONS LS=75 PS=60 PAGENO=1;
DATA LIKETEXTURE;
INPUT SAMPLES $ @;
 DO i=1 TO 19;
  INPUT LIKETEXTURE @;
  OUTPUT;
  END;
CARDS;
VEGAN   3 3 3 2 6 6 5 2 4 4 4 7 3 2 3 4 4 4 3
MNUGGETS 3 3 3 3 2 2 5 3 1 3 1 3 7 1 2 6 4 4 8
PROC PRINT DATA=LIKETEXTURE;
RUN;
PROC ANOVA DATA=LIKETEXTURE;
CLASS SAMPLES;
MODEL LIKETEXTURE=SAMPLES;
MEANS SAMPLES/TUKEY;
RUN;
```

Appendix D: Nutrition labels

Nutrition Facts

Serving Size (85g)
Servings Per Container

Amount Per Serving

Calories 100	Calories from Fat 10
	% Daily Value*
Total Fat 1g	**2%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 310mg	**13%**
Total Carbohydrate 0g	**0%**
Dietary Fiber 0g	**0%**
Sugars 0g	
Protein 22g	

Vitamin A 0% • Vitamin C 0%
Calcium 6% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Fig.1. Nutrition label of untreated SPI meat analog

Nutrition Facts

Serving Size 3 oz (3 nuggets) (85g)
Servings Per Container

Amount Per Serving

Calories 120	Calories from Fat 10
	% Daily Value*
Total Fat 1g	**2%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 400mg	**17%**
Total Carbohydrate 9g	**3%**
Dietary Fiber 0g	**0%**
Sugars 0g	
Protein 20g	

Vitamin A 0% • Vitamin C 0%

Calcium 6% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Fig.2. Nutrition label of M nuggets

Fig.3. Nutrition label of commercial vegan chicken-free nuggets

Fig.4. Nutrition label of commercial chicken nuggets

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