PHYSICAL AND CHEMICAL ATTRIBUTES OF A DEFATTED SOY FLOUR MEAT ANALOG

A Thesis
presented to
the Faculty of the Graduate School
University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree

Master of Science

by
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MAY 2007

The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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and hereby certify, that their opinion, it is worthy of acceptance.

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ACKNOWLEDGEMENTS

I would want to thank my advisor, Dr. Fu-Hung Hsieh, for his aid and support. During my graduate work, Dr. Hsieh has supported me with great academic advice.

Also, I would like to give a very special thank you to Dr. Ingolf Gruen and Dr. Mark Ellersieck for being on my committee. Their help was very valuable and greatly appreciated.

I would also like to thank Mr. Harold Huff for his guidance, technical assistance, time and friendship. He always provided as many resources as I have needed without question. Without Mr. Huff's help, this project would have been much more difficult for me to complete. However, thanks to his patience and willingness to help, I was able to succeed.

I also wish to express my appreciation to all of my friends at the University of Missouri, especially Hannah Edlen and Drew Brueggeman. Their assistance has been very helpful, without which I would have been hard pressed to complete my research on my own.

Finally, I would also like to thank my loving family, especially my parents and Dr. and Mrs. Stringer. Without their support and encouragement, I could not have possibly finished my graduate work and thesis at the Department of Food Science at the University of Missouri-Columbia.

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ABSTRACT

The objective of this study was to observe how the replacement of soy protein isolate in a meat analog with defatted soy flour would affect the physical and chemical characteristics of the product. A 4 × 3 × 3 (four mixes, three moisture contents, and three cooking temperatures) factorial experiment with 2 replications was conducted. The first set of information acquired was the extruder responses. To observe any differences in texture, a texture profile analysis (TPA) was conducted. Color tests were performed to determine if the addition of defatted soy flour, moisture content, or cooking temperatures had an effect on the appearance of the final product. Finally, protein solubility was performed to interpret the changes in chemical bonds that would cause the differences in the textures of the samples. Statistical methods, such as generalized linear models (GLM) and analysis of variance (ANOVA) were used to determine the significance of the variables and the relationships among results.

CHAPTER 1

INTRODUCTION

Soy based foods have great nutritional benefits and functional properties associated with them (Morr and Ha, 1991). One of the types of soy foods produced are meat analogs. Meat analogs have been traditionally produced using soy protein isolates along with wheat gluten and other proteins. These soy protein isolates are highly processed and likely have lost many of the benefits that make soy foods appealing to consumers in the United States (Allred *et al*, 2004). Alternatively, soy foods that are consumed in Asian countries are generally made from soy flours or soybeans that have been through minimal processing. Some believe that the soy foods made from the minimally processed soy flours will contain a more health benefits than those composed from the highly processed soy protein isolates (Allred *et al*, 2004; Setchell and Cole, 2003).

Extrusion cooking is a process by which meat analogs are produced. This process has been used in processing food for a number of years. Other foods, such as breakfast cereals, some pastas, pet foods, and cheese puffs are also made by extrusion cooking. Meat analogs can be produced at either low moisture conditions, fewer than 35% by use of a single-screw extruder, or at high moisture conditions, above 50% by use of a twin-screw extruder (Lin *et al*, 2000; Lin *et al*, 2002). Extrusion is also the most common method used to give the soy product a meat-like texture (Burgess and Stanley, 1976; Hager, 1984).

During extrusion, the soy is texturized. The fibrous texture of the product has been thought to be caused by intermolecular disulfide bonding (Cumming *et al*, 1973). This explanation agrees with studies done by Kelley and Pressey (1966) and also Chiang and Sternberg (1974), who stated that spun soy fibers are held together by intermolecular disulfide bonds. Work done by Stanley and Burgess (1976) suggests that intermolecular peptide bonds formed during extrusion are responsible for the structure of the meat analog. A study performed by Hager (1984) did not show the pronounced decrease in disulfide levels that Stanley and Burgess (1976) had reported. Hager (1984) made the suggestion that peptide bond formation required a temperature of at least 180°C, while disulfide bonds were responsible at lower temperatures (110-150°C).

The purpose of this experiment is to determine what bonds are responsible for the texturization of a meat analog composed of defatted soy flour, soy protein isolate, wheat starch, and wheat gluten and what effects extrusion variables will have on bond formation in addition to the effects on the physical characteristics of the meat analog. The physical characteristics include texture profile analysis, moisture, and color.

A factorial experiment with four levels of defatted soy flour (0, 15, 30, and 45%), three levels of feed moisture (55, 60, 65% w.b.), and three cooking temperatures (165, 174, and 182°C) and two replications were conducted on an APV Baker MPF 50/25 twin-screw extruder.

CHAPTER 2

LITERATURE REVIEW

2.1 Soybeans

2.1.1 History:

Asian culture has used soy extensively for centuries as a food (Henkel, 2004). Soybeans have also been used in western culture as well, though more often as a livestock feed than for human consumption. In the last few decades, however, Western culture has slowly begun to accept soy as a product for human consumption. Dietary soy is still considered by some as a niche market (Henkel, 2004). However, retail sales of soyfoods have increased from less than $1 billion in 1992 to nearly $4 billion in 2002. This is great news for the United States, since soybeans are a huge cash crop for the U.S. (Henkel, 2004). Major soybean production in the United States started in 1920, when the National Soybean Growers Association was formed. In 1928, an agreement was made between soybean growers and industrial processors for a production of 50,000 acres of soybeans (Anonymous, 2007a). From 1940 – 1946, soybean production increased from 78 million bushels to 201 million bushels in the U.S. alone. At the time, Germany and Japan were major international soybean markets for the United States and by 1949 the United States was exporting 23 million bushels of soybeans (Anonymous, 2007b). According to the American Soybean Association (Anonymous, 2007b), soybeans are now a $14 billion commodity in the United States and its second largest cash crop. Now, more soybeans are grown in the United States than anywhere else in the world (Anonymous, 2007b).

2.1.2 Soy Protein:

Soybeans contain roughly 40% - 45% (w/w) of protein that is dependent upon the conditions under which they were grown (Berk, 1992; Lin, 1998). Soy protein is actually composed of several proteins that have a various range of different sizes. Most of the protein in soy is globulin, which is soluble in salt solution (Berk, 1992). Soy protein has been utilized by the food industry to serve as a replacement of animal protein (Lin *et al*, 2000; Maurice *et al*, 1976; Burgess and Stanley, 1976). One reason for this is because the protein value of soy is comparable to that of meat protein (deMan, 1990). Another reason that soy proteins have been used as a replacement for animal proteins is that the risk of heart disease can be decreased by the ingestion of soy proteins. The decreased risk of heart disease is most likely associated with a significant decrease in serum cholesterol, LDL cholesterol, and triglyceride concentrations (Anderson *et al*, 1995; Henkel, 2004). In addition, soybeans contain all of the amino acids that are essential to human nutrition and contain less fat than animal foods (Henkel, 2004).

2.1.3 Nutritional Benefits:

The major nutritional benefit of soy is its association with the decreased risk of heart disease. In 1999 the FDA allowed a health claim on food labels, when the food contains at least 25 grams of soy protein and is also low in saturated fat and cholesterol, which may help reduce the risk of heart disease (Henkel, 2004). In laboratory animals, consumption of soy protein in place of animal protein decreased the serum cholesterol concentrations. This cholesterol lowering effect has been recognized in laboratory animals for more than 80 years (Anderson *et al*, 1995). In addition, the ingestion of soy protein in place of animal protein resulted in less hypercholesterolemia and less atherosclerosis in laboratory animals (Carroll, 1982). In a report by Anderson *et al* (1995), the total cholesterol of 730 subjects in 38 studies decreased an average of 23.2 mg/dl. Also, in this report, the LDL cholesterol of 564 subjects in 31 studies decreased an average of 21.7 mg/dl while HDL cholesterol in 551 subjects in 30 studies increased 1.2 mg/dl. Triglyceride levels and VLDL levels also decreased in the subjects in these studies. This report shows that soy proteins can have a significant effect in reducing cholesterol in humans, which in turn lowers the risk of heart disease.

2.2 Wheat Gluten

The major contribution of wheat gluten is a supplementary role to help hold the fibers that are produced from the soy protein together in the matrix (Rizvi *et al*, 1980). This essentially makes the wheat gluten a binder in this system so the product will stick together and remain stable. Wheat gluten is a wheat protein concentrate and is prepared by removing starch from wheat flour. The remaining gluten is then dried in a manner to retain the native properties. Properties of wheat gluten include strengthening of dough, gas retention, and a controlled expansion of the product (Appendix B).

2.3 Wheat Starch

Wheat starch can improve the water holding capacity of products and also acts as a fat emulsion stabilizer. Starches can also have a variety of other functional properties in foods. In addition to having a high water holding capacity and acting as a fat emulsion stabilizer, wheat starch can also help to improve the shelf-life of a product. Another quality of wheat starch is that it has a bland taste, and will therefore not interfere with the taste of a product that it is being used in (Satin, 2007). This property is especially helpful when developing a product that may need to make use of one of the many other properties of wheat starch without influencing the product's overall taste.

2.4 Extrusion

2.4.1 Extruders and Extrusion Cooking

Extrusion processing of food materials was first used in 1935 with pasta (Maurice and Stanley, 1978). However, at that time, food extruders were used mainly for mixing and forming the product. Cooking was not an extruder function at the time (Maurice and Stanley, 1978). In the last 50 years, however, extrusion cooking has been utilized by the food industry (Lin, 1998). A few examples of extruded products are ready-to-eat cereals, pet foods, pastas, cheese puffs, and various other snack foods. Pastas and ready-to-eat cereals were originally made with a single-screw extruder that was used to mix and form the product. Now, extrusion cooking can be done with either twin-screw extruders or single-screw extruders. This modern method of extrusion can be defined as high-temperature short-time (HTST) food processing (Harper, 1989; Lin, 1998).

Extrusion cooking also has many advantages over other processing methods, such as:

1. Extruders are a flexible, straight-forward machine that helps reduce the risk of contamination.
2. Capability of thermal and mechanical processing of different types of materials.
3. Processing time is short and easily controlled.
4. Processing conditions are easily monitored and controlled (Cheftel *et al*, 1992; Lin, 1998).

Single-screw extruders have been in use longer than twin-screw extruders. These single-screw extruders were mainly used for the processing of pastas and cereals. Operation of a single-screw extruder is dependent on many factors, including the pressure that is built up at the die, barrel temperature, screw speed, feed rate, and the presence of barrel wall grooves (Harper, 1989). Moisture contents of products that are extruded by single-screw extruders are traditionally lower than products produced by twin-screw extruders.

In addition to the single-screw extruder, there are also two types of twin-screw extruders available, co-rotating and counter-rotating twin-screw extruders. In food processing, co-rotating extruders are more popular due to a better mixing capability and a higher capacity (Harper, 1989; Lin, 1998). Several processing parameters can affect the final product. In addition to the parameters listed for single-screw extruders (excluding barrel grooves), these parameters are the moisture content of the raw material, screw type, and screw configuration.

Overall, product quality can be controlled easier by the use of a twin-screw extruder. While single-screw extruders are sufficient for products such as pet foods and pastas, products that need better control and flexibility would be better produced using a twin-screw extruder (Harper, 1989).

2.4.2 Effects of Extrusion on Products

It is a known fact that extrusion has an effect on the final characteristics of any product due to the heat, shear force, and pressure that occurs during the extrusion process. The type and intensity of conditions that are present in extrusion can influence the amount of beneficial products contained in raw materials (Cumming *et al*, 1973; Mahungu *et al*, 1999). The extrusion process changes the physical structure of the raw particles and makes them more water soluble (Harper, 1988).

In addition to the chemical changes that can occur during extrusion, physical changes also take place. The most easily seen changes occur due to moisture that is added during extrusion and the cooking that takes place. Textural changes occur in the product depending on the moisture content and temperature. At different moisture contents and temperatures, the textural properties of the product will change (Lin *et al*, 2002; Maurice *et al*, 1976).

2.5 Texturization and Texture Profile Analysis

2.5.1 Texturization of Soy Protein

Traditional texturized vegetable proteins, such as tofu, have long been used in Asian countries such as China and Japan. With today's advancements in food processing, texturized meat analogs can be made into a product that more closely resembles meat (Lin, 1998). Some texturizing methods include extrusion texturization with soy flours and soy proteins with up to 30% moisture by single screw-extrusion and extrusion texturization with soy flours and soy proteins with >50% moisture by twin-screw extrusion (Noguchi, 1989). Most texturized vegetable proteins are produced using moderate moisture extrusion. Vegetable proteins texturized in this manner are required to be re-hydrated before serving, while vegetable proteins texturized by wet extrusion are ready to serve (Lin, 1998).

2.5.2 Texture Profile Analysis

There are several characteristics that are important to meat analogs that can be analyzed by texture profile analysis. Definitions of these physical and sensory properties are found in Table 2.1 (Larmond, 1976). Texture profile analysis is done using a texture analyzer and the profile is generated by using instrument readings for each parameter. The profile describes the overall textural property of the product. Texture profile analysis (TPA) had a major breakthrough with the development of the General Foods Texturometer (Bourne, 1978). This machine used a cylinder to compress the bite-size piece of food to approximately 25% of its original height two times. This was to imitate jaw action. The texturometer then used strain gauges and a strip-chart recorder to generate a force-time curve that portrayed the simulated masticatory action (Bourne, 1978).

Table 2.1: Sensory and physical textural characteristic definitions (Larmond, 1976)

Property	**Sensory**	**Physical**
Chewiness	Length of time required to masticate sample at a constant rate of force application to reduce it to a consistency suitable for swallowing	Energy required to masticate a food to a state ready for swallowing
Gumminess	Denseness that persists throughout mastication; energy required to get a semisolid food to a state ready for swallowing	Energy required to get a semisolid food to a state ready for swallowing
Hardness	Force required to compress a substance between molar teeth or between the tongue and palate	Force required to attain a given deformation
Cohesiveness	Degree to which a substance is compressed between the teeth before it breaks	Extent to which a material can be deformed before it ruptures
Springiness	Degree to which a product returns to its original shape once it has been compressed between the teeth	Rate at which a deformed material goes back to its undeformed condition after the deforming force has been removed

Table 2.2: Definitions of terms used in Texture Profile Analysis (TPA) (Bourne, 1978)

Term	Definition
Hardness	The height of the force peak on the first compression cycle ("first bite")
Cohesiveness	The ratio of the positive force areas under the first and second compressions (A_2/A_1)
Springiness	The height that food recovers during the elapsed time between the end of the first bite and the beginning of the second; originally called elasticity
Adhesiveness	Negative force area of the first bite (A_3) which represents the work necessary to pull the compressing plunger from the sample
Fracturability	The force at the first significant break in the curve; originally called brittleness
Chewiness	Hardness × Cohesiveness × Adhesiveness
Gumminess	Hardness × Cohesiveness

The definitions of the terms used in TPA are shown in Table 2.2 (Bourne, 1978). The textural parameters that were identified by General Foods and analyzed with the texturometer gave results that correlated quite well with values that were obtained from sensory measurements that used a hardness scale (Bourne, 1978).

2.6 Protein Solubility

Changes in protein structure can occur during extrusion. These changes can be investigated by looking at the change in the protein solubility in different solvents. In addition, protein-protein interactions during the extrusion process can be determined using protein solubility. Burgess and Stanley (1976) used sodium phosphate buffer to extract proteins in their native states. To dissolve proteins with hydrogen bonds, urea was used in addition to the buffer. Disulfide bonds were disrupted by the use of 2-mercaptoethanol and sodium dodecyl sulfate (SDS) was used to interrupt the non-covalent bonds and hydrophobic interactions (Burgess and Stanley, 1976). Prudencio-Ferreira and Areas (1993) used a five state system to separate the proteins of an extruded meat analog product made with soy. The concentrations of the agents used in the buffering system are as follows: 35mM sodium phosphate buffer at pH 7.6 with the addition of one or more of 8M urea, 0.1M sodium sulfite, 0.1M acrilonitrile, 0.1M 2-merceptoethanol, and 1.5% sodium dodecyl sulfate. The five states that were examined were:

State 1: proteins soluble in simple buffer without additives.

State 2: proteins insoluble by non-covalent forces (proteins that had non-covalent linkages). These proteins are soluble in the presence of urea.

State 3: proteins insoluble due to disulfide covalent forces (proteins with disulfide linkages). The amount of proteins with disulfide linkages were determined by calculating the difference between those that can be dissolved in buffer with both disulfide cleaving agents and urea and those that are able to be dissolved in buffer with urea.

State 4: proteins that are insoluble due to a combination of disulfide and non-covalent interactions (proteins that have both disulfide and non-covalent linkages). This is determined by subtracting the amount of those in state 2 from those that can be dissolved in buffer with urea.

State 5: proteins that could not be dissolved by any of the previous buffer systems.

Prudencio-Ferreira and Areas (1993) found that moisture content and extrusion temperature affect the amount of protein found in each of the five states. At the lowest temperature (140°C), they found that most of the protein in the product was made insoluble by disulfide linkages. When the temperature was increased, there was less protein detected at this state. In addition, protein insolubility came from a combination of non-covalent and disulfide linkages and increased as the temperature increased.

Kelley and Pressey (1966) and Chiang and Sternberg (1974) found that the protein structure of the soy fibers is held together by hydrogen and hydrophobic bonds as well as disulfide bonds. This agrees somewhat with the data presented by Prudencio-Ferreira and Areas (1993), as hydrogen bonds and hydrophobic interactions are non-covalent forces.

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw Materials

The ingredients used for making the meat analog were defatted soy flour, soy protein isolate, wheat starch, and wheat gluten. The Baker's Flour brand defatted soy flour and Pro-Fam® 974 soy protein isolate were purchased from Archer Daniels Midland Company (Decatur, IL). The Midsol-50 wheat starch and vital wheat gluten were provided *gratis* by MGP Ingredients, Inc. (Atchison, KS). Table 3.1 shows the proximate composition of the defatted soy flour and soy protein isolate. The proximate composition of wheat starch and wheat gluten is shown in Table 3.2.

There were four mixture ratios of product in this study. All components were weighed individually first and then mixed in a single speed double action mixer (Model 100DA70, Leland Southwest, Forth Worth, TX) for 20 min in order to produce a homogeneous material. The amount of wheat gluten and wheat starch remained constant throughout the mixtures.

Table 3.1: Proximate composition of defatted soy flour and soy protein isolate

Nutrient content (%)	Defatted Soy Flour	Soy Protein Isolate
Carbohydrates (including total dietary fiber)	30.0	--
Protein (min)	53.0	90.0
Fat	3.0	4.0
Fiber	18.0	--
Moisture (max)	9.0	6.0
Ash	--	5.0

*--: indicates the information was not shown in the data sheets (Appendix A)

Table 3.2: Proximate composition of vital wheat gluten and wheat starch

Nutrient content (%)	Vital Wheat Gluten	Wheat Starch
Carbohydrates	--	--
Protein	75.0	0.3
Fat	2.0	--
Fiber	1.0	--
Moisture	8.0	12.0
Ash	1.1	0.3

*--: indicates that information was not shown in the data sheets (Appendix B)

3.2 Extrusion

The meat analogs were produced by a co-rotating and intermeshing twin screw extruder (MPF 50/25, APV Baker Inc., Grand Rapids, MI). Since the product does not need to expand as a breakfast cereal might, only five barrel zones were used for this project. The screw profile of the extruder was 8D spacer, 2 paddle spacers, 2D single lead feed screw (SL), 2D twin lead feed screw (TL), 4×30 forward paddle (FP), 2D SL, 7×30 FP, 3.5D SL, 7×30 FP, 4×30 reversing paddle (RP), and 2D SL. The temperatures of the barrel zones were as follows: room temperature, 44°C, 100°C, 155°C, and the last zone was either 165, 174, or 182°C depending on the temperature level for the run. Temperature settings for each zone depended upon the purpose of that particular zone. The first zone, at room temperature, was the feeding zone where the dry flour entered into the extruder. Water was added in the second zone, which mixed with the dry flour to produce a dough. The last three zones were for mixing, kneading, and cooking the product. A cooling die was placed at the end of the extruder die plate to cool the product as it left the extruder. The dimensions of the cooling die were 30cm x 10cm x 1cm (L×W×H). A mixture of anti-freeze and water was circulated through a cooler (Neslab Instruments, Portmouth, NH, Model HX-200). The temperature of the cooler was set at 4°C. The cooling die serves to help build up pressure, prevent expansion of the product, shape the extrudate, and also helps to ensure continuity (Cheftel *et al*, 1992). In addition, the die dimensions are also important for the formation of the fibrous structures in the meat analog (Noguchi, 1989). Besides the temperatures, screw configuration will also affect how much shear and pressure is present in the extruder. This can affect the fiber formation in the meat analog. During preliminary testing, the defatted soy flour, soy protein isolate, wheat gluten, wheat starch mixture was able to form a testable product in a 15D co-rotating and intermeshing screw configuration. The screws that were used by the extruder had a diameter (D) of 50 mm. The homogenized flour mixture was held in a K-tron type T-35 twin screw volumetric feeder (K-tron Corp., Pitman, NJ). The feed rate was set at 9.07 kg/h (20 lb/h). Water was added at the second barrel zone of the extruder to mix with the flour to form dough in the final zones. A positive displacement pump was applied to add water to make the moisture content of the product 55%, 60%, and 65% in this study.

3.3 Experimental Design

Four levels of defatted soy flour were used in this study, along with three levels of moisture content and three different cooking temperatures to produce the meat analogs. Therefore, 36 ($4 \times 3 \times 3$) treatments were evaluated in this study and all the treatments were duplicated. The information for the three independent variables and their levels are shown in the Table 3.3. For the statistical analysis, the code numbers 1, 2, 3 and 4 were applied here.

Table 3.3: Three independent variables and their levels

Variables	**Level**			
Soy flour content (%)	0	15	30	45
Moisture content (%)	55	60	65	
Temperature (°C)	165	174	182	

Soy flour content is percentage on dry basis; moisture content refers to what the final moisture content to what the product was brought up.

Table 3.4: Dry mix composition

Ingredient	**Level (%)**			
Defatted Soy Flour	0	15	30	45
Soy Protein Isolate	60	45	30	15
Wheat Gluten	35	35	35	35
Wheat Starch	5	5	5	5

3.4 Analytical Methods

3.4.1 Moisture Content

Moisture content was analyzed before protein solubility studies were performed. Approximately 2 g of sample were weighed out in an aluminum weighing dish (weighed previously) and the weight of the sample plus the aluminum dish was recorded. The aluminum dishes containing the samples were then placed into a convection oven for 18 h at 105°C. After being removed from the oven, the samples were placed in a desiccator for approximately 2 h so that no moisture was reabsorbed into the sample from the atmosphere. After cooling, the samples in the aluminum dishes were reweighed and the weight lost was recorded as moisture.

3.4.2 Texture Profile Analysis

The texture profile analysis of the meat analog samples was done using a texture analyzer (Model TA-XT2, Texture Technologies Corp., Scarsdale, NY). Six cylindrical samples were cut from each sample and then placed one at a time on a metal support underneath the texture analyzer. A cylindrical probe with a diameter of 50 mm was attached to the texture analyzer. The samples were then compressed by 50% twice to simulate two bites from a human subject. The test speed was set at 2 mm/s. Each of the samples was approximately 10 mm tall and 10 mm in diameter. Six samples from each product were randomly cut using a metal hole punch. Values for hardness, springiness, cohesiveness, chewiness, gumminess, and adhesiveness were reported in this study.

3.4.3 Color Analysis

The Konica Minolta chromameter (Model CR-410, Konica Minolta, Inc., Mahwah, NJ) had to be standardized by a white chromatic reference tile whenever it was used. The values of the reference tile were L*=96.95, a*=0.15, and b*=1.92. The Konica Minolta chromameter was placed directly above each defatted soy flour – soy protein isolate or soy protein isolate meat analog sample. Since the chromameter was a hand held model, it had to be placed directly on the sample so that it could measure the color of the sample it analyzed. Three measurements for each sample were taken in this study. The Konica Minolta chromameter result gives three different color units: lightness (L*), redness (a*), and yellowness (b*). The L* value was from 0 for darkness to 100 for brightness. The +a* and –a* were representative of redness and greenness. The +b* and –b* corresponded to yellowness and blueness. Three color units were recorded in each measurement and would be analyzed using SAS.

3.4.4 Protein Solubility

Protein solubility was performed in several different buffers to find out what bonds were responsible for the texturization of the product. The buffers used and their properties (Burgess and Stanley, 1976; Lin, 1998; Lin *et al*, 2000) are shown in Table 3.5.

Table 3.5: Buffers used in protein solubility

Buffer	Property
Sodium phosphate buffer pH 7.6, 35mM	Extracts proteins in their native state
Sodium phosphate buffer 35mM + 8M Urea	Extracts native proteins as well as interrupts hydrogen bonds
Sodium phosphate buffer 35mM + 1.5% SDS	Extracts native proteins and breaks non-covalent bonds and hydrophobic interactions
Sodium phosphate buffer 35mM + 0.1M 2-mercaptoethanol	Extracts native proteins and breaks disulfide bonds
Sodium phosphate buffer 35mM + 8M Urea + 1.5% SDS	Extracts native proteins, interrupts hydrogen bonds, and breaks non-covalent bonds and hydrophobic interactions
Sodium phosphate buffer 35mM + 8M Urea + 0.1M 2-mercaptoethanol	Extracts native proteins, interrupts hydrogen bonds, and breaks disulfide bonds

The buffers were those that were used by Burgess and Stanley (1976). Other studies used buffers that contained urea (Chiang and Sternberg, 1974 and Hager, 1984), 2-merceptoethanol (Chiang and Sternberg, 1974), and SDS (Boatright and Hettiarachchy, 1995).

Preparation of the samples began with finely chopping the defrosted sample in a chopper (Wal-Mart model 106848) for 1 min. Approximately 1.5g of sample was weighed out and was extracted in 30ml of solvent for 2.5h at 35°C in a shaking water bath. Once being removed from the water bath, the samples were then centrifuged at 12,900 rpm (20000 g) for 40 min with a Beckman J2-21 M/E centrifuge (Schaumburg, IL).

A Bradford assay using a coomassie blue dye was done on the samples that were in phosphate buffer, phosphate buffer with urea, phosphate buffer with 2-mercaptoethanol, and phosphate buffer with urea and 2-mercaptoethanol. Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL) was used as an indicator and the samples were measured at 595 nm with a Genesis 20 spectrophotometer (Thermo Spectronic, Rochester, NY).

For the samples that used the phosphate buffer with SDS and the phosphate buffer with urea and SDS, a BCA Protein Assay Kit (Pierce, Rockford, IL) was used to determine the amount of solubilized protein. The BCA kit was used for the buffers with SDS due to its detergent properties. The SDS interferes with the Coomassie blue stain used in Bradford assays, therefore, a reagent with less sensitivity to SDS had to be used to determine the protein solubility in these samples (Robyt and White, 1990). The BCA reagent used was more tolerable to the SDS concentration in the buffer solution than the Coomassie blue reagent. The Coomassie plus protein assay kit had a 0.016% compatible substance concentration for SDS while the BCA kit had a compatible substance concentration of 5.0% for SDS. This means that a solution containing 0.016% SDS would be the highest concentration able to be read using the Coomassie Plus assay kit, and a solution containing 5.0% SDS would be the highest concentration able to be read using the BCA kit.

3.5 Statistical Analysis

All statistical analysis was done using SAS® version 9.1 to determine the significance of the variables. Analysis of variance (ANOVA) and general linear model (GLM) were both used along with the MEANS procedure.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Extruder Responses

4.1.1 Results and Discussion

The extruder responses from soy flour percentage for torque, product temperature, and die pressure are shown in Table 4.1. It can be seen that as soy flour percentage increased, the product temperature gradually decreased until the 30-45% range where the product temperature was nearly identical. Other experimental variables, such as the moisture content and cooking temperature, also had an effect on the extruder responses. The effects from moisture content and cooking temperature can be seen in Tables 4.2 and 4.3 respectively.

Table 4.1 Effect of Soy Flour on Extruder Responses

Soy Flour (%)	Product Temp. (°C)	Torque (%)	Die Pressure (kPa)
0	117.39^{a}	8.15^{a}	475.27^{a}
15	115.10^{b}	6.99^{b}	330.39^{d}
30	114.04^{c}	7.02^{b}	350.18^{c}
45	114.20^{c}	6.94^{c}	440.57^{b}

Differing superscripts within the same column indicate a significance at $p<0.001$

The defatted soy flour could have possibly reduced the dough viscosity, which would cause the percentage of torque and the die pressure to decrease. A reduction in dough viscosity is thought to be the cause in a reduction in die pressure and percentage of torque (Lin et al, 2000). The decrease in product temperature could possibly be due to the effect of replacing soy protein isolate with soy flour, which could reduce the viscosity, thus causing less friction and a lower torque, which, in turn, would result in a lower product temperature. Viscosity measurements of soy protein isolate and defatted soy flower confirm this as the viscosity of defatted soy flour was observed to be in the range of 115 to 125 cP, while the viscosity of soy protein isolate was observed to be in the range of 7500 to 8000 cP.

Table 4.2 Effect of Moisture Content on Extruder Responses

Moisture Content (%)	Product Temp. (°C)	Torque (%)	Die Pressure (kPa)
55	117.24^{a}	8.01^{a}	519.51^{a}
60	115.85^{b}	7.21^{b}	383.89^{b}
65	112.47^{c}	6.60^{c}	293.92^{c}

Differing superscripts within the same column indicate a significance at $p<0.001$

The reduction in the percentage of torque and die pressure as the moisture content increased was due to a reduction in the viscosity of the dough (Lin et al., 2000). The decrease in product temperature as the moisture content was increased was due to the reduced shear force and the mechanical energy input, since the water served as a lubricant (Lin et al, 2000).

Table 4.3 Effect of Cooking Temperature on Extruder Responses

Cooking Temperature (°C)	Product Temp. (°C)	Torque (%)	Die Pressure (kPa)
165	109.50^{c}	7.38^{a}	380.58^{b}
174	115.92^{b}	7.28^{b}	408.30^{a}
182	120.14^{a}	7.15^{c}	408.44^{a}

Differing superscripts within the same column indicate a significance at $p<0.001$

The product temperature increased as the cooking temperature increased because the cooking temperature has a direct effect on the temperature of the product. The percentage of torque decreased as the cooking temperature increased, due to a reduction in the dough viscosity (Lin et al., 2000). However, the reduction in die pressure that would be expected with a reduction in dough viscosity was not observed. This could be due to cooking temperature having a lesser effect on the dough viscosity than moisture content.

4.1.1.1 Product Temperature

4.1.1.1.1 Results

Additionally, extruder responses can also be seen from a combination of variables. Table 4.4 shows the effects for all combinations of soy flour and moisture content. Product temperature was the highest at 55% moisture content and 0% soy flour and the lowest at 65% moisture with the soy flour content in the 15-30% range. When the moisture content and soy flour percentage increased, a decrease in product temperature was observed. This was because both increasing moisture content and increasing soy flour percentage reduced the dough viscosity as stated earlier. Table 4.5 shows the trend for product temperature due to cooking temperature and soy flour percentage. Again, in general a lower product temperature was observed when increasing soy flour percentage and when lowering the cooking temperature. The highest product temperature was observed at a cooking temperature of 182°C and a 0% soy flour mixture. Table 4.6 shows the trend for product temperature due to cooking temperature and moisture content.

Table 4.4 Effect of Product Temperature from Soy Flour and Moisture Content

	Product Temperature (°C)		
	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	120.18^{a}_{A}	117.86^{a}_{B}	114.14^{a}_{C}
15	117.69^{b}_{A}	115.72^{b}_{B}	111.88^{b}_{C}
30	115.39^{c}_{A}	114.98^{c}_{B}	111.76^{b}_{C}
45	115.71^{c}_{A}	114.82^{c}_{B}	112.08^{b}_{C}

Superscripts indicate a significant effect at p<0.001 from soy flour; subscripts indicate a significant at p<0.001 effect from moisture content.

Table 4.5 Effect of Product Temperature from Cooking Temperature and Soy Flour

	Product Temperature (°C)		
	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	111.18^{a}_{C}	118.37^{a}_{B}	122.63^{a}_{A}
15	108.18^{d}_{C}	116.51^{b}_{B}	120.60^{b}_{A}
30	108.68^{c}_{C}	114.32^{c}_{B}	119.13^{c}_{A}
45	109.94^{b}_{C}	114.48^{c}_{B}	118.19^{d}_{A}

Superscripts indicate a significant effect from soy flour at p<0.001; subscripts indicate a significant effect at p<0.001 from cooking temperature.

Table 4.6 Effect of Cooking Temperature and Moisture on Product Temperature

	Product Temperature (°C)		
	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	112.98^{a}_{C}	116.35^{a}_{B}	122.40^{a}_{A}
60	110.43^{b}_{C}	116.42^{a}_{B}	120.69^{b}_{A}
65	105.07^{c}_{C}	115.00^{b}_{B}	117.33^{c}_{A}

Superscripts indicate a significant effect from moisture content at $p<0.001$; subscripts indicate a significant effect at $p<0.001$ from cooking temperature.

4.1.1.1.2 Discussion

The product temperatures reported by Lin (2000) are higher than those reported in this study, probably due to differing cooking temperatures and moisture contents in the studies. In addition, the viscosity of soy protein isolate was much higher than the viscosity of defatted soy flour. The increased viscosity would have caused more friction, which would in turn increase the temperature of the product. However, they do follow the same trends. When looking at the interaction of soy flour and moisture content on product temperature, the moisture content has a greater effect at low soy flour concentrations and a reduced effect at high soy flour concentrations. This could be due to the increased moisture content helping to reduce viscosity in high soy protein isolate mixes, where the viscosity would be greater. The product temperatures in both studies are higher when there is a lower moisture content and higher cooking temperature, decrease as the cooking temperature is decreased, and increase as the moisture content is decreased. A decrease in product temperature as the moisture content increased was due to a reduced shear force and mechanical energy input, because water serves as a lubricant

(Lin et al, 1997; Hayashi et al, 1993). The difference in the product temperatures themselves could be due to the studies having different mixtures for the meat analogs, or to the different cooking temperatures and moisture contents used in the studies, or a combination of both.

4.1.1.2 Torque

4.1.1.2.1 Results

Torque is a very important factor to consider in extrusion cooking. While the torque percentage during extrusion was quite low for this product, it is still an important factor to consider. From Tables 4.1 and 4.2 it can be seen that soy flour and moisture content had a fairly significant effect on torque compared with the effect of cooking temperature (Table 4.3). The effects for all soy flour and moisture content combinations are shown in Table 4.7.

Table 4.7 Effect of Soy Flour and Moisture Content on Torque

	Torque (%)		
	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	9.26^{a}_{A}	8.01^{a}_{B}	7.17^{a}_{C}
15	7.51^{c}_{A}	6.95^{c}_{B}	6.51^{b}_{C}
30	7.61^{b}_{A}	7.07^{b}_{B}	6.38^{c}_{C}
45	7.68^{b}_{A}	6.80^{d}_{B}	6.33^{c}_{C}

Superscripts indicate an effect from soy flour at $p<0.001$; subscripts indicate a significant effect from moisture content at $p<0.001$.

As seen from Table 4.7, the highest torque was observed when extruding the 0% soy flour mix with a moisture content of 55%. Torque decreased with an increase in moisture and remained fairly consistent with higher percentages of soy flour. This is most likely due to the moisture content contributing more to the torque decrease, while just the presence of soy flour helps decrease torque.

Table 4.8 shows the effect on torque for all soy flour mixes and cooking temperatures.

Table 4.8 Effect of Soy Flour and Cooking Temperature on Torque

	Torque (%)		
	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	8.16^{a}_{A}	8.21^{a}_{A}	8.06^{a}_{B}
15	7.13^{b}_{A}	6.90^{c}_{B}	6.95^{b}_{B}
30	7.13^{b}_{A}	7.04^{b}_{B}	6.88^{b}_{C}
45	7.10^{b}_{A}	6.98^{bc}_{B}	6.72^{c}_{C}

Superscripts indicate a significant effect at $p<0.001$ from soy flour; subscripts indicate a significant effect at $p<0.001$ from cooking temperature.

From looking at Table 4.8 it can be seen again that the highest torque occurred with the extrusion of the 0% soy flour mix. The overall highest torque was observed with a soy flour mix of 0% and a cooking temperature of 174°C. Once again, the presence of soy flour contributes to a decrease in torque, while the concentration of soy flour seems to have no effect. In general, as the percentage of soy flour increased and/or cooking temperature increased, torque dropped which was consistent with the results of previous sections.

The effects of moisture content and cooking temperature on torque are shown in Table 4.9.

Table 4.9 Effect of Cooking Temperature and Moisture Content on Torque

	Torque (%)		
	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	8.10^{a}_{B}	8.20^{a}_{A}	7.73^{a}_{C}
60	7.27^{b}_{A}	7.14^{b}_{B}	7.22^{b}_{A}
65	6.77^{c}_{A}	6.52^{c}_{B}	6.51^{c}_{B}

Superscripts indicate a significant effect from moisture content at $p<0.001$; subscripts indicate a significant effect at $p<0.001$ from cooking temperature.

As can be seen from Table 4.9, the highest torque percentage occurred at 174°C and 55% moisture content. Again, torque in general was lower when increasing either moisture content, with the lowest torque percentage being observed at 182°C and 65% moisture content. Cooking temperature showed varying results at different moisture contents.

4.1.1.2.2 Discussion

The percentage of torque on the extruder was greater in the study by Lin (2000). The highest torque percentage reported by Lin (2000) was 12% and the lowest was 7.1%. In this study, the torque percentage did not go above 10%, with most of the values ranging around 7%. This could again be due to the different mixtures for the meat analogs, the different cooking temperatures or moisture contents, or any sort of combination. Lin and others (2000) suggested that a decrease in dough viscosity could cause a decrease in torque. In addition, the decrease in torque with increasing moisture content and increasing temperature was also most likely due to a reduction in viscosity. It is possible that the higher soy flour content had an effect on the dough viscosity as well, thus resulting with a decrease in torque.

Die Pressure

4.1.1.3.1 Results

Die pressure is a variable that must be carefully observed to avoid backups in the extruder. Tables 4.1, 4.2, and 4.3 show the effects of die pressure from varying levels of soy flour concentration, moisture content, and cooking temperature respectively. The following table, Table 4.10, shows the effect of soy flour and moisture content concentration on die pressure.

Table 4.10 Effect of Soy Flour and Moisture Content on Die Pressure

	Die Pressure (kPa)		
	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	658.44^{a}_{A}	449.39^{a}_{B}	318.05^{a}_{C}
15	417.26^{c}_{A}	315.36^{d}_{B}	258.55^{b}_{C}
30	429.05^{c}_{A}	354.31^{c}_{B}	267.23^{b}_{C}
45	573.43^{b}_{A}	416.43^{b}_{B}	331.77^{a}_{C}

Numbers with different superscripts indicate a significant difference at $p<0.001$ for soy flour; numbers with different subscripts indicate a significant difference at $p<0.001$ for moisture content.

As can be seen from Table 4.10, higher die pressures are observed at the lowest moisture content level (55%). Soy flour has a different effect on the die pressure, with the larger values being observed at the extreme concentrations (0% and 45%).

In Table 4.11, the effects from soy flour concentration and cooking temperature can be seen.

Table 4.11 Effect of Soy Flour and Cooking Temperature on Die Pressure

	Die Pressure (kPa)		
	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	445.60^{a}_{B}	486.83^{a}_{A}	493.45^{a}_{A}
15	297.91^{d}_{C}	334.25^{d}_{B}	359.07^{c}_{A}
30	357.28^{c}_{A}	359.90^{c}_{A}	333.49^{d}_{B}
45	421.54^{b}_{B}	452.22^{b}_{A}	447.94^{b}_{A}

Numbers with a different superscript indicate a significant difference at $p<0.001$ for soy flour; numbers with a different subscript indicate a significant difference at $p<0.001$ for cooking temperature.

Again, the same trend with soy flour concentration on die pressure is observed, with the larger die pressure values being seen at the extreme soy flour concentrations (0% and 45%). It can also be seen, that with the exception of the 30% soy flour mixture that the higher die pressure values occur at higher cooking temperatures. Soy flour initially decreased die pressure, but at higher concentrations increased the die pressure.

Finally, Table 4.12 shows the effect that moisture content and cooking temperature have on die pressure.

Table 4.12 Effect of Moisture Content and Cooking Temperature on Die Pressure

	Die Pressure (kPa)		
	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	517.03^{a}_{B}	547.50^{a}_{A}	494.07^{a}_{C}
60	354.31^{b}_{C}	371.34^{b}_{B}	426.02^{b}_{A}
65	270.41^{c}_{B}	305.98^{c}_{A}	305.29^{c}_{A}

Numbers with a different superscript indicate a significant difference at $p<0.001$ for moisture content; numbers with a different subscript indicate a significant difference at $p<0.001$ for cooking temperature.

Table 4.12 shows that, with the exception of 55% moisture content and 182°C, higher die pressures are observed at lower moisture contents. The lowest die pressure was observed at 65% moisture content and 165°C cooking temperature and the highest die pressure was observed at 55% moisture content and a cooking temperature of 174°C.

4.1.1.3.2 Discussion

The die pressures in the study by Lin et al. (2000) had much larger values than the die pressure values obtained from this study. The largest die pressure reported by Lin et al. (2000) was 1727.3 kPa, which is much larger than the die pressures obtained in this study. Once again, this could be due to a number of factors, such as the mixture composition, the differences in moisture contents, or the differences in cooking temperatures. As with torque, Lin and others (2000) suggested that the decrease in die pressure was most likely due to a decrease in dough viscosity. The higher cooking temperatures and higher moisture contents would reduce the dough viscosity, thus decreasing the die pressure.

4.2 Effects on Texture

4.2.1 Results and Discussion

The texture properties of the meat analog changed with varying levels of soy flour and moisture content, as well as with differing cooking temperatures. Only three textural properties were observed: gumminess, chewiness, and hardness. The other texture properties, adhesiveness, cohesiveness, and springiness, showed some varying results and played a role in the three observed attributes. Table 4.13 shows the effect of soy flour concentration on all textural attributes. As can be seen from the table, as the soy flour concentration is increased, chewiness, hardness, and gumminess decrease, though not much difference is seen between the 15% and 30% mixes. While statistically there were differences between soy flour concentrations for adhesiveness, cohesiveness, and springiness, these differences were also observed in chewiness, hardness, and gumminess. As chewiness and gumminess were dependent on cohesiveness, it will be discussed in those sections. Adhesiveness will be addressed when concerning chewiness, as chewiness was dependent on adhesiveness as well.

Table 4.13 Effect of Soy Flour on Texture

Soy Flour (%)	Adhesiveness (mm g)	Gumminess (g)	Chewiness (mm g)	Hardness (g)	Cohesiveness	Springiness (mm)
0	-1.80^{a}	1704^{a}	1646^{a}	2676^{a}	0.637^{a}	0.969^{a}
15	-4.91^{b}	1185^{b}	1134^{b}	1933^{b}	0.610^{b}	0.958^{b}
30	-5.96^{b}	1192^{b}	1094^{b}	2042^{b}	0.580^{d}	0.919^{d}
45	-7.41^{c}	823^{c}	768^{c}	1396^{c}	0.596^{c}	0.928^{c}

Superscripts indicate a significant difference at $p<0.001$

The level of moisture content and cooking temperatures of the samples also had an effect on the texture attributes of the product. The effects of moisture content are shown in Table 4.14 and the effects of cooking temperature are shown in Table 4.15.

Table 4.14 Effect of Moisture Content on Texture

Moisture Content (%)	Adhesiveness (mm g)	Gumminess (g)	Chewiness (mm g)	Hardness (g)	Cohesiveness	Springiness (mm)
55	-4.356^{a}	1851^{a}	1747^{a}	3027^{a}	0.613^{a}	0.936^{c}
60	-6.147^{b}	1193^{b}	1127^{b}	1959^{b}	0.605^{b}	0.941^{b}
65	-4.559^{a}	635^{c}	608^{c}	1050^{c}	0.600^{c}	0.954^{a}

Superscripts indicate a significant difference at $p<0.001$

Table 4.15 Effect of Cooking Temperature on Texture

Cooking Temp. (°C)	Adhesiveness (mm g)	Gumminess (g)	Chewiness (mm g)	Hardness (g)	Cohesiveness	Springiness (mm)
165	-4.373^{a}	1220^{ab}	1146^{ab}	2014^{ab}	0.603^{b}	0.939^{b}
174	-4.949^{ab}	1257^{a}	1195^{a}	2065^{a}	0.606^{ab}	0.945^{a}
182	-5.740^{b}	1201^{b}	1140^{b}	1957^{b}	0.608^{a}	0.948^{a}

Superscripts indicate a significant difference at $p<0.001$

Figure 4.1 Effect of Moisture Content and Die Temperature on Hardness

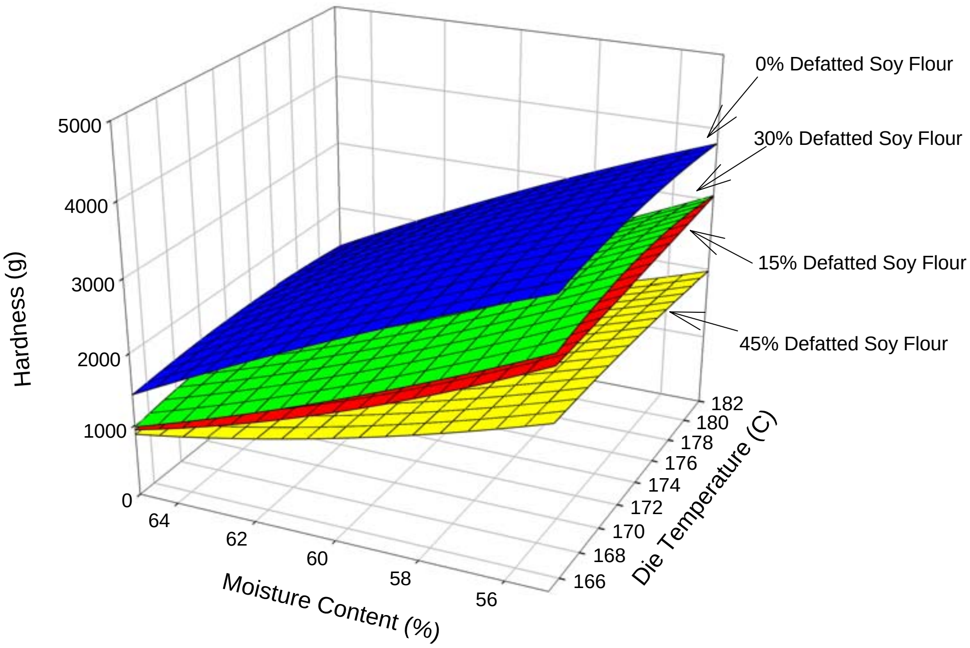


Figure 4.2 Effect of Moisture Content and Die Temperature on Chewiness

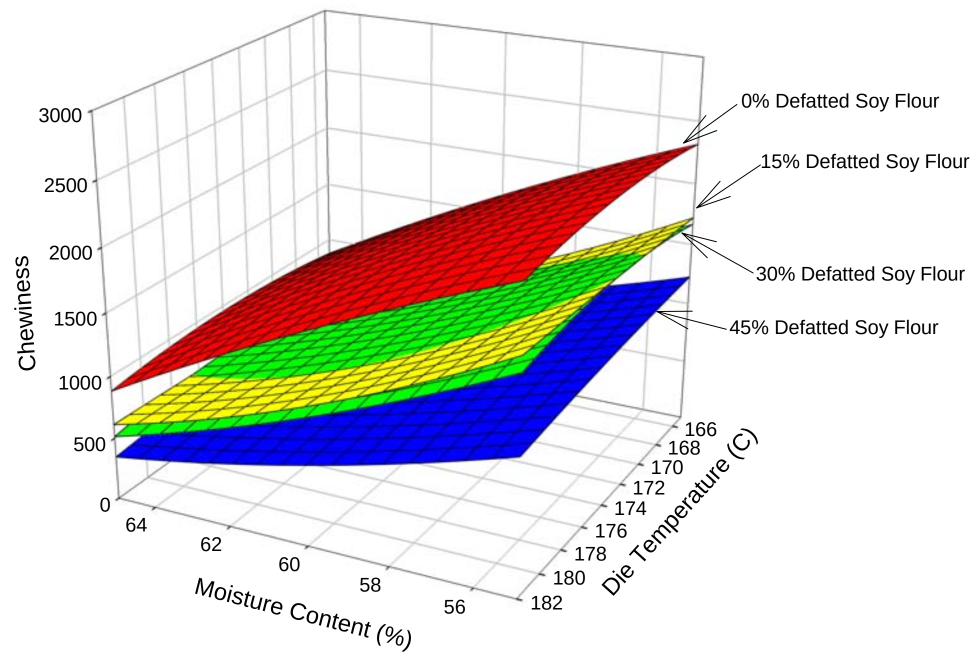
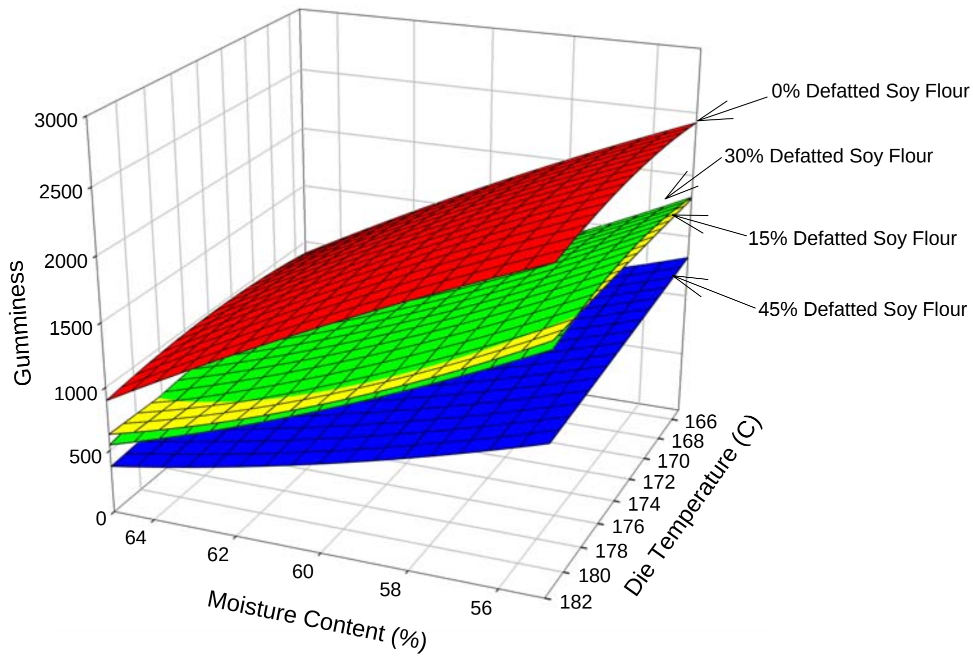


Figure 4.3 Effect of Moisture Content and Die Temperature on Gumminess



As can be seen from the Table 4.13 and Figures 4.1, 4.2, and 4.3, the mix that contained 0% defatted soy flour had the highest values for hardness, chewiness, and gumminess while the mix that contained 45% defatted soy flour had the lowest values for the same attributes. There was no significant difference seen between the 15% and 30% defatted soy flour mixes for these attributes.

The other texture attributes, with the exception of adhesiveness, followed different trends than hardness, chewiness, and gumminess. Adhesiveness seemed to follow a similar trend; with the 0% defatted soy flour mix having the larger value while the 45% defatted soy flour mix had the lower value. In addition, there was also no significant difference between the 15% and 30% mixes. Cohesiveness and springiness both showed significant differences between all mixes. In both cases the 0% defatted soy flour mix had the largest values, followed by the 15% mix. Also, both attributes had larger values for the 45% mix than they did for the 30% mix. In addition, decreased moisture content resulted in higher values for hardness, chewiness, and gumminess.

Table 4.14 shows that as the moisture content of the product increased, the gumminess, chewiness, and hardness of the product decreased. The cohesiveness and springiness seemed to remain fairly consistent even though the moisture increased. Adhesiveness was the only oddity observed, with the adhesiveness decreasing from 55% moisture to 60% moisture and then increasing from 60% moisture to 65% moisture. The adhesiveness values obtained at 55% moisture and 65% moisture were not statistically different.

The differences in texture that occurred from cooking temperature are shown in Table 4.15. Also, as can be seen from Figures 4.1, 4.2, and 4.3, cooking temperature did not have as great an effect on gumminess, chewiness, and hardness as the percentage of soy flour or moisture content. As with the case of moisture content, cooking temperature did not seem to affect cohesiveness and springiness very much. While the values that were obtained for these attributes were all statistically different, the values themselves did not differ very much from one to the other. Adhesiveness, however, seemed to be affected by the cooking temperature and amount of defatted soy flour, with the adhesiveness value decreasing as the cooking temperature and soy flour concentration increased.

4.2.1.1 Hardness

4.2.1.1.1 Results

Hardness is one of the six main texture profiles measured in this experiment. It is defined as the peak force during the first compression cycle (Bourne, 1978). Of the six attributes that were measured, hardness was one of the three attributes that showed major differences between samples. Table 4.16 shows the effect of moisture content and soy flour percentage on the hardness of the product.

Table 4.16 Effect of Moisture Content and Soy Flour on Hardness

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	3843^{a}_{A}	2735^{a}_{B}	1449^{a}_{C}
15	3000^{c}_{A}	1819^{c}_{B}	981^{b}_{C}
30	3154^{b}_{A}	1970^{b}_{B}	1012^{b}_{C}
45	2119^{d}_{A}	1312^{d}_{B}	757^{c}_{C}

Different superscripts indicate a significant difference for soy flour at $p<0.001$; different subscripts indicate a significant difference for moisture content at $p<0.001$

There are two trends that can be observed from this table. One is that as the moisture content increases, the hardness decreases. The other is that as the amount of soy flour increases, the hardness decreases. However, from 15% to 30% soy flour concentration, the rule to this case changes. Between the 15% and 30% soy flour mixes, the 30% soy flour mix has a larger hardness value than the 15% mix at all different moisture contents. However, it should be noted that, with the exception of the 65% moisture product, the values are larger for the 30% mix and they are statistically different, but not much larger. Cooking temperature can also cause a change in the hardness value as previously discussed.

Table 4.17 shows the effect of soy flour percentage and cooking temperature on hardness.

Table 4.17 Effect of Soy Flour and Cooking Temperature on Hardness

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	2656^{a}_{A}	2742^{a}_{A}	2629^{a}_{A}
15	1885^{b}_{A}	1929^{c}_{A}	1987^{b}_{A}
30	2005^{b}_{B}	2158^{b}_{A}	1963^{b}_{B}
45	1510^{c}_{A}	1430^{d}_{A}	1249^{c}_{B}

Different superscripts indicate a significant difference for soy flour at $p<0.001$; different subscripts indicate a significant difference for cooking temperature at $p<0.001$

It can be seen from the above table that the effect that soy flour has on the hardness of the product remains the same as from the previous table (Table 4.16). The hardness values decrease as the soy flour percentage of the mix increases from 0% to 15% and from 30% to 45%. In addition, the same trend that is observed for soy flour concentration and moisture, is also observed here, in that the 30% soy flour mix has a larger hardness value than that of the 15% mix for all cooking temperature, with the exception of the value obtained at 182°C. Again, while the 30% soy flour mix values are larger than those of the 15% soy flour mix, they are not statistically different, with the exception of the occurrence at the 174°C cooking temperature. The largest difference from the effect of cooking temperature on hardness occurs between 174°C and 182°C.

The hardness values at these two cooking temperatures are statistically different from one another, with the hardness values obtained at 165°C being statistically similar to the values obtained at 174°C and 182°C. When the percentage of soy flour in the mix is not taken into consideration, the hardness values of the product can change.

Table 4.18 shows the effect of moisture content and cooking temperature on the hardness of the product.

Table 4.18 Effect of Moisture Content and Cooking Temperature on Hardness

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	3029^{a}_{B}	3167^{a}_{A}	2884^{a}_{C}
60	1912^{b}_{A}	2016^{b}_{A}	1949^{b}_{A}
65	1100^{c}_{A}	1011^{c}_{B}	1038^{c}_{A}

Different superscripts indicate a significant difference for moisture content at $p<0.001$; different subscripts indicate a significant difference for cooking temperature at $p<0.001$

As seen in the above table, a greater hardness value is obtained at 55% moisture content for all cooking temperatures. Cooking temperature, however, shows a different trend. It can be seen that the highest hardness values are obtained at the 174°C cooking temperature for the 55% and 60% moisture content products, while for the 65% moisture content product the highest hardness value is obtained at 165°C. Also, the lowest hardness value for each moisture content product was observed at different cooking temperatures. For the 55% moisture content product, the lowest hardness value was obtained at 182°C, for the 60% moisture content product, the lowest hardness value was observed at 165°C, and for the 65% moisture content product, the lowest hardness value was obtained at 174°C.

4.2.1.1.2 Discussion

The hardness values obtained in this study observe the same pattern as the hardness values reported by Lin et al. (2000). As the moisture content of the product is increased, the hardness value decreases, and as the cooking temperature is increased, the hardness value decreases. However, the values in this study were much lower than those obtained by Lin et al. (2000). While the lowest value for hardness obtained by Lin (2000) was 3524 g, the lowest value obtained in this study was below 1000 g. This could be from the differences in the mixture composition or differences in the cooking temperatures and moisture contents in the studies.

4.2.1.2 Chewiness

4.2.1.2.1 Results

Chewiness is another texture attribute that was measured in the experiment and the second of three attributes that showed a large value difference between samples. Chewiness is defined as the product of gumminess and springiness, which is equal to the product of hardness, cohesiveness, and springiness (Bourne, 1978).

The effects of soy flour percentage and moisture content on chewiness are shown in Table 4.19.

Table 4.19 Effect of Soy Flour and Moisture Content on Chewiness

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	2347^{a}_{A}	1686^{a}_{B}	903^{a}_{C}
15	1770^{b}_{A}	1060^{b}_{B}	570^{b}_{C}
30	1711^{b}_{A}	1038^{b}_{B}	535^{b}_{C}
45	1159^{c}_{A}	724^{c}_{B}	423^{c}_{C}

Superscripts indicate a significant difference for soy flour at p<0.001; subscripts indicate a significant difference for moisture content at p<0.001.

From the table, it can be seen that as the soy flour content in the sample increases, the chewiness of the sample decreases. While there are no significant difference in the chewiness values between the 15% and 30% soy flour samples, at all moisture contents the 30% soy flour sample showed the lower chewiness value. Also, it can be seen that as the moisture content increased, the chewiness value decreased in all soy flour mixes.

The effects of soy flour concentration and cooking temperature on chewiness can be seen in Table 4.20.

Table 4.20 Effect of Soy Flour and Cooking Temperature on Chewiness

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	1616^{a}_{B}	1697^{a}_{A}	1624^{a}_{B}
15	1084^{b}_{B}	1143^{b}_{AB}	1174^{b}_{A}
30	1051^{b}_{B}	1160^{b}_{A}	1072^{c}_{B}
45	835^{c}_{A}	780^{c}_{A}	690^{d}_{B}

Superscripts indicate a significant difference for soy flour at p<0.001; subscripts indicate a significant difference for cooking temperature at p<0.001.

It can be seen from Table 4.20 that as the percentage of soy flour in the product increased, the chewiness value decreased. The one exception to this trend is observed between the 15% and 30% soy flour mixes at the 174°C cooking temperature. Here, while the values between the two were not significantly different, the 30% mix had a slightly larger chewiness value than the 15% mix. At the 165°C and 182°C cooking temperatures, the 15% and 30% soy flour mixes also did not have a significant difference. The effect of cooking temperature was quite different than that of the soy flour. For the 0% and 30% soy flour mixes, the chewiness values went up from 165°C to 174°C, but then decreased from 174°C to 182°C. The 15% soy flour mix had a steady increase in chewiness values from 165°C to 182°C, while the 45% soy flour mix had a steady decrease in chewiness values from 165°C to 182°C.

Table 4.21 shows the effects of moisture content and cooking temperature on chewiness.

Table 4.21 Effect of Moisture Content and Cooking Temperature on Chewiness

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	1727^{a}_{B}	1833^{a}_{A}	1680^{a}_{B}
60	1083^{b}_{B}	1165^{b}_{A}	1133^{b}_{AB}
65	630^{c}_{AB}	587^{c}_{A}	607^{c}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$; subscripts indicate a significant difference for cooking temperature at $p<0.001$.

Table 4.21 shows that as the moisture content of a sample is increased, the chewiness value of the sample decreases at all cooking temperatures. Cooking temperature has a different effect at different moisture contents however. At 55% and

60% moisture content, the chewiness values increase in value from 165°C to 174°C and then decrease again from 174°C to 182°C. However, at 65% moisture content, that trend is reversed, with the chewiness value decreasing from 165°C to 174°C and then increasing again from 174°C to 182°C.

4.2.1.2.2 Discussion

As with the hardness values, the chewiness values between this study and the study by Lin et al. (2000) observe similar trends. As the moisture content is increased, the chewiness value decreases and as the cooking temperature increases, the chewiness value decreases. Once again, the values obtained in the Lin (2000) study were greater than the values obtained in this study, and these differences could possibly be attributed to the difference in mixtures, moisture contents, or cooking temperatures, or a combination.

4.2.1.3 Gumminess

4.2.1.3.1 Results

Gumminess is the final textural attribute that showed large, significant differences between samples. According to Bourne (1978), gumminess is defined as "the product of hardness and cohesiveness." In addition, gumminess is also a factor used in calculating the chewiness of a sample. Table 4.22 shows the effect of soy flour and moisture content on gumminess.

Table 4.22 Effect of Soy Flour and Moisture Content on Gumminess

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	2448^{a}_{A}	1742^{a}_{B}	922^{a}_{C}
15	1854^{b}_{A}	1110^{b}_{B}	591^{b}_{C}
30	1863^{b}_{A}	1138^{b}_{B}	576^{b}_{C}
45	1241^{c}_{A}	780^{c}_{B}	449^{c}_{C}

Superscripts indicate a significant effect from soy flour at $p<0.001$; subscripts indicate a significant effect from moisture content at $p<0.001$.

The trend from the table shows that as the concentration of soy flour in the sample was increased, a lower gumminess value was obtained. The exceptions again occur between the 15% and 30% soy flour samples, with the values at 30% soy flour being larger than the values at 15% soy flour at 55% and 60% moisture content. However, the difference observed between the two values was not significant. It can also be seen that as the moisture content increased, the gumminess value decreased. This trend held true at all soy flour concentrations. In addition, as moisture content of the product increased, the gumminess value decreased for all soy flour concentrations, with the largest gumminess values being observed at 55% moisture content.

The effect from soy flour concentration and cooking temperature on gumminess can be seen in Table 4.23.

Table 4.23 Effect of Soy Flour and Cooking Temperature on Gumminess

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	1679^{a}_{A}	1755^{a}_{A}	1679^{a}_{A}
15	1141^{b}_{A}	1192^{b}_{A}	1223^{b}_{A}
30	1153^{b}_{B}	1264^{b}_{A}	1160^{b}_{B}
45	907^{c}_{A}	819^{c}_{AB}	745^{c}_{B}

Superscripts indicate a significant effect from soy flour at $p<0.001$; subscripts indicate a significant effect from cooking temperature at $p<0.001$.

From Table 4.23 it can be seen that as the concentration of soy flour was increased, the gumminess value of the product decreased. The two exceptions to this are seen between the 15% and 30% soy flour concentrations at 165°C and 174°C. At these points, the gumminess value is larger for the products with 30% soy flour concentration. When looking at the trend that cooking temperature has on the gumminess value, even more anomalies can be observed. While the gumminess values decrease for the product as the temperature increases for the 45% soy flour mix, the other mixes have different trends. The 15% soy flour mix shows that as the cooking temperature was increased, the gumminess values increase as well. For the 0% and 30% soy flour mixes, the gumminess value increased from 165°C to 174°C, but then decreased from 174°C to 182°C. However, since these trends tend to occur with all of the textural attributes that have been analyzed, and at the same instances, it may be that these trends are not unusual.

Next, the effects of moisture content and cooking temperature on gumminess will be observed. Table 4.24 shows the effect of moisture content and cooking temperature on gumminess values.

Table 4.24 Effect of Moisture Content and Cooking Temperature on Gumminess

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	1847^{a}_{B}	1927^{a}_{A}	1780^{a}_{C}
60	1152^{b}_{A}	1233^{b}_{A}	1192^{b}_{A}
65	660^{c}_{A}	612^{c}_{A}	632^{c}_{A}

Superscripts indicate a significant effect from moisture content at $p<0.001$; subscripts indicate a significant effect from cooking temperature at $p<0.001$.

It can be observed from Table 4.24 that the trend for moisture content holds. For each cooking temperature, as the moisture content was increased, the gumminess value decreased. However, for the 55% and 60% moisture content products, as the temperature increased from 165°C to 174°C, the gumminess values increased, but from 174°C to 182°C the gumminess values decreased again. At 65% moisture content the trend was reversed, with the gumminess value decreasing from 165°C to 174°C and then increasing again from 174°C to 182°C. This is the same trend that has been observed for all texture attributes when looking at the effects of moisture content and cooking temperature.

4.2.1.3.2 Discussion

The values for gumminess follow the same trend as the values for chewiness. In the study by Lin et al. (2000), the gumminess values decreased as the cooking temperature was increased and also decreased as the moisture content was increased. The gumminess values in this study follow the same trend, although most of the time there was a slight increase from the 165°C cooking temperature to the 174°C cooking temperature. Also, the gumminess values obtained in this study were lower than those found in the study by Lin et al. (2000). These differences could possibly be from the difference in the mixture compositions or the different cooking temperatures and moisture contents.

4.3 Effects on Color

4.3.1 Results and Discussion

The color properties of the soy flour meat analog were taken with a Konica Minolta (Model CR-410, Konica Minolta, Inc., Mahwah, NJ) chromameter. Values for L*, a*, and b* were obtained using the Minolta chromameter, with L* being lightness, a* being redness, and b* being yellowness. Values for color were determined the day after extrusion on samples that were taken from refrigeration storage. The values on the colorimeter for lightness range from 0 to 100. For lightness, values at the lower end indicate a darker product, while those with a value on the higher end are a lighter product. Positive a* values represented a red color in the product, while negative a* values represented a green color in the product. For b* values, a positive number represented a yellow color in the product while a negative b* value represented a blue color in the product. Effects on color from soy flour can be seen in Table 4.25.

Table 4.25 Effect of Soy Flour Concentration on Color

Soy Flour (%)	L*	a*	b*
0	60.85[c]	3.05[a]	13.99[d]
15	62.58[a]	2.73[b]	14.37[c]
30	62.08[b]	2.27[d]	15.78[b]
45	62.32[ab]	2.53[c]	16.91[a]

Superscripts indicate a significant difference at $p<0.001$.

As seen from the above table, the lightness (L*) of the extruded product increased from 0% to 15% and from 30% to 45% soy flour concentration, with a slight decrease in lightness from 15% to 30% soy flour concentration. In addition, the values for yellowness (b*) showed an increase from each concentration level. This implies that as the amount of soy flour in the product increases, the yellowness of the final extruded product will increase. The redness value (a*) shows a decrease for each concentration level from 0% to 30% and a slight increase in value from 30% to 45% soy flour concentration. The implication from this is that as the concentration of soy flour is increased, the final extruded product has less red color.

Effects on the color values from moisture content can be seen on Table 4.26.

Table 4.26 Effect of Moisture Content on Color

Moisture Content (%)	L*	a*	b*
55	60.33^{c}	2.84^{a}	15.73^{a}
60	61.45^{b}	2.65^{b}	15.25^{b}
65	64.10^{a}	2.44^{c}	14.81^{c}

Superscripts indicate a significant difference at $p<0.001$.

As can be seen from Table 4.26, as the moisture content of the product was increased, the lightness (L*) increased while the redness (a*) and yellowness (b*) of the product decreased. This shows that as the moisture content is increased, the product becomes lighter in color and does not have such strong red and yellow hues. The cooking temperature of the product will also have an effect on the color.

The effects on the color from cooking temperature can be seen in Table 4.27.

Table 4.27 Effect of Cooking Temperature on Color

Cooking Temperature (°C)	L*	a*	b*
165	61.41^{c}	2.78^{a}	15.13^{b}
174	62.05^{b}	2.60^{b}	15.28^{a}
182	62.42^{a}	2.55^{b}	15.38^{a}

Superscripts indicate a significant difference at $p<0.001$.

As seen in Table 4.27, as the cooking temperature was increased, the lightness (L*) and yellowness (b*) of the product increased while the redness (a*) decreased. This trend was also observed in the effects that soy flour concentration had on product color. Between the 174°C and 182°C temperatures, there were no significant differences between the yellowness and redness of the products. Because the differences between the values at the two temperatures were small, they were not enough to indicate a statistically significant change. In addition, from looking at the values in Tables 4.25 and 4.26, it can be seen that the cooking temperature had the smallest changes in color differences. The color values for soy protein isolate were 91.23 for lightness, 2.15 for redness, and 14.37 for yellowness. The color values of defatted soy flour were 99.6 for lightness, -1.15 for redness, and 16.56 for yellowness.

4.3.1.1 Lightness

4.3.1.1.1 Results

Lightness is one of the values obtained from the color measurement of the product. Represented by L* in the Konika Minolta machine, the values for lightness can range from 0 – 100. Values that are closer to 0 have a darker appearance, while those products with values closer to 100 have a lighter appearance. The two way interactions between soy flour concentration and moisture content are shown in Table 4.28.

Table 4.28 Effect of Soy Flour Concentration and Moisture Content on Lightness

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	60.78^{a}_{B}	59.70^{b}_{C}	62.09^{c}_{A}
15	60.39^{a}_{C}	62.14^{a}_{B}	65.22^{a}_{A}
30	59.69^{b}_{C}	61.80^{a}_{B}	64.77^{ab}_{A}
45	60.47^{a}_{C}	62.15^{a}_{B}	64.34^{b}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

From looking at Table 4.28 it can be seen that the lightest product was obtained at 65% moisture content with a soy flour concentration of 15%. This trend was observed in the one-way interactions with both soy flour concentration and moisture content. The lightest product in the one-way interaction with soy flour was found to be at 15% soy flour concentration, while the lightest product in the one-way interaction with moisture content was found to be at 65% moisture content. From the samples that were collected with 60% moisture content, the lightest sample had a soy flour concentration of 45%. However, the lightness value for the sample that contained 15% soy flour was almost identical to that of the sample that contained 45% soy flour. In addition, in the one-way interactions for soy flour concentration, the samples that contained 45% soy flour had the next highest value for lightness, so the results are not completely unexpected. For the products with 55% moisture content, the highest lightness values occurred at 0% soy flour concentration and 45% soy flour concentration. In addition, all values from products with 55% soy flour concentration have lower lightness values than those at higher moisture contents. The results are likely due from less water in the product, causing the cooking to darken the product. Looking at the lightness values from 55% to 65%, lightness increases as moisture content increases.

Table 4.29 shows the effect of cooking temperature and soy flour concentration on the lightness (L*) value of meat analog products.

Table 4.29 Effect of Soy Flour Concentration and Cooking Temperature on Lightness

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	60.96^{b}_{A}	60.90^{b}_{A}	60.71^{c}_{A}
15	61.77^{a}_{C}	62.65^{a}_{B}	63.33^{a}_{A}
30	61.39^{ab}_{B}	62.48^{a}_{A}	62.38^{b}_{A}
45	61.52^{a}_{C}	62.18^{a}_{B}	63.25^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From Table 4.29 it can be observed that an increase in cooking temperature caused the lightness (L*) of the product to increase when observed in trends with the amount of soy flour in the product. The lowest value for lightness was observed at a cooking temperature of 182°C and 0% soy flour concentration. However, with the exception of the 182°C and 0% soy flour concentration and 182°C and 30% soy flour concentration, the largest values for lightness were observed at 182°C. For the products that contained 0% soy flour, the lightness values were very similar. In the 15% and 45% soy flour products, the lightness values increased in between each temperature. For the

30% soy flour product, the lightness value increased in between the 165°C and 174°C cooking temperatures, but decreased slightly between 174°C and 182°C.

The lightness values from the effects of moisture content and cooking temperature are shown in Table 4.30.

Table 4.30 Effect of Moisture Content and Cooking Temperature on Lightness

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	60.06^{c}_{B}	60.28^{c}_{AB}	60.65^{c}_{A}
60	60.86^{b}_{C}	61.40^{b}_{B}	62.08^{b}_{A}
65	63.30^{a}_{B}	64.48^{a}_{A}	64.52^{a}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

Once again, it can be seen that as the cooking temperature of the product increases, the lightness (L*) value of the product increases. The same trend holds true for moisture content; as the moisture content increases, the lightness value increases for the product. Both of these trends can be seen in Tables 4.26 and 4.27 as well as Tables 4.28 and 4.29 when looking at the effects for cooking temperature and moisture content respectively. In all cases, the lightness value increases as the temperature increases from 165°C to 182°C for all moisture contents. In addition, the lightness value increases for all moisture contents from 55% to 65% for each cooking temperature, with the largest L* value being observed at 182°C cooking temperature with 65% moisture content.

4.3.1.1.2 Discussion

Cooking temperature, moisture content, and soy flour concentration all affected the lightness value of the extruded meat analog products. Moisture content seemed to have had the greatest effect on the lightness, as when the moisture content was increased from 55% to 65%, the lightness value increased. When the soy flour concentration was increased from 0% to 15%, the lightness value increased and then decreased when the concentration was increased to 30% or 45%. The cooking temperature also had a slight effect on the lightness values. As the cooking temperature increased, the lightness values increased slightly.

In a study by Collins and Pangloli (1997), when defatted soy flour was added to a noodle mix, the lightness values decreased as the concentration of soy flour increased. However, this could be due to the defatted soy flour replacing wheat flour in the mixture and that the mixture also contained either sweet potato flour or sweet potato puree. In another study by Singh et al (2005), soy flour had an L value of 84.68, indicating that it had a high lightness value. In a study by Rababah et al (2006), as the amount of SPI in fortified biscuits increased, the lightness value decreased. When looking at the studies by Singh et al (2005) and Rababah et al (2006), it seems likely that the addition of defatted soy flour had a slight effect on the lightness value of the product. In addition, the lightness values obtained in this study from defatted soy flour were higher than the lightness values for soy protein isolate.

4.3.1.2 Redness

4.3.1.2.1 Results

Redness is another value that is obtained from the color measurement of a product. When the redness value (a*) is positive, it indicates a red color in the product. A higher positive value indicates a redder color in the product. If the redness value (a*) is negative, it indicates a green color present in the product. The effects from soy flour concentration and moisture content on redness are shown in Table 4.31.

Table 4.31 Effect of Soy Flour Concentration and Moisture Content on Redness

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	3.26^{a}_{A}	3.17^{a}_{A}	2.73^{a}_{B}
15	2.93^{b}_{A}	2.66^{b}_{B}	2.60^{b}_{B}
30	2.44^{d}_{A}	2.29^{c}_{A}	2.07^{d}_{B}
45	2.72^{c}_{A}	2.50^{b}_{B}	2.36^{c}_{B}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

The trends in this table illustrate that as the soy flour concentration in the product was increased, the redness value for the product decreased, and as the moisture content of the product increased, the redness value decreased. The notable exception is the values for the 30% soy flour products. Here, the redness values were lower than the values obtained for 15% soy flour products and 45% soy flour products for all moisture contents. However, seeing as each mixture of soy flour/soy protein isolate was run on different days, this could be due to a small difference in the extrusion process.

The trends from the concentration of soy flour concentration and cooking temperature can be observed in Table 4.32.

Table 4.32 Effect of Soy Flour Concentration and Cooking Temperature on Redness

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	3.06^{a}_{A}	3.04^{a}_{A}	3.06^{a}_{A}
15	2.95^{ab}_{A}	2.65^{b}_{B}	2.59^{b}_{B}
30	2.34^{c}_{A}	2.23^{c}_{A}	2.23^{c}_{A}
45	2.78^{b}_{A}	2.48^{b}_{B}	2.32^{c}_{B}

Superscripts indicate a significant difference for soy flour concentration at p<0.001, subscripts indicate a significant difference for cooking temperature at p<0.001.

From Table 4.32 it can be seen that the same trend holds true for the soy flour concentration as in Table 4.31. The highest redness value for all cooking temperatures is observed in the products containing 0% soy flour, while the lowest redness values are observed in the products that contain 30% soy flour. With the exception of the products with 30% soy flour, the redness values decreased as the soy flour concentration increased. In addition, the redness values also decreased as cooking temperature was increased for all soy flour concentrations except the 0% concentration. The values obtained for redness in the 0% soy flour concentrations were all very similar, with little or no difference in the values between cooking temperatures. The relationships between cooking temperature and moisture content in regards to the redness value of the products can be seen in Table 4.33.

Table 4.33 Effect of Moisture Content and Cooking Temperature on Redness

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	2.91^{a}_{A}	2.85^{a}_{AB}	2.75^{a}_{B}
60	2.80^{a}_{A}	2.61^{b}_{B}	2.55^{b}_{B}
65	2.63^{b}_{A}	2.34^{c}_{B}	2.35^{c}_{B}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From Table 4.33, it can be seen that as the cooking temperature of the product increased, the redness (a*) value of the product decreased. One exception was observed at 65% moisture content, between the redness values of 174°C and 182°C, where the redness value was observed to be slightly larger in the product that had a cooking temperature of 182°C. Additionally, it can be seen that the redness values for products from each of the cooking temperatures decreased as moisture content was increased. This shows that the moisture content has an effect on the redness of the product, when coupled with the cooking temperature.

4.3.1.2.2 Discussion

The results show that the addition of defatted soy flour had the greatest effect on the redness of the product. From a 0% concentration of defatted soy flour to a 30% concentration of defatted soy flour, the redness value dropped, with increases in the redness value occurring at the 45% defatted soy flour concentration. Moisture content and cooking temperature also played a role in the redness values, with moisture content decreasing the redness value as the moisture was increased and cooking temperature slightly decreasing the redness value as the temperature was increased.

In the study by Collins and Pangloli (1997), redness values for the mixtures containing defatted soy flour and either sweet potato flour or 10% sweet potato puree did not differ much. An increase in redness was seen between the 0% soy flour and 5% soy flour mixtures that contained sweet potato flour or sweet potato puree. However, a decrease in redness was observed between the 5% soy flour mixture and 10% soy flour mixture that contained sweet potato flour. This is an opposite trend from what occurred in this study, which may be due to the defatted soy flour replacing wheat flour instead of soy protein isolate. In the study by Singh et al (2005), defatted soy flour had a redness value of -2.62, which would lead one to the conclusion that as soy flour is added to a mixture, a lower redness value should be observed. In the study by Rababah et al (2006), an increase in the SPI in fortified biscuits increased the redness value. When looking at the studies by Singh et al (2005) and Rababah et al (2006), it seems likely that replacing soy protein isolate with defatted soy flour helped cause a decrease in the redness values of the meat analogs when coupled with increased cooking temperatures and moisture contents. This is further supported by the redness values obtained for defatted soy flour and soy protein isolate in this study. While the soy protein isolate had a positive redness value, defatted soy flour had a negative redness value, which lead to less redness in the final product.

4.3.1.3 Yellowness

4.3.1.3.1 Results

Yellowness (b*) is the other color that can be given a numerical value from chromameters. Like redness (a*), values for yellowness can be either positive or negative. The higher the positive value for b* obtained from a chromameter, the more yellow color in the product. A negative value for b* indicates a blue color in the product, with a larger negative value indicating a more blue color present in the product. The effects on the yellowness of products from the combination of soy flour concentration and moisture content can be seen in Table 4.34.

Table 4.34 Effect of Soy Flour Concentration and Moisture Content on Yellowness

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	15.15^{c}_{A}	13.99^{d}_{B}	12.83^{d}_{C}
15	14.77^{d}_{A}	14.32^{c}_{B}	14.01^{c}_{C}
30	15.83^{b}_{A}	15.85^{b}_{A}	15.66^{b}_{B}
45	17.17^{a}_{A}	16.84^{a}_{B}	16.72^{a}_{B}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

It can be observed that the yellowness of products increased with an increase in the amount of soy flour that was used in the product. Again, one exception for this trend occurred between the product combinations of 0% soy flour and 55% moisture content and the product that contained 15% soy flour concentration and 55% moisture content. For the products that contained 60% and 65% moisture content, the yellowness value increased as the concentration of soy flour in the product increased. In each of the products with a set amount of soy flour, the yellowness value decreased with an increase in moisture content, with the one exception being in the 30% soy flour product in between the 55% and 60% moisture contents. The largest value for yellowness was observed at 55% moisture content and 45% soy flour concentration, while the lowest yellowness value was observed at 65% moisture content with a 0% soy flour concentration. These trends indicate that as soy flour concentration in the mixture is increased and as the moisture content decreases, the products have a more yellow color. Table 4.35 shows the effect that soy flour concentration and cooking temperature have on the yellowness of the products.

Table 4.35 Effect of Soy Flour and Cooking Temperature on Yellowness

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	13.85^{d}_{B}	14.01^{d}_{AB}	14.11^{d}_{A}
15	14.29^{c}_{B}	14.36^{c}_{AB}	14.45^{c}_{A}
30	15.59^{b}_{B}	15.74^{b}_{B}	16.02^{b}_{A}
45	16.78^{a}_{B}	17.02^{a}_{A}	16.94^{a}_{AB}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From this table, it can be seen again that as the concentration of soy flour increases, the yellowness (b*) value increases for all cooking temperatures. In addition, it can be seen that as the cooking temperatures increase, the yellowness value differs very little. In effect, cooking temperature had no effect on the yellowness of the final product.

However, the increase in yellowness values between the cooking temperatures is very small when compared to the yellowness values between the various soy flour concentrations, which would indicate the soy flour concentration having a larger effect on the yellowness than cooking temperature. The smallest yellowness value obtained was observed at 165°C with the 0% soy flour product, while the largest yellowness value occurred at 174°C with the 45% soy flour product, followed closely by the 45% soy flour product with a 182°C cooking temperature.

Table 4.36 shows the effect that moisture content and cooking temperature have on the yellowness value of a product.

Table 4.36 Effect of Moisture Content and Cooking Temperature on Yellowness

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	15.59^{a}_{B}	15.83^{a}_{A}	15.77^{a}_{A}
60	15.17^{b}_{B}	15.21^{b}_{B}	15.37^{b}_{A}
65	14.61^{c}_{C}	14.81^{c}_{B}	15.00^{c}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

Observing the above table, it can be seen that as the moisture content increases, the yellowness (b*) value for the product at each cooking temperature decreases. The trend in the yellowness value decreasing as moisture content increases was also observed in Table 4.26 and Table 4.34. Furthermore, cooking temperature again seems to have little to no effect on yellowness. At every moisture content, as the cooking temperature was increased the yellowness value increased. Again, one exception occurred with the

55% moisture content product in between the 174°C and 182°C cooking temperatures where the yellowness value was lower at 182°C than it was for 174°C. In addition, the cooking temperature does not show as much of an effect on the yellowness of the product as the concentration of soy flour and moisture content.

4.3.1.3.2 Discussion

The concentration of defatted soy flour seemed to have the greatest affect on the yellowness value of the extruded meat analog product. This can be seen when the defatted soy flour concentration was increased from 0% to 45%, the yellowness of the meat analog increased. As the cooking temperature was increased, the yellowness value of the product did not change much at all, suggesting that cooking temperature has little to no effect on the yellowness. The affect of cooking temperature on the yellowness of the product was much less dramatic, resulting in only a slight increase in the yellowness value. Moisture content had an inverse affect on the yellowness value of the product. As the moisture content was increased, the yellowness value of the product decreased. This trend was observed when the moisture content was increased from 55% to 65%, the yellowness value of the product dropped.

In the study by Collins and Pangloli (1997), the yellowness values of pasta decreased as the amount of defatted soy flour in the pasta was increased, with the wheat flour having a yellowness of 17.90. In the study by Singh et al (2005), defatted soy flour had a yellowness value of 11.70. The trend in the study by Collins and Pangloli (1997) is most likely due to the wheat flour having a higher yellowness value than defatted soy flour. In a study by Rababah et al (2006), the yellowness value of biscuits fortified with SPI increased as the amount of SPI in the biscuits increased. Looking at the studies presented by Singh et al(2005) and Rababah et al (2006), it seem likely that an increase in defatted soy flour would lead to an increase in the yellowness value of the product. The yellowness values for defatted soy flour were higher than the yellowness values obtained from soy protein isolate and support this theory.

4.4 Protein Solubility

4.4.1 Results and Discussion

Six buffers were used to study the protein solubility of the soy flour meat analogs. These buffers were; phosphate buffer, pH 7.6, 35mM (PB), phosphate buffer with 8M urea (PB+Urea), phosphate buffer with 1.5% sodium dodecyl sulfate (PB+SDS), phosphate buffer with 0.1M 2-mercaptoethanol (PB+ME), phosphate buffer with 8M urea and 1.5% sodium dodecyl sulfate (PB+Urea+SDS), and phosphate buffer with 8M urea and 0.1M 2-mercaptoethanol (PB+Urea+ME). Each of the buffers served a different purpose in determining the solubility of the protein. These purposes can be seen in Table 3.4. The percent of total soluble protein obtained from each buffer for the amount of soy flour concentration is shown in Table 4.37.

Table 4.37 Effect of Soy Flour Concentration on the Percent of Soluble Protein

Soy Flour (%)	PB	PB+SDS	PB+Me	PB+Urea	PB+Urea +SDS	PB+Urea +Me
0	1.45^{b}	5.97^{b}	7.16^{b}	17.18^{a}	13.11^{a}	21.41^{c}
15	1.59^{b}	3.91^{c}	7.89^{b}	12.96^{b}	12.34^{a}	22.69^{bc}
30	1.82^{a}	7.08^{ab}	8.70^{b}	16.43^{a}	13.59^{a}	24.29^{b}
45	2.07^{a}	7.61^{a}	16.01^{a}	16.49^{a}	12.44^{a}	32.01^{a}

Differing superscripts indicate a significant difference at $p<0.001$

From the above table, it can be seen that as the amount of soy flour in the mixture is increased, more protein is soluble. This could be due to the products with higher soy flour mixes having less fiber formation, causing more of the protein to be soluble. A couple of exceptions in this trend appear in the 15% soy flour mixture in the PB+SDS, PB+Urea, and PB+Urea+SDS buffers. At each of these points, the amount of soluble protein is less than the amount that was present in the 0% soy flour mixture in the same buffer solutions. Another exception occurs in the 45% soy flour mixture in the PB+Urea+SDS buffer. The percentage of soluble protein was less in the 45% mixture than it was in the 30% soy flour mixture. Since these differences occurred in the buffers containing urea, sodium dodecyl sulfate, or both, it is possible that those chemicals had an effect on the absorbance reading. Also, much more disulfide bonds are broken in the 45% soy flour mix, which is evident in the PB+Me and PB+Urea+Me buffers.

Table 4.38 shows the effect of moisture content on the protein solubility of the products.

Table 4.38 Effect of Moisture Content on the Amount of Soluble Protein

Moisture Content (%)	PB	PB+SDS	PB+Me	PB+Urea	PB+Urea +SDS	PB+Urea +Me
55	1.60[b]	5.53[b]	10.19[ab]	14.08[b]	12.39[b]	24.54[b]
60	1.73[ab]	6.34[ab]	9.15[b]	14.41[b]	11.85[b]	25.19[a]
65	1.86[a]	6.56[a]	10.55[a]	18.80[a]	14.38[a]	25.57[a]

Superscripts indicate a significant difference at $p<0.001$.

The data presented in Table 4.38 shows that as moisture content increased, the percentage of soluble protein in the product increased. This could again be due to less fiber formation in the products with higher moisture content, which would cause more soluble protein. Again, there were exceptions to this trend. The exceptions were between the products with 55% and 60% moisture content in the PB+Me and PB+Urea+SDS buffers, where the percent of soluble protein was lower in the 60% moisture content product than in the 55% moisture content product. Unlike the exceptions from Table 4.37, the exceptions in Table 4.38 occurred in the PB+Me and PB+Urea+SDS buffers.

The effect that cooking temperature has on the amount of soluble protein is shown in Table 4.39.

Table 4.39 Effect of Cooking Temperature on the Amount of Soluble Protein

Cooking Temperature (°C)	PB	PB+SDS	PB+Me	PB+Urea	PB+Urea +SDS	PB+Urea +Me
165	1.69[a]	5.92[ab]	10.75[a]	14.63[b]	12.42[b]	25.73[a]
174	1.70[a]	5.79[b]	8.06[b]	15.50[ab]	12.64[b]	24.02[b]
182	1.82[a]	6.74[a]	11.09[a]	17.18[a]	13.58[a]	25.56[a]

Superscripts indicate a significant difference at $p<0.001$.

In Table 4.39 it can be seen that as the cooking temperature is increased, the amount of soluble protein in the product is increased. However, this is in contrast to what was reported by Lin (1998), where it was stated that the extractable proteins in each solvent decreased as the product temperature increased. As with the previous two tables, however, there are exceptions. These exceptions occur in between the 165°C and 174°C cooking temperatures in the PB+SDS, PB+Me, and PB+Urea+Me buffers. The amount of soluble protein in the products from the 174°C cooking temperature contained a lower percentage of soluble protein than the products from the 165°C cooking temperatures. In addition, the amount of soluble protein in the 165°C product in the PB+Urea+Me buffer also had a higher percentage of soluble protein than the product from the 182°C cooking temperature. However, this is the only case of a product from the 165°C cooking temperature had a higher percentage of soluble protein than a product from the 182°C cooking temperature.

4.4.1.1 Phosphate Buffer

4.4.1.1.1 Results

The phosphate buffer that was used was a 35 mM, pH 7.6 sodium phosphate buffer. Also, this concentration of sodium phosphate buffer has been used in many other protein solubility studies as well (Burgess and Stanley, 1976; Prudencio-Ferreira and Areas, 1993; Lin, 1998), and provides good starting references when faced with any problems in the studies. From Table 3.4, it can be seen that the sodium phosphate buffer is used to break the bonds of the native proteins, such as peptide bonds. Disulfide bonds, hydrogen bonds, hydrophobic interactions, and non-covalent bonds are not broken or disrupted with the sodium phosphate buffer alone.

Table 4.40 shows the effect that soy flour concentration and moisture content have on the percentage of soluble protein in the product.

Table 4.40 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	1.37^{c}_{A}	1.39^{b}_{A}	1.60^{c}_{A}
15	1.50^{bc}_{A}	1.59^{b}_{A}	1.70^{bc}_{A}
30	1.65^{b}_{B}	1.90^{a}_{A}	1.92^{b}_{A}
45	1.91^{a}_{B}	2.09^{a}_{AB}	2.23^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

From the table, it can be seen that as the concentration of soy flour and moisture content are increased, the amount of soluble protein in the product is increased in the sodium phosphate buffer. It can also be seen that the amount of soluble protein present at 65% moisture content for a specific soy flour concentration is higher than that of the 55% moisture content for the next highest soy flour amount (ie: 65% moisture content, 0% soy flour has more soluble protein than 55% moisture content, 15% soy flour). The effect from soy flour and cooking temperature is shown in Table 4.41.

Table 4.41 Effect of Soy Flour and Cooking Temperature on Soluble Protein in Phosphate Buffer

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	1.33^{c}_{A}	1.44^{c}_{A}	1.59^{c}_{A}
15	1.61^{b}_{A}	1.60^{bc}_{A}	1.59^{c}_{A}
30	1.83^{ab}_{A}	1.77^{b}_{A}	1.86^{b}_{A}
45	1.99^{a}_{B}	2.01^{a}_{AB}	2.23^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

As can be seen from Table 4.41, as both soy flour concentration and cooking temperature increased, the amount of soluble protein in the product increased. However, there were exceptions to this observation. These exceptions occurred in the products with 15% soy flour and the solubility decreased as the cooking temperature was increased. In addition, in the products that contained 30% soy flour, the percent of soluble protein decreased as the cooking temperature was increased from 165°C to 174°C, but then increased as the cooking temperature was raised from 174°C to 182°C. The lowest amount of soluble protein was observed in products that contained 0% soy flour at a cooking temperature of 165°C, while the highest amount of soluble protein was observed in products that contained 45% soy flour at a cooking temperature of 182°C.

In Table 4.42, the effects from cooking temperature and moisture content on the amount of soluble protein in sodium phosphate buffer can be seen.

Table 4.42 Effect of Cooking Temperature and Moisture Content on Soluble Protein in Phosphate Buffer

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	1.52^{b}_{B}	1.53^{b}_{B}	1.77^{a}_{A}
60	1.64^{b}_{A}	1.81^{a}_{A}	1.76^{a}_{A}
65	1.90^{a}_{A}	1.77^{a}_{A}	1.91^{a}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

Looking at Table 4.42, it can be seen that the trends in soluble protein are not consistent. In the products that had a moisture content of 55%, the percentage of soluble protein in the product increased as the cooking temperature of the product was increased. However, in the products that had a moisture content of 60%, the percentage of soluble protein increased as the cooking temperature was increased from 165°C to 174°C, but then decreased as the cooking temperature was increased from 174°C to 182°C. Then, in the products that had a moisture content of 65%, the percentage of soluble protein decreased as the cooking temperature was increased from 165°C to 174°C, and then increased as the cooking temperature was increased from 174°C to 182°C. Therefore, at lower temperatures, moisture content has an effect on the solubility of protein, but at higher temperatures, moisture content has no effect on the solubility of protein. In the study by Prudencio-Ferreira and Areas (1993), it was found that protein insolubility came from a combination of non-covalent and disulfide linkages and increased as the temperature increased. This would indicate that the protein solubility should decrease as the cooking temperature is increased, but that was not the case in this part of the experiment.

4.4.1.1.2 Discussion

The amount of protein extracted with phosphate buffer was less than the amounts extracted from the other solvents. This was due mostly to the removal of phosphate buffer soluble proteins during the production of the soy protein isolate (Berk, 1992b). The amount of soluble protein extracted from the phosphate buffer is comparable to that found by Lin (1998) but was quite lower than the 8.3% solubility found by Burgess and Stanley (1976) and the 6.96% solubility found by Prudencio-Ferreira and Areas (1993). This difference could have come from the type of method used to extract the proteins. Lin (1998) used a Micro Protein Determination Kit, while Burgess and Stanley (1976) and Prudencio-Ferreira and Areas (1993) used a micro-Kjeldahl technique.

4.4.1.2 Phosphate Buffer with 2-Mercaptoethanol

4.4.1.2.1 Results

In previous studies (Burgess and Stanley, 1976; Prudencio-Ferreira and Areas, 1993) the addition of 2-mercaptoethanol was used to facilitate the breaking of disulfide bonds in addition to the bonds of native proteins that are broken by the sodium phosphate buffer. With the addition of 2-mercaptoethanol to the sodium phosphate buffer, it can be determined how much of the protein in the product has disulfide bonds.

Table 4.43 shows the effect of soy flour concentration and moisture content on the amount of soluble protein from sodium phosphate buffer and 2-mercaptoethanol.

Table 4.43 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer with 2-Mercaptoethanol

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	7.04^{b}_{AB}	6.36^{b}_{B}	8.10^{c}_{A}
15	8.53^{b}_{A}	7.70^{b}_{A}	7.74^{ce}_{A}
30	8.58^{b}_{B}	6.33^{b}_{C}	11.20^{b}_{A}
45	16.65^{a}_{A}	16.22^{a}_{A}	15.18^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

In Table 4.43, it can be seen that, for the most part, as the amount of soy flour was increased in the products, the higher the percentage of soluble protein that was present. However, the exception to this occurred in the products that had a moisture content of 60%, when the amount of soy flour was increased from 15% to 30%. As the soy flour was increased, the amount of soluble protein was decreased. As the amount of soy flour was increased from 30% to 45%, however, the amount of soluble protein increased. In addition, it can be seen that as the amount of moisture content was increased, the amount of soluble protein in the products was decreased. Again, there are exceptions to this. These exceptions occur in the products containing 0% and 30% soy flour. As the moisture content was increased from 60% to 65%, the amount of soluble protein increased. This trend was observed in the products that had 15% soy flour concentration as well, but to a lesser extent.

Table 4.44 shows the effect of soy flour concentration and cooking temperature on the amount of soluble protein in the meat analog products.

Table 4.44 Effect of Soy Flour and Cooking Temperature on Soluble Protein in Phosphate Buffer with 2-Mercaptoethanol

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	10.00^{b}_{A}	1.84^{c}_{B}	9.65^{b}_{A}
15	8.97^{b}_{A}	6.91^{b}_{B}	8.09^{b}_{AB}
30	8.66^{b}_{A}	8.54^{b}_{B}	8.90^{b}_{A}
45	15.35^{a}_{B}	14.96^{a}_{B}	17.73^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

Looking at Table 4.44 it can be seen that the amount of soluble protein decreased in each product as the cooking temperature was increased from 165°C to 174°C and then increased as the cooking temperature was raised from 174°C to 182°C. Also, with the exception of the products from the 174°C cooking temperature, the amount of soluble protein was decreased when the soy flour concentration was increased from 0% to 15%, and then increased as the amount of soy flour concentration was increased from 30% to 45%. In the products from the 174°C cooking temperature, the amount of soluble protein increased as the amount of soy flour in the product was increased.

Table 4.45 shows the effect of moisture content and cooking temperature on the amount of soluble protein in sodium phosphate buffer with 2-mercaptoethanol.

Table 4.45 Effect of Moisture Content and Cooking Temperature on Soluble Protein in Phosphate Buffer with 2-Mercaptoethanol

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	10.51^{b}_{B}	7.67^{a}_{C}	12.41^{a}_{A}
60	9.57^{b}_{A}	7.63^{a}_{B}	10.26^{b}_{A}
65	12.17^{a}_{A}	8.89^{a}_{C}	10.60^{b}_{B}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

As seen in Table 4.45, the lowest amounts of soluble protein in the sodium phosphate buffer and 2-mercaptoethanol occur in the 174°C cooking temperature for all moisture contents. The trend that is observed in this table is that as the cooking temperature is increased from 165°C to 174°C, the amount of soluble protein in the product is decreased, but when the cooking temperature is increased from 174°C to 182°C, the amount of soluble protein in the product increased. However, in the products with 65% moisture content, when the cooking temperature was increased from 174°C to 182°C, the amount of soluble protein did not increase above its levels in the 165°C cooking temperature, whereas the products from the 55% and 60% moisture contents has a higher amount of soluble protein at 182°C than at 165°C.

4.4.1.2.2 Discussion

The amount of soluble protein extracted from the phosphate buffer and 2-mercaptoethanol solution are slightly lower than the values obtained by Lin (1998). While the amount of soluble proteins extracted by Lin (1998) were around 10-15%, with most being around 12%, the amount of soluble protein extracted in this study ranged from roughly 6-15%, with most being around 8%.

4.4.1.3 Phosphate Buffer with Sodium Dodecyl Sulfate

4.4.1.3.1 Results

The addition of sodium dodecyl sulfate (SDS) to the sodium phosphate buffer was to break non-covalent bonds as well as hydrophobic interactions in addition to breaking the bonds of the native proteins that are present. SDS was used for this purpose in the studies by Burgess and Stanley (1976), Prudencio-Ferreira and Areas (1993), Kelley and Pressey (1966), Chiang and Sternberg (1973), and Lin (1998).

The effects from soy flour concentration and moisture content on the amount of soluble protein in phosphate buffer with SDS can be seen in Table 4.46.

Table 4.46 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer with Sodium Dodecyl Sulfate

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	5.35^{b}_{A}	6.31^{ab}_{A}	6.28^{b}_{A}
15	3.64^{c}_{B}	5.07^{b}_{A}	3.03^{c}_{B}
30	6.19^{ab}_{B}	6.96^{a}_{AB}	8.12^{a}_{A}
45	6.98^{a}_{B}	7.05^{a}_{B}	8.82^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

As can be seen from Table 4.46, as the moisture content of the products was increased, the amount of soluble protein increased. One exception to this occurred in the product containing 15% soy flour as the moisture content was increased from 60% to 65% and another, lesser, exception occurred in the product containing 0% soy flour as the moisture content was increased from 60% to 65%. In all products, the amount of soluble protein increased as the moisture content was raised from 55% to 60%. Also, as the amount of soy flour was increased in the product, the amount of soluble protein increased. Again, there was one exception to this trend and it occurred as the soy flour concentration was increased from 0% to 15% for all moisture contents.

The effect from soy flour concentration and cooking temperature can be seen in Table 4.47.

Table 4.47 Effect of Soy Flour and Cooking Temperature
on Soluble Protein in Phosphate Buffer with Sodium Dodecyl Sulfate

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	6.66^{a}_{A}	5.58^{b}_{B}	5.69^{b}_{AB}
15	2.45^{b}_{B}	3.09^{c}_{B}	6.20^{ab}_{A}
30	6.72^{a}_{A}	6.99^{a}_{A}	7.56^{a}_{A}
45	7.85^{a}_{A}	7.50^{a}_{A}	7.51^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at p<0.001, subscripts indicate a significant difference for cooking temperature at p<0.001.

In looking at Table 4.47, it can be seen that there are two different trends. One trend occurs in the products containing 0% and 45% soy flour, where the amount of soluble protein decreases as the cooking temperature is increased. The other trend occurs in the products containing 15% and 30% soy flour, where the amount of soluble protein in the products is increased as the cooking temperature is increased. In addition, for the products with the 165°C and 174°C cooking temperature, the amount of soluble protein decreased as the amount of soy flour was increased from 0% to 15%, but then increased as the amount of soy flour was raised from 15% to 30% and 30% to 45%. For the products with the 182°C cooking temperature, the amount of soluble protein increased as the amount of soy flour was increased, with only a small decrease in soluble protein from the 30% soy flour concentration to the 45% soy flour concentration.

The effect of moisture content and cooking temperature on the amount of soluble protein can be observed in Table 4.48.

Table 4.48 Effect of Moisture Content and Cooking Temperature on Soluble Protein in Phosphate Buffer with Sodium Dodecyl Sulfate

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	5.29^{a}_{A}	5.16^{b}_{A}	6.17^{a}_{A}
60	6.07^{a}_{AB}	5.68^{b}_{B}	7.30^{a}_{A}
65	6.41^{a}_{A}	6.53^{a}_{A}	6.74^{a}_{A}

Superscripts indicate a significant difference for moisture content at p<0.001, subscripts indicate a significant difference for cooking temperature at p<0.001.

As can be seen from Table 4.48, the largest percentages for soluble protein were obtained at the 182°C cooking temperature for each moisture content. For the products at 55% and 60% moisture content, the amount of soluble protein decreased as the cooking temperature was increased from 165°C to 174°C and then increased as the cooking temperature was raised from 174°C to 182°C. At 65% moisture content, the amount of soluble protein increased as the cooking temperature increased. In addition, for the products with 165°C and 174°C cooking temperatures, as the moisture content was increased, the amount of soluble protein in the products increased. For the products with the 182°C cooking temperature, the amount of soluble protein increased as the moisture content was raised from 55% to 60%, but then decreased as the moisture content was increased from 60% to 65%.

4.4.1.3.2 Discussion

The soluble protein that was extracted with phosphate buffer and 1.5% SDS was comparable to the amount extracted by Lin (1998), with the majority of extracted protein being around 7%. Again, however, these values are nowhere near the amount extracted by Burgess and Stanley (1976) in phosphate buffer with 1.5% SDS. The amount of protein extracted by the method of Burgess and Stanley (1976) was 13.9%, which is nearly twice the average value obtained in this study. However, this could again be due to the difference in methods.

4.4.1.4. Phosphate Buffer with Urea

4.4.1.4.1 Results

Urea was another important chemical used in the protein solubility study. The urea was used to interrupt hydrogen bonds. Burgess and Stanley (1976), Prudencio-Ferreira and Areas (1993), and Lin (1998) are a few of the researchers who used urea in protein solubility studies for this manner. The urea used was an 8M concentration.

Table 4.49 shows the effect that soy flour concentration and moisture content had on the amount of soluble protein in the phosphate buffer and urea solvent.

Table 4.49 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer with Urea

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	14.69^{a}_{B}	15.35^{a}_{B}	21.51^{a}_{A}
15	13.13^{a}_{A}	11.91^{b}_{A}	13.86^{b}_{A}
30	14.38^{a}_{B}	14.86^{ab}_{B}	20.08^{a}_{A}
45	14.16^{a}_{B}	15.56^{a}_{B}	19.77^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

As can be seen from Table 4.49, with the exception of the products with 15% soy flour, as the moisture content increased, the amount of soluble protein in the product increased. For the products with 15% soy flour, the amount of soluble protein decreased as the moisture content was increased from 55% to 60% and then increased as the moisture content was raised from 60% to 65%. However, as the amount of soy flour was increased, no significant differences could be detected in the amount of soluble protein except for the products that had 15% soy flour. At each moisture content, the products with 15% soy flour had less soluble protein than the other products with differing soy flour concentrations at that specific moisture content.

The effect of soy flour concentration and cooking temperature on the amount of soluble protein in the phosphate buffer and urea solvent can be seen in Table 4.50.

Table 4.50 Effect of Soy Flour and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	13.90^{ab}_{B}	15.50^{a}_{B}	22.15^{a}_{A}
15	12.16^{b}_{AB}	15.88^{a}_{A}	10.86^{c}_{B}
30	15.95^{a}_{A}	15.90^{a}_{A}	17.47^{b}_{A}
45	16.53^{a}_{AB}	14.72^{a}_{B}	18.24^{b}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From looking at Table 4.50, no specific trend can be seen. At a 0% soy flour concentration, it can be seen that as the cooking temperature was increased the amount of soluble protein in the product increased. For products with 15% soy flour concentration, the amount of soluble protein increased as the cooking temperature was increased from 165°C to 174°C, but then decreased as the cooking temperature was increased from 174°C to 182°C, the amount of soluble protein decreased and was below the amount of soluble protein observed at 165°C. At a 30% soy flour concentration, the amount of soluble protein decreased slightly as the cooking temperature was increased from 165°C to 174°C but then increased as the cooking temperature was increased from 174°C to 182°C. For the products with a 45% soy flour concentration, the amount of soluble protein decreased as the cooking temperature was raised from 165°C to 174°C and then increased as the cooking temperature was raised from 174°C to 182°C. In addition, as the soy flour concentration was increased at 165°C cooking temperature, the amount of soluble protein decreased as the soy flour concentration was increased from 0% to 15%, but then increased as the amount of soy flour was increased to 30% and 45%. At the

174°C cooking temperature, the amount of soluble protein increased as the amount of soy flour was increased until the soy flour concentration was increased from 30% to 45%, when the amount of soluble protein decreased. For the products with a cooking temperature of 182°C, the amount of soluble protein decreased as the amount of soy flour was increased from 0% to 15%, but then increased as the amount of soy flour was increased to 30% and 45%.

The effect of moisture content and cooking temperature on the amount of soluble protein in the phosphate buffer and urea solvent can be seen in Table 4.51.

Table 4.51 Effect of Moisture Content and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	12.89^{b}_{A}	14.09^{b}_{A}	15.29^{b}_{A}
60	13.45^{b}_{B}	13.44^{b}_{B}	16.38^{b}_{A}
65	17.56^{a}_{A}	18.97^{a}_{A}	19.88^{a}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From Table 4.51, it can be seen that as the cooking temperature of the product was increased, the amount of soluble protein in the product increased. One exception of this trend occurs at 60% moisture content between the 165°C and 174°C cooking temperatures. Between these two cooking temperature at this moisture content, the amount of soluble protein decreased slightly, but then increased as the cooking temperature was increased from 174°C to 182°C. In addition, as the moisture content was increased in the products, the amount of soluble protein in the product increased. Again, one exception to this trend occurs. This exception is in the products with a 174°C cooking temperature as the moisture content was raised from 55% to 60%. The amount of soluble protein in the 60% moisture sample was lower than the amount of soluble protein in the 55% moisture content sample.

4.4.1.4.2 Discussion

The percentage of extracted proteins from phosphate buffer with 8M urea was lower than the percentage of extracted proteins by Lin (1998). Most of the samples from Lin (1998) had over 20% of the proteins extracted, while in this study the amount of extracted proteins was mostly under 20%. Burgess and Stanley (1976) had an average extractability of 14.8% for phosphate buffer and 8M urea. The value obtained by Burgess and Stanley (1976) is closer to the majority of values obtained in this study.

4.4.1.5. Phosphate Buffer with Urea and 2-Mercaptoethanol

4.4.1.5.1 Results

Both urea and 2-mercaptoethanol were added to the sodium phosphate buffer solution to aid in the breaking of disulfide bonds (2-mercaptoethanol) and in the interruption of hydrogen bonds (urea). This solvent was used by other researchers such as Burgess and Stanley (1976), Prudencio-Ferreira and Areas (1993), and Lin (1998). In all of these studies, the urea and 2-mercaptoethanol were used for the same purposes.

The effect of soy flour concentration and moisture content on the amount of soluble protein in the products can be seen in Table 4.52.

Table 4.52 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer with Urea and 2-Mercaptoethanol

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	24.80^{c}_{A}	18.24^{d}_{C}	21.20^{d}_{B}
15	20.93^{b}_{B}	22.77^{c}_{AB}	24.38^{c}_{A}
30	18.45^{b}_{B}	27.95^{b}_{A}	26.48^{b}_{A}
45	33.00^{a}_{A}	31.81^{a}_{B}	30.25^{a}_{B}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

As can be seen in Table 4.52, with the exception of the products with a 55% moisture content, as the amount of soy flour in the products was increased, the amount of soluble protein present increased. At 55% moisture content, the amount of soluble protein decreased as the amount of soy flour was increased to 15% and 30%, but then had a large increase as the amount of soy flour was increased from 30% to 45%. At the 0% soy flour concentration, the amount of soluble protein decreased as the moisture content went from 55% to 60% and then increased as the moisture content went from 60% to 65%. In the products with 15% soy flour concentration, the amount of soluble protein increased as the moisture content in the product increased. The products with 30% soy flour concentration had an increase in the amount of soluble protein as the moisture content was increased from 55% to 60% and then had a slight decrease as the moisture content increased from 60% to 65%. Products with a soy flour concentration of 45% had a decrease in the amount of soluble protein as the moisture content increased.

The effect of soy flour concentration and cooking temperature on the amount of soluble protein can be seen in Table 4.53.

Table 4.53 Effect of Soy Flour and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea and 2-Mercaptoethanol

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	22.20^{c}_{A}	20.61^{b}_{A}	21.44^{c}_{A}
15	22.86^{c}_{A}	22.18^{b}_{A}	23.04^{bc}_{A}
30	25.82^{b}_{A}	22.46^{b}_{B}	24.61^{b}_{A}
45	32.04^{a}_{AB}	30.86^{a}_{B}	33.16^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From Table 4.53 it can be seen that as cooking temperature was increased from 165°C to 174°C, the percentage of soluble protein in the products decreased, but as the cooking temperature was increased from 174°C to 182°C, the amount of soluble protein in the products increased. In addition, it can be seen that at each cooking temperature, as the amount of soy flour in the product increased, the amount of soluble protein in the product increased. The effect of moisture content and cooking temperature on the amount of soluble protein in a product can be seen in Table 4.54.

Table 4.54 Effect of Moisture Content and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea and 2-Mercaptoethanol

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	26.19^{a}_{A}	22.77^{b}_{B}	24.67^{a}_{A}
60	25.48^{a}_{A}	24.31^{ab}_{A}	25.79^{a}_{A}
65	25.52^{a}_{A}	25.00^{a}_{A}	26.22^{a}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

In Table 4.54 it can be seen that for all moisture contents, as the cooking temperature increases from 165°C to 174°C, the amount of soluble protein in the product decreased. However, when the cooking temperature is increased from 174°C to 182°C, the amount of soluble protein in the product increases, but at the 55% moisture content, the amount of soluble protein at 182°C is still less than the amount present at 165°C. It can also be seen that as the moisture content is increased in the product, the amount of soluble protein in the product increases. Again, there is an exception to this trend which occurs at the 165°C cooking temperature. As the moisture content is increased from 55% to 60%, the amount of soluble protein decreases, but then has a slight increase as the moisture content is raised from 60% to 65%.

4.4.1.5.2 Discussion

Phosphate buffer with urea and 2-mercaptoethanol extracted around 60-75% of soluble protein in the study by Lin (1998). In the study by Prudencio-Ferreira and Areas (1993), the amount of protein extracted was over 95%. However, the amount extracted in this study was significantly lower than those percentages. The average extractability for Burgess and Stanley (1976) was 28.7%. The values in this study are closer to the values obtained by Burgess and Stanley (1976) than those obtained by Lin (1998) and Prudencio-Ferreira and Areas (1993).

4.4.1.6. Phosphate Buffer with Urea and Sodium Dodecyl Sulfate

4.4.1.6.1 Results

As with the sodium phosphate buffer with urea and 2-mercaptoethanol, the sodium phosphate buffer with urea and sodium dodecyl sulfate (SDS) was used by other researchers. The purpose of this solvent was to break the bonds of native proteins, interrupt the hydrogen bonds (urea), and to break non-covalent bonds and hydrophobic interactions (SDS). The concentration of the SDS in the solvent was 1.5% and the urea was again used in an 8M concentration. The effect of soy flour concentration and moisture content on the amount of soluble protein in the product can be seen in Table 4.55.

Table 4.55 Effect of Soy Flour and Moisture Content on Soluble Protein in Phosphate Buffer with Urea and Sodium Dodecyl Sulfate

	Moisture Content (%)		
Soy Flour (%)	55	60	65
0	13.23^{a}_{B}	11.89^{a}_{C}	14.23^{a}_{A}
15	13.27^{a}_{A}	12.00^{a}_{AB}	11.77^{c}_{B}
30	12.36^{a}_{B}	13.02^{a}_{B}	15.42^{ab}_{A}
45	10.72^{b}_{B}	10.49^{b}_{B}	16.12^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for moisture content at $p<0.001$.

In Table 4.55, it is hard to see any trends. However, at a soy flour concentration of 0% and 45%, it can be seen that as the moisture content is increased from 55% to 60% the amount of soluble protein in the product is decreased, while as the moisture content is increased from 60% to 65%, the percentage of soluble protein increases. At the 15% soy flour concentration, it can be seen that as the moisture content is increased, the amount of soluble protein is decreased. For the products with 45% soy flour, the amount of soluble protein decreased slightly as the moisture content was increased from 55% to 60% and then increased as the moisture content was increased from 60% to 65%. For the products with a moisture content of 55%, as the soy flour concentration increased from 0% to 15%, the amount of soluble protein in the product increased slightly, but then as the amount of soy flour was increased from 15%, the amount of soluble protein decreased. In products that had a moisture content of 60%, as the amount of soy flour increased, the percentage of soluble protein increased until the soy flour was increased from 30% to 45%, at which point the amount of soluble protein decreased. Products that had a moisture content of 65% showed a decrease in the amount of soluble protein as the amount of soy flour was increased from 0% to 15%. After that point, as the amount of soy flour was increased, the percentage of soluble protein in the product increased. The effect of soy flour concentration and cooking temperature on the amount of soluble protein in a product is shown in Table 4.56.

Table 4.56 Effect of Soy Flour and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea and Sodium Dodecyl Sulfate

	Cooking Temperature (°C)		
Soy Flour (%)	165	174	182
0	13.42^{a}_{A}	12.52^{ab}_{A}	13.41^{ab}_{A}
15	12.02^{b}_{A}	12.36^{ab}_{A}	12.66^{b}_{A}
30	13.39^{a}_{A}	13.52^{a}_{A}	13.89^{ab}_{A}
45	10.83^{b}_{C}	12.17^{b}_{B}	14.34^{a}_{A}

Superscripts indicate a significant difference for soy flour concentration at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

As can be seen from Table 4.56, the amount of soluble protein in a product increases as the cooking temperature is increased for products that contain 15%, 30%, and 45% soy flour. For the products that contain 0% soy flour, the amount of soluble protein decreases as the cooking temperature is increased from 165°C to 174°C and then increases again as the cooking temperature is increased from 174°C to 182°C. At all cooking temperatures, as the amount of soy flour is increased from 0% to 15%, the amount of soluble protein in the product decreases. For products with 165°C and 174°C cooking temperatures, the amount of soluble protein in the product decreases again as the amount of soy flour is increased from 30% to 45%. For products with the 182°C cooking temperature, the amount of soluble protein has another increase as the amount of soy flour is increased from 30% to 45%.

The effect of moisture content and cooking temperature on the amount of soluble protein in the product can be seen in Table 4.57.

Table 4.57 Effect of Moisture Content and Cooking Temperature on Soluble Protein in Phosphate Buffer with Urea and Sodium Dodecyl Sulfate

	Cooking Temperature (°C)		
Moisture Content (%)	165	174	182
55	12.47^{b}_{B}	11.95^{b}_{A}	12.76^{b}_{A}
60	10.97^{c}_{B}	12.01^{b}_{AB}	12.58^{b}_{A}
65	13.80^{a}_{B}	13.97^{a}_{B}	15.39^{a}_{A}

Superscripts indicate a significant difference for moisture content at $p<0.001$, subscripts indicate a significant difference for cooking temperature at $p<0.001$.

From Table 4.57 it can be seen that for products with a moisture content of 60% or 65%, as the cooking temperature increases the amount of soluble protein in the product increases. For products with a moisture content of 55%, the amount of soluble protein decreases as the cooking temperature is increased from 165°C to 174°C. However, the amount of soluble protein in the product increases when the cooking temperature is increased from 174°C to 182°C. In addition, for products with a cooking temperature of 165°C or 182°C, the amount of soluble protein decreased as the moisture content was increased from 55% to 60% and then increased as the moisture content was increased from 60% to 65%. Products with a cooking temperature of 174°C had an increase in the percentage of soluble protein as the moisture content of the product was increased.

4.4.1.6.2 Discussion

Compared with others (Lin, 1998; Burgess and Stanley, 1976), the extractability values obtained by using phosphate buffer with urea and SDS were much more consistent between studies. Burgess and Stanley (1976) reported an average extractability of 13.9%, while Lin (1998) had values ranging from 8% to 20%, with the average being

13.2%. In this study, most of the values were between 10% and 14%, with an average around 12.8%.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Extrusion Responses

The addition of defatted soy flour reduced the viscosity of the dough in the extruder, helping to reduce the percentage of torque. The reduction in viscosity also helped lower product temperature by reducing shear stress. Higher moisture content added lubrication to the system which also helped reduce torque percentage. This is due again to a reduction in dough viscosity. Increased cooking temperature raised the product temperature due to the direct effect cooking temperature has on product temperature.

5.2 Texture Profile Analysis

The addition of soy flour reduced adhesiveness, which reduced chewiness. This is because adhesiveness has a direct effect on the chewiness of the product. The presence of soy flour also reduced hardness, gumminess, and cohesiveness as well. The reduction in gumminess is likely due to the correlation between gumminess and cohesiveness. A higher moisture content also reduced the hardness, gumminess, and chewiness. Adhesiveness was the lowest at 60% moisture content and increased at 65% moisture. This likely caused less of a reduction in chewiness. Increased cooking temperature did not have much of an effect on gumminess, hardness, and chewiness, but it did cause a reduction in adhesiveness.

5.3 Color

The addition of soy flour increased the lightness value and yellowness value of the product and decreased the redness value. This is due to the higher lightness and yellowness values of defatted soy flour when compared to soy protein isolate. The reduction in the redness value is also due to defatted soy flour having a lower redness value than soy protein isolate. Higher moisture contents increased the lightness. This was most likely due to less solids present in the final product which would have decreased the lightness value. The reduction in the redness and yellowness values was also likely due to less solids, which would have diluted any strong red or yellow color in the product. Cooking temperature did not have much of an effect on any of the color values obtained. While there were significant differences noted, the differences themselves were not very large.

5.4 Protein Solubility

Increased defatted soy flour concentration increased the protein solubility in most of the buffers, with the buffers containing SDS being the exception. The increase in protein solubility is likely due to more soluble protein being present in the product due to less fiber formation. Increased moisture content also showed similar results, and this is also likely due to more soluble protein being present due to less fiber formation in the final product. Increased cooking temperature also showed an increase in the amount of soluble protein in some buffers. This is the opposite of what was expected based on the reports of others. Increased cooking temperature should have showed a reduction in the amount of soluble protein because the increased cooking temperature usually results in more fiber formation in the final product.

5.5 Summary and Suggestions

The research objectives of this study to illustrate the relationships among the extruder responses, textural properties, color, and chemical properties were achieved. The amount of defatted soy flour that replaces soy protein isolate in a meat analog can have an effect on every attribute observed. Even at the same extruding conditions, the soy flour affects the textural, color, and chemical attributes of the final product. When the extrusion parameters were changed, the same attributes were affected in similar ways. For example, when the moisture content of a product was increased, no matter what the composition of the mix, the textural properties in the products all behaved the same. The chemical properties of the products were much harder to predict. This could be due to different types of bonds being more prevalent in one mix than in another, or could be due to some small variation in extrusion runs affecting the bond formations.

The effect of the cooling die on the textural properties of the product is an important factor. Since the cooling die is what sets the uncoiled protein strands in place to make a fibrous-like structure, setting the die at a cooler temperature might have some effect on the final product. In addition, since only one cooling die was used in this study, other lengths of cooling die could be used to determine which one is best suited for making a soy flour meat analog product.

The chemical properties of the meat analog need to be further studied. From all of the differences that occurred between the trends of the solvents and extrusion parameters, it is difficult to determine a set trend that should occur. Other methods of determining the amount of soluble protein in a product could be used to see if similar trends are observed or if a more accurate method is available. Since no solvent obtained close to 100% solubility, other types of solvents could be used to determine if they are better suited to break bonds in the product or be more easily read by a spectrophotometer or other method.

APPENDIX A

Soy Protein Isolate and Defatted Soy Flour Nutrient Content

PRODUCT DETAILS: PRINT CLOSE WINDOW

ADM

Product: PRO-FAM® 974

Product Trademark: PRO-FAM®

Product Code:

General Description:
PRO-FAM® 974 soy protein is part of the NutriSoy® brand family of soy proteins. Application: Dairy-free products and milk replacers, snack foods, processed meats, emulsified meats, sausage-type meats. Characteristics: Highly soluble, highly functional, easily dispersible.

Product Characteristics:		
Characteristic:	**Value:**	**Characteristic Qualifier:**
% Protein (N x 6.25) (mfb) Minimum	90	%
Ash	5	%
Fat (by acid hydrolysis)	4%	
Moisture	6.0%	Max
pH (1:10 disp. in water)	7.0-7.4	

Packaging: Bag

Technical Documents:

Contact Information:
Archer Daniels Midland Company [Specialty Ingredients - Soy Protein Isolates]
P.O. Box 1470
Decatur, IL 62525
800.637.5850
217.451.8067
specialtyingredients@admworld.com
http://www.admworld.com

Disclaimer:
The information contained herein is correct to the best of our knowledge. The recommendations or suggestions contained in this database are made without guarantee or representation of the results. We suggest that you evaluate these recommendations and suggestions in your own laboratory prior to use. Our responsibility for claims arising from breach of warranty, negligence, or otherwise, is limited to the purchase price of the material. Freedom to use any patent owned by ADM or others is not to be inferred from any statement contained herein.

PRODUCT DETAILS: PRINT CLOSE WINDOW

ADM

Product: BAKERS FLOUR

Product Trademark:

Product Code:

General Description:
Application: Breads, cakes, donuts, sweet doughs, cookies, macaroni, dry mixes, pizza crusts, tortillas, pancake and waffle mixes. Characteristics: Light, heat-treated flour.

Product Characteristics:		
Characteristic:	**Value:**	**Characteristic Qualifier:**
% Protein (N x 6.25) (mfb) Minimum	53 %	%
Calories	270	per 100 gm
Carbohydrates (including TDF)	30	%
Fat (by acid hydrolysis)	3%	
Moisture	9%	Max
Total Dietary Fiber	18	%

Technical Documents:

Contact Information:
Archer Daniels Midland Company [Flours - Defatted Soy Flour/Grits]
PO Box 1470
Decatur, IL 62525
800.637.5850
217.451.8067
specialtyingredients@admworld.com
http://www.admworld.com

Disclaimer:

APPENDIX B

Vital Wheat Gluten and Wheat Starch Nutritional Information

MGP INGREDIENTS, INC.

TECHNICAL DATA

Supporting Your Innovations

Vital Wheat Gluten

Product Description

Vital Wheat Gluten is a wheat protein concentrate that is prepared by removing starch from wheat flour and carefully drying the remaining high protein gluten in such a manner as to retain the native properties of the wheat gluten. Vital Wheat Gluten provides exceptional functionality for the baking industry with the following properties: dough strengthening, gas retention and controlled expansion which results in uniformly shaped products, water absorption and retention, and a natural flavor and color. Vital Wheat Gluten can be used in such products as breads, rolls, cereals, breadings, batter mixes, pasta, meat, fish, poultry products, pet foods, sausage products, and pizza toppings.

Specifications	Ranges
Protein (N x 5.7)	75% min.
Moisture	8.0% max.
Ash	1.1% max.
Fat	1.0-2.0%
Fiber	1.0% max.
Color	Pale Cream
Odor	Clean, Fresh
Microbiological	
Standard Plate Count	10,000/g max.
Mold and Yeast	200/g max.
E. coli	Negative
Salmonella	Negative in 25g

Packaging and Storage

Vital Wheat Gluten is packed in multi-ply kraft paper bags with net weight of 50 lbs. (22.7 kg.) Vital Wheat Gluten can also be shipped in tote bags with net weight of 2000 lbs. (907.2 kg) or in bulk using trucks or rail cars. Store under cool, dry and sanitary conditions for maximum stability. Shelf life is one year when stored under these conditions.

Labeling Ingredient Statement

Wheat Gluten

The information and recommendations in this sheet are based on our experience and analysis using standard procedures, and are believed to be accurate and reliable. However, they serve merely as typical guides, and are presented in good faith for the benefit of our customers. No guarantee, expressed or implied, is made regarding accuracy of the analysis, patent infringement, liabilities, or risks involved from the application of our products.	Issued:	6/12/00
	Revised:	7/30/03
	Approved:	R&D/QC

MGP INGREDIENTS, INC.

TECHNICAL DATA

Supporting Your Innovations

MIDSOL 50

PRODUCT SPECIFICATIONS

PRODUCT DESCRIPTION

- Highly refined wheat starch for general use in foods.
- High water holding capacity and is an excellent fat emulsion stabilizer.
- Provides a very bland flavor and brilliant whiteness.

RECOMMENDED USES

Midsol 50 provides functionality in many different products applications. Examples include cakes, icings, glazes, cookies, pie crusts, baby food, puddings, salad dressings, sauces, gravies, soups and other cereal products.

PROPERTY	SPECIFICATIONS
Moisture (%)	12.0% Max
Protein (%)	0. 3 Max.
pH	5.0 - 6.5
Ash (%)	0.3 Max.
Color	White
Odor	Bland
Flavor	Bland
Sieve Analysis % on USBS 200 Mesh	20 Max.
Aerobic Plate Count	10,000/g Max.
Mold and Yeast	200/g Max.
Coliforms	<100/g
E. Coli	Negative

PACKAGING and STORAGE

Midsol 50 is packed in multi-ply Kraft bags with net weight of 50 lbs. (25 kg). It is also available in tote bags, and for bulk shipment. The product should be stored in a cool, dry, and sanitary area to achieve maximum stability. Shelf life is one year when stored under these conditions.

INGREDIENT LABELING STATEMENT

Wheat Starch

Issued 2/1/00
Revision 4/07/03
Approved
Mkt/R&D/QC

APPENDIX C

Sample SAS Input File

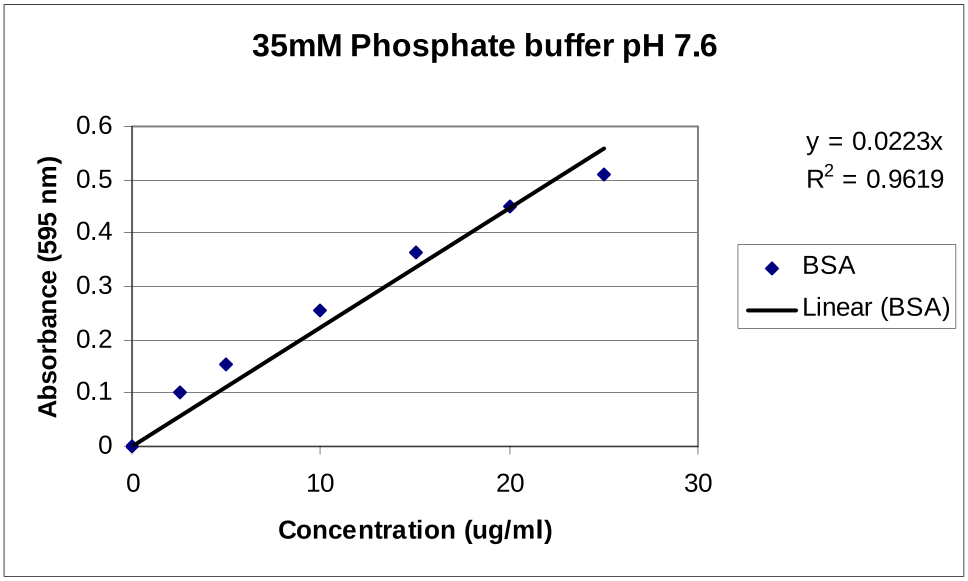
```
data one;
title1 'Soy Extrusion Data';
options pagesize=52;
infile 'c:\matt\mattextall.dat' missover;
input code mix water temp rep brl9 brl8 brl7 brl6 die_t p9 p8 p7 p6 p5 rpm torque psi feed;
if temp=330 then temp=329;
proc sort; by code;
proc means; by code;
proc sort; by mix water temp rep;
proc glm; classes mix water temp rep;
model die_t p9 p8 torque psi= rep mix|water|temp;
lsmeans rep mix|water|temp / s p;
run;
```

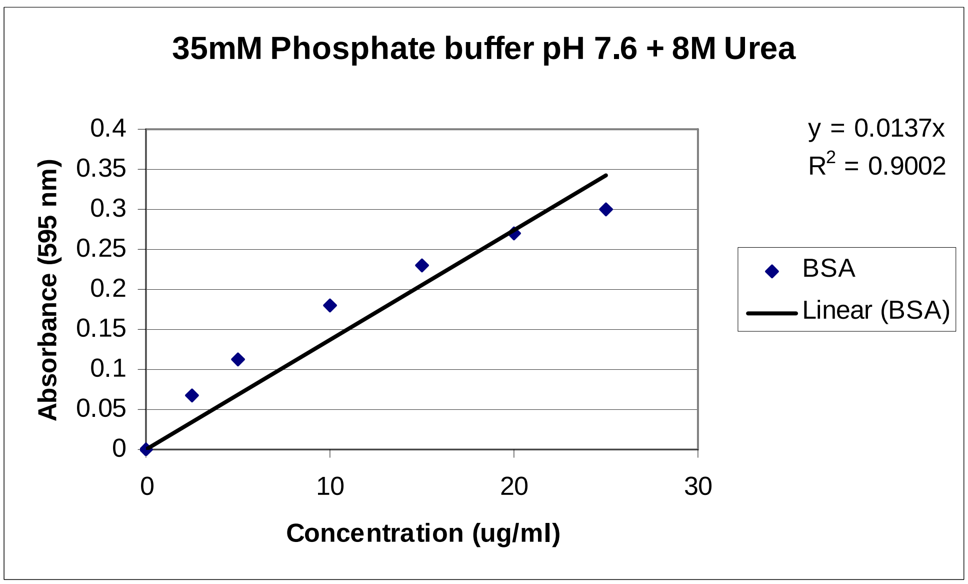
$t\text{-}tab \times \sqrt{2\ SE^2}$

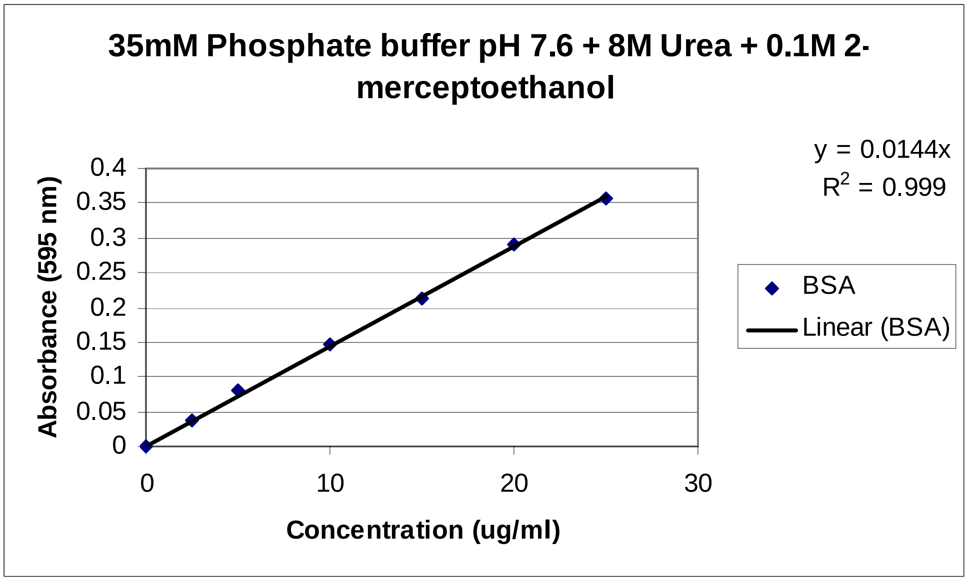
Error
df

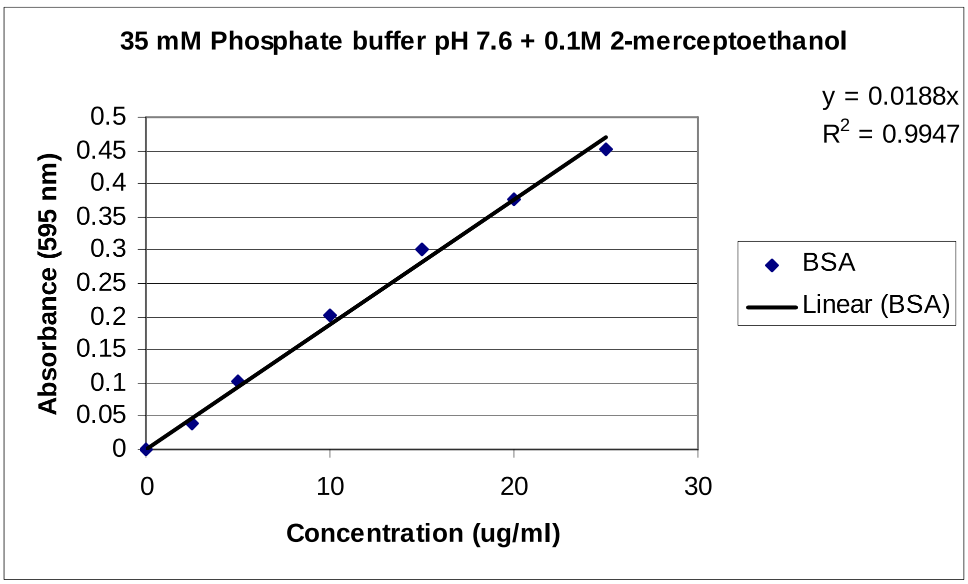
APPENDIX D

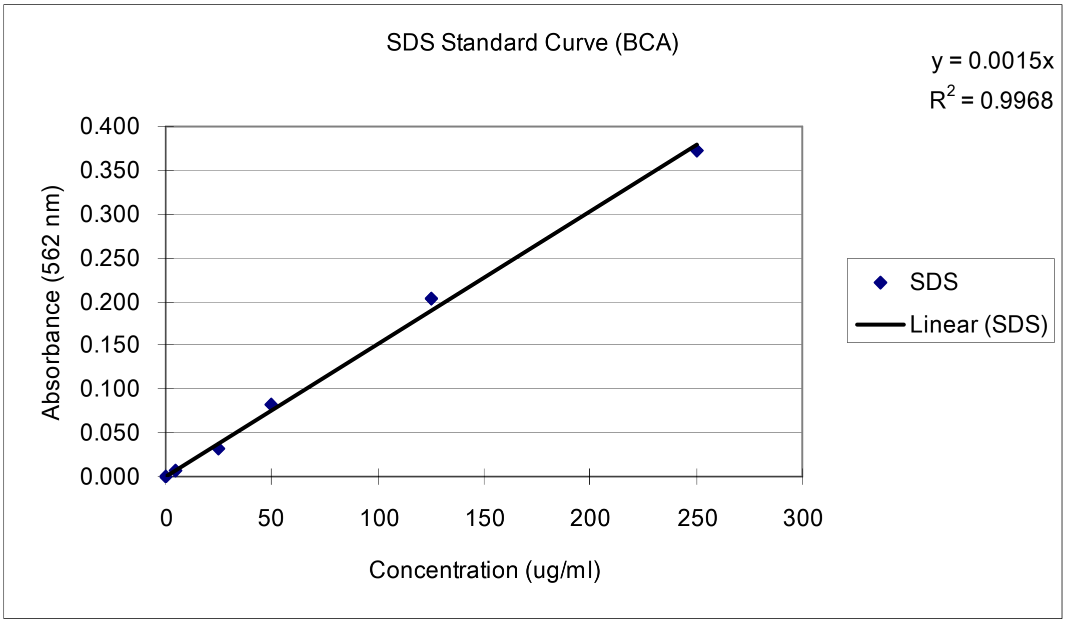
Protein Solubility Standard Curves

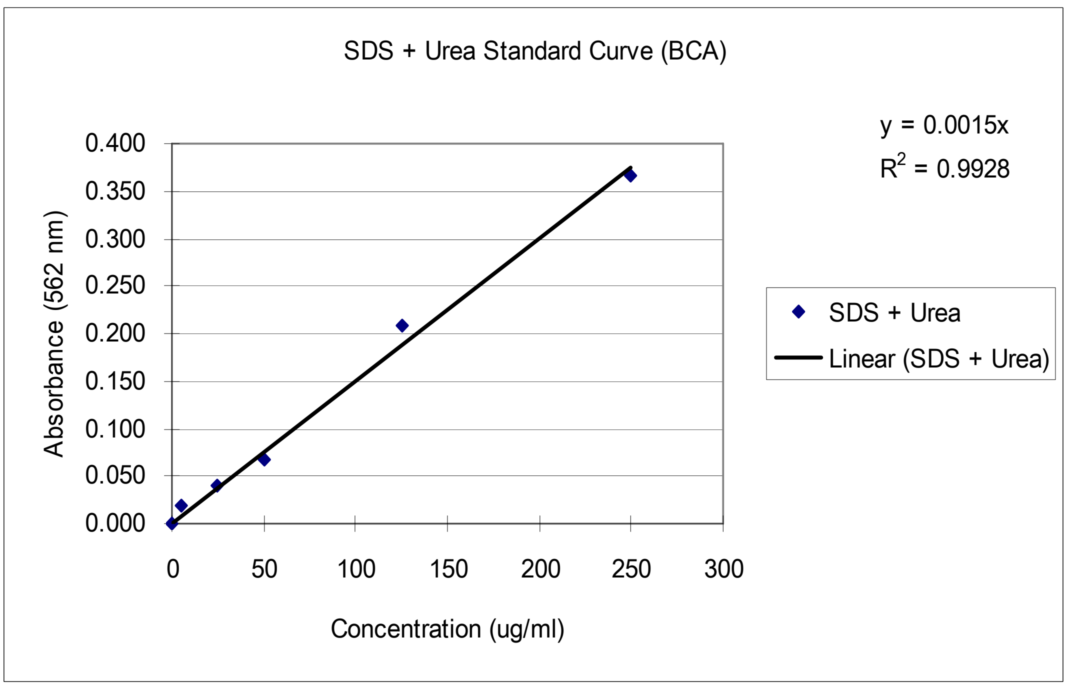

35mM Phosphate buffer pH 7.6
y = 0.0223x
R^2 = 0.9619
BSA
Linear (BSA)
Absorbance (595 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
Concentration (ug/ml)


35mM Phosphate buffer pH 7.6 + 8M Urea
y = 0.0137x
R^2 = 0.9002
BSA
Linear (BSA)
Absorbance (595 nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
Concentration (ug/ml)


35mM Phosphate buffer pH 7.6 + 8M Urea + 0.1M 2-merceptoethanol
y = 0.0144x
R2 = 0.999
BSA
Linear (BSA)
Absorbance (595 nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
Concentration (ug/ml)


35 mM Phosphate buffer pH 7.6 + 0.1M 2-merceptoethanol
y = 0.0188x
R2 = 0.9947
BSA
Linear (BSA)
Absorbance (595 nm)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
Concentration (ug/ml)


SDS Standard Curve (BCA)
y = 0.0015x
R2 = 0.9968
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Absorbance (562 nm)
0
50
100
150
200
250
300
Concentration (ug/ml)
SDS
Linear (SDS)


SDS + Urea Standard Curve (BCA)
y = 0.0015x
R2 = 0.9928
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Absorbance (562 nm)
0
50
100
150
200
250
300
Concentration (ug/ml)
SDS + Urea
Linear (SDS + Urea)

REFERENCES

Allred, C. D., Allred, K. F., Ju, Y. H., Goeppinger, T. S., Doerge, D. R., and Helferich, W.G. 2004. Soy processing influences growth of estrogen-dependent breast cancer tumors in mice. Carcinogenesis. 25 (9): 1649-1657.

Anderson, J. W., Johnstone, B. M., and Cook-Newell, M. E. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. The New England Journal of Medicine. 333 (5): 276-282.

Anonymous. 2007a. www.soygrowers.com/soyindustry/started.htm. Last accessed 4/14/07.

Anonymous. 2007b. www.soygrowers.com/soyindustry/nextyears.htm. Last accessed 4/14/07.

Berk, Z. 1992a. The soybean. Ch. 1 in the *Technology of production of edible flours and protein products from soybeans*. Berk, Z. Ed. FAO of the United Nations, Rome, Italy. 1-16.

Berk, Z. 1992b. Isolated soybean protein. Ch. 6 in the *Technology of production of edible flours and protein products from soybeans*. Berk, Z. Ed. FAO of the United Nations, Rome, Italy. 82-95.

Boatright, W. L., and Hettiarachchy, N. S. 1995. Effects of lipids on soy protein isolate solubility. JAOCS. 72(12): 1439-1444.

Bourne, M. C. 1978. Texture profile analysis. Food Technology. July. 62-72.

Burgess, L. D. and Stanley, D. W. 1976. A possible mechanism for thermal texturization of soybean protein. J. Inst. Can. Sci. Technol. Aliment. 9 (4): 228-231.

Carroll, K. K. 1982. Hypercholesterolemia and atherosclerosis: effects of dietary protein. Fed. Proc. 41: 2792-2796.

Cheftel, J. C., Kitagawa, M., and Quenguiner, C. 1992. New protein texturization processes by extrusion cooking at high moisture levels. Food Rev. Intern. 8, 235-275.

Chiang, J. P. and Sternberg, M. 1974. Physical and chemical changes in spun soy protein fibers during storage. Cereal Chemistry. 51: 465-471.

Claughton, S. M. and Pearce, R. J. 1989. Protein enrichment of sugar-snap cookies with sunflower protein isolates. J. Food Sci. 54: 354.

Collins, J. L. and Pangloli, P. 1997. Chemical, physical, and sensory attributes of noodles with added sweetpotato and soy flour. J. Food Sci. 62 (3): 622-625.

Cumming, D. B., Stanley, D. W., and deMan, J. M. 1973. Fate of water soluble soy protein during thermoplastic extrusion. J. Food Sci. 38: 320-323.

deMan, F. M. 1990. Protein. Ch. 3 in *Principles of Food Chemistry*, 2nd ed. An AVI Book. Van Nostrand Reinhold. New York. 89-142.

Hager, D. F. 1984. Effects of extrusion upon soy concentrate solubility. J. Agric. Food Chem. 32: 293-296.

Harper, J. M. 1988. Effects of extrusion processing on nutrients. Ch. 14 in the *Nutritional Evaluation of Food Processing*. 3rd ed. Karmas, E. and Harris, R. (eds.). Wiley, N.Y. 365-391.

Harper, J. M. 1989. Food extruders and their applications. Ch. 1 in *Extrusion Cooking*. Mercier, C., Linko, P., and Harper, J. M. (eds.). American Association of Cereal Chemists, Inc., St. Paul, MN. 1-15.

Hegarty, P. V. J. and Ahn, P. C. 1976. Nutritional comparisons between a soy-based meat analog and ground beef in the unheated and heated states. J. Food Sci. 41: 1133-1136.

Henkel, J. 2004. Soy: health claims for soy protein, questions about other components. U. S. FDA. http://www.fda.gov/fdac/features/2000/300_soy.html Last accessed date: 11/14/2006.

Hua, Y., Huang, Y., Qiu, A., and Liu, X. 2004. Properties of soy protein isolate prepared from aqueous alcohol washed soy flakes. Food Research International 38(2): 273-279.

Kelley, J. J. and Pressey, R. 1966. Studies with soybean protein and fiber formation. Cereal Chemistry. 43: 195-206.

Larmond, E. 1976. The texture profile. Ch. 18 in *Rheology and Texture in Food Quality*. deMan, J.M., Voisey, P.W., Rasper, V.F., and Stanley, D.W. (eds.). The AVI Publishing Company, Inc., Westport, CT. 546-553.

Lin, S. 1998. Meat analog development. Ph.D. Dissertation, University of Missouri – Columbia, Columbia, MO.

Lin, S., Huff, H. E., and Hsieh, F. 2000. Texture and chemical characteristics of soy protein meat analog extruded at high moisture. J. Food Sci. 65 (2): 264-269.

Lin, S., Huff, H. E., and Hsieh, F. 2002. Extrusion process parameters, sensory characteristics, and structural properties of a high moisture soy protein meat analog. J. Food Sci. 67 (3): 1066-1072.

Mahungu, S. M., Diaz-Mercado, S., Li, J., Schwenk, M., Singletary, K., and Faller, J. 1999. Stability of isoflavones during extrusion processing of corn/soy mixture. J. Agric. Food Chem. 47 (1): 279-284.

Maurice, T. J. and Stanley, D. W. 1978. Texture-structure relationships in texturized soy protein. IV. Influence of process variables on extrusion texturization. Can. Inst. Food Sci. Technol. J. 11(1): 1-6.

Maurice, T. J., Burgess, L. D., and Stanley, D. W. 1976. Texture-structure relationships in texturized soy protein. III. Textural evaluation of extruded products. Can. Inst. Food. Sci. Technol. J. 9(4): 173-176.

Messina, M., Barnes, S., and Setchell, K. D. 1997. Phyto-estrogens and breast cancer. The Lancet. 350 (9083): 971-972.

Morr, C. V. and Ha, E. Y. W. 1991. Processing to prevent formation of off-flavors in soy products. Comments Agric. & Food Chemistry. 2(4): 247-260

Noguchi, A. 1989. Extrusion cooking of high-moisture protein foods. Ch. 11 in *Extrusion Cooking*. Mercier, C., Linko, P., and Harper, J.M. (eds.). American Association of Cereal Chemists, Inc. St. Paul, MN. 343-370.

Patterson, A. 2005. Isoflavones: new frontier in nutrition. U. S. Soyfoods Directory. http://www.soyfoods.com/nutrition/isoflavone.html Last accessed date: 11/12/2006.

Prudencio-Ferreira, S. H. and Areas, J. A. G. 1993. Protein-protein interactions in the extrusion of soya at various temperatures and moisture contents. J. Food Sci. 58 (2): 378-384.

Rababah, T. M., Al-Manasneh, M. A., and Ereifej, K. I. 2006. Effect of chickpea, broad bean or isolated soy protein additions on the physiochemical and sensory properties of biscuits. J. Food Sci. 71 (6): 438-442.

Rizvi, S. S. H., Blaisdell, J. L., and Harper, W. J. 1980. Thermal diffusivity of model meat analog systems. J. Food Sci. 45: 1727-1731.

Robyt, J. F. and White, B. J. 1990. Qualitative and quantitative methods for determining biological molecules. Ch. 7 in *Biochemical Techniques Theory and Practice*. Waveland Press, Inc. Prospect Heights, IL. 213-252.

Satin, M. 2007. Functional properties of starches. FAO Agricultural and Food Engineering Technologies Service. www.fao.org/AG/magazine/pdf/starches.pdf. Last accessed 4/12/07.

Setchell, K. D. R. and Cole, S. J. 2003. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. J. Agric. Food Chem. 51 (14): 4146-4155.

Singh, N., Kaur, M., and Singh, S. K. 2005. Physiochemical and functional properties of freeze-dried and oven dried corn gluten meals. Drying Technology. 23: 975-988.

Yorks, T. P. 1978. Energy use in soy-bean meat analog manufacture: a comparison with beef. J. Sci. Food Agric. 29: 895-902.

Zhuo, X., Melby, M. K., and Watanabe, S. 2004. Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. Nutritional Epidemiology. 134: 2395-2400.