

VILLAGE SCALE TECHNOLOGY FOR A NOVEL SNACK FOOD
PRODUCT BASED ON HIGH PROTEIN LEGUMES

By

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(Under the Direction of Manjeet Chinnan)

ABSTRACT

Village Texturizer is a small scale instrument that texturizes plant protein by press texturization, expanding utilization of high protein legumes. Ten formulations containing soy, peanut and/or cowpea flours were created through formulation software. Based on protein content and amino acid requirements for children (10-12 yrs), 5 formulations were texturized at 3 moisture contents (30, 35, 40%), 2 temperatures (300, 350°F), 4 pressures (110, 120, 130, 140 psi). Shape retention decreased the number of viable samples to 26 which density, moisture, texture and water activity were analyzed. Eight samples were selected by comparison to a control (full fat soy) for protein solubility, digestibility and amino acid profile analysis. Formulation/parameter sets best suited for meeting the protein and amino acid requirements were: formulation 8 (80% defatted soy, 20% cowpea), 35% moisture, 300°F, 120 psi; formulation 5 (70% full fat soy, 30% peanut (28% fat)), 35% moisture, 300°F, 140 psi.

INDEX WORDS: press texturization, soy flour, peanut flour, cowpea flour, physiochemical properties, formulation software, protein analysis, children ages 10 to 12

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DEDICATION

This thesis is dedicated to my parents, Richard and Vickie Smith, for always telling me that I can accomplish anything and for being my biggest fans.

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INTRODUCTION

While meat is traditionally consumed as the primary source of protein within the United States, many nations rely on high protein vegetables to meet their protein requirements. This has led to the utilization of legumes such as soybeans, peanuts and cowpeas in traditional dishes in developing nations. However, there lies the possibility of increasing the use of these high protein legumes by texturization which offers products that resemble meat. Plant protein texturization processes include fiber spinning, extrusion, steam texturization and press texturization. Extrusion, the most common form of texturized plant protein processing, is achieved by placing a plant protein mixture into an extruder that heats and mixes causing the proteins to denature and form new cross links resulting in a fibrous structure. The mixture is then forced through a die which expands upon release forming a texturized product. Extrusion processing requires ingredients with low or no fat, limiting the raw ingredients that can be used. Press texturization on the other hand has been shown to be effective with full fat soy indicating that fat would not be a barrier in texturization.

Press texturization has been the processing method include in several patents dating as early as 1970. The initial patent called for the use of steam and pressure to textured dough composing of 40-60% flour and 60-40% water. As pressure was released during the texturization process, the moisture in the dough vaporized and the texturized product expanded resulting in a spongy type product. Another patent from 1970 focused on the ability to create a “meat-like protein product” from a protein mix that contained at least 30% protein and 20 to 60% moisture content. The mixture is masticated at “temperatures substantially above the boiling point of water” before “extruding the mix at elevated pressure and temperature through an orifice

into a medium of lower pressure and temperature.” However, another patent, “Texturizing of Proteins,” established that the mastication of the mix before extrusion was not necessary. Texturizing of the protein mix would occur if compressed between two hot plates and then released. The method calls for a protein content of at least 50% and a moisture content of 15 to 30%.

The Village Texturizer is an apparatus that was used in Korean villages to create puffed snack foods by street vendors. A steel plate sets on four legs, allowing for heating from underneath by means of coal briquettes. In the center of the plate, a milled chamber is used to contain the mixture for cooking. A steel lid, shaped to fit like a “plug” in the milled chamber is placed on top and pressure is applied via a piston controlled by a torque wrench. The mix is compressed between the two steel plates while being heated creating a pressurized atmosphere in which the protein denatures and the water is superheated. When the pressure is released, the superheated water instantly vaporizes and escapes the product leaving a fibrous, texturized product.

In 1973, the Village Texturizer was used to create a puffed product from “rice-like pellets of sweet potatoes”. Following research focused on the use of the texturizer to develop a soy based snack food by exploring various combinations of temperature (300 and 350°F), pressure (100, 200, 300, 400, 500, 600 and 700 psi) and time (5, 10 and 15 sec) as well as protein (50 and 36.7%) and moisture content (30 and 40%). This study showed that this method of texturization can be performed on full fat soy flour.

The objective of this study was to develop a formulation that meets the protein requirements of children ages 10-12 years that texturizes most similar to full fat soy protein flour at 40% moisture. This was accomplished by developing ten formulations that meets the protein

requirements for press texturization using formulation software. Of the ten formulations developed, five were selected to texturize and examine the physicochemical properties.

The preliminary study examined full fat soy flour and cowpea flour processed on the Village Texturizer for density and moisture in order to replicate the results of the full fat soy from previous research as well as determine the ideal processing parameters for cowpea flour. Full fat soy processed at the ideal processing parameters was used as a standard by which to compare the texturized products of the created formulations. The formulations created using formulation software included the following ingredients: soy flour (full fat or defatted) (50-95%), partially defatted peanut flour (12 or 28% fat) (0-30%) and/or cowpea flour (0-70%). Of the ten formulations, five were selected according to their protein content and amino acid profile. Each formulation was brought to three different moisture levels (30, 35 and 40%) and texturized at two different temperatures (300 and 350°F) and four different pressures (110, 120, 130 and 140 psi) resulting in 120 samples.

Each sample that was texturized on the Village Texturizer was examined for shape retention, density and moisture content. Using the results of these tests, twenty six samples were selected to determine and compare texture profile and water activity to the full fat soy from the preliminary study. This allowed for the selection of eight samples to continue with for protein analysis including: amino acid profile, protein solubility and protein digestibility. By comparing the results of the protein analysis to soy and to the requirements of children, the two most ideal formulation/parameter sets were determined to be: formulation 8 consisting of 80% defatted soy flour, 20% cowpea flour brought to 35% moisture processed at 300°F under 120 psi; and formulation 5 consisting of 70% full fat soy flour, 30% partially defatted peanut flour (28% fat) brought to 35% moisture processed at 300°F under 140 psi.

SECTION I
LITERATURE REVIEW
TEXTURIZED PLANT PROTEINS

The food processing industry has spent many years developing ways to utilize plant proteins in place of animal proteins (Gutcho, 1977). However, this endeavor has meet with certain challenges. The increase in demand for meat has been shown to correlate with an increase in affluence and development while plant proteins have traditionally been consumed more frequently by poorer subsections of the population (Harper, 1981). For instance, cowpea, or black eyed peas was once known as “poor man’s meat” (Vanchina, Chinnan, & McWatters, 2006). Animal protein has increased in price and decreased in availability (Gutcho, 1977; Harper, 1981). In addition, plant proteins lack the texture, flavor and appearance of meat. However, the use of plant protein as a substitution for meat or animal proteins has several advantages: lower cost, greater supply, and less environmental stress (Harper, 1981).

Texturized plant proteins (TPP) have been developed to meet the global need of protein while offering products that resemble meat. TPP should absorb water and fat, resulting in a composition comparable to meat, as well as comprise of structural integrity through the processing procedure (Harper, 1981). There are two basic groups of TPP products: meat extenders and meat analogs. Meat extenders have distinct fiber formation and highly expanded character, are usually produced by a single high temperature/pressure extrusion process and upon rehydration have been used to extend meat products such as in pizza toppings, meat sauces and fabricated food formulations (Gutcho, 1977; Harper, 1981). In contrast, meat analogs are produced through multiple extrusion processes, specially cooled dies or in some cases fiber

spinning. This type of TPP can be use in the place of meat but must be dense, contain minimal air pockets, layered fiber conformation and retain “meat-like character” throughout additional processing (Harper, 1981). The goal for this type of TPP is to be as similar to meat as possible in regards to sensory attributes (Gutcho, 1977; Harper, 1981).

Plant protein texturization processes include fiber spinning, extrusion, steam texturization and press texturization (Harper, 1981). Fiber spinning is a process which the “relatively pure vegetable protein”, which is basic and called spinning dope, is forced through a spinneret into an acidic bath to coagulate (Harper, 1981). The fibers are stretched, washed, colored and flavored compacted through a heated forming extruder to form a meat analog. Extrusion, the most common form of TPP processing, is achieved by placing a plant protein mixture into an extruder that heats and mixes causing the proteins to denature and form new cross links resulting in a fibrous structure. The mixture is then forced through a die and expands upon release forming a texturized product. Extrusion processing requires ingredients with low or no fat, limiting the raw ingredients that can be used. Steam texturization is the texturizing of vegetable proteins in a steam environment in which the protein expands and texturizes upon release of the pressure (Harper, 1981). Pressure texturization is discussed in detail later in this chapter.

MEALS FOR MILLIONS

Meals for Millions (MFM), established in 1946, was a non-profit organization founded for the purpose of “transfer(ing) emerging protein foods technology to the protein deficient developing countries” that merged with the American Freedom from Hunger Foundation, founded by President John F Kennedy, in 1979 (Anon., 2001). On behalf of the MFM organization, Sterner and Sterner performed research in 1976 focusing on two techniques to minimize the costs of extruding vegetable proteins (Sterner & Sterner, 1976). The first technique

explored methods for preventing the vaporization of water in an extruder during the texturizing of vegetable proteins. The second technique, which is the focus of this research, focused on the creation of protein rich, low-cost, non-extruded, texturized snack foods (Sterner & Sterner, 1976).

PRESS TEXTURIZATION

In 1964, John K McAnelly of Swift & Company (Chicago, IL) received a patent entitled “Bland, textured product from the water insoluble portion of defatted cooked soybean flour”. This method of processing soybean flour called for the use of steam and pressure to textured dough composing of 40-60% flour and 60-40% water. McAnelly explains that to have more than 60% water results in dough that is too fluid to handle while dough containing more than 60% flour resulted in a product that was crumbly. As pressure was released during the texturization process, the moisture in the dough vaporized and the texturized product expanded resulting in a spongy type product (McAnelly, 1964).

Atkinson established in his 1970 patent the ability to create a “meat-like protein product” from a protein mix that contained at least 30% protein and 20 to 60% moisture content (based on the weight of the protein mix). The material used for the protein is recommended to be a “solvent extracted oil seed protein material” (Atkinson, 1970). The mixture is masticated at “temperatures substantially above the boiling point of water” before “extruding the mix at elevated pressure and temperature through an orifice into a medium of lower pressure and temperature” (Atkinson, 1970). However, another patent, “Texturizing of Proteins” was obtained by Touba in 1970 which established that the mastication of the mix before extrusion was not necessary (Touba, 1970). Texturizing of the protein mix would occur if compressed between two hot plates and then released. The method calls for a protein content of at least 50%

and a moisture content of 15 to 30% (Touba, 1970). This form of non-extrusion texturization is known as press texturization (Harper, 1981).

The Village Texturizer is an apparatus that was used in Korean villages to create puffed snack foods by street vendors. It functions much in the same way that Touba described in his 1970 patent. A steel plate sets on four legs, allowing for heating from underneath by means of a coal briquette. In the center of the plate, a milled chamber is used to contain the mixture for cooking. A steel lid, shaped to fit like a “plug” in the milled chamber is placed on top and pressure is applied via a piston which is controlled by a torque wrench. The mix is compressed between the two steel plates while being heated creating a pressurized atmosphere in which the protein denatures and the water is superheated. When the pressure is released, the superheated water instantly vaporizes and escapes the product leaving a fibrous, texturized product.

In 1973, MFM began testing The Village Texturizer by creating a puffed product from “rice-like pellets of sweet potatoes” (Stern & Stern, 1976). Stern and Stern then used the texturizer to develop a soy based snack food by exploring various combinations of temperature (300 and 350°F), pressure (100, 200, 300, 400, 500, 600 and 700 psi) and time (5, 10 and 15 sec) as well as protein (50 and 36.7%) and moisture content (30 and 40%). For full fat flour (protein 36.7%), the conditions at which the product puffed while retaining its shape was concluded to be: mixture containing 40% moisture; pressure of 400 psi; 5 sec of heating; and a temperature of 300°F (Stern & Stern, 1976). Stern and Stern (1976) showed that this method of texturization can be performed on full fat soy flour while extrusion does not allow for texturizing of high fat products (Harper, 1981).

The texture formed during press texturization has been shown to occur in stages (Taranto & Rhee, 1978). The first stage occurs after 2 seconds of texturization in which the cell wall

fragments, isolated protein bodies and cell fragments fuse together. The second stage, in which the protein bodies continue to deform and fuse, occurs after 4 seconds. Even though some protein bodies have broken down, the bulk of the flour protein is not available for reaction which is evident by the presence of the protein bodies' membrane. Stage three, 6 seconds of texturization, shows the cell membrane and protein bodies' membrane continuing to break down. The protein bodies' matrix proteins and the protein-carbohydrate matrix are released. After 8 seconds, the protein-carbohydrate matrix continues to form. Protein bodies have come together to form protein spheres which are encapsulated by cell membrane fragments. In the words of Taranto & Rhee (1978), "the cell contents appear to have been reformed into protein packets surrounded by carbohydrates." Finally, after 10 seconds, the cellular fragments have fused together forming a well defined protein-carbohydrate matrix and formation of the fibers of the protein matrix is evident. While not all the protein bodies had fused together, the process is near completion as indicated by the severe deformation of the protein bodies and break down of cell wall fragments that fuse with the protein-carbohydrate matrix (Taranto & Rhee, 1978).

NOVEL LEGUME BASED SNACK FOODS

The soy based product developed by Sterner and Sterner (1976) using the Village Texturizer is just an example of legume based snack products that are being created with the intent of providing access to healthier snack food products, not only in the United States, but in developing countries as well. U.S patent "Nutritionally balanced protein snack food prepared from legume seeds" describes the creation of a snack food that is composed of legume paste that is supplemented with methionine, cut into the desired shape and fried resulting in a "potato chip-like" snack (Rockland & Radke, 1978). "Reduced-flatulence, legume-based snack foods" is another patent in which the objective is to create a healthy, legume based snack food

(Kazemzadeh, 2001). This particular patent describes a product that is also a chip like snack. However, the appeal is the reduction in soluble sugars by processing aids and extrusion of the dough before either baking, frying or toasting, thereby reducing the likelihood of causing flatulence in the consumer (Kazemzadeh, 2001). A third example of a legume based snack food is the “Bean-nut popping beans” (Ehlers & Sterner, 2000). This product is composed of nunas, legume found in the Andes, which have been hybridized with a kidney cultivar resulting in a bean that can be popped via hot air, microwave, hot skillet or deep frying in oil (Ehlers & Sterner, 2000).

COWPEA

Over the past 30 years, Cowpeas (*Vigna unguiculata*) have been extensively researched due to its health benefits and availability to developing nations which has led to improvements in the growth and consumption of cowpeas. Cowpeas were originally found in Africa, probably somewhere near Ethiopia, and are one of the oldest crops in the world (Gómez, 2004; Phillips, McWatters, Chinnan, Hung & Beuchat, 2003). As this protein rich legume spread across the world, many different names for the crop developed including: (English) black-eye pea, southern, crowder pea; (Afrikaans) akkerboon, swartbekboon, and koertjie (Gómez, 2004). In the United States, the cowpea is an under-utilized source protein, consumed primarily in the south in the form of a boiled vegetable. However, in Africa, cowpea seeds are incorporated into traditional dishes, including homemade weaning foods, as well as used to make foods such as akara and moin-moin (Gómez, 2004). Cowpeas provide an inexpensive protein to developing nations. In Africa alone there are millions of farmers, primarily women, that grow cowpeas which around two million Africans consume (Gómez, 2004).

Cowpeas are high protein (24%) and low fat (1.3%) in composition and widely available in Africa, South America, Latin America and the Southeastern part of the United States (Davis, Oelke, Oplinger, Doll, Hanson & Putman, 1991). In addition, cowpeas are composed of approximately 60% carbohydrates (Phillips, 1993). The protein is composed of 66.6% globulins and 24.9% albumins (Chan & Phillips, 1994). Of particular interest, the cowpea protein has a higher level of the amino acids lysine and tryptophan than that of cereal grains. In the same respect, cowpea protein is lower in methionine and cystine than animal proteins. This allows cowpea to be used not only as a supplement to cereal proteins but as a meat extender as well (Davis, Oelke, Oplinger, Doll, Hanson & Putman, 1991). In 1981, Akpapunam & Markakis analyzed autoclaved cowpea flour for protein efficiency ratio (PER: 1.71 ± 0.13), Amino Acid Score (54), and percent apparent protein digestibility (90%) (Akpapunam & Markakis, 1981).

Cowpeas have been shown to benefit the health in several different ways. Legume starch which includes cowpeas, are digested slower than cereal starches and therefore change the body's glucose level and insulin levels slower (Phillips, McWatters, Chinnan, Hung & Beuchat, 2003). This is especially beneficial to people suffering from diabetes and insulin resistance. In addition, starchy legumes are a good source of dietary fiber as well as vitamins and minerals such as folate, thiamin, riboflavin, potassium, magnesium and phosphorus (Iqbal, Khalil, Ateeq, & Khan, 2006). Despite the amount of potassium, magnesium and phosphorus available from cowpeas, the high ratio of potassium to magnesium and phosphorus can prevent absorption and utilization of the two latter minerals (Iqbal, Khalil, Ateeq & Khan, 2006).

Even with the obvious economic and health benefits of cowpea, there are issues with the legume that limit its consumption. One such drawback is the Hard To Cook (HTC) defect. HTC defect is a result of storage of the cowpea seeds at high temperatures and/or high humidity

causing the protein to change from water soluble to water insoluble. Consequently, the seeds remain hard even after soaking and cooking (Liu, Phillips, Hung, Shewfelt, & McWatters, 1992). This requires a lengthy preparation process and high amount of labor (Gómez, 2004). In addition, the nutritional quality of the cowpea seeds is diminished and the texture is less than ideal for consumption in cowpeas that exhibit the HTC defect (Tuan & Phillips, 1992). Cowpeas also have a beanie flavor that is undesirable to some consumers (Gómez, 2004).

Anti-nutritional factors of cowpeas also play a part in under utilization and consumption. Cowpea composition includes indigestible sugars, such as oligosaccharides, raffinose, stachyose, and verbascose. Humans do not produce the α -galactosidase enzyme that is needed to digest these sugars which leads to flatulence when the sugars pass through the small intestine and ferment thus producing gas (Sosulski, Elkowicz, & Reichert, 1982). Germination has been shown to reduce the flatulence causing sugars, mainly oligosaccharides, because the sugars are water soluble. However, changes to the compositional and functional properties of cowpeas due to higher germination time and temperature needed to reduce the oligosaccharides decreases the acceptability of products produced with cowpeas (Phillips, McWatters, Chinnan, Hung & Beuchat, 2003). In addition to the flatulence causing sugars, trypsin inhibitors and tannins affect protein digestibility by inhibiting digestive enzymes (Plahar, Annan, & Nti, 1997). Both of these anti-nutritional factors can be controlled by conditioning of cowpeas. By decortication of the cowpea seeds, most of the tannins are removed while the trypsin inhibitors, which are heat labile, are destroyed when processed at temperatures greater than 100°C and at least 20% moisture (Phillips, McWatters, Chinnan, Hung & Beuchat, 2003).

SOY BEANS

Soybeans (*Glycine max*), an oilseed, are a good source of vegetable protein containing up to 48% protein and 22% oil (Friedman & Brandon, 2001). Originating in China, the soybean plant (*Glycine max*) was brought to the American colonies in 1785 by Samuel Brown in the form of soy sauce and soy noodles. In today's market, the addition of soy to food products has become very prevalent in food products such as cheese, drinks, miso, tempeh, tofu, salami and vegetarian meat substances; and can be found in various forms including infant formulas, flours, protein isolates and concentrates and textured fibers (Friedman & Brandon, 2001). The use of soy in today's market is driven primarily by soy's functional properties that meet certain needs in processed food while being available at lower costs than ingredients from animal origins (Lusas & Riaz, 1995).

Water and fat absorption, emulsification, aeration (foam volume and stability), flavor binding, color control and imparting of texture are soy's most useful functional properties (Lusas & Riaz, 1995). The thermosetting proteins found in soy flour are large in molecular weight and allow for firming of the product after heating. In addition, the whipping proteins do not heat set like that of the albumin in egg whites. By processing soy protein, the functional properties can be altered to meet certain needs. For example, heating reduces solubility while grinding improves interactions between ingredients by increasing surface area (Lusas & Riaz, 1995).

As in cowpeas, soybeans also contain anti-nutritional factors, including the Kunitz inhibitor of trypsin and the Bowman-Birk inhibitor of chymotrypsin and trypsin, flatulence inducing oligosaccharides (Friedman & Brandon, 2001). By processing the soybeans, the digestibility of the protein is improved; however, unnatural amino acids may form as well such as fructisyl-lysine (protein-carbohydrate browning reaction induced by heat) and D-amino acids

(induced by high pH). Allergies to soy are also of concern when including soy as an ingredient. In Sweden, a survey conducted between 1993-1996, showed that several children who experienced an allergic reaction to peanut were also found to show allergies to soy (Friedman & Brandon, 2001).

The consumption of soy has shown to lower plasma cholesterol as well as helps prevent cancer, diabetes and obesity (Friedman & Brandon, 2001). Soy's wide use can be contributed to many factors including nutritional benefits, availability, low cost, high quality and known suitability as human food (Harper, 1981). It is considered high in the amino acid lysine, which is low in cereals, while being low in the sulfur amino acids methionine and cysteine (Friedman & Brandon, 2001).

PEANUTS

Peanuts (*Arachis hypogaea*), which are grown on about 1.5 million acres of land in the United States alone, are a good source of vitamin E, folate, niacin and essential (linoleic) fatty acid (Yeh, Phillips, Resurreccion, & Hung, 2002). There are four varieties of peanuts, Virginia, Runner, Spanish and Valencia, all of which are ideal for different forms of preparation (Woodroof, 1983). For instance, runners are used in the production of peanut butter while the Virginia is popular for use in confections and roasting because the kernel is larger (Woodroof, 1983). This groundnut is universally popular in used not only as an ingredient in various foods because of its high protein content, bland flavor and light tan color but is consumed a snack as well (Prinyawiwatkul, Beuchat, Phillips, & Resurreccion, 1995; Venkatachalam & Sathe, 2006). Peanuts are one of the most consumed edible nuts and is an important source of vegetable protein and vegetable oil (Venkatachalam & Sathe, 2006; Yeh, Phillips, Resurreccion, & Hung, 2002).

Peanuts are composed of, on average, 28.5% protein (87% globulins) and 47.5% lipids (Woodroof, 1983). Peanut protein is rich in lysine but a poor source of methionine and cysteine. Due to the high protein content of peanuts, the addition of defatted peanut flour to vegetable and cereal proteins from formation of an extruded product has been extensively studied (Prinyawiwatkul, Beuchat, Phillips, & Resurreccion, 1995). This does not mean that the inclusion of large amounts of defatted peanut flour for extrusion is ideal. Extruded products show high expansion but poor structural integrity when the formulation is composed of peanut flour and other oilseed flours. In contrast, when peanut flour and soy flour is used to produce an extruded product, the results are a more acceptable product. Studies have shown that extruding a product that is composed solely of defatted peanut flour produces a fibrous, meat like extrudate. However, due to the gelatinization of the starch breaking the protein matrix, texturization was not complete (Prinyawiwatkul Beuchat, Phillips, & Resurreccion, 1995).

In 2003, Zenere performed experimented with the development of a peanut press cake for human consumption (Zenere, 2003). Peanut press cakes are the result of extraction of the oil from peanuts for use as a vegetable oil in Nigeria. Generally, the peanut press cakes are not suitable for human consumption due to the inclusion of the skins and hulls. By removing the skins and hulls and using food-grade peanuts, the by product of the oil extraction can be used to make a high protein snack food. Zenere devised a chip like snack composed of defatted peanut flour and either soy or wheat flour, cornstarch, peanut butter and sugar (Zenere, 2003).

Peanuts provide a variety of health benefits to humans. Peanuts contain, on average, 2.8% crude fiber (Woodroof, 1983). Diets high in fiber has shown to decrease energy density which increases satiety and decreases intake (Salas-Salvado, Bullo, Perez-Heras, & Ros, 2006). This can be explained by several facts. First, a greater amount of mastication is required for

foods high in fiber which decreases the rate of intake and increases the feeling of satiety. Second, consumption of soluble fiber results in the formation of a gel causes gastric distension and increases the rate at which the stomach empties. By reducing the rate at which the stomach empties in the small intestine, the amount of insulin after eating is decreased (Salas-Salvado, Bullo, Perez-Heras, & Ros, 2006). Third, soluble fiber inhibits the absorption of macronutrients in the large intestine, for example fats and protein, which increases the amount of fecal energy loss (Salas-Salvado, Bullo, Perez-Heras, & Ros, 2006). All these factors show the potential of high fiber diets decreasing obesity and controlling type diabetes. Diets high in soluble fiber have also been shown to reduce total and low-density lipoprotein (LDL) cholesterol in blood serum thus preventing cardiovascular disease (Salas-Salvado, Bullo, Perez-Heras, & Ros, 2006). Peanuts have also been shown to be a good source of antioxidants especially after roasting (Anon., 2008b; Talcott, Passeretti, Duncan, & Gorbett, 2005).

PROTEIN

Of the biological macromolecules, protein is the most abundant and can be found in every cell. Comprised of a combination of 20 different amino acids, proteins are essential to proper functioning of the human body. The functionality of a protein is dependent on the number of amino acids and the order of the amino acids within the polypeptide chain. For this reason, proteins serve several different functions within the human body, such as, enzymes, structural components of cells, cell signaling, immune response, cell cycle, etc (deMan, 1999; Fennema, 1996; Nelson & Cox, 2000).

On the most basic level, the amino acids are the building blocks of proteins. All the amino acids have a central chemical composition that is the same, with the exception of proline which is cyclic, and contains an α -carbon that is covalently bonded to a hydrogen atom, a

carboxyl group, an amino group and a side chain. The side chain of an amino acid determines how the structure of the protein and therefore determines the function of the protein. Amino acids are divided into five groups according their side chain, or more specifically, the side chain's polarity: nonpolar, polar, aromatic, positively charged (basic) and negatively charged (acidic) (Nelson & Cox, 2000). The most hydrophobic group is the nonpolar which includes one of the two sulfur containing amino acids, methionine. The most hydrophilic amino acids are those that are either positively or negatively charges, of which includes lysine (basic) (Nelson & Cox, 2000).

Amino acids are also separated into three groups according the human body's ability to synthesize the amino acid. These two groups are indispensable (essential), dispensable (non-essential) and conditionally indispensable amino acids. The indispensable amino acids are those amino acids that must be consumed in the diet because the human body is unable to synthesize the carbon skeleton from simpler molecules. The indispensable amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Dispensable amino acids are those amino acids that can be synthesized in the human body from other amino acids or other nitrogenous precursors. These amino acids are alanine, aspartic acid, asparagine, glutamic acid and serine. There are some amino acids are considered conditionally indispensable meaning they can be synthesized from other amino acids in the human body but can be disrupted under specific pathophysiological conditions and include arginine, cysteine, glutamine, glycine, proline and tyrosine.

Proteins are divided into three groups based on the behavior in an ultracentrifuge and electrophoretic properties (deMan, 1999). These groups are as follows: simple protein, hydrolysis results in amino acids only, includes albumins (water-soluble) and globulins (almost

water insoluble); conjugated protein, includes amino acids as well as nonprotein material, includes phosphoproteins, lipoproteins, etc.; derived protein, result of chemical or enzymatic reactions (deMan, 1999).

Aside from the biological importance, proteins serve a variety of purposes within the structure of foods including viscosity; water, fat and flavor binding; emulsification; foaming, etc. (Vanchina, Chinnan & McWatters, 2006). Proteins are also the component of foods of concern in the texturizing process. Texturization of proteins is essentially the thermal denaturation of proteins causing the irreversible loss of the globular formation of the protein (Harper, 1981). The globular formation is caused by the interactions between the side chains for the amino acids. The globular formation begins to unfold due to disruption of the hydrogen, ionic and disulfide bonds and van Waals' forces as the temperature and water increases. The reactive sites of the proteins then begin to reform bonds and interactions as which point the texturizing process tries to realign the proteins so as to force reformation to maintain the linear form of the denatured protein. In extrusion, this is completed by the screw action (Harper, 1981).

Table 1.1 Amino acid composition and score for children (age 10-12) of cowpea, soy flour and peanut flour

Indispensable Amino Acids	FAO ⁴ Age 10-12 (g/100g)	Cowpea ¹ (g/100g)	AA Score	Soy flour ² (g/100g)	AA Score	Peanut flour ³ (g/100g)	AA Score
Arginine		7.5 ± 0.04		7.3			
Histidine	1.80	3.1 ± 0.03	100	2.5	100	2.2	100
Isoleucine	2.50	4.5 ± 0.03	100	4.7	100	2.6	100
Leucine	5.50	7.7 ± 0.08	100	7.7	100	5.9	100
Lysine	5.10	7.5 ± 0.04	100	5.8	100	3.4	66
Methionine + Cystine	2.50	2.7 ± 0.04	88	2.3	92	1.64	66
Phenylalanine + Tyrosine	4.70	7.5 ± 0.06	100	5.1	100	4.3	91
Threonine	2.70	3.8 ± 0.05	100	3.6	100	2.4	89
Tryptophan	0.70	0.7 ± 0.02	100			1.0	100
Valine	3.20	5 ± 0.06	100	5.2	100	2.9	92

¹ (Iqbal, Khalil, Ateeq & Khan, 2006)

² (Oomah & Mazza, 1993)

³ (Seligson & Mackey, 1984)

⁴ (*Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, 2002)

FORMULATION SOFTWARE

With the advancements in technology of the past 20-25 years, tools to assist with the process of food formulations have also developed. One such tool is computer software with the ability to develop a food formulation that meets certain desired needs in less time and cost. These needs include nutrient requirements for a determined population at least possible cost. Pearson's Square or simultaneous equations methods can be used to determine simple diets however, are unable to figure in ranges and cost (Anon., 2006). However, Linear Programming, which is now used widely in least cost formulation programs, figures in variations of the nutrient content of the ingredients used due to variations between batches and laboratory or human error. An example of a linear programming model as stated by the least cost formulation software company Winfeed Limited (Cambridge, UK) is as follows (Anon., 2006):

$$\begin{aligned} \text{Minimise } & \sum_{j=1}^n c_j x_j \rightarrow (j = 1, 2, 3, \dots, n) \\ & \sum_{j=1}^n a_{ij} x_j \leq b_i \\ & \sum_{j=1}^n a_{ij} x_j \geq b_i \\ & \sum_{j=1}^n x_j = 1 \\ & x_j \geq 0 \end{aligned}$$

Where:

c_j = Cost per unit for j th ingredient

x_j = Quantity of j th ingredient

a_{ij} = Quantity of i th nutrient per unit of j th ingredient

b_i = Requirement for i th nutrient in the diet

Despite the benefit of using linear programming, this method results in only a 50% confidence that the formulation will meet the nutrient needs for the population of concern. There are two ways to controlling this concern: using a safety margin in the linear programming; and using Stochastic Programming (Anon., 2006). The use of a safety margins essentially figures that the formulation is not suffice to meet the nutrient requirements and therefore increases the amount by 5-10%. This is only an acceptable solution in the case that the ingredients do not have the composition expected. However, in cases when the nutrients of the ingredients have been determined correctly, using a safety margin that automatically increases the amount of the ingredients is equal to profit loss and therefore not an ideal solution (Anon., 2006). The second solution, Stochastic Programming, is better option.

Stochastic Programming increases the probability that the formulation will contain just the right amount of the ingredients to meet the desired nutrient level by modifying the constraints used in the Linear Programming using $P > \phi_i$ so that the model is as follows (Anon., 2006):

$$\begin{aligned} \text{Minimise } & \sum_{j=1}^n c_j x_j \rightarrow (j = 1, 2, 3, \dots, n) \\ & P\left(\sum_{j=1}^n a_{ij} x_j \leq b_i\right) \geq \phi \\ & P\left(\sum_{j=1}^n a_{ij} x_j \geq b_i\right) \geq \phi \end{aligned}$$

Stochastic Programming is a more economical choice than Linear Programming by increasing the confidence level that the nutrient requirements are met from 50% in Linear Programming to 99.99% confidence (Anon., 2006).

Regardless of the program used, the basic process is the same. The initial step is to develop the desired ingredient list. In some cases, certain ingredients may be entered in the

software when received. However, many of the ingredients and the nutrient profiles of the ingredients must be entered manually and thereby the quality of the formulation is only as good as the information entered by the user (Rossi, 2004). Once the ingredients are entered and selected, the nutrient profile for the formulation must be specified in the program. The nutrient profile need not be complete as long as the nutrients of interest are identified. The software then analyzes the desired nutrient profile and cost for the product and determines whether the profile is feasible with the desired ingredient list, what amount of each ingredient that should be used and cost of the formulation (Rossi, 2004). The software also allows the user to minimize cost by finding ingredients that may meet the same nutrient requirements but may cost less (Rossi, 2004). The software used for this research is Creative Formulation Concepts, LLC's formulation and management software Concept4® (Annapolis, MD).

METHODOLOGY FOR EVALUATING TEXTURIZED PRODUCT

Density

In order to determine how the microstructure of a particular food the density of that food is measured (Singh & Heldman, 2001). There are three types of density: solid density, particle density and bulk density. Bulk density is of interest in the case of press texturized products. Bulk density accounts for the void space in between particles in the product (Singh & Heldman, 2001). Taranto *et. al* (1978) compared soy and cottonseed flour that was processed via extrusion or press texturization. The density of the press texturized soy flour was determined to be 194 g/L (Taranto, Cegla, & Rhee, 1978). However, it is unclear as to the method by which the density was determined. A consumer study conducted for the acceptability of a mixed extruded product containing cowpea/peanut/corn/banana showed that products with the lowest bulk density were most acceptable to the consumers (Jain, 2007). However, the product resulting from press

texturization is more likely to be used as a meat analog or meat substitute. The requirements for such a product includes having a layered fiber conformation and must be dense, void of air pockets (Harper, 1981).

Bulk density has been determined by volumetric displacement using glass beads (0.125-0.170 mm) as a replacement medium (Hwang & Hayakawa, 1980; Sokhey, Ali, & Hanna, 1997). Glass beads were poured into a 500 mL spout-less beaker and the density of the glass beads was standardized by filling the measuring cup, leveling the surface with a metal straight edge food scrapper and weighing. The density was calculated using the following equation:

$$\rho_{gb} = \frac{W_{gb}}{V_{gb}}$$

Where, ρ_{gb} is the density of the glass beads; W_{gb} is the weight of the glass beads; V_{gb} is the volume of the glass beads. The calculated densities were then average and used as the known density of the glass beads.

The sample was then cut into small pieces and layered with glass beads, tapping at least forty times between each layer, until the tared cup was completely filled. Excess glass beads were then scrapped off the top using a scrapper and the sample and glass beads were weighed. Density of the sample was determined using the following equation:

$$\rho_s = \frac{W_s}{W_{gbd}} \times \rho_{gb}$$

Where, ρ_s is the density of the sample; W_s is the weight of the sample used; W_{gbd} is the weight of the glass beads displaced; and ρ_{gb} is the density of the glass beads.

Calculation of the percent density reduction allows for determining to what extend the product of press texturization has puffed. Percent density reduction is calculated by determining

the bulk density of the uncooked dough and the texturized product and using the following equation:

$$\%reduction = \frac{\rho_d - \rho_s}{\rho_d} \times 100\%$$

Where, ρ_d is the density of the uncooked mix and ρ_s is the density of the sample.

Moisture Content

Moisture content is water content of a food (Figure & Teixeira, 2007). Determination of moisture content allows for the verification of moisture loss due to press texturization. As discussed earlier, the protein is texturized when the superheated water within the dough flashes off as pressure is released and the product expands. Therefore, determining moisture content (wet basis) via drying in a vacuum oven and using the following equation indicates how successful the process was at vaporizing the water within the dough.

$$\%mc = \frac{W_i - W_d}{W_i} \times 100\%$$

Where, W_i is the initial weight of the sample and W_d is the weight of the dried sample. Moisture content is also non-linearly related to water activity by means of moisture sorption isotherm curve which are dependent of the substance and temperature (Fennema, 1996).

Water Activity

Water activity is method to measure the amount of water bound within the internal structure of the food material by determining how much is available to vaporize, freeze, act as a solvent or determine how much is chemically bound within the food structure (Figure & Teixeira, 2007). While products of the Village Texturizer have been traditionally consumed immediately after preparation, in order develop a product that can be produced at a single location and sold days after processing, water activity of the product must be determined to allow

for proper storage and handling. Water activity has been determined to allow for the prediction of food safety and stability (Fennema, 1996).

The most accurate method to determine water activity is the use of a dew point hygrometer. This instrument has a mirror that is positioned over a closed chamber that is cooled until the dew point is reached within the chamber and dew forms on the surface (Figure & Teixeira, 2007). The dew point is then used to calculate the relative humidity of the chamber which is related to water activity by the following equation:

$$RH = aw \times 100\%$$

By reducing the water activity of a food to 1 or below, the rate at which microbial, chemical and biochemical reactions occur is greatly reduced (Figure & Teixeira, 2007). As the water activity is reduced even further, these reactions occur at a slower and slower pace thereby extending the shelf life of the product. Microbial growth is particularly susceptible to water activity, of particular interest is that the lower limit for growth of most molds is a water activity of 0.8 (Figure & Teixeira, 2007).

Texture Profile Analysis

In 1978, Taranto *et al.* compared soy and cottonseed products produced by the Village Texturizer (Hand Press) with soy and cottonseed products from an extruder (Taranto, Cegla & Rhee, 1978). In this experiment, 25 grams of either defatted soy mixture or cottonseed mixture containing 30% moisture (soy) or 25% moisture (cottonseed) were texturized for 10 seconds under 2.1×10^5 kg/m² of pressure and 195°C (soy) or 200°C (cottonseed) cooking temperature. The products were examined under a scanning electron microscope (SEM) in order to compare the structure resulting from the texturizing processes. In all four cases, the structure of the products appeared fibrous indicating that the products had similar surface morphology.

However, rheological testing (Ottawa Texture Measuring System cell connected to a Model 1122 Instron Universal Testing Machine) showed the press texturized product had higher natural log of stress values and resilience values than the extruded products indicating greater structural integrity. Enzyme (protease) digestion showed that while both processes are successful in texturizing due to cellular disruption followed by fusion of cellular components neither texturizing process resulted in complete disruption or fusion. Taranto *et al.* go on to speculate that not only is extrusion not required to texturize protein but that the shearing caused by the screw may actually be destroying the texture that is being formed (Taranto, Cegla & Rhee, 1978).

Rheological testing was carried out according to the method described in Cegla *et al.* (1978). This method calls for the hydration of 35 g of sample with 70 g of water for 1 hour at ambient temperature with stirring every 10 minutes. The sample was then autoclaved for 30 min at 15 psig at 121°C. The samples were allowed to cool for 1 hour before being tested with an Ottawa Texture Measuring System cell connected to an Instron Universal Testing Machine, Model 1122. Load rate was 50 mm/min and compression was stopped 1.5 mm from the wire grid. The force-deformation curves were used to determine point of inflection stress and resilience giving an overall profile of the structural integrity of the product (Cegla, Taranto, Bell, & Rhee, 1978).

Another possible method for determining texture is the use of the compression test development for the evaluation of akara (Hung, Chinnan, & McWatters, 1988) using universal testing machine (Model 5542, Instron, Inc, Canton, MA). A sample (1 cm x 1 cm) is cut from the product and compressed twice to 25% of its original height at a crosshead speed of 50 mm/min and a chart speed of 200 mm/min.

A third possible method is the use of a Kramer Shear cell. Because the product is much thinner than akara, this compression test may not be feasible for TPA. Among other uses, the Kramer Shear texture analysis has been used to determine the tenderness of meats (Anon., 2008a).

Protein Digestibility

Protein Digestibility determines the quality of a food as a protein source by examining the proportion of nitrogen absorbed after ingestion (Fennema, 1996). Even though the actual amino acid composition of the protein is important in the determination of the quality of a protein, how well the human body is able to digest and use the amino acids present is also of concern. The digestibility of a protein can be affected by protein conformation, antinutritional factors, binding and processing (Fennema, 1996). The conformation of the protein determines how easily it is hydrolyzed by proteases. In general, proteins that have denatured slightly are hydrolyzed more completely than proteins in the native conformation. However, proteins that have been denatured extensively and insoluble fibrous proteins are harder to hydrolyze than proteins that have only slightly been denatured (Fennema, 1996). Antinutritional factors, such as trypsin inhibitors found in cowpeas as well as chymotrypsin inhibitors, inhibit hydrolysis by pancreatic proteases. Lectins (one type of glycoproteins) inhibit absorption of amino acids by binding to mucosa cells. Other antinutritional factors include tannins and phytates. These factors are found in most plant protein isolates and concentrations but less prevalent in heat treated legumes and oilseeds due to heat lability of certain antinutritional factors such as lectins and Kunitz type chymotrypsin inhibitors. Additionally, if the proteins bind with other non-protein molecules, such as polysaccharides and dietary fiber, hydrolysis can be hindered. Under heat during processing and alkaline pH, lysyl residues undergo chemical alterations reducing

their digestibility. Digestibility can also be hindered by the reaction of reducing sugars with ϵ -amino groups induced by heat known as the Maillard reaction (Fennema, 1996).

Digestibility of a protein can be determined via *in vivo* and *in vitro* methods. *In vivo* requires controlling the food consumed by rats and collecting feces that is then analyzed for nitrogen content. *In vitro* determination can be conducted more easily by the addition of an enzyme to the sample to which pancreatin and pepsin are added to initiate and complete the enzymatic reactions (Clark, 2003). The denatured proteins of the digested sample have a reduced solubility and therefore can be recovered by Trichloroacetic acid (TCA) Precipitation to concentrate the protein and then analyzed for percent nitrogen content (Bollag & Edelstein, 1991; Clark, 2003). Polypeptides with higher molecular weight are not soluble in TCA solutions and are considered non-digestible after treatment with protease.

Protein Denaturization

Since the globular conformation of proteins are dependent on the interactions of the amino acid side chains in the protein, any change to the environment of the protein, such as pH, ionic strength, temperature, solvent composition, heat, breaks the side-chain interactions causing the protein structure to become more linear or denature (Fennema, 1996). When globular proteins denature and then react with other denatured proteins, bonds such as S-S linkage, salt bridges and hydrophobic bonds form between the proteins which decreases the solubility of the protein (Fennema, 1996). By measuring the reduction in protein solubility, the extent of protein denaturization can be determined. Two methods for determining protein solubility are Protein Dispersibility Index (PDI) and Nitrogen Solubility Index (NSI) (Anon., 1997, 1999; Morr, German, Kinsella, Regenstein, Vanburen, Kilara, Lewis & Mangino, 1985). Both methods call for the hydration of the sample by stirring. However, NSI requires a slow speed which results in

lower results than PDI. The slurry is then allowed to separate and the upper layer is removed, centrifuged and analyzed for nitrogen content (Anon., 1997, 1999). The PDI is calculated by the following equation:

$$\text{PDI} = \frac{\% \text{ water - dispersible protein} \times 100}{\% \text{ total protein}}$$

NSI is calculated by:

$$\text{NSI} = \frac{\% \text{ water - soluble protein} \times 100}{\% \text{ total nitrogen}}$$

Amino Acid Profile

Determination of the amino acid profile of a product allows for the calculation of the Protein Digestibility Corrected Amino Acid Score (PDCAAS). Calculation of the PDCAAS reveals the overall quality of the protein in the final product. PDCAAS is determined by multiplying the digestibility by the uncorrected amino acid score. A PDCAAS of 1.0 is considered the highest score. This method takes into the account the digestibility of the proteins. In order to separate the amino acids for determination reverse chromatography is used (Anon., 2007). The protein sample must first be hydrolyzed in order to free the amino acids the make up the protein. The hydrolysates are then derivatized making the amino acids easier to separate. These hydrolysates are retained on the column allowing the amino acids to be easier to detect by either by UV absorbance or fluorescence. Waters' Corporation AccQ•Tag™ HPLC Method is a simple step derivatization that prepares the amino acids to be detected via fluorescence by a high performance liquid chromatograph (HPLC). The AccQ•Tag™ Kit contains the following reagents: AccQ•Fluor Borate Buffer which adjust the pH for ideal derivatization; AccQ•Fluor Reagent Powder that acts as the derivatizing agent; and AccQ•Fluor Reagent Diluent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)) which reconstitutes the reagent for

derivatization (Anon., 2007). The HPLC uses high pressure pumps to increase the rate at which the protein molecules move down the column thus limiting spreading of protein bands and increasing resolution (Nelson & Cox, 2000).

SUMMARY

Press texturization is a simple alternative to the use of an extruder in order to texturize vegetable proteins such as cowpeas, soybeans and peanut. Previously studies have determined the appropriate texturizing conditions for a soy product. By using formulation software, several formulations that include cowpeas, soy and/or peanut can be devised to be tested on the Village Texturizer for consumption by children in the age range of 10 to 12 years. The ideal formulation will produce a product that puffs under the right processing conditions and provides a high protein snack food. The quality of this product can be established by analyzing density, water activity and texture profile analysis. By evaluating the protein digestibility, solubility and amino acid profile, it is possible to determine the ability of the texturized product to meet the nutritive requirements of children.

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SECTION II

PRELIMINARY WORK¹

¹Smith HE, Chinnan MS. To be submitted to Int. J of Food Science and Technology

INTRODUCTION

In 1976, Sterner and Sterner (Sterner & Sterner, 1976) used the Village Texturizer (Figure 2.1) to determine the appropriate processing parameters needed to press texturize defatted soy (50% soy protein) and full fat soy (36.7% soy protein). Defatted soy was tested because it met the criteria set by Touba in his patent “Texturizing of Protein” where he determined that protein material could be texturized successfully between two hot plates as long as the material contained at least 50% protein and 30% moisture (Touba, 1970). However, the purpose behind Sterner and Sterner’s research was to use the Village Texturizer to devise a method of preparing texturized vegetable protein products at a much lower cost, in which case, texturizing full fat soy is more applicable (Sterner & Sterner, 1976). The parameters of interest were pressure (100, 200, 300, 400, 500, 600 and 700 psi), temperature (300 and 350°F) and time (5, 10 and 15 s). In our study the Village Texturizer was modified to better control and record the parameters by installing an electric heating element to the bottom of the steel plate and “instruments” to measure temperature and pressure simultaneously. In this study, the pressure reported was the pressure at which the lever was held over the desired period of time. However, the scope of the current study includes the pressure reached within the chamber as opposed to the pressure of the lever. For this reason, the Village Texture was modified to house a temperature/pressure probe within the actual cooking chamber. The objective of this study is to replicate the results of texturization of full fat soy flour at 40% moisture as reported by Sterner and Sterner (1976) while determining the ideal internal pressure to achieve texturization as well as to determine the ideal processing parameters for the texturization of cowpea flour at 40% moisture.

MATERIALS AND METHODS

For defatted soy product, Sterner and Sterner (Sterner & Sterner, 1976) determined the most ideal cooking parameters, by visually observing shape retention and “puff” or expansion, to be 350°F, 5-10 s at a pressure between 400 and 600 psi when the soy mixture contained 30% moisture. In the case of full fat soy product, the ideal cooking parameters were 40% moisture cooked at 300°F under a pressure of 400 psi for 5 s.

For the purpose of replicating the results reported by Sterner and Sterner (1976), full fat soy flour (Bob’s Red Mill, Stone ground whole grain soy flour, 35% protein, Milwaukie, OR) was purchased from Kroger, Inc (Griffin, GA). Flour was sieved using sieve #40 (large), #100 (medium), and #400 (fine). Particle size of sieved flour was determined to be 59.5% large (>0.425), 40.7% medium (0.180 – 0.425 mm) and <1% fine (0.045-0.180 mm) (Vanchina, Chinnan, & McWatters, 2006). Moisture content of the flour was determined and water added resulting in a mix with 40% moisture content.

A mixture composed of cowpeas and water was tested under the same conditions as soy to determine the appropriate cooking parameters using the Village Texturizer. Cowpea flour was prepared from Great Value Brand Black-eyed Peas (cowpeas) purchased from Wal-Mart Stores, Inc (Griffin, GA) which were soaked, dried, dehulled and ground in a coffee grinder (Black & Decker, Towson, MD). In order to control the particle size of the cowpea flour, the resulting cowpea flour was sieved the same as the soy flour and recombined to produce a cowpea flour composed of 65% medium (0.180 – 0.425 mm) particles and 35% large (0.425 – 1.000 mm) particles which was determined to be the ideal particle size composition for akara (Vanchina et al., 2006). Akara, fried cowpea paste, is a traditional snack in Ghana. Moisture content of the flour was determined and water added resulting in a mix with 40% moisture content.

Soy or cowpea mix (40% moisture, 25 g) was pressed into a patty and placed in the texturizer. Pressure was then applied to the lid forming a sealed cooking chamber. Once the desired cooking pressure was achieved, pressure was maintained for a determined amount of time. The handle was released allowing water to instantly vaporize and escape. Cooking pressures studied were 110, 120, 130 and 140 psi at 5 and 10 s. Pressure and time combinations were conducted at 300°F for soy and cowpea, and 350°F for cowpea. Soy was not texturized at 350°F due to Sterners' determination that the ideal cooking temperature for full fat soy was 300°F (Sterner & Sterner, 1976).

Density Measurement

Bulk density was determined by volumetric displacement using glass beads as the replacement medium (Hwang & Hayakawa, 1980). The glass beads were poured into a stainless steel measuring cup which was determined to have a volume of 118 mL. The density of the glass beads was standardized by filling the measuring cup, leveling the surface with a metal straight edge food scrapper and weighing four times. Density was calculated using the following equation:

$$\rho_{gb} = \frac{W_{gb}}{V_{gb}}$$

Where, ρ_{gb} is the density of the glass beads; W_{gb} is the weight of the glass beads; V_{gb} is the volume of the glass beads. The calculated densities were then averaged and used as the known density of the glass beads.

The sample was then cut into small pieces and layered with glass beads, tapping and shaking gently between each layer, until the tared cup was completely filled. Excess glass beads were then scrapped off the top using the food chopper and the sample and glass beads were weighed. Whenever possible, the entire sample was used to determine density. In some cases

the whole sample would not fit within the cup, therefore, the weight of the sample used was determined by subtracting the weight of the material not used from the weight of the total sample. Density of the sample was determined using the following equation:

$$\rho_s = \frac{W_s}{W_{gbd}} \times \rho_{gb}$$

Where, ρ_s is the density of the sample; W_s is the weight of the sample used; W_{gbd} is the weight of the glass beads displaced; and ρ_{gb} is the density of the glass beads.

Percent density reduction was calculated using the following equation:

$$\%reduction = \frac{\rho_d - \rho_s}{\rho_d} \times 100\%$$

Where, ρ_d is the density of the uncooked mix and ρ_s is the density of the sample.

Moisture Measurement

Moisture content was determined on ground samples via forced air oven (Linberg/Blue M, Mechanical Oven, Asheville, NC, USA). Approximately 5 g of each sample was weighed in moisture tin cups and dried at 60°C for one hour. Samples were allowed to cool completely in a desiccator before reweighing. Moisture content was determined using the following equation:

$$\%mc = \frac{W_i - W_d}{W_d} \times 100\%$$

Where, W_i is the initial weight of the sample and W_d is the weight of the dried sample. Moisture content was determined in duplicate and averaged for reporting.

RESULTS

Preliminary results have shown that the parameters established by Sterner and Sterner (1976) for the village texturizer do result in a texturized soy product via a non-extrusion method. These parameters are as follows: moisture content of mix 40%, temperature 300°F, time 5 s and pressure of 400 psi. However, the pressure reported in the 1976 study reflects the pressure

applied to the piston by the torque wrench, not the actual cooking pressure that the mix is subjected to in the cooking cavity. When the pressure and temperature probe was installed in the base of the cooking cavity, pressure was shown to only reach approximately 150 psi.

Texturized product was visually examined for shape retention before determining density and moisture loss. Percent reduction in density is an indication as to the extent the product puffed where the greater the percent reduction the better the texturization. While reduction in density for cowpea cooked at 350°F was greater than cowpea cooked at 300°F, all samples disintegrated during texturizing. Therefore, cowpea cooked at 350°F was not further analyzed. The greatest soy density reduction (75%) occurred at 120 psi and 5 s while resulting in the best shape retention. Moisture analysis showed a 25% reduction due to texturizing. The greatest density reduction (54%) was achieved at 130 psi, 10 s and 300°F for cowpea. However, shape retention was more ideal at 130 psi, 5 s while still resulting in 46% and 19% density and moisture reduction, respectively.

CONCLUSION

Results show the most ideal texturizing conditions at 300°F: soy 120 psi, 5 s; cowpea 130 psi, 5 s. By determining the which set of parameters soy and cowpea flour texturize successfully, this gives a better idea at which parameters formulations containing soy, peanut and/or cowpea flours will texturize best. Full fat soy was shown to texturize more than cowpea, as evidenced by the greater reduction in density, and will therefore be used as a standard by which to compare new formulations.

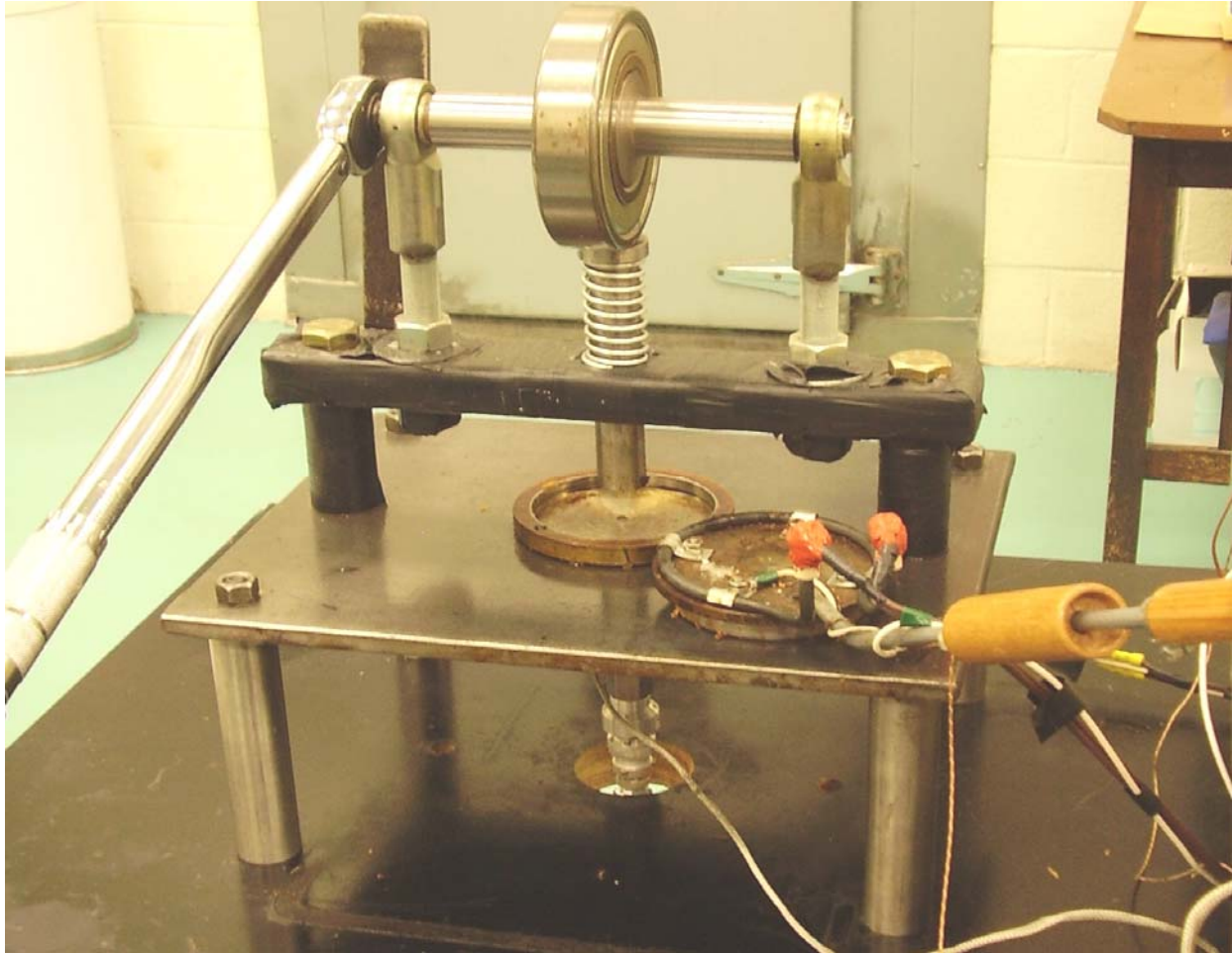


Figure 2.1 The Village Texturizer

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SECTION III

DEVELOPMENT OF FORMULATIONS CONSISTING OF SOY, PEANUT AND COWPEA BLENDS PROCESSED WITH THE VILLAGE TEXTURIZER¹

¹Smith HE, Chinnan MS. To be submitted to Int. J of Food Science and Technology

ABSTRACT

Ten formulations utilizing soybean flour (full fat and defatted) (0-95%), peanut flour (12 and 28% fat) (0-30%) and cowpea flour (0-70%) were created using formulation software. The formulations were analyzed for protein, fat content and amino acid profiles in order to select five formulations for processing by press texturization on the Village Texturizer. All samples were processed at two temperatures (300 and 350°F), three moisture levels (30, 35 and 40%) and four pressures (110, 120, 130 and 140 psi) and compared to full fat soy processed at 300°F, 120 psi for 5 seconds. Shape retention showed that all formulations processed best at 300°F while only the formulation with the highest fat content processed well at all processing parameters. Density measurements indicated that the addition of peanut flour hinders expansion and moisture analysis showed a decrease in moisture loss with an increase in pressure.

INTRODUCTION

Patents beginning in 1964 describe methods by which plant proteins can be texturized using elevated pressure and temperature that occurs between two hot plates and involving dough that contained moisture between the levels of 20%-60% (Atkinson, 1970; McAnelly, 1964; Touba, 1970). Upon the release of the pressure, the superheated water vaporizes and escapes from the product leaving fibrous spongy like product. This method of texturizing plant proteins is referred to as press texturization (Harper, 1981)

Atkinson established in his 1970 patent the ability to create a “meat-like protein product” from a protein mix that contained at least 30% protein and 20 to 60% moisture content (based on the weight of the protein mix). The material used for the protein is recommended to be a “solvent extracted oil seed protein material”. The mixture is masticated at “temperatures substantially above the boiling point of water” before “extruding the mix at elevated pressure and temperature through an orifice into a medium of lower pressure and temperature”. However, another patent, “Texturizing of Proteins” was obtained by Touba in 1970 which established that the mastication of the mix before extrusion was not necessary (Touba, 1970). Texturizing of the protein mix would occur if compressed between two hot plates and then released. This method calls for a protein content of at least 50% and a moisture content of 15 to 30% (Touba, 1970). The texture formed during press texturization has been shown to occur in stages (Taranto & Rhee, 1978). The first stage occurs after 2 seconds of texturization in which the cell wall fragments, isolated protein bodies and cell fragments fuse together. The second stage, in which the protein bodies continue to deform and fuse, occurs after 4 seconds. Even though some protein bodies have broken down, the bulk of the flour protein is not available for reaction which is evident by the presence of the protein bodies’ membrane. Stage three, 6 seconds of

texturization, shows the cell membrane and protein bodies' membrane continuing to break down. The protein bodies' matrix proteins and the protein-carbohydrate matrix are released. After 8 seconds, the protein-carbohydrate matrix continues to form. Protein bodies have come together to form protein spheres which are encapsulated by cell membrane fragments. In the words of Taranto & Rhee (1978), "the cell contents appear to have been reformed into protein packets surrounded by carbohydrates." Finally, after 10 seconds, the cellular fragments have fused together forming a well defined protein-carbohydrate matrix and formation of the fibers of the protein matrix is evident. While not all the protein bodies had fused together, the process is near completion as indicated by the severe deformation of the protein bodies and break down of cell wall fragments that fuse with the protein-carbohydrate matrix (Taranto & Rhee, 1978).

The Village Texturizer is an apparatus that was used in Korean villages to create puffed snack foods by street vendors. It functions much in the same way that Touba described in his 1970 patent. A steel plate sets on four legs, allowing for heating from underneath by means of a coal briquette. In the center of the plate, a milled chamber is used to contain the mixture for cooking. A steel lid, shaped to fit like a "plug" in the milled chamber is placed on top and pressure is applied via a piston which is controlled by a torque wrench. The mix is compressed between the two steel plates while being heated creating a pressurized atmosphere in which the protein denatures and the water is superheated. When the pressure is released, the superheated water instantly vaporizes and escapes the product leaving a fibrous, texturized product.

In 1973, Meals for Millions (MFM) began testing The Village Texturizer by creating a puffed product from "rice-like pellets of sweet potatoes" (Stern & Stern, 1976). Stern and Stern then used the texturizer to develop a soy based snack food by exploring various combinations of temperature (300 and 350°F), pressure (100, 200, 300, 400, 500, 600 and 700

psi) and time (5, 10 and 15 sec) as well as protein (50 and 36.7%) and moisture content (30 and 40%). For full fat flour (protein 36.7%), the conditions at which the product puffed while retaining its shape was concluded to be: mixture containing 40% moisture; pressure of 400 psi; 5 sec of heating; and a temperature of 300°F (Stern & Stern, 1976). Stern and Stern (1976) showed that this method of texturization can be performed on full fat soy flour while extrusion does not allow of texturizing of high fat products (Harper, 1981).

Modifications to the Village Texturizer were needed in order to better control the processing conditions. Heating elements were attached underneath the steel plate around the base of the milled chamber as well as to the top of the lid and were controlled electronically using temperature controller (Barnant, Model 621-8620, Barrington, IL). A temperature/pressure probe was installed through the center of the milled chamber in order to determine the actual pressure and temperature within the chamber during processing. Use of the pressure/temperature probe showed that the pressure within the chamber during processing is not as high as the processing pressure reported in Stern and Stern (1976) which was a measurement of the pressure applied to the lid by the piston. Pressures measured by the pressure/temperature probe indicated that 150 psi within the milled chamber during processing was towards the upper limit achieved by manual application of the lever.

Developing a product that is composed of a combination of soy flour (defatted or full fat), partially peanut flour (12% fat or 28% fat) and/or cowpea flour texturized on the Village Texturizer could potentially provide a high protein snack food that can be easily processed in developing nations. Formulation software is one method by which to determine the ability a combination of ingredients to meet the nutrient parameters of interest. However, the information provided by the software is only as good as the information input by the user. Formulation

software requires the user to input the macronutrient composition and any other information of interest of each ingredient (Rossi, 2004). Initial formulations were constructed by use of a least cost formulation software in which the feasibility of meeting the required protein content was indicated. The macronutrient and indispensable amino acid profile of the ingredients allowed for the determination of formulations that meet the protein requirements of the processing procedure as well as the indispensable amino acid requirements of children ages 10 to 12 years. The objective of this study is to determine the processing parameters at which 5 formulations, at three moisture levels, meeting the protein requirements for press texturization and amino acid requirements of children ages 10 to 12 years texturize while retaining shape and to compare the formulation/processing parameter sets to a control composed of full fat soy flour, brought to 40% moisture and processed at 300°F under 120 psi as determined to be ideal by previous research, for density and moisture content.

MATERIAL AND METHODS

Flours

Cowpeas, *Vigna unguiculata*, were obtained from SeedGrow Inc. (Meridian, CA). The cowpeas were immediately transferred to barrier bags that were heat sealed and stored at 4°C before processing. Approximately 3.4 kg of cowpeas were placed in a plastic container and covered with water to soak for 18 hours at 4°C. The cowpeas were then drained and spread into a single layer on a metal pan and dried at 60°C for approximately 12 hours in a forced air oven (Linberg/Blue M, Mechanical Oven, Asheville, NC, USA). Dried cowpeas were cracked using a hand cranked nut grinder in order to loosen the hulls before sorting the cowpea seeds from the hull by running through a seed blower. Visual examination was used to remove any remaining hulls from the cowpea seeds.

The cowpea seeds were hammer milled using (Model, 6 x 14, Champion Products Inc., Eden Prairie, MN) using a 2.54 mm screen. The resulting cowpea flour was sieved through sieves #40, 100 and 400 (USA Standard Testing Sieve, ASTM E II Specification, Fisher Scientific, Pittsburgh, PA) for 10 minutes with a mechanical shaker (RX-86, W.S. Tyler, Mentor, OH). The final cowpea flour was produced by using medium particles (0.180 – 0.425 mm) particles and large particles (>0.425 mm) in a 65:35 ratio by weight (Vanchina, Chinnan, & McWatters, 2006). Mixed cowpea flour was repackaged in barrier bags and stored at 4°C until used.

Both defatted soy flour and full fat (organic) soy flour was obtained from Hodgson Mills Inc (Effingham, IL). Immediately upon receipt, the flours were repacked in barrier bags, heat sealed and stored at 4°C.

Partially defatted peanut flour (12% fat, light roast and 28% fat light roast) was obtained from Golden Peanut Company, Alpharetta, GA. Both flours were received in plastic containers and therefore just placed in 4°C environmental chamber until used.

Formulations

Formulations to be tested on the press texturizer were developed using the formulation software Creative Formulation Concepts, LLC, Concept 4.0, Level 2 (Annapolis, MD). Each of the 10 formulations contained at least two and up to three ingredients: cowpea; full fat soy flour or defatted soy flour; 12% fat peanut flour (light roast) or 28% fat peanut flour (light roast). Formulations produced were evaluated electronically for percent protein, percent fat and grams amino acid per 100 g protein.

Texturization

Each formulation was mixed with the appropriate amount of water to give mixtures at 30, 35 and 40% moisture content resulting in 15 formulation/moisture level combinations to be texturized. The mixes were weighed out into 25 g samples and flattened with a fondant rolling pin (3 mm thick) before placing into the milled chamber of the Village Texturizer. Mixtures were texturized once at four pressures (110, 120, 130 and 140 psi) and two temperatures (300°F and 350°F) for 5 seconds giving a total of 120 formulation/moisture/pressure/temperature combinations tested. While the mixtures were held at the desired pressure for 5 seconds, the come up time ranged from 2 to 3 seconds giving a total processing time of up to 8 seconds.

Density

Bulk density was determined by volumetric displacement using glass beads as the replacement medium (Hwang & Hayakawa, 1980). The glass beads (0.850 - 0.250 mm) were poured into a stainless steel measuring cup which was determined to have a volume of 118 mL. The density of the glass beads was standardized by filling the measuring cup, leveling the surface with a metal straight edge food scraper and weighing four times. The calculated densities were then averaged and used as the known density of the glass beads. Density was calculated using the following equation:

$$\rho_{gb} = \frac{W_{gb}}{V_{gb}}$$

Where, ρ_{gb} is the density of the glass beads; W_{gb} is the weight of the glass beads; V_{gb} is the volume of the glass beads

The sample was then cut into small pieces and layered with glass beads, tapping and shaking gently between each layer, until the tared cup was completely filled. Excess glass beads were then scrapped off the top using the food chopper and the sample and glass beads were weighed. Whenever possible, the entire sample was used to determine density. In some cases it

was necessary to determine the weight of the sample not used in order to determine the weight of the sample placed within the cup.

$$\rho_s = \frac{W_s}{W_{gbd}} \times \rho_{gb}$$

Where, ρ_s is the density of the sample; W_s is the weight of the sample used; W_{gbd} is the weight of the glass beads displaced; and ρ_{gb} is the density of the glass beads. Percent density reduction was calculated using the following equation:

$$\%reduction = \frac{\rho_d - \rho_s}{\rho_d} \times 100\%$$

Where, ρ_d is the density of the uncooked mix and ρ_s is the density of the sample. Density of each sample was compared to the density of full fat soy texturized product determined in the preliminary study.

Moisture Content

Moisture content was determined on ground samples using a forced air oven (Linberg/Blue M, Mechanical Oven, Asheville, NC, USA). Approximately 5 g of each sample was weighed in moisture tin cup and dried at 60°C for one hour. Samples were allowed to cool completely in a desiccator before reweighing.

$$\%mc = \frac{W_i - W_d}{W_d} \times 100\%$$

Where, W_i is the initial weight of the sample and W_d is the weight of the dried sample. Moisture content was determined in duplicate and averaged for reporting.

RESULTS AND DISCUSSION

Formulation Limitations

Limitations were set for the formulations based on previous studies. Sterner and Sterner (1976), showed that full fat soy with a protein content of 36.7% was successfully textured via press

texturization, therefore this was set as the minimum amount of protein for the formulation.

Formulation 3 does not meet the protein requirements and therefore was not further considered for texturization. In the same study, defatted soy flour, 50% protein, was texturized as well (Sterner & Sterner, 1976). The maximum protein limit was set at 50% accordingly. However, due to the ingredients used, the only formulation that would reach the 50% maximum was that containing 100% peanut flour (12% fat) (full fat contains 46.67% protein). This leads to another limitation, percent peanut flour. Previous research showed that up to 30% partially defatted peanut flour could be added to a formulation for texturization through extrusion and the product have a bulk density and shear strength similar to two commercially available extruded snack foods (Suknark, 1998). Consequently, peanut flour was limited to 30% of the formulation for this study. The recommended grams of amino acid per gram of protein for the indispensable amino acids were not set as limits in the software but were compared and are discussed later in the Amino Acid section.

Formulation Trends

By reviewing the percent protein estimated for the formulations, a trend can be seen regarding the percent of protein and the ingredients used. Due to the difference in fat content, full fat soy flour contains less percent protein than defatted soy flour; similarly, 12% fat peanut flour contains more percent protein compared to 28% fat peanut flour. Therefore, a formula that contains 30% peanut flour (12% fat) will have higher percent protein content than the same formula using 30% peanut flour (28% fat). The same holds true for the use of defatted soy flour in place of full fat soy flour, percent protein is higher. Of the five ingredients (full fat soy flour, defatted soy flour, 28% peanut flour, 12% peanut flour and cowpea flour), cowpea contains less protein per gram. The effect is that an increase or decrease in cowpea inversely affects the percent protein of the product.

Not surprisingly, almost all the formulations were shown to be deficient in the percent of methionine (Table 3.1). The highest calculated methionine content was 2.58 g/100 g protein and resulted from the formulation containing 70% cowpea and 30% peanut flour (12% fat). However, this formulation gives the lowest calculated protein content, 31.80%, which falls below the minimum percent protein. The remaining formulations give a calculated methionine content of between 2.10 and 2.44 g/100 g protein. This indicates that no formulation which contains a combination of soy, peanut and cowpea flour will meet the recommended requirements of methionine. In addition, this same formulation of 30% peanut flour (12% fat) and 70% cowpea was the only formulation to meet the recommended 0.7 g/100 g protein of tryptophan (calculated value 0.85 g/100 g protein).

Another amino acid influenced is lysine. The amount of defatted soy flour and cowpea is directly related to the grams lysine/100 g of protein. Because peanut flour is considered deficient in lysine, an increase in percent of peanut flour decreases the amount of lysine while cowpea, high in lysine, increases the lysine content of the formulation. Therefore, the benefits of including cowpea in the formulation are an increase in lysine, methionine and tryptophan, while peanut and soy flour increase protein content and tryptophan.

Recommended Formulations

The ingredient ratio of the formulations and the calculated protein, fat and indispensable amino acid content of each formulation are compared in Table 3.1. Based on the limitations of the formulations and the trends of protein and amino acid content, the formulations recommended for texturization experimentation are formulations 5, 6, 8, 9 and 10. These formulations meet the minimum requirement of 36.7% protein, with the exception of formulation 5, and are deficient in only methionine(*Food and Nutrition Report: DRI Report*). Formulation 5 contains full fat soy and partially defatted peanut flour (28% fat, light roast) and has a calculated protein content of 35.55%

and fat content of 22.40%. This formulation is of particular interest because the higher fat soy and peanut flour requires less processing to produce making them more readily available in developing nations. By replacing the full fat soy in formulation 5 to defatted soy flour, formulation 6 has a higher protein content of 44.67% and a lower fat content of 8.40%. Formulations 8 and 10 vary in the ratio of defatted soy flour to cowpea flour. However, the decrease in cowpea flour in formulation 10 increases the gram per 100 grams of protein of Methionine+Cystine while formulation 8 contains a higher percentage of protein. The only formulation containing soy, peanut and cowpea flour is formulation 9.

Visual Observations

The ability of the formulations to cook under the processing parameter combinations was determined by visually examining the product immediately after processing (figures 3.1 - 3.5). In the case of processing combinations that included using the temperature 350°F, the products were unable to retain shape retention upon release of the pressure. This shows that use of the higher temperature is not appropriate for processing. Formulation 5 (figure 3.1) processed at 350°F resulted in better shape retention than any other formulation processed at the same temperature. However, the product had a tendency to explode on one side rendering the shape retention unacceptable. Formulation 6 (figure 3.2) required a moisture content of at least 40% in order to produce dough that was able to be processed; at 30% and 35% moisture content, the dough was too dry and crumbly to process. Texturing of this dough was only successful at 300°F under the pressures 120, 130 and 140 psi. For formulation 8 (figure 3.3), the ideal moisture content for processing was 35%. A moisture content of 30% resulted in a dry dough while a moisture content of 40% caused the product to burst in the center once the pressure was released. Formulation 9 (figure 3.4) resulted in shape retention similar to that seen on formulation 6 where 40% moisture content was ideal and moisture 30% and 35%

were too dry to process. In addition, products of formulation 10 (figure 3.5) were visually similar to those of formulation 8. Formulations 8 and 10 were similar in composition where the only difference was the ratio of defatted soy flour to cowpea flour. By using the visual examination of the products, 26 samples were determined to texturize successfully: formulation 5, 300°F, moistures 30, 35 and 40%, pressures 110, 120, 130 and 140 psi; formulation 6, 300°F, 40% moisture, pressure 120, 130 and 140 psi; formulation 8, 300°F, 35% moisture, pressures 110, 120, 130 and 140 psi; formulation 9, 300°F, 40% moisture, pressure 120, 130 and 140 psi; and formulation 10, 300°F, 35% moisture, pressures 110, 120, 130 and 140 psi.

Formulation 5 contained the highest calculated fat content (22.40%) and lowest protein content (35.33%). Because formulation 5 resulted in the best shape retention, there is an indication that the higher fat content assisted in the ability of the dough to be processed across a broader range of processing conditions. This may be a result of the soybean and peanut oil within the flours ability to improve elasticity of the protein carbohydrate matrix.

Density

Bulk density was determined for each of the 26 samples that retained shape upon texturization and percent reduction was calculated to establish to what extent the product was texturized. Because bulk density accounts for the void space in between particles (Singh & Heldman, 2001), a greater percent reduction in bulk density indicates that the protein-carbohydrate matrix was formed successfully and the escaped water vapor has caused the product to expand (Harper, 1981; Taranto & Rhee, 1978).

Full fat soy was texturized at 300°F and 120 psi for 5 seconds as determined to be the ideal texturizing conditions in the preliminary study and was used as a standard by which to compare the formulation samples. Table 3.2 shows the density and percent reduction in density for each of the

samples. Initial densities of the doughs are as follows: formulation 5, moisture 30%, 1.34 g/mL; moisture 35%, 1.18 g/mL; moisture 40%, 1.21 g/mL; formulation 6, 40% moisture, 1.20 g/mL; formulation 8, 35% moisture, 1.28 g/mL; formulation 9, 40% moisture, 1.27 g/mL; formulation 10, 35% moisture, 1.29 g/mL. Texturization of full fat soy resulted in a density of 0.4063 g/mL and a percent reduction in density due to texturization of 75.15%. Density determinations of samples which retained shape retention upon processing ranged from 0.679 g/mL (Formulation 10, 40% moisture, 140 psi) to 0.981 g/mL (Formulation 6, 40% moisture, 130 psi) corresponding with a percent reduction in density of 47.38% to 18.16%, respectively. Calculation of percent reduction in density showed that while texturization was successful for all 26 samples, expansion was greatest at 110 psi for formulation 5 (35% moisture); 120 psi for formulations 5 (40% moisture), 6 (40% moisture) and 8 (35% moisture) while formulations 5 (30% moisture) and 9 (40% moisture) saw the greatest expansion at 130 psi. Formulation 10 (35% moisture) had the greatest expansion at 140 psi. None of the formulations texturized resulted in expansion as great as that seen in the full fat soy sample (percent reduction 75.15%). Generally, the samples which contained only soy and cowpea flour (formulations 8 and 10) expanded better than samples containing peanut flour, with the exception of formulation 5 at 30% moisture which also contained the highest percent calculated fat.

Moisture

Determination of the percent reduction in moisture due to press texturization showed a general trend of decreased moisture loss with an increase in pressure with the only exception being formulation 8 (35% moisture) (Table 3.3). One theory is that the increase in pressure causes more of the water within the product to become trapped within the product upon release of the pressure (Aboagye & Stanley, 1985). The sample with the greatest percentage reduction in density (formulation 10, 35% moisture, 140 psi) was also the sample that had the lowest percentage

reduction in moisture. Also, the samples (formulations 8 and 10) containing only defatted soy flour and cowpea flour retained more moisture than the other formulations. Formulation 5 lost the most moisture through processing across all three moisture levels (30, 35 and 40%). The higher the fat content of the flours the higher the percent reduction of moisture. This leads to the conclusion that the fat within the flour is preventing the water from being absorbed and trapped as well as it is within formulations with lower fat contents.

CONCLUSION

The use of formulation software allowed for the creation of 10 possible formulations that met the protein requirement for press texturization of as well as determination of the expected amino acid profile of each formulation. Of the 10 formulations, five formulations were selected because of the most ideal expected amino acid profile or, as is the case for formulation 5, because the ingredients would be more available in developing nations. Of the formulations texturized, formulation 5 is the only one that was successfully texturized across all the processing parameters. Formulations 6, 8, 9 and 10 did not result in dough that could be texturized at a moisture level of 30%, furthermore, formulations 6 and 9 were too dry at a moisture level of 35% as well. The results show that all formulations had better shape retention at a temperature of 300°F.

Density and moisture determinations were conducted on only those samples which held their shape upon texturization: formulation 5 (30, 35 and 40% moisture), formulation 6 (40% moisture), formulation 8 (35% moisture), formulation 9 (40% moisture) and formulation 10 (35% moisture). Percent reduction in density showed that formulations 8 and 10 expanded better than formulations 5, 6 and 9 which contained peanut flour. The one exception was formulation 5 at 30% moisture. While all samples were shown to expand, none of the processing parameter

combinations resulted in a product with as high a percent reduction in density as that of full fat soy flour (75%). Percent reduction in moisture showed that an increase in pressure resulted in a decrease in moisture loss. Also, the higher the fat content of the dough, the greater the reduction in moisture due to press texturization.

Table 3.1 Formulation compositions and calculated indispensable amino acid profile (amino acid content reported as g/100 g protein)

	<i>FAO</i>	Soy (full-fat)	1	2	3	4	5	6	7	8	9	10
Formulation												
Soy full fat		100%	65%				70%					
Soy defatted				68%		70%		70%	95%	80%	50%	65%
Peanut 12% fat			30%	30%	30%	30%					30%	
Peanut 28% fat							30%	30%	5%			
Cowpea			5%	2%	70%					20%	20%	35%
Fat		20.00%	16.66%	3.63%	4.51%	3.60%	22.40%	8.40%	1.40%	0.26%	3.86%	0.45%
Protein		33.33%	37.87%	47.21%	31.80%	47.67%	35.33%	44.67%	46.33%	42.13%	43.13%	38.73%
Indispensible amino acid content												
Methionine + Cystine	2.5	2.30	2.13	2.12	2.58	2.10	2.10	2.10	2.27	2.38	2.19	2.44
Lysine	5.1	5.80	4.89	5.04	5.57	5.03	4.98	5.15	5.70	6.01	5.15	6.17
Tryptophan	0.7	1.64	1.37	1.44	0.85	1.45	1.44	1.48	1.62	1.54	1.32	1.45
Threonine	2.7	3.60	3.14	3.22	3.14	3.23	3.20	3.27	3.54	3.63	3.20	3.64
Isoleucine	2.5	4.70	3.86	4.02	3.62	4.05	3.99	4.14	4.62	4.68	3.94	4.65
Histidine	1.8	2.50	2.38	2.39	2.64	2.39	2.38	2.42	2.48	2.56	2.46	2.63
Valine	3.2	5.20	4.30	4.47	4.03	4.49	4.44	4.59	5.09	4.94	4.38	5.16
Leucine	5.5	7.70	7.00	7.14	6.86	7.15	7.10	7.23	7.62	7.69	7.09	7.69
Arginine		7.30	4.41	4.98	3.96	4.99	4.81	5.33	6.99	7.33	4.78	7.33
Phenylalanine + Tyrosine	4.7	5.10	4.86	4.87	5.97	4.85	4.84	4.88	5.07	5.36	5.08	5.63

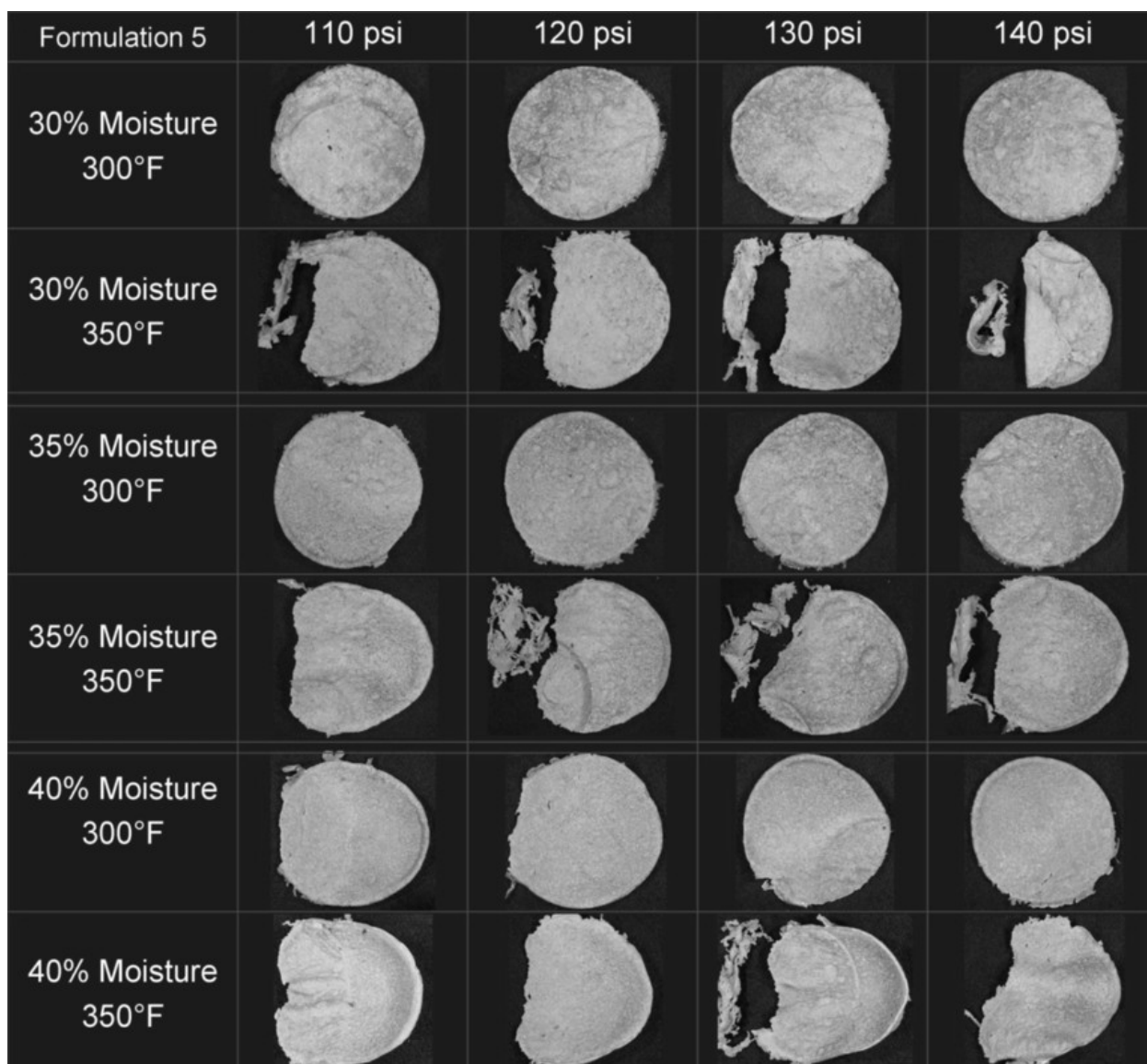


Figure 3.1 Formulation 5 processed under various conditions: pressure 110, 120, 130 and 140 psi; moisture level 30, 35 and 40%; and temperatures 300 and 350°F

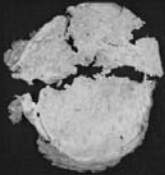
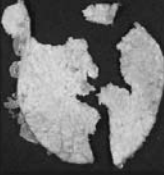


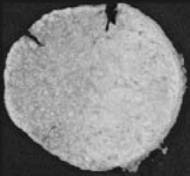
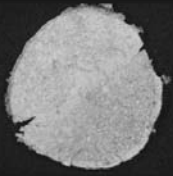
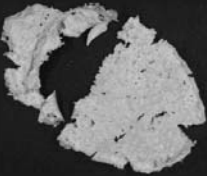
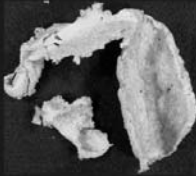

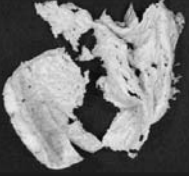
Formulation 6	110 psi	120 psi	130 psi	140 psi
30% Moisture 300°F				
30% Moisture 350°F				
35% Moisture 300°F				
35% Moisture 350°F				
40% Moisture 300°F				
40% Moisture 350°F				

Figure 3.2 Formulation 6 processed under the following conditions: pressure 110, 120, 130 and 140 psi; moisture level 35 and 40%; and temperatures 300 and 350°F. (Processing parameters containing no image were not processed due to insufficient moisture needed to create a dough)









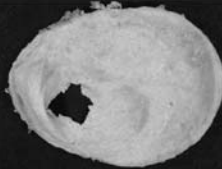
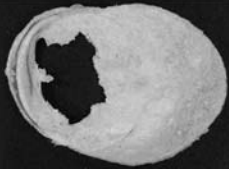
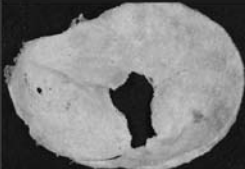


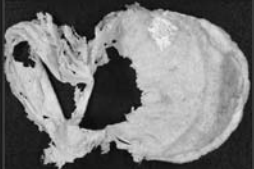
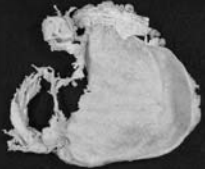
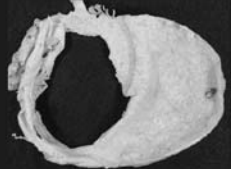
Formulation 8	110 psi	120 psi	130 psi	140 psi
30% Moisture 300°F				
30% Moisture 350°F				
35% Moisture 300°F				
35% Moisture 350°F				
40% Moisture 300°F				
40% Moisture 350°F				

Figure 3.3 Formulation 8 processed under the following conditions: pressure 110, 120, 130 and 140 psi; moisture level 35 and 40%; and temperatures 300 and 350°F. (Processing parameters containing no image were not processed due to insufficient moisture needed to create a dough)







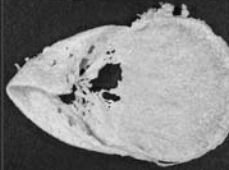
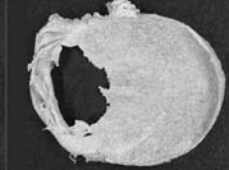
Formulation 9	110 psi	120 psi	130 psi	140 psi
30% Moisture 300°F				
30% Moisture 350°F				
35% Moisture 300°F				
35% Moisture 350°F				
40% Moisture 300°F				
40% Moisture 350°F				

Figure 3.4 Formulation 9 processed under the following conditions: pressure 110, 120, 130 and 140 psi; moisture level 40%; and temperatures 300 and 350°F. (Processing parameters containing no image were not processed due to insufficient moisture needed to create a dough)






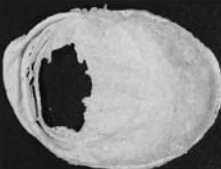
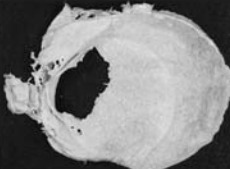

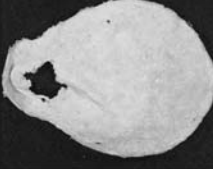


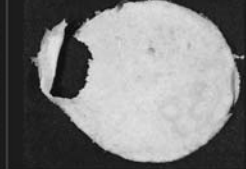

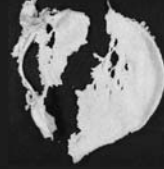
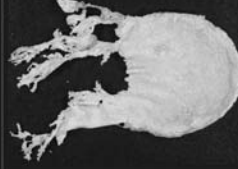
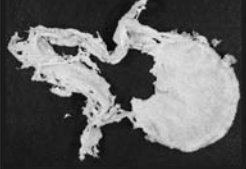
Formulation 10	110 psi	120 psi	130 psi	140 psi
30% Moisture 300°F				
30% Moisture 350°F				
35% Moisture 300°F				
35% Moisture 350°F				
40% Moisture 300°F				
40% Moisture 350°F				

Figure 3.5 Formulation 10 processed under the following conditions: pressure 110, 120, 130 and 140 psi; moisture level 35 and 40%; and temperatures 300 and 350°F. (Processing parameters containing no image were not processed due to insufficient moisture needed to create a dough)

Table 3.2 Density measurements of samples which met the required shape retention upon processing with the Village Texturizer

Formulation	Temperature	Moisture	Pressure (psi)	Density (g/mL)	% Reduction in Density	% Difference in Density vs. Control
Soy (full fat)	300°F	40%	120	0.406	75.15	
5	300°F	30%	110	0.799	40.26	65.19
			120	0.864	35.45	72.02
			130	0.791	40.89	64.25
			140	0.833	37.77	68.82
		35%	110	0.763	35.10	61.04
			120	0.813	30.90	66.67
			130	0.920	21.81	77.43
			140	0.952	19.09	80.31
		40%	110	0.948	21.70	79.94
			120	0.767	36.60	61.49
			130	0.877	27.56	61.49
			140	0.844	30.24	73.30
6	300°F	40%	120	0.808	32.57	66.16
			130	0.981	18.16	82.82
			140	0.907	24.30	76.26
8	300°F	35%	110	0.851	33.23	70.76
			120	0.738	42.11	57.97
			130	0.862	32.38	71.86
			140	0.864	32.20	72.09
9	300°F	40%	120	0.957	24.87	80.81
			130	0.822	35.47	67.72
			140	0.953	25.25	80.39
10	300°F	35%	110	0.930	27.93	78.34
			120	0.918	28.87	77.23
			130	0.728	43.57	56.71
			140	0.697	47.39	50.20

Table 3.3 Moisture measurements of samples which met the required shape retention upon processing with the Village Texturizer

Formulation	Temperature	Moisture	Pressure (psi)	% Moisture	% Reduction in Moisture
5	300	30%	110	22.30	25.68
			120	23.97	20.10
			130	25.28	15.72
			140	26.01	13.29
		35%	110	27.82	20.52
			120	28.37	18.94
			130	28.54	18.45
			140	29.04	17.02
		40%	110	32.67	18.33
			120	33.47	16.33
			130	33.63	15.92
			140	33.60	16.00
6	300	40%	120	33.63	15.92
			130	34.53	13.67
			140	34.27	14.34
8	300	35%	110	32.24	7.88
			120	30.80	11.99
			130	32.96	5.84
			140	29.56	15.55
9	300	40%	120	32.51	18.73
			130	35.92	10.19
			140	36.47	8.82
10	300	35%	110	30.79	12.02
			120	31.08	11.19
			130	32.01	8.55
			140	33.74	3.60

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SECTION IV

TEXTURE AND WATER ACTIVITY ANALYSIS OF SOY, PEANUT AND COWPEA BLENDS PROCESSED WITH THE VILLAGE TEXTURIZER¹

¹Smith HE, Chinnan MS. To be submitted to Int. J of Food Science and Technology

ABSTRACT

Texture and water activity can be used to determine the chemical structure of foods. A total of 26 samples containing soy flour (full fat or defatted) (50-95%), partially defatted peanut flour (12 or 28% fat) (0-30%) and/or cowpea flour (0-70%) which achieved shape retention upon texturizing with the Village Texturizer were analyzed and compared to a control composed of full fat soy flour as well as a commercial vegan burger product. The samples were soaked and steamed before analysis with the Ottawa Texture Measuring System. Stress required to extrude the samples through a six-wire base was calculated. Samples containing peanut flour were harder and required more stress to extrude (1.98-4.68 N/cm²). Water activity was shown to be higher for samples containing peanut flour (0.949-0.976) as well. The 8 samples with the least percent difference of texture from the full fat soy control were determined for future protein analysis.

INTRODUCTION

Textural analysis of foods has been used as method to determine the effects that the macro and microstructure of a food substance has on the external structure (deMan, 1999). The chemical structure of a food directly effects physical structure and properties. Food texture analysis is used to determine three things: the effect of mechanical action such as harvesting; flow properties of the food during processing, handling and storage; and the mechanical behavior when consumed. While there are a variety of terms used to describe texture, many of the terms are poorly defined (consistency, hardness, firmness, brittleness, stickiness) and some have no “objective physical meaning” (body, crisp, greasy, brittle, tender, juicy, mealy, flaky, crunchy, etc.) (deMan, 1999). However, there have been instruments designed to quantify the texture of foods. The use of these instruments has made it possible to define texture by several terms: hardness, which describes the force required for deformation; cohesiveness, measures the strength of the internal bonds; viscosity, measures the rate of flow; elasticity, describes the ability of a product to return to its original condition after deformation; and adhesiveness, which measures the work needed to overcome the “attractive forces between the surface of the food and the surface of other materials in which the food comes in contact” (deMan, 1999). In addition, adhesiveness can be broken up into three terms: brittleness, measures the force required to fracture the product; chewiness, measures the energy required for mastication; and gumminess, energy required for disintegration of the product (deMan, 1999).

The Ottawa Texture Measuring System (OTMS) produces shear stress as the product is forced to extrude through a base containing either holes or wires and also replicates the biting process. While this method is traditionally used for fruits and vegetables, previous studies examining the rheological measurements of cottonseed blends texturized on the Village

Texturizer used the OTMS to determine the stress required to extrude the product through a six-wire base (Cegla, Taranto, Bell, & Rhee, 1978). For the purposes of this study, a modified version of the method devised by Cegla *et al.* was used (1978). Aboagye and Stanley (1985) also report using the OTMS in addition to the bending test to analyze defatted peanut flour which had been press texturized at 140, 175 and 210°C. The results for both the bending test and the OTMS showed an increase in the resistance to deformation as the temperature increased. The bending test also showed an increase in resistance to deformation as a function on moisture, where an increase in moisture resulted in an increased resistance (Aboagye & Stanley, 1985). They contributed this direct relation to the increase in protein solubilization of the globular proteins which reorganizes to form the fibrous structure of the final product (Aboagye & Stanley, 1985).

Water activity is a method to measure the amount of water bound within the internal structure of the food material by determining how much is available to vaporize, freeze, act as a solvent or determine how much is chemically bound within the food structure (Figure & Teixeira, 2007). While products of the Village Texturizer have been traditionally consumed immediately after preparation, in order to develop a product that can be produced at a single location and sold days after processing, water activity of the product must be determined to allow for proper storage and handling. Water activity has been determined to allow for the prediction of food safety and stability (Fennema, 1996).

Water activity also has an effect on the texture of a food product (deMan, 1999). By looking at the range of water activity as three regions it is possible to see the correlation between water activity and texture. Region 1 is of low water activity (0-0.25); within this water activity range, products are hard, dry and crisp. Region 2 ranges from 0.25 to about 0.78 and products

are described as being dry, flexible and firm. High water activity is region 3 (0.78-1.0); within this region products are described as being soft, moist, swollen, sticky, etc. (deMan, 1999).

The most accurate method to determine water activity is the use of a dew point hygrometer. This instrument has a mirror that is positioned over a closed chamber that is cooled until the dew point is reached within the chamber and dew forms on the surface (Figure & Teixeira, 2007). The dew point is then used to calculate the relative humidity of the chamber which is related to water activity by the following equation:

$$RH = aw \times 100\%$$

By reducing the water activity of a food to 1 or below, the rate at which microbial, chemical and biochemical reactions occur is greatly reduced (Figure & Teixeira, 2007). As the water activity is reduced even further, these reactions occur at a slower and slower pace thereby extending the shelf life of the product. The reaction most susceptible to water activity is microbial, of particular interest is that the lower limit for growth of most molds is a water activity of 0.8 (Figure & Teixeira, 2007).

Previous research consisted of the development of ten formulations consisting of full fat or defatted soy flour (50-95%), partially defatted peanut flour, 12 or 28%, (0-30%) and/or cowpea flour (0-70%). Analysis of the formulations resulted in five being selected for texturization according to the calculated protein content and amino acid profile: formulation 5 (70% full fat soy flour and 30% peanut flour, 28% fat); formulation 6 (70% defatted soy flour and 30% peanut flour, 28% fat); formulation 8 (80% defatted soy flour and 20% cowpea flour); formulation 9 (50% defatted soy flour, 30% peanut flour (12% fat) and 20% cowpea flour); and formulation 10 (65% defatted soy flour and 35% cowpea flour). The formulations were brought to three moisture contents (30, 35 and 40%) and processed at two temperature (300 and 350°F)

under four pressures (110, 120, 130 and 140 psi). All formulation/processing parameter sets were shown to have poor shape retention at 350°F; therefore, 350°F was determined not to be a viable processing temperature. In addition, formulations 6, 8, 9 and 10 resulted in dough too dry for processing at 30% moisture as well as formulation 6 and 9 being too dry at 35% moisture. The objective for this study is to determine the texture and water activity of samples that were processed and achieved shape retention in the earlier study and compare to a control composed of full fat soy flour at 40% moisture and texturized at 300°F under 120 psi, as determined to be ideal for texturization in the preliminary study, as well as a commercially available texturized soy product.

MATERIALS AND METHODS

Samples

Duplicates of the samples that retained shape upon texturizing in the previous chapter (formulation 5, 300°F, 30, 30 and 40% moisture, 110, 120, 130 and 140 psi; formulation 6, 300°F, 40% moisture, 120, 130 and 140 psi; formulation 8, 300°F, 35% moisture, 110, 120, 130 and 140 psi; formulation 9, 300°F, 40% moisture, 120, 130 and 140 psi; and formulation 10, 300°F, 35% moisture, 110, 120, 130 and 140 psi) were processed for texture and water activity analysis.

Full fat soy flour was brought to 40% moisture and texturized at 300°F under 120 psi for 5 sec as determined to be the most appropriate processing parameters in the preliminary study and was used as a control to compare the texture and water activity of the samples.

Commercially available texturized soy product (Vegan Burger, Morningstar Farms, Kellogg Co., Battle Creek, MI) was also analyzed in order to determine the difference between the samples and consumer acceptable product already available on the market.

Texture

The method used for texture analysis was modified from the method described by Cegla *et al.* (1978). Samples were prepared for texture analysis by cutting the sample into 14 pieces and soaking for 1 hour in excess water so that the sample/water level was 140 mL in a 150 mL beaker. The samples were then strained and reweighed to determine the weight of the water absorbed. The beakers containing both the samples and the water they were soaked in were then placed into a pressure cooker containing a canning rack for added stability of the beakers. Once the pressure (15psig) was achieved in the pressure cooker, the samples were allowed to steam for 30 min at 15 psig. The beakers were then removed from the pressure cooker and allowed to cool slightly then strained and allowed to further cool to room temperature before analyzing. If the weight of the steamed sample was greater than 53 g, small pieces were removed until the desired weight of 53 g was achieved.

Each sample was tested in triplicate on an universal testing machine (Model 5542, Instron, Inc, Canton, MA) equipped with an eight-wire (50cm² cross sectional area) Ottawa Texture Measuring System (OTMS). The Ottawa cell cross head rate was 50 mm/min and stopped 1.5 mm from the wire grid.

Water Activity

Water activity was conducted on samples ground with a water activity meter (Model Series 3TE, Decagon Devices, Inc, Pullman, WA). Disposable sample cups were filled three quarters full with ground sample at room temperature and placed in the water activity meter. Samples were read in triplicate.

RESULTS AND DISCUSSION

Texture

Analysis of the force required in the Ottawa Test using an analysis of variance (ANOVA) table in SAS (SAS Institute Inc, Cary, NC) showed that there was no significance difference between the samples, control or commercial product. Therefore, the eight samples with the least percent difference between the sample and the control (soy full fat) were selected for further analysis of protein solubility, protein digestibility and amino acid profiles in a future study. The samples with the least percent difference of stress in comparison to the control were: formulation 5, 30%, 140 psi and 35% 140 psi; formulation 8, 35%, 110, 120, 130 and 140 psi; formulation 9, 40%, 120 psi; and formulation 10, 35%, 110 psi. Of particular interest in formulation 8 (80% defatted soy flour and 20% cowpea flour), all four processing pressures fell within the cutoff of percent difference in stress to the control. This indicates that formulation 8 is most similar in texture to the full fat soy control.

As an overall trend, the stress measurement seen in formulations containing peanut flour (5, 6, and 9), both 28% and 12% fat, are higher than that seen in formulations containing only soy and cowpea flour (8 and 10). A higher stress measurement shows that the product is more elastic. This supports the conclusion in the previous chapter that the higher fat content of formulation 5 increases the elasticity of the protein carbohydrate-matrix. Because formulation 5 was analyzed for texture at all moistures, the stress measurements show an increase in stress with an increase in moisture. This can be contributed to the increase in moisture available for protein solubilization (Aboagye & Stanley, 1985). Protein solubilization of the globular proteins plays a crucial role in the breakdown of the proteins during the first phase of press texturization and

facilitates the protein-protein interactions that result in the fibrous structure (Aboagye & Stanley, 1985).

The solubilization of globular proteins also explains the differences in the stress measurement between formulations. Formulations 5 and 6 vary only in the use of full fat soy flour (5) and defatted flour (6), which resulted in a decrease in fat and an increase in protein in formulation 6. However, this also results in a higher percentage of percent of globular proteins in the formulation. This is reflected in the higher stress measurements of formulation 6 versus formulation 5 at 40% moisture.

Water Activity

Water activity measurements were analyzed using least square difference (LSD) method in SAS (SAS Institute Inc, Cary, NC). There was a significant difference in water activity between the three moisture levels, with an increase in moisture level of the original dough resulting in an increase in water activity of the final product. This supports the idea from the moisture analysis that the texturizing process traps more of the moisture preventing its escape upon release of the pressure during processing. Another possible explanation is that the higher the initial moisture content of the dough, the higher the final moisture of the product. In addition, the samples containing peanut flour (5, 6 and 9) had a higher water activity than those samples containing only defatted soy and cowpea; this may be a result of the higher fat content of the samples containing peanut flour. In comparison to the full fat soy control, the most similar samples (no significant difference) were formulation 5 at 40% moisture and all pressures and 35% moisture at 140 psi. All formulations were significantly different from the commercial product.

CONCLUSION

Texture measurements of stress indicated that a higher fat content results in a more elastic the product. There also appears to be a direct relationship between the moisture content of the dough and the elasticity of the product with higher moisture content resulting in higher stress measurements. Water activity analysis showed that the addition of peanut flour to the formulation, which results in a higher fat content, increases the water activity. In addition, the higher the initial moisture content of the dough texturized, the higher the water activity of the final product. The addition peanut flour, thereby increasing the fat content of the product, increased both the elasticity of the product as well as the water activity.

The eight formulation/processing parameter sets most similar in texture to the full fat soy flour control, and therefore of interest for further analysis of protein, was determined to be: formulation 5 (70% full fat soy flour and 30% partially defatted peanut flour, 28%), 30 and 35% moisture, 140 psi; formulation 8 (80% defatted soy flour and 20% cowpea flour), 35% moisture, 110, 120, 130 and 140 psi; formulation 9 (50% defatted soy flour, 30% partially defatted peanut flour (12% fat) and 20% cowpea flour) 40% moisture, 120 psi; and formulation 10 (65% defatted soy flour and 35% cowpea flour) 35% moisture, 110 psi.

Table 4.1 Stress values for extrusion of soy, peanut and/or cowpea processed with the Village Texturizer which achieved shape retention

Formulation	Moisture	Pressure (psi)	Stress (N/cm ²)	Percent Difference in Stress vs Control
Control (Soy full fat)	40%	120	1.462	
Commercial			4.649	
5	30%	110	2.412	49.05
		120	2.312	45.04
		130	2.208	40.67
		140	2.185	39.65
	35%	110	2.189	39.82
		120	3.282	76.73
		130	3.478	81.60
		140	2.030	32.51
	40%	110	3.976	92.46
		120	2.849	64.34
		130	2.222	41.23
		140	4.016	93.24
6	40%	120	4.684	104.84
		130	2.547	54.13
		140	4.222	97.11
8	35%	110	2.114	36.46
		120	1.286	12.84
		130	1.445	1.16
		140	1.408	3.77
9	40%	120	1.988	30.49
		130	2.606	56.22
		140	2.590	55.68
10	35%	110	1.394	4.79
		120	6.940	130.39
		130	2.883	65.39
		140	4.619	103.83

Table 4.2 Water activity of soy, peanut and/or cowpea processed with the Village Texturizer which achieved shape retention^a

Formulation	Moisture	Pressure (psi)	Water Activity
Control (Soy full fat)	40%	120	0.973 <i>bc</i>
Commercial			0.984 <i>a</i>
5	30%	110	0.952 <i>hij</i>
		120	0.955 <i>g</i>
		130	0.949 <i>ijk</i>
		140	0.951 <i>hij</i>
	35%	110	0.964 <i>ef</i>
		120	0.965 <i>ef</i>
		130	0.964 <i>ef</i>
		140	0.970 <i>cd</i>
	40%	110	0.976 <i>b</i>
		120	0.976 <i>b</i>
		130	0.976 <i>b</i>
		140	0.976 <i>b</i>
6	40%	120	0.964 <i>ef</i>
		130	0.953 <i>hi</i>
		140	0.964 <i>ef</i>
8	35%	110	0.948 <i>jk</i>
		120	0.942 <i>l</i>
		130	0.950 <i>ijk</i>
		140	0.934 <i>m</i>
9	40%	120	0.967 <i>de</i>
		130	0.963 <i>f</i>
		140	0.965 <i>ef</i>
10	35%	110	0.946 <i>kl</i>
		120	0.951 <i>hij</i>
		130	0.952 <i>hij</i>
		140	0.958 <i>g</i>

^a Values are an average of three measurements. Mean values in a column not followed by the same letter are significantly different at $\alpha=0.05$

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SECTION V

PROTEIN ANALYSIS OF SOY, PEANUT AND COWPEA BLENDS PROCESSED WITH THE VILLAGE TEXTURIZER¹

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ABSTRACT

Eight samples were selected for having a texture profile similar to that of full fat soy with 40% moisture texturized at 300°F/149°C under 120 psi/8.2 atm. The samples were then analyzed for protein solubility by nitrogen solubility index method, protein digestibility by TCA precipitation and indispensable amino acid content by HPLC. Protein solubility was shown to increase with an increase in initial moisture content while protein digestibility showed no dependence on moisture content. An increase in pressure resulted in an increase in protein solubility and a decrease in protein digestibility. Amino acid profile was similar across all samples. The two formulation/parameter sets best suited for meeting the protein and amino acid requirements for children (10 to 12 years) are formulation 8 (80% defatted soy flour and 20% cowpea flour), 35% moisture, 120 psi and formulation 5 (70% full fat soy flour and 30% partially defatted peanut flour, 28% fat), 35% moisture, 140 psi.

INTRODUCTION

Of the biological macromolecules, protein is the most abundant and can be found in every cell. Comprised of a combination of 20 different amino acids, proteins are essential to proper functioning of the human body. The functionality of a protein is dependent on the number of amino acids and the order of the amino acids within the polypeptide chain. For this reason, proteins serve several different functions within the human body, such as, enzymes, structural components of cells, cell signaling, immune response, cell cycle, etc (deMan, 1999; Fennema, 1996; Nelson & Cox, 2000).

On the most basic level, the amino acids are the building blocks of proteins. All the amino acids have a central chemical composition that is the same, with the exception of proline which is cyclic, and contains an α -carbon that is covalently bonded to a hydrogen atom, a carboxyl group, an amino group and a side chain. The side chain of an amino acid determines how the structure of the protein and therefore determines the function of the protein. Amino acids are divided into five groups according their side chain, or more specifically, the side chain's polarity: nonpolar, polar, aromatic, positively charged (basic) and negatively charged (acidic) (Nelson & Cox, 2000). The most hydrophobic group is the nonpolar which includes one of the two sulfur containing amino acids, methionine. The most hydrophilic amino acids are those that are either positively or negatively charges, of which includes lysine (basic) (Nelson & Cox, 2000).

Amino acids are also separated into three groups according the human body's ability to synthesize the amino acid. These two groups are indispensable (essential), dispensable (non-essential) and conditionally indispensable amino acids. The indispensable amino acids are those amino acids that must be consumed in the diet because the human body is unable to synthesize

the carbon skeleton from simpler molecules. The indispensable amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Dispensable amino acids are those amino acids that can be synthesized in the human body from other amino acids or other nitrogenous precursors. These amino acids are alanine, aspartic acid, asparagine, glutamic acid and serine. There are some amino acids are considered conditionally indispensable meaning they can be synthesized from other amino acids in the human body but can be disrupted under specific pathophysiological conditions and include arginine, cysteine, glutamine, glycine, proline and tyrosine.

Proteins are divided into three groups based on the behavior in an ultracentrifuge and electrophoretic properties (deMan, 1999). These groups are as follows: simple protein, hydrolysis results in amino acids only, includes albumins (water-soluble) and globulins (almost water insoluble); conjugated protein, includes amino acids as well as nonprotein material, includes phosphoproteins, lipoproteins, etc.; derived protein, result of chemical or enzymatic reactions (deMan, 1999).

Aside from the biological importance, proteins serve a variety of purposes within the structure of foods including viscosity; water, fat and flavor binding; emulsification; foaming, etc. (Vanchina, Chinnan, & McWatters, 2006). Proteins are also the component of foods of concern in the texturizing process. Texturization of proteins is essentially the thermal denaturation of proteins causing the irreversible loss of the globular formation of the protein (Harper, 1981). The globular formation is caused by the interactions between the side chains for the amino acids. The globular formation begins to unfold due to disruption of the hydrogen, ionic and disulfide bonds and van Waals' forces as the temperature and water increases. The reactive sites of the proteins then begin to reform bonds and interactions as which point the texturizing process tries

to realign the proteins so as to force reformation to maintain the linear form of the denatured protein. Texturization of plant proteins has the potential to alter the over protein solubility, digestibility and amino acid profile of the final product.

Previous research consisted of the development of ten formulations consisting of full fat or defatted soy flour (50-95%), partially defatted peanut flour, 12 or 28%, (0-30%) and/or cowpea flour (0-70%). Analysis of the formulations resulted in five being selected for texturization according to the calculated protein content and amino acid profile: formulation 5 (70% full fat soy flour and 30% peanut flour, 28% fat); formulation 6 (70% defatted soy flour and 30% peanut flour, 28% fat); formulation 8 (80% defatted soy flour and 20% cowpea flour); formulation 9 (50% defatted soy flour, 30% peanut flour (12% fat) and 20% cowpea flour); and formulation 10 (65% defatted soy flour and 35% cowpea flour). The formulations were brought to three moisture contents (30, 35 and 40%) and processed at two temperature (300 and 350°F) under four pressures (110, 120, 130 and 140 psi). All formulation/processing parameter sets were shown to have poor shape retention at 350°F; therefore, 350°F was determined not to be a viable processing temperature. In addition, formulations 6, 8, 9 and 10 resulted in dough too dry for processing at 30% moisture as well as formulation 6 and 9 being too dry at 35% moisture.

Further analysis of the samples achieving shape retention (formulation 5, 30, 35 and 40% moisture; 110, 120, 130 and 140 psi: formulation 6; 40% moisture; 120, 130 and 140 psi: formulation 8; 35% moisture; 110, 120, 130 and 140 psi: formulation 9; 40% moisture; 120, 130 and 140 psi: formulation 10; 35% moisture; 110, 120 130 and 140 psi: all formulations at 300°F) were analyzed for texture and water activity. The texture of each formulation/processing parameter set was compared to the texture of the full fat soy control (40% moisture, 300°F, 140 psi as determined to be ideal for texturization by preliminary research) and the eight most similar

to the control were selected for protein analysis. The objective for this study is to determine the protein content, fat content, protein solubility, protein digestibility and amino acid profile for the following formulation/processing parameter sets as determined by previous research:

formulation 5, 30 and 35% moisture, 140 psi; formulation 8, 35% moisture, 110, 120, 130 and 140 psi; formulation 9, 40% moisture, 120 psi; formulation 10, 35% moisture, 110 psi.

MATERIALS AND METHODS

Samples

All samples were processed at 300°F for 5 sec under the moisture level and pressure that resulted in texture results most similar to full fat soy: formulation 5, 30 and 35% moisture, 140 psi; formulation 8, 35% moisture, 110, 120, 130 and 140 psi; formulation 9, 40% moisture, 120 psi; and formulation 10, 35% moisture, 110 psi. All samples were ground and dried in a forced air oven (Linberg/Blue M, Mechanical Oven, Asheville, NC, USA) at 60°C for 1 hour.

Nitrogen Solubility

Nitrogen solubility was conducted in duplicate using the Nitrogen Solubility Index (NSI) AOCS Official Method Ba 11-65 (Anon., 1997). Five grams of each sample was weighed into a 500 mL Erlenmeyer flask and 200 mL of distilled water was added slowly while stirring with a glass stir rod to ensure the sample was dispersed in the water. The flasks were then placed in a shaking water bath set at 30°C (VWR, West Chester, PA) and shaken at 120 shakes per min for 2 h. Samples were then transferred to 250 mL volumetric flask and brought to level with distilled water. Two drops of silicone antifoam agent (Dow Corning AF) was added and the samples mixed thoroughly. The samples were allowed to stand for 5 min before decanting 40 mL into a 50 mL centrifuge tube and centrifuged for 10 min at 1500 rpm. Twenty five mL of the filtrate

was filtered from glass fiber and collected in a 50 mL beaker. The samples were then frozen overnight at -20°C and freeze dried for 96 h (Model Genesis SQ 25, Gardiner, NY). Dried samples, as well as the original samples, were analyzed for nitrogen content by the Dumas combustion method using a LECO FP-2000 Nitrogen Analyzer (St. Joseph, MI). Nitrogen Solubility Index was calculated using the following equation:

$$\text{NSI} = \frac{\% \text{ water soluble nitrogen}}{\% \text{ total nitrogen}} \times 100$$

Protein Digestibility

Protein digestibility was determined *in vivo* by Trichloroacetic acid (TCA) precipitation (Clark, 2004) and in duplicate. Samples containing 250 mg of protein was suspended in 17 mL of 0.1 HCl for 10 min then placed in a shaking water bath at 37°C and 70 shakes per min for 20 min. The pH was then adjusted to 1.9 using 1N NaOH before adding 0.0013 g of pepsin (ACROS). Samples were then incubated at 37°C and 70 shakes per min for an additional 30 min. Ten mL of 0.1N sodium phosphate buffer was added and the pH adjusted to 7.5. Pancreatin (SIGMA, 4X USP), 0.010 g, was added and the samples incubated at 37°C, 70 shakes per min, for 6 h. Once the samples were removed from the water bath, 5.0 g of TCA was added and the samples transferred to a 50 mL centrifuge tube for centrifuging at 12500 RPM for 20 min. The clear supernatant was removed and frozen overnight at -20°C before freeze drying for 96 h (Model Genesis SQ 25, Gardiner, NY). Dried samples were analyzed for nitrogen content by the Dumas combustion method using a LECO FP-2000 Nitrogen Analyzer (St. Joseph, MI). Protein digestibility was calculated using the following equation:

$$\text{Protein Digestibility} = \frac{\% \text{ digested nitrogen}}{\% \text{ total nitrogen}} \times 100$$

Amino Acid Analysis

Oil was extracted from dried, ground samples using (INFO). Samples, 0.03 g, were weighed into pyrolyzed hydrolysis tubes and 0.5 mL of internal standard solution (Norleucine, 2.5 mM in 0.1 HCl) was added. Double deionized water, 1.5 mL, was added before adding 3 mL of Dithiodipropionic acid dissolved in 0.2 N NaOH. Five mL of 2.5% phenol in 12N HCl was added and the tubes topped with O-rings and stopcocks to seal the tubes.

Hydrolysis was achieved by de-aerating each tube by vacuuming (4, 30 sec cycles) and flushing with Argon (3, 30 sec cycles) while continuous vortexing and leaving the tubes under high vacuum. Samples were heated for 75 min at 145°C and then allowed to cool to room temperature. One mL of each sample was added to a pyrolyzed 25 mL volumetric flask and diluted with double deionized water to level. Samples were inverted and mixed before filtering 3 mL through 0.2 µm nylon filter attached to a 5 mL syringe into a sample vial that was capped and frozen overnight at -20°C to await derivatization.

Samples were removed from the freezer and allowed to thaw to room temperature before derivatizing. Ten µL of each sample was added to a tube and 70 µL of AccQFlour Borate buffer was added and the mixture vortexed. Twenty µL of AccQFlour Reagent was added and the mixture vortexed again. After waiting for 1 min, the entire mixture was added to a low volume insert for HPLC and inserted in a 4 mL vial then heated at 55°C for 10 min. Samples were then separated by HPLC (INFO) and analyzed for the indispensable amino acid profile.

RESULTS AND DISCUSSION

Protein Solubility

Protein solubility (Table 5.1), determined by nitrogen solubility index, showed that 76% of the protein in the full fat soy control was soluble in water. Formulations containing cowpea

(8, 9 and 10) had lower protein solubility than formulation 5 which only contains soy and peanut flour. The samples that had a protein solubility closest to that of soy was: formulation 5 (35%, 140 psi) and formulation 10 (35%, 110) with a protein solubility of 45.40% and 45.86%, respectively. Formulation 8 was the only formulation where the same moisture level was texturized across all pressures for protein solubility analysis; therefore, it was used to observe the possible effects processing pressure has on protein solubility. Pressures 110, 120 and 130 psi showed a gradual increase in protein solubility (35.10, 39.67 and 42.26% respectively) however, the processing pressure of 140 psi showed a sudden decrease in protein solubility (30.16%) which was the lower protein solubility seen across all the samples. This indicated that an increase in pressure does increase protein solubility up to 130 psi. Comparison of formulation 5, 30% moisture, 140 psi (40.26%) to formulation 5, 35% moisture, 140 psi (45.40%) showed an increase in protein solubility with an increase in initial moisture content. While a reduction in protein solubility is indicative of an increased protein denaturation and restructuring (Whitaker & Tannenbaum, 1977), there was also difference in the percentage of globular proteins available for denaturation during the texturization of the samples. This is dependent on the overall percent protein of the formulation as well as the ingredients used. Protein solubility seemed to show similar trends as that seen with water activity, especially for formulation 8, 35% moisture, where water activity increased with increase in pressure from 110 to 130 psi but dropped for further increase in pressure (140 psi). The increase in protein solubility for formulation 5 in comparison to the other formulations may be a result of the fat content of the samples. Due to the fat binding the water within the sample upon texturization the globular proteins may not be forming as many bonds during the restructuring of the proteins.

Protein Digestibility

Digestibility of the protein within the samples showed the opposite trend in relation to pressure to that of protein solubility. Formulation 8 showed that an increase in pressure resulted in a decrease in digestibility through 130 psi and an increase for the processing pressure of 140 psi (Table 5.1). Formulation 5 showed that moisture content of the dough did not alter the digestibility of the final product (30%: 84.25, 35%: 84.46). All of the samples resulted in a digestibility higher than the full fat soy control (78.10).

Amino Acid

Determination of the amino acid profile and protein and fat content of each sample showed that all the formulation/parameter sets could be processed for the intention of meeting the requirements of children ages 10 to 12 years (Table 5.2). As expected, formulation 5 had the highest percentage of fat (19.41 and 19.42%); however, the higher fat ingredients would be more readily available in developing nations as opposed to defatted or partially defatted flours while still meeting the desired amino acid profile. The drawback is that with a higher percentage of fat, formulation 5 also has a lower percentage of protein (38.26 and 37.47%). Formulation 10 also has lower percentage of protein (38.77%) due to the higher percentage of cowpea flour but meets the methionine/cysteine (3.46) requirements of children as does formulation 9 (3.30) which is unexpected according the calculated amino acid profile from earlier research where the calculated methionine/cysteine content, according to formulation software (Creative Formulation Concepts, LLC, Concept 4.0, Level 2, Annapolis, MD), of formulation 9 and 10 were 1.28 and 1.42 g per 100 g of protein, respectively. All other formulations do not meet the methionine/cysteine requirement. Formulation 8 (35% moisture, 120 psi) has the highest percentage of protein (44.52%) and is highest in the amino acids Threonine (5 g/100 g protein),

Valine (5.97 g/100 g protein), Isoleucine (5.15 g/100 g protein), and Leucine (8.98 g/100 g protein) and Phenylalanine/Tyrosine (11.73 g/100 g protein). Formulation 8 is also low in fat (1.67) due to use of defatted soy and cowpea flour although the results are higher than the calculated fat (0.26%) from Section III. The full fat soy control has a protein content (38.96%) similar to formulations 5 and 10 and a fat content (14.35%) slightly lower than formulation 5. Overall, the indispensable amino acid results were as expected or slightly higher.

CONCLUSIONS

By comparing the protein content and indispensable amino acid profile of the formulations, formulation 8 (35% moisture, 120 psi), consisting of 80% defatted soy flour and 20% cowpea flour, appears to be the best choice as a formulation meeting the protein and amino acid requirements of children (10-12 years), with the exception of methionine/cysteine, while maintaining a low fat content. This formulation/parameter set resulted in a protein solubility of 39.67% which is just slightly lower than the median (40.26%) of the protein solubility of all the samples and a protein digestibility of 10.80% as compared to the median of 12.41%. However, formulation 8 also utilizes defatted soy which may not always be easily attainable in developing nations. On the other hand, formulation 5 (35% moisture, 140 psi), which is composed of 70% full fat soy flour and 30% partially defatted peanut flour (28% fat), while containing lower percentage of protein and a higher fat content, also meets the amino acid requirements of children (10 to 12 years) with the exception of methionine/cysteine, but utilizes full fat soy flour and 28% fat peanut flour which may be more accessible in developing nations. The protein solubility and digestibility of this sample was shown to be of among the highest of the samples (45.40 and 15.54, respectively). Therefore, the two formulation/parameter sets best suited for meeting the protein and amino acid requirements for children (10 to 12 years) are formulation 8

(80% defatted soy flour and 20% cowpea flour), 35% moisture, 120 psi and formulation 5 (70% full fat soy flour and 30% partially defatted peanut flour, 28% fat), 35% moisture, 140 psi.

Table 5.1 Nitrogen solubility and *in vivo* protein digestibility of soy, peanut and/or cowpea processed with the Village Texturizer

Formulation	Moisture	Pressure (psi)	NSI ^a	Percent Difference NSI vs Control	Digestibility ^b	Percent Difference Digestibility vs Control
Soy	40%	120	76.03 <i>a</i>		78.10 <i>e</i>	
5	30%	140	40.26 <i>c</i>	61.51	84.25 <i>f</i>	7.58
	35%	140	45.40 <i>b</i>	50.46	84.46 <i>f</i>	7.83
8	35%	110	35.10 <i>d</i>	73.67	87.59 <i>fg</i>	11.46
		120	39.67 <i>cd</i>	62.86	89.20 <i>gh</i>	13.28
		130	42.26 <i>bc</i>	57.09	92.06 <i>g</i>	16.42
		140	30.16 <i>d</i>	86.38	88.84 <i>gh</i>	12.87
9	40%	120	35.64 <i>d</i>	72.71	88.29 <i>g</i>	12.26
10	35%	110	45.86 <i>b</i>	49.51	85.68 <i>fg</i>	9.26

^a Values are an average of three measurements. Mean values in a column not followed by the same letter are significantly different at $\alpha=0.05$

^b Values are an average of two measurements. Mean values in a column not followed by the same letter are significantly different at $\alpha=0.05$

Table 5.2 Determined protein content, fat content and indispensable amino acid profile for soy, peanut and/or cowpea processed with the Village Texturizer (amino acid content reported as g/100 g protein)^a

Formulation	<i>FAO</i> ¹	5		8			9		10	Soy
Moisture		30%	35%	35%			40%		35%	40%
Pressure		140 psi	140 psi	110 psi	120 psi	130 psi	140 psi	120 psi	110 psi	120 psi
% Protein (Dry) ^b		38.26	37.47	42.99	44.52	41.33	42.14	42.67	38.77	38.96
% Fat (Dry)		19.42 <i>a</i>	19.41 <i>a</i>	1.01 <i>e</i>	1.67 <i>d</i>	0.95 <i>e</i>	1.24 <i>e</i>	2.63 <i>c</i>	0.16 <i>f</i>	14.35 <i>b</i>
Histidine ^b	1.8	3.74	3.76	3.83	3.99	3.78	3.79	3.96	4.07	3.28
Arginine ^b		10.15	10.23	8.71	9.08	8.77	8.82	10.44	8.76	7.52
Threonine ^b	2.7	4.27	4.40	4.60	5.00	4.68	4.79	4.50	4.86	3.96
Valine ^b	3.2	4.52	4.61	4.52	5.97	4.60	5.04	4.38	4.41	3.89
Methionine + Cysteine	2.5	1.55 <i>cd</i>	1.47 <i>cd</i>	1.74 <i>cd</i>	1.93 <i>c</i>	1.50 <i>cd</i>	1.50 <i>cd</i>	3.30 <i>a</i>	3.46 <i>a</i>	1.42 <i>d</i>
Lysine ^b	5.1	5.77	6.04	7.63	7.29	7.38	7.47	5.81	7.16	6.33
Isoleucine ^b	2.5	4.62	4.40	4.58	5.15	4.70	4.81	4.22	4.41	3.98
Leucine ^b	5.5	8.25	7.83	8.05	8.98	8.38	8.50	8.41	8.75	6.72
Phenylalanine + Tyrosine ^b	4.7	11.61	11.03	10.68	11.73	10.92	11.03	11.47	10.91	9.06

^a Values are an average of two measurements. Mean values in a row not followed by the same letter are significantly different at $\alpha=0.05$

^b Values are an average of two measurements. Mean values in a row are not significantly different at $\alpha=0.05$.

¹ *Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, 2002)

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SECTION VI

SUMMARY AND CONCLUSIONS

Formulation software was used to develop ten formulations containing defatted or full fat soy flour (50-95%), partially defatted peanut flour, 12 or 28% fat (0-30%) and/or cowpea flour (0-70%) that met the protein requirements needed for texturization on the Village Texturizer as well as the amino acid requirements of children ages 10 to 12 years. The formulations were then analyzed for calculated protein and fat content as well as amino acid profile and five formulations were chosen to process: formulation 5 (70% full fat soy flour and 30% peanut flour, 28% fat); formulation 6 (70% defatted soy flour and 30% peanut flour, 28%); formulation 8 (80% defatted soy flour and 20% cowpea flour); formulation 9 (50% defatted soy flour, 30% peanut flour (12%) and 20% cowpea flour); formulation 10 (65% defatted soy flour and 35% cowpea flour). Each formulation was brought to three different moisture levels (30, 35 and 40%) and processed at two temperatures (300°F and 350°F) under four pressures (110, 120, 130 and 140 psi). Formulation 5, which contained the highest calculated fat content, was the only formulation to be successfully mixed at all three moisture levels. Formulations 6 and 9 could only be mixed at a moisture level of 40% while formulations 8 and 10 were mixed at 35 and 40%. Processing at 350°F resulted in poor shape retention for all formulations and was thereby concluded not to be an appropriate temperature for texturization. For formulations 8 and 10 at a moisture level of 40%, the shape retention was poor. Therefore, the following formulation/parameter sets were tested for density, moisture, texture and water activity: formulation 5, moisture 30, 35 and 40%, pressure 110, 120, 130 and 140 psi; formulation 6, moisture 40%, pressure 120, 130 and 140; formulation 8, moisture 35%, pressure 110, 120, 130,

and 140 psi; formulation 9, moisture 40%, pressure 120, 130, and 140 psi; formulation 10, moisture 35%, pressure 110, 120, 130 and 140 psi.

Percent reduction in density showed that formulations 8 and 10 expanded better than formulations 5, 6 and 9 which contained peanut flour. The one exception was formulation 5 at 30% moisture. While all samples were shown to expand, none of the processing parameter combinations resulted in a product with as high a percent reduction in density as that of full fat soy flour (75%). Percent reduction in moisture showed that an increase in pressure resulted in a decrease in moisture loss. Also, the higher the fat content of the dough, the greater the reduction in moisture due to press texturization. Texture measurements of stress indicated that a higher fat content results in a more elastic the product. There also appears to be a direct relationship between the moisture content of the dough and the elasticity of the product with higher moisture content resulting in higher stress measurements. Water activity analysis showed that the addition of peanut flour to the formulation, which results in a higher fat content, increases the water activity. In addition, the higher the initial moisture content of the dough texturized, the higher the water activity of the final product. The addition of peanut flour, thereby increasing the fat content of the product, increased both the elasticity of the product as well as the water activity. Based on the results of the texture analysis, eight samples were selected to determine protein solubility, protein digestibility and amino acid profile: formulation 5, moisture 30 and 35%, 140 psi; formulation 8, 35% moisture, 110, 120, 130 and 140 psi; formulation 9, 40% moisture, 120 psi; and formulation 10, 35% moisture, 110 psi.

By comparing the protein content and indispensable amino acid profile of the formulations, formulation 8, consisting of 80% defatted soy flour and 20% cowpea flour, (35% moisture, 120 psi) appears to be the best choice as a formulation meeting the protein and amino

acid requirements of children (10-12 years), with the exception of methionine/cysteine, while maintaining a low fat content. This formulation/parameter set resulted in a protein solubility of 39.67% which is just slightly lower than the median (40.26%) of the protein solubility of all the samples and a protein digestibility of 89.20% as compared to the median of 87.59%. However, formulation 8 also utilizes defatted soy which may not always be easily attainable in developing nations. On the other hand, formulation 5 (35% moisture, 140 psi), while containing lower percentage of protein and a higher fat content, also meets the amino acid requirements of children (10 to 12 years) with the exception of methionine/cysteine, but utilizes full fat soy flour (70%) and 28% fat peanut flour (30%) which may be more accessible in developing nations. The protein solubility (45.40) of this sample was shown to be of among the highest of the samples while digestibility is lowest (84.46). Therefore, the two formulation/parameter sets best suited for meeting the protein and amino acid requirements for children (10 to 12 years) are formulation 8, 35% moisture, 120 psi and formulation 5, 35% moisture, 140 psi.