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Characterization and utilization of extruded-expelled soybean flours

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Characterization and utilization of extruded-expelled soybean flours

by

Adrianna Ashley Heywood

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Program of Study Committee:
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ABSTRACT

Extrusion-expelling (EE) is an alternative oilseed processing technique to solvent extraction. The objective of both processing techniques is to obtain oil and meal products. The products of EE processing are a crude oil and low-fat meal (flour) (LFSF). Extrusion-expelling processing is a relatively new process (developed in 1987) and the meal currently produced is used primarily in the animal feed industry. Due to these two reasons, the literature available on LFSF functional properties and utilization in food products is limited. The present work was divided into two parts; the first part was focused on LFSF with a wide range of protein dispersibility indexes (PDI) and residual oil (RO) levels; the second part was focused on LFSF from value-enhanced soybean varieties. The objective of both parts of the present work was to characterize the functional properties of the respective LFSF and utilize the LFSF in a food system. Functional properties of LFSF from part one (LFSF with a wide range of PDI and RO) showed that the greater the PDI, the more functional the protein. However, the functional properties of the LFSF in the PDI/RO range of 42/8 to 67/11 were relatively equal. In general, this range of LFSF behaved similar to defatted soy flour (DFSF) at a PDI of 71. In a batter system (cake doughnuts), LFSF behaves differently from DFSF in that the performance of LFSF is relatively unpredictable compared to DFSF. However, when compared the doughnuts made with DFSF were compared to those doughnuts made with LFSF were relatively equal in certain quality and sensory characteristics.

Part two of the present work utilized six varieties of value-enhanced soybeans. Value-enhanced soybeans are those soybeans which have had some trait altered either through traditional plant breeding or through biotechnological means. Two commodity soybean varieties were also used in this study. Functional characteristics were not affected by trait alteration. For objective two, LFSF from different value-enhanced soybeans were texturized to produce texturized soy protein (TSP). Texturized soy protein was rehydrated, and added to a beef patty at a 30% level. The TSP-extended patties were then subjected to instrumental analyses and sensory evaluation. The results showed that the value-enhanced soybean varieties did not negatively impact the beef patty. The exception, however, was that the TSP-extended products had added soy flavor. This increase in soy flavor was detected with all TSP-extended patties and was not affected by soybean variety.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Soybean processing by extrusion-expelling (EE) is gaining in popularity for several reasons, including, low capital investment, ease of using extrusion-expelling equipment and feasibility of processing identity-preserved (IP) soybeans. Extrusion-expelling processing results in low-fat soybean meal (LFSM) and crude soybean oil. This meal can be further processed into flour or re-extruded to form texturized soy protein (TSP). Historically, the LFSM has been used as animal feed. However, these products may be used for human food consumption if processed properly (e.g. using food-grade soybeans as a starting material, processing in a food grade facility). To date, there is a very limited amount of published material, which reports on functional properties of LFSM or its utilization in food products. Furthermore, products manufactured from LFSM (i.e. low-fat soybean flour (LFSF) and TSP) have only begun to be investigated for basic functional properties in order to evaluate their usefulness in food products or industrial applications.

Identity-preserved soybeans include a category of soybeans referred to as value-enhanced soybeans. Value-enhanced soybeans are soybeans that have undergone some trait alteration either through traditional plant breeding or genetic engineering. One soybean variety that falls into this category is the lipoxygenase triple-null soybean. Several researchers have investigated this soybean variety in both model systems and food systems. However, there are many more value-enhanced soybean varieties that have not been investigated. Finally, lipoxygenase triple-null and these additional varieties have not been investigated to any degree when processed with EE technology.

The research reported in this document strives to provide the groundwork for future investigations into LFSF functional properties and utilization in food and industrial applications. Additionally, this investigation into value-enhanced soybean varieties processed with EE technology attempts to provide an indication of the usefulness of these soybean varieties by defining functional properties and identifying application in food systems.

Dissertation organization

This dissertation begins with a general introduction section that covers the general research problem and dissertation organization. Following this section, a literature review is included. In this literature review, background information is found which relates back to the research problem. Chapters three through six are manuscripts, which will be or have been submitted to both the Iowa Agriculture and Home Economics Experiment Station for editing and the *Journal of American Oil Chemists' Society* for publication. The format of all four manuscripts follows that of the *Journal of American Oil Chemists' Society*; including an abstract, introduction, experimental procedures, results and discussion, references, and tables and figures following the text. This dissertation concludes with a chapter offering comments on general conclusions and suggestions for future work.

CHAPTER 2. LITERATURE REVIEW

Introduction

Soybeans have long been used in Asian diets as an important source of nutrition. It has taken Western cultures longer to accept the taste of soybean products. For this reason, the majority of the crushed soybean products in the U.S. have been used for animal feeding purposes. Today, this trend is changing with nearly 37 million bushels of soybeans used for human consumption in 2001 and an anticipated 10% growth by 2005 (1). One reason for this expanding market is the recently approved (October 26, 1999) Food and Drug Administration (FDA) claim indicating soy protein's role in decreasing the incidence of coronary heart disease (2). This claim can be used with any food product that provides 6.25 g of soybean protein per serving and adheres to requirements for a food low in fat, saturated fat, and cholesterol. Many food processors are now very interested in using soy protein in their formulations so that they may use this claim on their products. In order for soy protein-based ingredients to meet this demand, however, the importance of understanding soy protein functionality is necessary. In addition to examining functional properties in model systems, these proteins must be utilized in food systems and the hypothesized functionality assessed in real world applications.

Extrusion-Expelling of Soybeans

Traditional soybean processing includes separating of the oil from the crushed meal using solvent extraction, most frequently with hexane as the solvent. The end result of this process is a defatted meal with less than 0.5% fat (<3% if measured by the acid-hydrolysis method). There are drawbacks in choosing this form of soybean processing.

Substantial investment in facilities and equipment is required (ca. in excess of 50 million dollars). Secondly, the process of solvent extraction is hazardous due to the high flammability/explosive potential of hexane, which is the commonly used solvent. For these reasons, this process has typically been left to large companies to process soybeans.

An additional issue with the solvent extraction process is that the large corporations have difficulty manufacturing soy products using IP soybeans. This difficulty is encountered due to the requirement to maintain separation of all identity-preserved soybeans throughout the entire manufacturing process (storage to shipping). This means that the processor needs to have an extensive and accurate record-keeping method, maintain several different storage bins for each soybean variety, keep a record of the cleaning of the system between runs of different soybean varieties, and test frequently to ensure that no cross-contamination has occurred. Associated with these storage logistic difficulties is the issue of low supply. Traditional solvent extraction plants sustain daily processing figures of approximately 2000 tons soybeans a day. Identity-preserved soybeans, although gaining in the acres planted, today is estimated to be 1 million acres (3). The estimated total U.S. soybean acres planted in the year 2000 growing season was 7.5 million acres (4). Because one acre of IP soybeans typically yields approximately 1 ton of soybeans per acre and each acre yields approximately 40 bushels (equivalent to 2400 pounds, which is just over 1 short ton (4), one can see that the quantity of IP soybeans will not provide enough soybeans for several larger processors operating 24 hr/day and 7 days a week.

To alleviate the aforementioned issues, the EE process is being used. Extrusion-expelling was developed by Nelson *et al.* (5) at the University of Illinois in partnership with Triple ‘F’ Feeds Insta-Pro Division (Des Moines, IA). This processing technique has the advantages of lower capital investment ($200,000 to $500,000 versus 50-100 million dollars) compared to solvent extraction facilities, much safer processing with relatively "low tech" equipment and a daily processing capacity of from 6 to 120 tons of soybeans. Finally, EE processors are very capable of producing soybean products starting from IP soybeans. This is because these small-scale producers often have smaller quantities of soybeans to ease record-keeping duties, several small storage bins that they can easily clean out after they have been occupied with different soybean varieties. These processors can quickly and easily clean the equipment after each run of different soybean varieties. The final product from EE processing is a low-fat meal with six to ten percent residual oil and crude protein contents of approximately 51% (dry moisture basis) (5).

Extrusion-expelling soybeans involves disrupting of the oil cells by extruding and then extracting the oil with an expeller, or screw press. The beauty of the EE processing system is that, when using the same EE equipment, manipulation of the pretreatments (using hulled or dehulled soybeans, moisture content of starting soybeans, etc.) and of the processing equipment itself results in low-fat meals and oils with different characteristics. Figure 1 is a flow diagram representing a common EE process. A very thorough overview of the process has been written by Crowe (6). In this overview, Crowe describes the EE process and how alterations to the pretreatments and equipment results in meal with different characteristics.

Soybean products

Soybean processing typically is carried out by removing the oil via solvent extraction resulting in a defatted soy meal that may be further be processed into soy grits, soy flour, soy concentrate, soy isolate or texturized soy protein (TSP) (7). The least processed and most

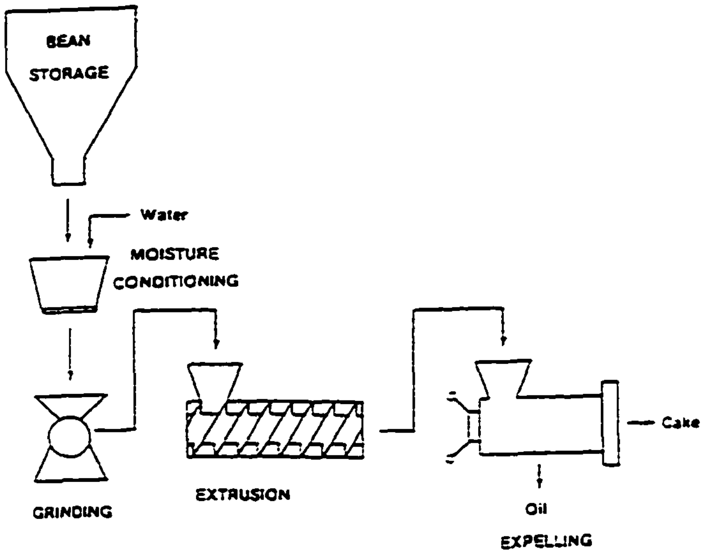

Figure 1: Flow diagram of EE processing technology (Source: Ref. 5).

economical ingredients are soy grits and soy flour. Soy grits and soy flour differ only in the size of the particle. Soy grits pass through US sieve Nos. 10 to 80 whereas soy flour is finer than an 80-mesh sieve. The soy flour most commonly used for food products is 100- or 200-mesh sized material (8). A soy flour of 100 mesh is required to have ≥ 90% of the flour pass through a US sieve No. 100; and a 200-mesh flour has the same requirements with a US sieve No. 200.

Soy flour can be produced in a number of different forms: defatted, low-fat, full-fat, and relecithinated. The proximate composition of soy flour depends upon the process utilized to obtain the product. The commercially available soy flours are classified by fat content. Defatted soy flour is defined as containing <0.5% fat, followed by low-fat at 5-6%, full-fat at 19-21% and relecithinated at 6% or 15% (Table 1) (4, 9). Commercially available LFSF has been found to deviate from the aforementioned 5-6% fat definition with some containing up to 11-12% fat (6). This deviation is due to the conditions of the EE equipment during processing (6).

Table 1: Proximate compositions of defatted, low-fat, full-fat soy flours and 15% lecithinated soy flour[a,b]

	Defatted	Low-fat	Full-fat	15% Fat Relecithinated
Protein (%)	51	51	40	50
Crude Oil (%)	0.5	7	21	16

[a] Source: Ref 4, 9

[b] All values are on a moisture-free basis (mfb)

The demand for commercially available lecithinated soy flour at the two aforementioned fat levels (6 or 15 %) has prompted the market to sell the flour at these two levels. Lecithinated-soy-flour production is a proprietary process by each individual producer. The basic premise of lecithinated soy flour is taking defatted soy flour and adding back refined lecithin to the desired level to aid in dispersing of the soy flour, reduce dustiness of the soy flour, replace egg yolks, and add fat to the product (10).

Soy protein concentrates (SPC) and soy protein isolates (SPI) are the most refined forms of soy protein. This additional processing leads to increased cost, with soy isolates priced higher than soy concentrates. Soy protein concentrates are prepared to contain a minimum of 65% protein while SPI are prepared to contain a minimum of 90% protein (4). Beyond the advantage of the higher protein content in both SPC and SPI compared to soy flour, the soluble carbohydrates that are believed to contribute to the flatulence and beany off-flavors in soybean products are removed (11). The elimination of these off-flavors can lead to the expansion of potential end-uses for these soybean products, particularly in Western markets where these flavors are considered undesirable.

Texturized soy protein may be prepared from soy flour (defatted, low-fat, full-fat or relecithinated), SPC, SPI, soy grits or soy flakes. The process begins with the starting material being pre-conditioned with water, steam, and any other necessary ingredients (sometimes flavors and colors are added if the TSP is used for meat analogs). This plastisized dough enters the extruder where the soy proteins are unfolded and re-aligned into, "...a laminar stretched and twisted condition into an appearance of a meat-like structure." (12).

Uses of Soy Flour

The use of soy flour in food systems ranges from bakery products to beverages. Table 2 summarizes the uses of soy flour and the functional properties required of each.

The extent of soy flour usage is limited by several factors. The first limitation is the decreased amount of protein present compared to SPC and SPI. This decreased amount of protein means that to obtain a desired protein level, one must use more flour or use a concentrate or isolate. Beyond the economic impact that this may cause, increased amounts of flour or concentrate may impact other quality attributes of the product (i.e. texture, moisture, flavor). A second deterrent lies in the fact that there are soluble carbohydrates (raffinose and stachyose) present in the soy flour that cause flatulence and for obvious reasons this limits usage. Thirdly, unless soy flour has been sufficiently heat treated, anti-nutritional factors may be present. For example, trypsin inhibitors will decrease the digestibility of the soy protein; the result has been linked to hypertrophy of the pancreas with

Table 2: Summary of uses for soy flour and functional properties required [a]

Use	Functional requirement(s)
Baked Goods	Solubility, emulsification, gelation, foaming, water-binding
Meat Products	Emulsification, water binding, fat-binding
Beverages	Solubility, emulsification, water-binding
Dry cereal and infant foods	Solubility, emulsification,
Pasta and macaroni products	Water-binding, dough formation

[a] Source: Ref. 8, 13

a resultant loss of essential amino acids (14). Urease, hemagglutinins and lectins are also anti-nutritional factors associated with soy flours that have not been sufficiently heat-treated. The last and most likely limitation for using soy flour in many food formulations is the presence of soluble components that impart beany, painty, grassy off-flavors. Lipoxygenase is the most prominent contributor to producing such compounds. Lipoxygenase has been demonstrated to catalyze the hydroperoxidation reaction of the *cis-cis* 1,4-pentadiene containing fatty acids (15). There are three lipoxygenase (LOX) isozymes present in the whole soybean; these are referred to as LOX-1, LOX-2 and LOX-3. Of the three isozymes, LOX-2 has been hypothesized to be the main isozyme responsible for off-flavors (16).

One caveat of ensuring that these problems do not occur is that, through heat treatment of the soybean, the functionality is decreased, e.g., solubility is negatively correlated to heat treatment. There have been studies performed which show the ideal heat/moisture conditions required to maintain destruction of anti-nutritive or undesirable factors and yet prevent an intense decline in functionality (17).

Soy Protein in Cake Doughnuts

Soy protein is used in the baking industry for functional properties in the formulation and finished baked product (i.e. moisture absorption, emulsification, foaming). In the snack food industry, namely in doughnut production, the addition of soy protein has been shown to decrease fat uptake by doughnuts (18, 19, 20, 21). Defatted, relecithinated, and full-fat soy flours may be used at 4-10% levels to improve crust color, tenderness, and shape, and increase moisture content leading to longer shelf-life (7). The mechanism of this phenomenon is unknown, but may be due to soluble protein forming a film on the doughnut and acting as a barrier to fat uptake (22). The degree of decreased fat uptake is related to the protein dispersibility index (PDI) of the soy flour that is utilized. At a PDI of 50 and above, fat absorption is a function of the quantity of added protein. At lower PDI levels, fat absorption is a function of the quantity of the soluble protein available (22). These results indicate that there is apparently something occurring in the protein structure upon denaturation that prevents it from forming a theoretical fat barrier. Research on doughnuts has focused on doughnut formulations made with defatted soy flour. Work done at Iowa State University has focused on doughnuts made with full-fat soy flour. A standard cake-doughnut formula made with up to 10% full-fat soy flour decreased fat absorption and retained sensory attributes comparable to control cake doughnut made with no soy flour (21).

Soy Protein in Meat Products

Soy flours, concentrates and isolates can be extruded to form TSP. Texturized soy protein has increased in popularity as meat alternatives. The market for both meat analogs (meat-like substitutes) and TSP-extended meat products has increased due to reasons ranging from health concerns to economic incentives. Numerous studies have shown that TSP has meat-like texture (23, 24) in a variety of applications. The majority of the work that conducted on TSP has focused on texturization of defatted soy flour, soy concentrates or soy isolates. Work performed by Crowe (25) showed that soy flour coming from EE technology was an acceptable starting material for making TSP for an extended meat product.

Much of the research carried out on TSP and meat products has centered on incorporation of TSP at a low level (e.g. 20-30% of total product weight) into an all-meat product. The formation of a meat analog is much more challenging due to the requirement to maintain a muscle-like texture with a plant-derived product (26). Research has proven that the incorporation of TSP into meat patties results in a more tender, juicy patty product and decreases cooking losses when compared with all-meat patties (27). These effects are due to increased water- and fat-binding abilities of TSP versus muscle protein used of meat products; the more water or fat that a patty is capable of absorbing, the more juices that are going to be retained leading to perception of a juicy, tender product. Additionally, the more juices that are absorbed, the lower the cooking losses observed. There is no defined level of TSP that can be incorporated into meat patties. Studies utilizing TSP from soy flour focused on amounts ranging from 20-30% of the beef patty being TSP; beyond this, deleterious effects of soy flavor were observed (28, 29)

Extruded-Expelled Soybean Meal in Food Products

The EE process is a relatively new system and has had limited usage in producing commercial food ingredients so there are few publications on employing EE-produced meal in food products. Crowe (25) examined the usefulness of low-fat soy meal with various PDI levels and residual oil (RO) contents for making TSP. EE soy meal, when milled into soy flour, was a useful starting material for producing TSP. Furthermore, this TSP was found to be acceptable for human food applications. In work done by Kulkarni *et al.* (30), white pan bread made with EE LFSF resulted in bread with decreased loaf volume and increased loaf weight due to water retention of the EE soy flour. They also found that internal characteristics, such as symmetry, volume, and crumb color; eating qualities, such as aroma, taste and mouthfeel; and overall quality were ranked significantly lower in bread made with EE soy flour than in the traditional white pan bread.

Functionality Testing

Functional properties of proteins have been defined by Kinsella (31) as "...any physicochemical property which affects the processing and behavior of protein in food systems, as judged by the quality attributes of the final product." Functional characteristics are extremely important to examine before a new protein is used in a food system. It should be noted that functional properties are performed in model systems, and the absolute function of the protein in a food system remains unknown until it is incorporated into the intended food system. There are several potential interactions that the protein may face in a food system; for instance, interaction of protein with sugar and/or salt to name a two. However, performing protein functionality testing on the bench-top is still a critical step to take before incorporating a protein to a food system.

Functionality testing is different when compared to other common testing methodologies due to the lack of universally approved methodology. The few methods that are generally accepted by all researchers are those approved by Association of Official

Analytical Chemists (AOAC), American Association of Cereal Chemists Society (AACC) and/or American Oil Chemists' Society (AOCS) for nitrogen solubility index (NSI), protein dispersibility index (PDI) and water-holding capacity (WHC). Although the NSI and PDI methodologies are often followed, the water-holding capacity approved method is not often adhered to. This is due to the time-consuming steps that must be followed. To resolve this dilemma of not having approved methodologies for the majority of these functionality tests, methodologies have been developed that are "frequently followed." The problem that results from not having generally approved methods is that results of the tests cannot accurately be compared between laboratories, and frequently not within the same laboratory. Furthermore, functionality tests are sensitive to the person performing the test and the equipment used to perform the test. When performing functionality testing the following are the most common tests used.

Protein Solubility

Protein solubility indicates how soluble a protein is in a given solution, usually water. Tests are measured over a wide pH range (commonly pH 2-12). Testing the protein's solubility over a range of pHs is what differentiates this test from the protein dispersibility index (PDI). If PDI testing follows the approved methods (i.e. AACC, AOAC, AOCS), the pH of the protein solution is maintained at the inherent pH of the protein sample. The pH is a major contributor to protein solubility with the protein having improved solubility farther from the isoelectric point (pI) of the protein. The PI of proteins is defined as the pH where there is minimum net charge on the protein (33). Soy protein has a pI range of 4.2 to 4.6 (8), thus solubility is low at the pH range of 4 to 5. Protein solubility is also affected by factors such as soybean varietal differences, storage conditions, relative humidity, types and concentrations of ions, freezing and thawing, and temperature (34).

Protein solubility is an indicator of predicting the overall degree of functionality of proteins (35). This is because the more soluble the protein, the more protein available to impart functionality onto the food system.

Other functionality tests used for interpreting of water-protein interactions include PDI and NSI. PDI has been mentioned previously. However, NSI is a method of testing the solubility of the protein at a specific pH. The difference between NSI and PDI lies in the methodology used for measurement. PDI uses a higher speed for blending water and protein. Because of this difference, PDI generally gives higher values. PDI and NSI are sometimes incorrectly used interchangeably.

Emulsification

Emulsification testing is commonly performed in functionality work. Using the broad definition, an emulsion is a two-phase system where a gas is dispersed in a liquid (38). This definition allows for the inclusion of foams, which will be discussed later. An emulsion is an oil-in-water or water-in-oil dispersion, where the dispersed (or discontinuous) phase is either oil or water in a continuous phase of either water or oil, respectively.

Emulsions are especially important in the meat industry for comminuted meat products and in the baking industry. Emulsification testing may include emulsification capacity (EC), emulsification stability (ES), and/ or emulsification activity (EA). Each of these emulsification tests characterizes the emulsification properties of the protein; EC is a measure of the maximum amount of oil that a protein solution can emulsify before the inversion point is reached; ES is a measure of the ability of a protein suspension to resist breakdown due to certain phenomena, such as coalescence, flocculation, and gravitational creaming (38). EA generally is a measure of the "...area of interface stabilized per unit weight of protein" (39). EA measurements may also be considered to be estimates of the average fat globule size (39).

The charge on the protein, the temperature of the system, protein concentration, and the viscosity of the emulsion affect emulsification characteristics. These factors are among the most prominent in affecting emulsification characteristics on a protein.

Foaming

Foaming is a third commonly used functionality test to define foaming characteristics: foaming capacity (FC) and foaming stability (FS). The literature also refers to foaming as whippability. Technically, the two are different and depend on the type of test used to obtain the information. Whippability refers to when a high-speed blending motion is used to entrain air, whereas foaming refers to when air or gas is forced through a protein solution. The function of foaming is important in food systems, such as whipped toppings, beverages, and leavening of baked products. Foams help to enhance the texture, consistency, and overall appearance of a variety of food systems (40). The definitions of each of these characteristics are similar to the emulsification characteristics—FC describes the maximum foam formed from a protein solution, FS describes the resistance of the foam to leak liquid or destabilization.

Vani and Zayas (40) state that foaming behavior is affected by "...amino acid sequence and disposition, molecular size, shape, conformation and flexibility, surface polarity, charge, and hydrophobicity." In addition, solubility of the protein is a critical factor; the more soluble the protein, the more desirable foaming conditions will be present. The presence of lipid associated with the protein decreased both FC and FS (32). Foaming capacity and stability are at a maximum at or near the pI of the protein due to the development of a strong, viscous protein film capable of forming at the air-water interface.

Water-holding and Fat-binding capacities

Water-holding (WHC) and fat-binding (FBC) capacities are two additional functionality tests that characterize protein functionality. Water-holding and fat-binding capacities measure the amount of water or fat (oil), respectively, that is retained within a protein matrix against centrifugal force. Water-holding capacity is particularly important in meat systems because it affects textural attributes (i.e. tenderness, juiciness, cohesiveness) of the meat analog or plant-protein-extended meat product. A high water-holding capacity is important in baked products because it extends the shelf life of the product by retaining moisture. Fat-binding capacity is also important in meat systems because it relates to cooking loss by retaining some of the fat lost during cooking. These two functionality tests are commonly described together; although the mechanism for each is quite different in the fact that one relies on hydrophilic interactions (WHC) and the other hydrophobic interactions (FBC). In general, WHC and FBC have inverse relationships when related to the PDI of the protein. As PDI increases (the protein has received less heat treatment), the WHC increases due to hydrophilic (polar) amino acid binding sites present. When the PDI of the protein is lower (the protein has received substantial heat treatment), the FBC increases due to more hydrophobic binding sites being exposed. There is some contradiction in the literature about this phenomenon. Hutton and Campbell (41) state "Proteins usually bind less water at high temperatures than at low temperatures, but if protein conformation changes with heating, it could override the effect of temperature on water absorption." Additionally, these two researchers found that FBC increased as temperature decreased (41). Hutton and Campbell (41) found that soy flours in a series of PDI's (85, 70, 55 and 15), WHC increased for samples of 85-55 and decreased for the 15 PDI sample. Kinsella *et al.* (42) suggested that the unfolding of proteins exposes more hydrophilic amino acid binding sites and thus increased the WHC. However, they do not explain why, at an extremely low PDI of 15, the WHC decreased again. This may be due to the presence of an optimum amount of protein denaturation and beyond this level the denaturation is too destructive to maintain any functional properties. The amino acid composition, protein conformation, protein concentration, ionic concentration, pH, and temperature factors also play roles in affecting the WHC and FBC.

Other functionality tests

There are other functionality tests used to investigate the functionality potential of a protein. Surface hydrophobicity studies typically examine the degree of hydrophobic amino acid side chains that are on the surface of the protein. This measure is said to be related to solubility, emulsification, water- and fat-binding capacities, and potentially to foaming (13). Gelation is another important functionality test that is commonly used. Gelation is important to food systems, especially in tofu. Gelation is a process wherein protein is heated, denatured and resets. Water is attracted to the polar amino acid side chains (hydrophilic) and a rigid, three-dimensional network forms. The functional property of gelation is studied with more concentrated forms of protein due to the interaction of carbohydrate moieties influencing the gelation patterns with less concentrated forms of protein (i.e. flours). Another test is apparent viscosity. Observing apparent viscosity gives information regarding the fluid flow properties of the protein. Knowledge of flow properties can aid in "processing and process design, new product development, designing of quality control tests, and mouth-feel and physical appearance…" (32). Film-forming properties are important to gaining insight into the ability of a protein solution to form a film. The objective of studying film-forming properties is to observe the potential for a protein to form edible films that form a barrier and thus prolong the shelf life of selective foods (43). Although these two tests are not discussed in much of the protein functionality literature, they are conducted in order to fully characterize the protein in some circumstances.

Value-Enhanced Soybeans

The number of value-enhanced soybean varieties in the marketplace is increasing. The term value-enhanced soybeans, refers to any soybean which has had an alteration of a trait or a trait added either through traditional plant breeding or biotechnology (44). Examples of value-enhanced soybean are listed in Table 3.

In today's agri-economy, value-enhanced crops are gaining in popularity due to the premium that may be received by raising such crops. Value-enhanced soybeans, although gaining in acres harvested, still represent a small amount of the soybeans that are harvested today.

Table 3:Examples of value-enhanced soybean varieties and their proposed benefits[a, b]

Soybean Variety	GMO/TB[b]	Proposed benefit
Round-Up Ready®[c]	GMO	Herbicide resistance
High Oleic	GMO	Increased shelf-life, health implications
High Sucrose	TB	Decreased flatulence, increased sweetness
Low Linolenic	TB	Increased shelf-life
Lipoxygenase-null	TB	Decreased off-flavors
High Cysteine	TB	Increased protein content, increased functionality
Low Saturated Fatty Acid	TB	Health implications

[a] Source: Ref. 43, 44, 45.

[b] GMO=genetically modified, biotechnological means for altering traits, TB= traditional plant breeding as a means to alter traits.

[c] Round-Up Ready® (Monsanto) soybeans are not food-grade soybeans.

Research has focused on the detection of these alterations in soybeans. Although this is important work for the purpose of maintaining identity preservation, the effect that these soybeans have when implemented in food systems must be studied in order to understand the potential value that they may have in a variety of food, feed and nonfood applications. For food applications, there needs to be significantly more work conducted on both functional characterization work and the effect that these value-enhanced soybeans have on sensory properties in food products.

The work that is available on value-enhanced soybean varieties in food systems is primarily work on the lipoxygenase-null (LOX-null) soybean variety. This soybean in a non-genetically modified (it has been obtained through traditional plant breeding) and is hypothesized to reduce the amount of beany, grassy, painty off-flavors in foods that contain soy products.

Davies *et al.* (45) were among the first to use a variety of the lipoxygenase-null soybeans in food products. This group of researchers found that with the elimination of just one of the lipoxygenase isozymes, there was a decrease in the off-flavors attributed to traditional soybeans in soymilk. Torres-Penaranda *et al.* (47), working with soymilk and tofu produced with LOX-null soybeans, found less beany flavor when compared to soymilk and tofu made with traditional, food-grade soybeans (normal soybeans). Chin (48) found that the substitution of 20% TSP originating from LOX-null SPC in beef patties reduced the soy flavor of the extended patty when compared to an extended patty with SPC from a normal soybean, however, the reduction was not significant. It should be noted that the author hypothesized that there was no significant difference found due to "...the complex flavor of beef" (49). Rahardjo *et al.* (49) found that using a LOX-null soybean line in the production of spray-dried soymilk (SDSM) to be added to pork sausage patties resulted in no statistical sensory difference when compared to the same SDSM originating from normal soybeans. Finally, King *et al.* (50) used bread, meat patties and a soy beverage to compare soy protein from LOX-null and normal soybeans added either as soy flour, acid-washed concentrate, ethanol-washed concentrate or isolate. This group of researchers found that the type of soy flour added (LOX-null versus normal) to bread did not differ in beany flavor imparted to the bread. In meat patties with SPC added, the type of soybean used (LOX-null versus normal) did not differ in beany flavor. Finally, in a beverage with soybean isolate from LOX-null and normal soybeans, the beany flavor was not affected by the type of soybean isolate added. Similar to Chin (48), these researchers note that the complexity of the food systems used (yeast bread and meat) may have been too "intense and complex" and therefore did not allow for the subtle differences in beany flavor to be detected. The work conducted on value-enhanced soybeans in food beyond using LOX-null soybeans is very limited.

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CHAPTER 3: FUNCTIONAL PROPERTIES OF LOW-FAT SOYBEAN FLOUR PRODUCED BY AN EXTRUSION-EXPELLING SYSTEM[1]

A paper submitted to the *Journal of American Oil Chemists' Society*

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ABSTRACT: Low-fat soy flour (LFSF) obtained by extrusion-expelling (EE) processing was investigated for functional properties. Flours with various levels of protein dispersibility index (PDI) and residual oil (RO) content were investigated: low, 14.3±5 / 6.8±0.0; mid, 41.6±3 / 7.8±1.8; and high, 66.6±4 / 11.20±1.5. The protein solubilities of all three LFSF were minimal at pH 4.0 and increased at more alkaline and acidic pH levels. Emulsification capacity (EC) was measured at three pH levels (5.5, 6.7, and 8.0). At each pH level, the low LFSF samples showed the least EC compared to the mid and high LFSF samples, with no significant difference between the mid and high LFSF samples at pH 6.7 and 8.0. Emulsification stability (ESI) and activity (EAI) decreased from low LFSF to high LFSF. Water-holding capacity (WHC) was lowest for high LFSF, with no significant differences between the other soy flour treatments. Fat binding capacity (FBC) was highest for DFSF, with no significant differences between LFSF treatments. Foam stability (FS) increased as

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PDI and RO increased with significant differences between all LFSF samples. This study shows that, in general, low LFSF was less functional than the other soy flours tested and there was no significant difference in the functionality of mid and high LFSF samples.

INTRODUCTION

Research efforts to find value-added uses for soybean protein in food and nonfood products have been going on for quite some time. Soy protein is a promising substitute for animal protein in foods because it contains all of the essential amino acids required for the human diet, has good sensory attributes, and has health benefits including lowering cholesterol and risk reduction of some cancers (1,2). In nonfood applications, soy protein has been used in wood adhesives as a partial replacement for petroleum-based ingredients, and in other applications, such as biodegradable plastics and paper coatings and sizings (3). These value-added uses for soy proteins are based on the functional properties of the protein that add key characteristics to the food or nonfood product that is being formulated. Important functional properties include emulsification, foaming, gelation, water-holding and fat-binding capacities, etc. Recently, researchers have sought to add additional value to soy protein by using alternative processing techniques and genetic engineering or traditional plant breeding to incorporate new, desirable characteristics or to reduce undesirable characteristics inherent in the soybean. In this paper, the potential for adding value to soy through an alternative processing technique, namely extrusion-expelling (EE) processing technology, will be discussed.

Traditional industrial soybean processing involves solvent extraction of the oil with subsequent desolventizing and drying of the meal. The meal is then further processed via grinding and sizing steps to produce meal, grits or flour. An alternative soybean processing technique is the EE process developed by Nelson *et al.* (4) at the University of Illinois in conjunction with Insta-Pro International (Triple 'F' Feeds, Des Moines, IA). Extrusion-expelling processing relies on the mechanical extraction of soybean oil and thus does not use any solvents in the extraction process. The meal remaining can then be processed in a manner that produces products similar to those made from traditional soybean processing, i.e., meal, grits, and flour. Extrusion-expelling equipment produces low-fat soy flour (LFSF). Low-fat soy flour typically has 5-6% residual oil (RO) (4). There are a significant number of small soybean processors, those that process between 6 and 120 T of soybeans per day, utilizing this technology due to low capital investment costs, enhanced extraction capabilities compared with cold pressing, and the ability to produce oxidatively stable oils and meals low in free fatty acids (4).

To begin the EE process, soybeans are dried, cracked, and dehulled. These beans then enter a dry extruder, which has a variety of restrictions thus producing heat through friction to inactivate antinutritional factors. Upon exiting the extruder, the soybeans are in a semi-solid state with most of the cell walls ruptured so as to free the oil. This mixture then enters the expeller (otherwise known as a screw press), where the oil is pressed out and the meal ejected in large, solid pieces. A mill (generally a roller mill) is used to break these large pieces into smaller particles that may either be consumed (as animal feed) or further ground into flour for human consumption (4).

Some researchers have hypothesized that EE meal may have better functional characteristics than defatted soybean meal due to the approximately 5-6% RO: defatted soybean meal contains less than 0.5 % RO. Functional characteristics are properties that promote improved behavior in food systems and industrial applications of protein. However, limited research has been published on the functional characteristics of LFSF coming from the EE process. The objective of this study was to characterize the LFSF produced from the EE processing system, in terms of the following functional properties, protein solubility, foaming and emulsification characteristics, and water-holding and fat-binding capacities.

EXPERIMENTAL PROCEDURES

Preparation of low-fat soy flour. Low-fat soy meal was processed at Iowa Soy Specialties (Vinton, IA). This meal was taken to the Center for Crops Utilization Research Center (CCUR) at Iowa State University where it was milled into flour using a hammer mill (Fitzpatrick Company, Elmherst, IL) to approximately 100 U.S. mesh size. The processing parameters used are described in detail by Crowe (5). The flours produced were categorized into three protein dispersibility index (PDI) (%)/RO (%) groupings: low LFSF, 14.3±5/6.8±0; mid LFSF, 41.6±3/7.8±1.8; and high LFSF, 66.6±4/11.2± 1.5. One commercially available DFSF was used as a control in this study (Archer Daniels Midland, Decatur, IL). This flour had PDI and RO parameters of 71 and 0.6.

Proximate analysis. Proximate analyses for crude protein (Perkin Elmer Series II Nitrogen Analyzer, Perkin Elmer, Norwalk, CT), moisture (AOCS Ba-38) (6), fat (AACC 30-25) (7), and ash (AOAC 942.05) (8) were performed. An outside laboratory (Woodson-Tenant, Des

Moines, IA) performed the analyses for PDI by using the fast-stir method (AOCS Ba 10-65) (6).

Solubility. A sample (250 mg) was dispersed in 25 mL of distilled water and was placed into a 50-mL centrifuge tube. This solution was adjusted to the appropriate pH with 1N HCl or NaOH, shaken at 120 rpm at 25°C and centrifuged at approximately 30,500 g for 30 min. The resulting supernatant was filtered through Whatman No.1 filter paper and nitrogen determination was performed on 10 mL of the filtered supernatant following Kjeldahl procedures (9). Protein solubility was calculated using the following:

$$\text{Protein Solubility (\%)}: \quad \frac{\text{Supernatant protein concentration (mg/ml)} \times 25}{\text{Sample wt (mg)} \times [\text{sample protein content}/100]} \times 100 \qquad [1]$$

Emulsification capacity (EC). A modified procedure of McWatters and Holmes (10) was followed. A 2% protein suspension in water was prepared. The pH of this suspension was altered to either 5.5, not altered (natural pH used, 6.7) or 8.0 with 1N NaOH or 1N HCl in order to observe effects of pH on EC. The 2% protein suspension (25 mL) was placed in a 500-mL plastic beaker. The suspension was continuously blended with a hand-held mixer (Bamix, Switzerland) at high speed (approximately 12,000 rpm) with soybean oil at a flow rate of 1 g/sec. This mixture was continuously blended until the inversion point (water in oil) was observed. Emulsification capacity was determined as the maximum amount of oil emulsified on a per gram protein basis.

Emulsification activity index (EAI) and stability index (ESI). A 2% protein suspension (21 mL) was blended with 7 mL of soybean oil for 1 min using a Waring Blender outfitted with a micro container (110 mL size, Fisher Scientific, Pittsburgh, PA) at low speed. This emulsion was immediately serially diluted with 0.1% SDS at a 500X dilution factor, and the absorbance measured at 500 nm. The diluted emulsion was then incubated at 95°C in a water bath and the absorbance of the emulsion measured at time zero and after 10 min. The EAI and ESI were calculated using the absorbance measured at time zero (A_0) and at 10 min (A_{10}). Calculations as defined by Pearce and Kinsella (11) were used to calculate EAI and ESI.

$$\mathrm{EAI}\ (\mathrm{m}^2/\mathrm{g}) = 2T/\Phi C \qquad [2]$$

C=weight of protein per unit volume of aqueous phase before emulsion is formed; $T=2.303 * A/l$ (A=absorbance at time zero, l=pathlength of cuvette); $\Phi = C-A-E(B-C)/C-A + (B-C)\ ((1+E)D_0/D_s-E)$ where A=mass of beaker, B=mass of beaker plus emulsion; C=mass of beaker plus dry matter; D_0=density of oil; D_s=density of protein solution; and E=concentration of solutes (mass per unit mass of solvent).

$$\mathrm{ESI}\ (\mathrm{min}) = A_0 \times \Delta t/\Delta A \qquad [3]$$

$\Delta t = 10$ min and $\Delta A = A_0 - A_{10}$.

Foaming capacity (FC) and foaming stability (FS). A 0.5% protein suspension (80 mL) was added to a glass column with a fritted glass disk (medium pore size) on the bottom. Nitrogen gas was purged through the column at a flow rate of 100 mL/min. Foaming capacity and foaming stability were calculated based on the equations described by Sorgentini *et al.* (12).

$$FC = V_f\ (\text{ml}) \,/\, f_r\ (\text{ml/min}) \times t_f\ (\text{min}) \qquad [4]$$

V_f=fixed volume of foam, 150 ml; f_r=flow rate of N_2 gas, 100 ml/min; t_f=time to reach fixed foam volume.

$$FS = 1 \,/\, V_{max} \times t_{1/2}\ (\text{ml}^{-1}\text{min}^{-1}) \qquad [5]$$

V_{max}=volume of liquid incorporated in foam at V_f; $t_{1/2}$=time to drain half of liquid incorporated into foam.

Water-holding capacity (WHC). A modified method of Lin and Zayas (13) was used to determine WHC. Low-fat soy flour (5 g) was weighed and dispersed into 95 mL of distilled water and mixed with a magnetic stir bar for 20 min at 25°C. Three-50-mL centrifuge tubes were filled with the flour-water solution and centrifuged at approximately 1080 g for 30 min. After disposing of the supernatant, the WHC was calculated as the difference in weight of the hydrated flour and the original weight of the flour. Water-holding capacity was expressed as gram of water per gram of protein.

Fat-binding capacity (FBC). The fat-binding capacity was determined by stirring a 5% soy flour solution with 50 mL of corn oil (Hy-Vee Brand, West Des Moines, IA) for 30 min and allowed to stand for 30 min at room temperature (25°C). This mixture was then placed into two 50-mL centrifuge tubes and centrifuged for 30 min at approximately 1080 g. After disposing of the excess oil, the FBC was calculated as the weight of residue divided by the original weight (14). Fat-binding capacity was expressed as grams of oil per gram of protein.

Data analysis. Production of soy flour and functionality tests followed a completely randomized design. The General Linear Model (GLM) procedure was used to analyze all functionality tests. TUKEY was used for multiple comparisons and significance was determined at the $P<0.05$ level. Statistical analysis was carried out using SAS statistical software (SAS Institute Inc., Version 8.0, Cary, NC, 1999).

RESULTS AND DISCUSSION

Proximate analysis. Table 1 shows the proximate compositions of all flours used in this experiment. The objective of the researchers was to obtain LFSF in three distinct PDI/RO ranges. However, based on equipment capabilities, the amount of material that was obtained in each category was unequal. Thus, two flours comprise low LFSF, seven flours comprise mid LFSF and six flours comprise high LFSF. One commercially available DFSF was used as the control (Archer Daniels Midland, Decatur, IL). Moisture content decreased with PDI in the LFSF. This was due to increased heating of these low-PDI/RO flours, thus driving off more moisture. The protein contents were lower in the LFSF's compared to the protein contents of DFSF. This trend deviates slightly from published values for each soy flour (15) where LFSF and DFSF is said to have protein contents of 52 and 56% (dmb), respectively, and much lower moisture contents for LFSF, 2.5%and higher moisture for DFSF, 7.3%. Carbohydrate content was calculated by difference. These values were slightly lower than typical carbohydrate contents of soy flour (15). Protein dispersibility index was an indirect measure of the amount of heat treatment applied to each soy flour: the more intense the heat treatment, the lower the PDI. The PDI measurement has also been found to correlate with protein functionality (16). If there is a decrease in PDI, there is a concomitant decrease in functionality observed. When EE processing was used, the fat content was correlated to the PDI and thus to the heat treatment. The preconditioning step (extrusion) was used to disrupt the oil cell thus allowing more oil to be expelled (4). When a less intense heat treatment (or lowering of time spent in extruder) was used, the degree of oil cell disruption was decreased and the amount of oil retained in the soy meal increased.

Protein solubility. All three LFSF and the DFSF showed minimum solubility at pH 4.0 and increased solubility at increased alkaline and acidic pH levels (Fig. 1). These curves indicate that the protein solubility of the flour is affected by the degree of heat treatment and pH. The low LFSF received the most heat treatment, while the high LFSF received the least. The results for the high LFSF and DFSF show that these two products are equal in solubility at pH 8.0.

Protein solubility is considered to be one of the most important functionality tests because it is an indication of how the protein will perform in other functionality tests (17).

Protein dispersibility index is related to the solubility of the protein. Thus, the higher the PDI the more soluble a protein is. Furthermore, the solubility of a protein may indicate how useful this protein will be in food systems. Therefore, mid, high LFSF and DFSF would be more functional than low LFSF in a food system based on solubility.

Emulsification capacity. Emulsification capacity is defined as the maximum amount of oil that is emulsified by a protein solution (16). As the pH and PDI/RO levels rose, emulsification capacity increased (Fig. 2). Emulsification capacity is affected by protein solubility (10). As a protein approaches the pI, there is a decrease in net electrical charge and thus minimum solubility and reactivity can be found (18). In this system at pH 5.5, proteins are less soluble and therefore have decreased capacities to act as surface-active agents and absorb at the oil/water interface. This decrease in surface activity leads to decreased EC. Among low, mid and high LFSF, as the PDI/RO increases there was also an increase in EC. These data suggest that EC increases with protein samples with less denatured protein (as indicated by PDI). Another hypothesis is that RO may play a part on EC. As the RO content increases, the hydrophobicity of the protein increases due to the increased oil content, a hydrophobic material, and in turn allows a greater amount of oil to be emulsified.

Significant differences were found between all soy flours at pH 5.5. However, at pH 6.7 and 8.0, the only significant differences were those between the low LFSF and all other soy flour samples. There was no significant difference between the DFSF and mid and high LFSF samples.

The viscosity of these emulsions was not measured. However, emulsions that resulted in an EC of less than 100 g oil/g protein could be considered simply a suspension, not an emulsion, due to the extremely low viscosity. The inversion point of these emulsions was difficult to identify due to very low viscosity.

Emulsification activity and stability. The EAI is a measure of the area of interface that is stabilized per unit weight of protein. Emulsification activity index may also be interpreted as the size of an oil globule. Emulsification stability index is a measure of the emulsion's resistance to breakdown (11). The EAI was highest in low LFSF and lowest in DFSF (Table 2). There was no significant difference between mid, high LFSF and DFSF. The ESI showed the same trend as EAI with a decreased ESI when moving from low to mid and high LFSF to DFSF; again no significant difference between the mid, high LFSF versus the DFSF. These results indicated that the low LFSF had more activity and stability in emulsions, yet decreased EC compared to the rest of the flour samples. If the results of EAI are interpreted as oil globule size then low LFSF has the largest oil globule size thus decreasing the stability of the emulsion. However, low LFSF has the highest ESI between all treatments. One explanation for these results is that low LFSF had smaller peptides due to the denaturation that occurred during processing and may react with the lipids present, forming a protein-lipid complex with surface activity. Thus, the low LFSF had increased ESI and EAI compared to the other LFSF and DFSF. However, low LFSF did not have enough undenatured protein to effectively emulsify relatively large amounts of lipids compared to the other flour samples and therefore had a lower EC.

Water-holding capacity and fat-binding capacity. The WHC and FBC results are shown in Table 3. Residual oil is hypothesized to play a role in both of these tests. Water-holding capacity was significantly decreased in the high LFSF. Comparing the high LFSF data with the DFSF results shows a significant difference in the two readings although the PDI readings were very similar. This result could be attributed to the amount of RO present in high LFSF sample. The mean RO for the high PDI-LFSF samples is 11%, much higher than the DFSF (0.6%). The presence of this additional fat (a hydrophobic material) could result in less available hydrophilic binding sites available for holding water by the protein.

The DFSF had a much greater degree of fat-binding capacity than any of the LFSF samples (Table 3). The mechanism for fat binding by soy protein has not been elucidated though fat binding is commonly attributed to the physical entrapment of fat by the protein (18). Thus, it can be theorized that the RO that is present in LFSF is blocking the hydrophobic binding sites usually available for binding hydrophobic substances. The DFSF theoretically has all the hydrophobic binding sites available for uptake of hydrophobic materials. The greater the amount of heat treatment that is given to a protein, the more hydrophobic the protein becomes due to a greater number of hydrophobic groups being exposed through the unfolding of the protein's three-dimensional structure. The results obtained from this study show a trend that deviates from this accepted theory. However, the results obtained here emphasize results by Hutton and Campbell (19) that showed that soy protein decreases in FBC with increased heat.

Foaming capacity and stability. Foaming capacity is a measure of the maximum level of foam generated by a solution, while foaming stability is a measure of the resistance of the foam to destabilization (18). The amount of foam that a protein can produce is important, but the more substantial investigation is the stability of the foam. Thus, although FC data is presented (Table 4), the FS data will be focused on. The lower the FS value, the more stable the foam is. The data show a very large variation in FS between DFSF and LFSF. Defatted soy flour produced very stable foams, with symmetrical, evenly distributed foam bubbles. The size of the bubbles is significant because this is an indication of stability (20). The less stable the foam, the larger the bubbles. As with WHC and FBC, EE LFSF foaming properties are dependent not only on the PDI of the flour but could also be influenced by RO content. Hydrophobicity enhances foaming stability (21). Thus, these results could again suggest that the hydrophobicity of these LFSFs is increased. When FS of LFSF is compared to DFSF, an interfering effect of RO could play a role in the decreased FS of the samples, particularly in the case of the high LFSF, which had a relatively equal PDI.

The functionality of LFSF with PDI and RO levels (dwb) between 42/8 and 67/11 and DFSF with a PDI of 71 have similar functional properties. In general, low PDI/RO LFSF is not as functional a protein compared with LFSF with higher PDI/RO levels and DFSF. Processing of LFSF may impact the resultant flour in such a way that the greater the PDI, the more RO present, and the greater the hydrophobicity. This increase in hydrophobicity will affect functional properties such as WHC, FBC, emulsification and foaming characteristics.

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Table 1. Proximate Composition of Low-fat Soy flours and Defatted Flour[a]

Component (%)	High LFSF[b]	Mid LFSF[c]	Low LFSF[d]	DFSF[e]
Moisture	6.9	5.6	4.1	9.4
Crude protein	49.6	50.9	50.2	53.2
Fat[f]	11.2	7.8	6.8	0.6
Ash	5.7	5.9	6.0	5.0
Carbohydrate[g]	26.4	30.3	33.2	32.5
PDI	66.6	41.6	14.3	71.3

[a] Results are expressed on a dry weight basis.
[b] Mean of six flours.
[c] Mean of seven flours.
[d] Mean of two flours.
[e] ADM Bakers Nutrisoy.
[f] Ether extract.
[g] By difference.

Figure 1. Protein Solubility Curve for LFSF and DFSF

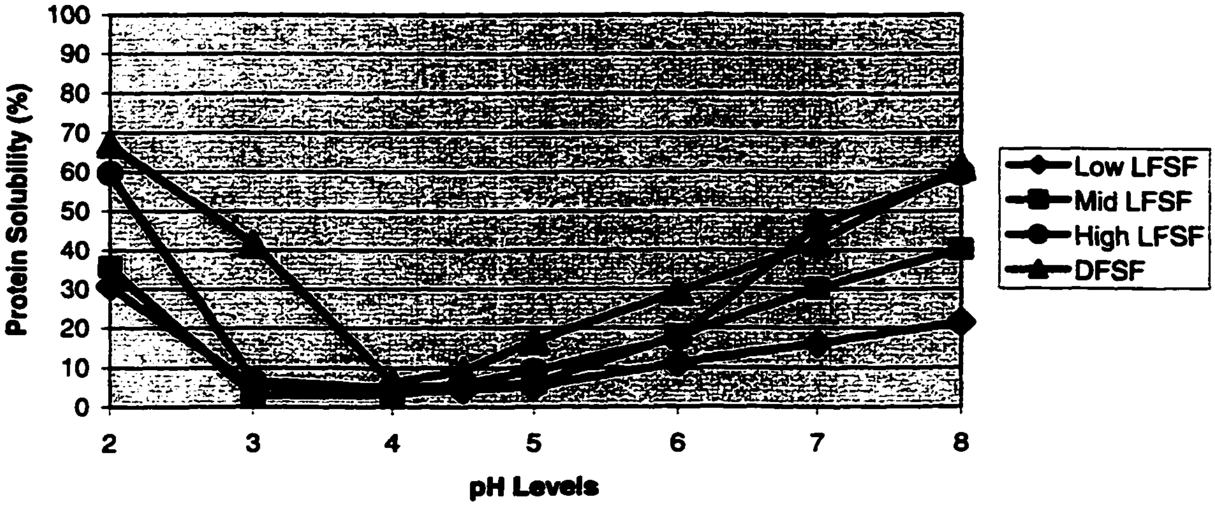

Figure 2. Emulsification Capacity of LFSF and DFSF

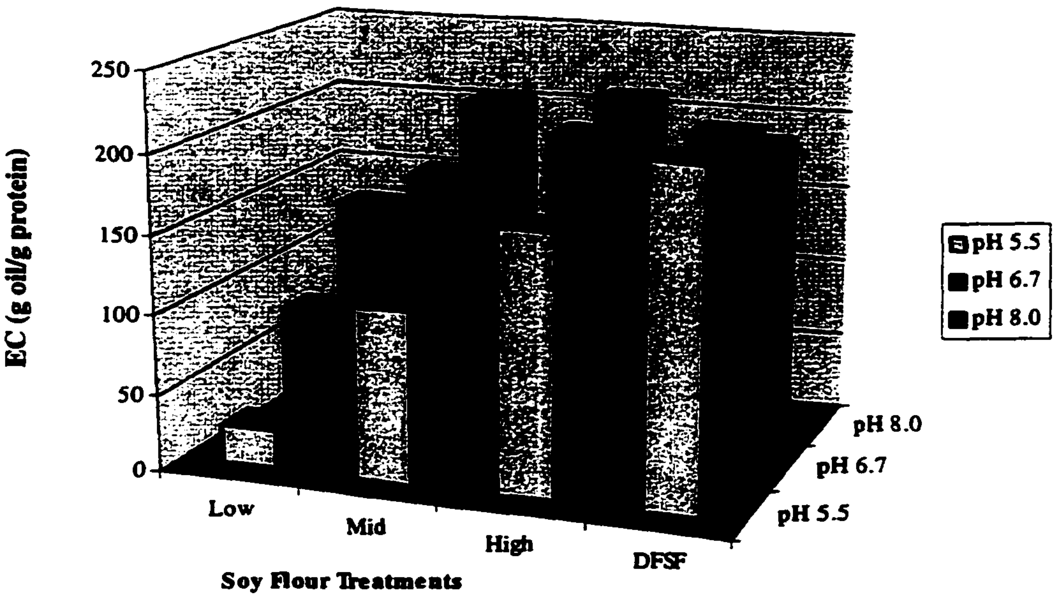

Table 2. Emulsification Activity (EAI) and Stability (ESI)[a]

Treatment	EAI (m^2g^{-1})[b]	ESI (min)[b]
Low LFSF	15.4[b]	12.8[c]
Mid LFSF	12.1[a]	11.4[b]
High LFSF	11.2[a]	10.3[a]
DFSF	10.8[a]	10.4[ab]

[a] Values followed by same letter are not significantly different at the $P<0.05$ level.

[b] EAI=emulsification activity index; ESI=emulsification stability index.

Table 3. Water and Fat Holding Capacities of LFSF and DFSF [a]

Treatment	Water-holding capacity (g water/g protein)	Fat-binding capacity (g oil/g protein)
Low LFSF	6.75[b]	1.66[a]
Mid LFSF	6.19[b]	1.74[a]
High LFSF	4.79[a]	1.84[a]
DFSF	6.70[b]	2.22[b]

[a] Values followed by same letter are not significantly different at the $P<0.05$ level.

Table 4. Foaming Properties of LFSF and DFSF [a]

Treatment	Foaming capacity (mL of foam / mL of N_2 x min)	Foaming stability (^{c}mL^{-1} x min^{-1})
Low LFSF	0.81^{a}	0.37^{d}
Mid LFSF	0.85^{b}	0.14^{c}
High LFSF	0.88^{c}	0.11^{b}
DFSF	0.85^{b}	0.01^{a}

[a] Values followed by same letter are not significantly different at the $p<0.05$ level.

CHAPTER 4: LOW-FAT SOYBEAN FLOUR ADDITION IN A STANDARD CAKE DOUGHNUT FORMULATION

A paper to be submitted to the *Journal of American Oil Chemists' Society*

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ABSTRACT: Low-fat soy flours (LFSF) were added to a standard cake doughnut formulation to determine the effect in chemical, physical and sensory properties. Three LFSF were produced using the extrusion-expelling technology and were compared with a commercially available defatted soy flour (DFSF). The LFSF varied in protein dispersibility index (PDI) and residual oil (RO) contents. The PDI/RO levels were: low, 18.2/6.5%; mid, 44.9/7.1%; high, 67.8/11.8%; the control DFSF was 73/0.6%. The soy flours were added at three levels: 3, 5, and 8% (flour weight basis). Low-fat soy flour did not necessarily follow the same trend as DFSF for chemical and physical analyses. Furthermore, the results from LFSF were very unpredictable, particularly with the mid LFSF and high LFSF. A trained sensory panel found that type of flour and level of addition both played an integral role in response for oiliness, darkness, tenderness and moistness. Soy flavor, however, was affected by flour type alone. Low-fat soy flour maintains certain quality and sensory characteristics when added to a standard cake doughnut formulation, however, does not behave as consistently and predictably as DFSF.

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INTRODUCTION

Soy flour is used in commercial bakery doughnut mixes for functionality purposes other than improved flavor and texture (1). The primary purpose for soy flour is to decrease the amount of oil uptake by the doughnut during frying (2). By reducing the amount of oil that is lost during processing there is an economic benefit for the processor. In addition to the benefit of oil reduction, soy flour also aids in improved gas retention and controlling crust color and volume (3). Typical usage levels of soy flour in commercial doughnut mix range from 1 to 3% of the total flour in the formulation (4). However, there have been additional research efforts investigating the potential of using larger volumes of soy flour to further reduce costs (5,6).

The mechanism for reduced fat absorption is not known. It has been hypothesized that the heat-denatured proteins form a barrier on the outer surface that prevents oil absorption (1). Martin and Davis (1) concluded that, "...the major effect of soy flour on fat absorption by cake doughnuts was a function of the quantity of protein added to the batter." This is in conflict with the theory that solubility plays a role in determining how functional a protein is in a food system.

Soy flour is also used as an egg replacer in doughnut mixes. Low (6) found that a 30% substitution of dried whole egg solids with full-fat soy flour reduced costs with no deleterious quality effects on the doughnuts.

Low-fat soy flour (LFSF) is a product of extrusion-expelling (EE) technology. Typically, LFSF has a residual oil (RO) content of 6% and a protein content of 50% (dmb) (7). Extrusion-expelling technology is gaining in popularity because of low capital investment, the economic feasibility to process identity-preserved (IP) soybeans and ease of operation and maintenance of equipment.

Current research has focused on the use of defatted soy flour (DFSF) in a standard cake doughnut formulation. There has been no work published on the use of LFSF from an extrusion-expelling process in cake doughnut formulations. Due to the common use of DFSF in cake doughnut formulations, it would be interesting to observe the effect LFSF had on these formulations. The objective of the present study was to determine the effects on certain chemical, physical and sensory attributes that added LFSF had on a standard cake doughnut formulation and compare these to the effect that DFSF had on the same standard cake doughnut formulation.

EXPERIMENTAL PROCEDURES

Soy flour preparation. Low-fat soy flour was produced at Iowa Soy Specialties (Vinton, IA) using their EE equipment. Three LFSF treatments were produced with different protein dispersibility indexes (PDI) and RO levels: low, 18.2/6.5; mid 44.9/7.1; and high 67.8/11.8. One commercial DFSF was used as a control in this study. The PDI/RO level for this sample was 73/0.6

Batter preparation. The formula for the cake doughnuts used in this study is found in Table 1. Sucrose and oil were mixed and beaten for 1 min in a bowl using a countertop mixer (KitchenAid model K5SS, Denver, CO). All dry ingredients were blended together to form a homogeneous mixture. Soy flour was added at 3, 5 or 8% addition levels (flour weight basis). The sucrose and oil mixture was then added to the dry ingredients, and water was added and all mixed for 2 min on speed number 1. The temperature of the added water was controlled in order to maintain a batter temperature of 22-24°C. The batter mixture was then allowed to stand for 10 min before being placed into the doughnut fryer (Doughnut Robot Mark II Fryer, Belshaw Brothers, Seattle, WA) hopper. The batter was again allowed to stand for 5 min after transport to the doughnut hopper.

Doughnut frying. Doughnuts were fried in commercial partially hydrogenated soybean oil (Iowa Doughnut Supply, Urbandale, IA). Frying oil was changed when free fatty acid (FFA) values surpassed 0.75% as measured by AOCS standard method, Ca 5a-40 (8). Settings for the doughnut fryer included: doughnut weight of 2.5 (machine cutter setting), oil temperature set at 190°C and a frying time of 150 sec. Doughnuts were automatically turned halfway through frying (75 sec). After frying, the doughnuts were allowed to air cool on metal racks at room temperature before further analyses were performed.

Sample preparation. Random doughnut samples were crumbled and then lyophilized (Unitrap II, Virtis Co., Gardiner, NY) for further analysis. Dry ice was then added to lyophilized samples and the mixture was ground in a commercial coffee grinder at a ratio of 1:1 to ensure that no fat was lost during grinding.

Physical determinations. Representative doughnut samples were evaluated for weight, height and width. After the doughnuts had cooled to room temperature, one whole doughnut was lightly padded with a paper towel to remove any excess fat, placed on a scale (Denver Instruments Co., Denver, CO) and weighted. Height and width measurements were taken by first cutting the doughnuts in half, vertically, followed by measuring the cross-section height and width. The weight, height and width measurements were taken on three doughnuts per replication (two replications).

Color measurements: Color measurements were taken when the doughnuts had reached room temperature. Doughnuts were cut into quarters. A Hunter Lab spectrocolorimeter (Hunter Lab, model 6100, Reston, VA) was used for color determinations. The spectrocolorimeter was standardized with a white tile (No. LS-12414, X=78.67, Y=83.31, Z=86.40) and a black tile. L, a, and b values were determined with settings including a 10° standard observer and cool fluorescent light (CFW). Color measurements were taken on three doughnuts per replication, with three readings taken per doughnut. The same procedure was followed for interior and exterior color.

Texture profile analysis (TPA): Texture profile analysis was performed using a texture analyzer (Texture Technologies, model TA-XT2, Scarsdale, NY). Doughnuts were cut into 2-cm cubes with the crust removed. A 40-mm aluminum anvil was used with a compression rate of 80% and a test speed of 3.3 mm/sec. Hardness, gumminess, chewiness, cohesiveness, and springiness values were recorded. Texture measurements were taken on four doughnuts per replication.

Composition. Protein, moisture, and fat measurements were taken of representative doughnut samples. Moisture was determined on both the batter and the final doughnut. Batter and doughnut samples were weighed before lyophilization and after. Moisture was then determined as the loss in weight. Fat determinations were made on both the batter and doughnuts using petroleum ether extraction methods (AACC 30-25) (9). Protein determinations were made using a nitrogen analyzer (Perkin Elmer, Model 2410 Series II, Norwalk, CT) (AOCS Ba 4e-93)(8). Fat absorption calculations were made by subtracting the fat in the batter from the fat in the doughnut. Protein and fat measurements were taken on three doughnuts per replication, while moisture measurements were taken on two doughnuts per replication.

Sensory evaluation. A 12-member trained panel evaluated doughnuts for soy flavor, moistness, oiliness, tenderness, darkness, and gumminess. Panelists were trained in two 1-hour training sessions. Panelists were volunteers from the Food Science and Human Nutrition Department at Iowa State University. Sensory evaluation was carried out in the Food Sciences Building at Iowa State University. Individual sensory panel booths with partitions and food serving doors were used. Doughnuts were made in the morning, and panels were held the same afternoon. This allowed for doughnuts to cool to room temperature before evaluation. Panelists were served four doughnut samples at each session, two per plate. Panelists were also encouraged to swallow the samples and rinse their mouths with room-temperature water between bites. A 15-cm linescale, in paper format, was used for panelists to rate each attribute from 0 cm (no indication of attribute present) to 15 cm (intense amount of attribute present). Sensory evaluation followed a completely randomized design.

Statistical analysis. Chemical, physical and sensory data were subjected to General Linear Modeling (GLM) using SAS statistical software (SAS Institute, Inc., Version 8.0 for Windows, 1999, Cary, NC). Main effect means (soy flour and level) and interaction means (flour*level) were analyzed for differences. When F-values for interactions were significant at the $P<0.05$ level, interaction means are presented. If no significant interaction was present, significant main effect means are presented. Significance in all instances was determined at the $P<0.05$ for all data analysis. Tukey's HSD was used for multiple comparison analysis.

RESULTS AND DISCUSSION

Physical determinations. Doughnut weight was reduced for doughnuts made with mid LFSF and DFSF when the amount of soy flour added was increased (Fig. 1). There was little change in weight for low LFSF. High LFSF increased in weight with addition of up to 8% addition. The average weight of doughnuts made with LFSF was significantly higher than the average weight of doughnuts made with defatted soy flour ($P<0.001$). This may be an indication of the RO in the LFSF before incorporation into the doughnut formula. Low-fat soy flours had RO in the starting soy flour material, thus adding weight to the material, whereas the defatted soy flour did not.

The amount of soy flour added did not significantly affect the height of the doughnut, however, the type of soy flour added did have a significant effect on doughnut height (Fig. 2). Doughnuts produced with high LFSF at the 5 and 8% addition level have greater average height compared with the doughnuts made with low LFSF and DFSF at the same addition levels. One reason for this increase in height may be that the protein and lipid in the high LFSF are interacting with each other instead of with the water present, thus allowing for more gluten formation. Spink *et al.* (2) found that gluten formation in doughnuts made with up to 30% protein substitution with a protein material of 44% protein (dmb) was inhibited due to a lack of gluten hydration. Gluten was unable to hydrate due to the water absorption capacities of the added protein materials. The height of these doughnuts was inhibited due to this lack of gluten formation thus structure formation (2). Earlier work performed on LFSF of the same PDI/RO levels found high LFSF had a significantly decreased level of water holding capacity (10).

No statistical differences were noted for width measurements. Width measurements ranged from 2.6 to 3.0 cm.

Color measurements. Decreased 'L' values and increased 'a' and 'b' values were found for the exterior of the doughnuts when compared with the doughnut interior color measurements (Table 2). Hunter Lab spectrophotometric values are represented by: 'L' 0=black and 100=white; 'a' +=red and - =green; and 'b' +=yellow and -= blue. These results were expected due to Maillard browning, which occurs upon heating of proteins and sugars. This reaction takes place between a protein, reducing sugar, and water. The result of this reaction is browning. Doughnuts with decreased 'L' value and increased 'a', and 'b' values were darker, redder and more yellow in color. Doughnuts produced with DFSF showed consistent results whereas doughnuts made with LFSF showed inconsistent results. There are fewer differences in the interior color measures compared with exterior color measurements. This was due to the aforementioned Maillard reaction predominantly taking place on the exterior of the doughnut.

Texture profile analysis. Doughnuts made with mid LFSF at the 5% level were significantly softer compared with most of the other doughnut treatments (Fig. 3). On the other hand, doughnuts produced with high LFSF at the 5% level were significantly harder when compared with doughnuts made with mid LFSF at the 5% level, doughnuts made with low LFSF at the 8% level, doughnuts made with high LFSF at the 8% level, and doughnuts made with DFSF at the 8% level. The hardness value for doughnuts produced with high LFSF at the 5% level also relates to the larger height for this doughnut compared with the other doughnut treatments. The control doughnuts made with DFSF and low LFSF had very consistent hardness values, whereas mid LFSF and high LFSF treatments were inconsistent.

Doughnuts were generally more cohesive at the 3% soy flour addition levels for all doughnut treatments (Fig. 4). Defatted soy flour consistently decreased in cohesiveness values with increased levels of soy flour addition.

Gumminess and chewiness values for doughnuts produced from low LFSF decreased when soy flour addition levels increased. Gumminess values for DFSF remained consistent with increased levels of soy flour addition. Gumminess and chewiness values significantly decreased in doughnuts produced from mid LFSF when moving from 3 to 5% and increased when moving from 5 to 8% addition levels (Figs. 5 and 6).

No interaction or main effects were significant for springiness. Springiness values ranged from 0.90 to 0.99 cm.

Composition. Doughnuts produced from mid LFSF and high LFSF increased in moisture level when moving from 3 to 5% addition levels and then decreased with moving from 5 to

8% addition levels (Fig. 7). At the 3% level, doughnuts made with DFSF were significantly more moist compared with the low LFSF and mid LFSF doughnut treatments.

Doughnuts made with DFSF contained decreasing amounts of fat when addition levels increased (Fig. 8). Doughnuts made with LFSF were expected to have more fat than doughnuts made with DFSF due to increased amounts of fat in the starting soy flour material. At the 5 and 8% addition levels, this was found to be the case.

Protein contents showed little variation, with the exception of a very high protein content for DFSF at the 8% addition level. This is in accordance with the higher protein content of the DFSF when compared with the LFSF (53% versus approximately 50%).

Finally, fat absorption data showed several interesting trends (Fig. 1). First, DFSF showed a trend of reduced fat absorption as the amount of DFSF increased in the doughnut formulation. Doughnuts made with low LFSF and mid LFSF behaved similarly when the level of added soy flour was increased from 5 to 8%. Both of these LFSFs increased in their fat absorption levels when soy flour addition moved from 5 to 8%. High LFSF tended to increase in fat absorption going from 3 to 5% addition, but then sharply decreased as addition levels increased to 8%. Low (6) found similar results in that fat absorption increased slightly with select full-fat soy flours. Despite the fact that at the 5% addition level, low LFSF and mid LFSF are very similar to the DFSF in fat absorption levels, overall none of the LFSF was as effective at lowering fat absorption in cake doughnuts as the DFSF.

Sensory evaluation. There was a significant interaction between flour and level for all responses except for gumminess and soy flavor (Tables 3 and 4). Neither flour type nor addition level affected soy flavor responses, thus no significant differences were found. Soy flavor mean scores for all treatments ranged from 0.9 to 1.9 cm, however. In general, for sensory evaluation results, panelists did not detect differences between doughnuts produced with the same soy flour at different levels. The gumminess values for doughnuts made with high LFSF were significantly lower than the mid LFSF and DFSF treatments. One explanation for the high LFSF result is, again, the potential decrease in interference high LFSF plays in gluten formation, thus allowing for complete gluten formation.

Low-fat soy flour behaves differently in a cake doughnut formulation when compared with DFSF. The reason for this is unknown, but may be related to the RO in LFSF. Defatted soy flour showed consistent results (e.g. decreased fat absorption with an increased amount of DFSF added, decreased oiliness and moisture measurement from sensory evaluation studies), while LFSF showed unpredictable results when compared with DFSF. In the present work, LFSF behaved with no consistency when addition levels increased. However, LFSF maintained certain quality attributes when subjected to sensory evaluation when compared with the DFSF control. Observing the important attribute of fat absorption, when comparing LFSF with DFSF, high LFSF had the best performance, with an overall decreased fat absorption of 13%.

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Table 1. Standard Cake Doughnut Formula

Ingredient	Amount (g)
Sucrose	300.00
Soybean Oil	86.4
Flour, A.P. (sifted)	781.36
Eggs, dried whole	35.97
Baking Powder	18.00
Salt	4.50
Cornstarch	2.93
Soy lecithin powder	0.98
Non-fat dry milk	44.21
Soy flour[a]	specified level
Water	639.50

[a] LFSF added at 3, 5 and 8% levels, flour weight basis.

Figure 1. Weight of Doughnuts made with 3, 5, and 8% Soy flour Additions

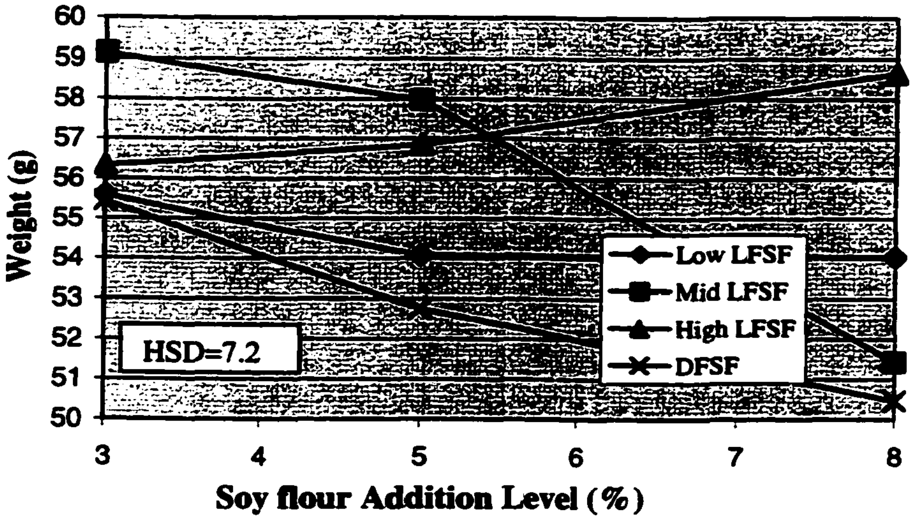


Figure 2. Height of Doughnuts made with 3, 5, and 8% Soy flour Additions

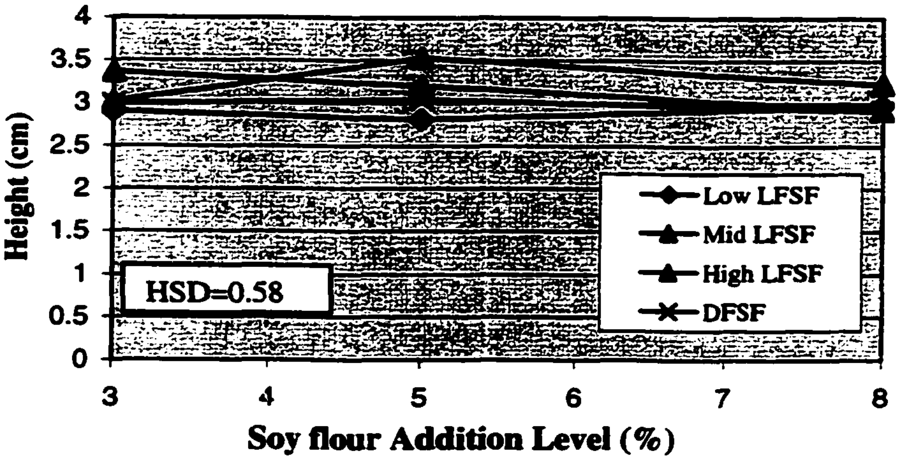


Table 2. Color Measurements for Doughnuts with 3, 5 and 8% Soy flour Additions [a]

		Exterior			Interior		
Flour[b]	Level	L	a	b	L	a	b
Low LFSF	3	54.38^{bc}	6.93^{bc}	18.99^{bc}	63.19^{abc}	0.01	12.59^{ab}
Low LFSF	5	55.67^{c}	5.32^{a}	17.88^{ab}	61.29^{ab}	-0.43	11.47^{a}
Low LFSF	8	48.21^{a}	7.56^{bcd}	17.16^{ab}	61.84^{abc}	-0.49	11.94^{ab}
Mid LFSF	3	50.93^{ab}	7.62^{bcd}	17.66^{ab}	69.33^{c}	-0.53	12.61^{ab}
Mid LFSF	5	49.10^{a}	7.13^{bc}	17.29^{ab}	66.46^{abc}	-0.50	12.55^{ab}
Mid LFSF	8	48.33^{a}	7.51^{bcd}	17.15^{ab}	59.46^{a}	-0.45	11.46^{a}
High LFSF	3	51.56^{abc}	7.59^{bcd}	17.91^{abc}	66.35^{abc}	-0.51	11.90^{ab}
High LFSF	5	54.42^{bc}	6.27^{ab}	18.27^{abc}	63.09^{abc}	-0.54	11.81^{ab}
High LFSF	8	47.88^{a}	7.85^{cd}	16.60^{a}	68.01^{bc}	-0.51	12.21^{ab}
DFSF	3	51.37^{abc}	8.58^{d}	17.97^{abc}	68.31^{bc}	-0.34	12.86^{ab}
DFSF	5	50.38^{ab}	7.69^{bcd}	17.88^{ab}	64.62^{abc}	-0.29	12.46^{ab}
DFSF	8	55.05^{bc}	7.70^{cd}	19.81^{c}	69.06^{bc}	-0.47	13.29^{b}
HSD		4.8	1.4	1.9	8.0	NS	1.6

[a] Values in same column followed by same letter are not significantly different at the $P<0.05$ level; L, a, and b values represent Hunter Lab Spectrophotometric values.

[b] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6.

Figure 3. Hardness of Doughnuts made with 3, 5, and 8% Soy flour Additions

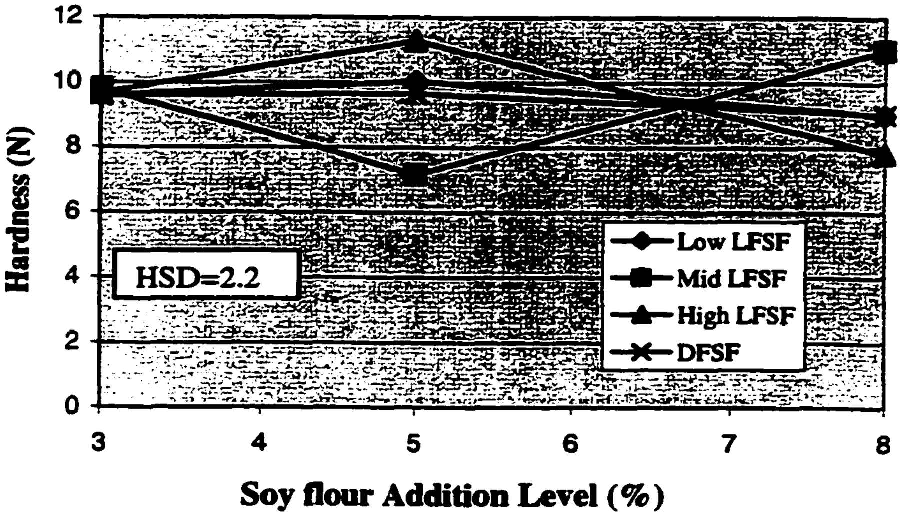


Figure 4. Cohesiveness of Doughnuts made with 3, 5, and 8% Soy flour Additions

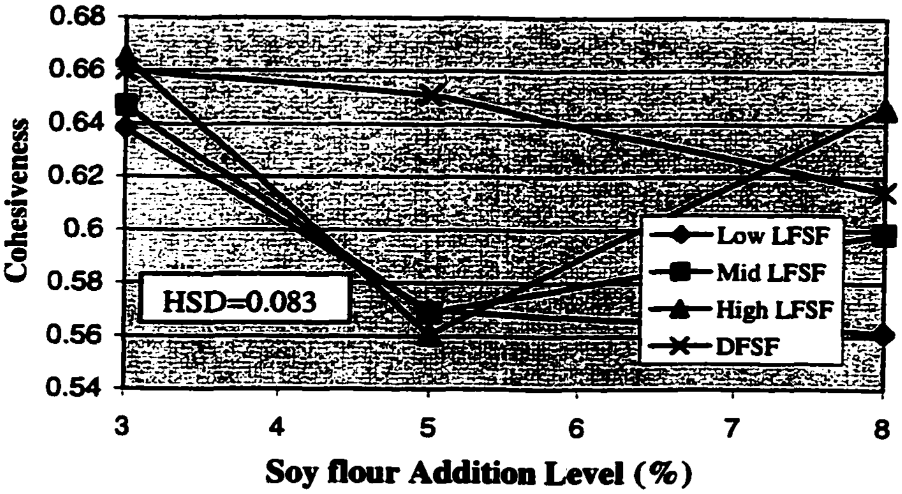


Figure 5. Gumminess of Doughnuts made with 3, 5, and 8% Soy flour Additions

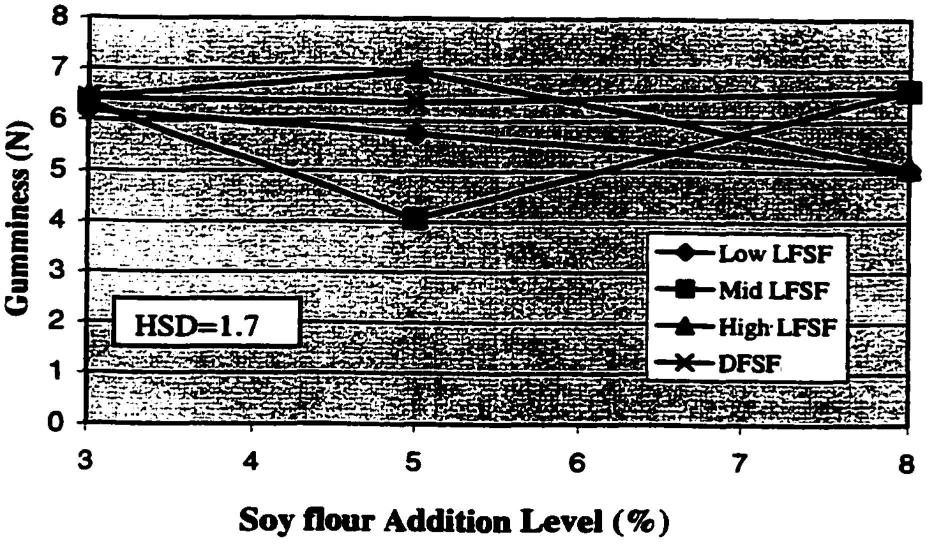


Figure 6. Chewiness for Doughnuts made with 3, 5, and 8% Soy flour Additions

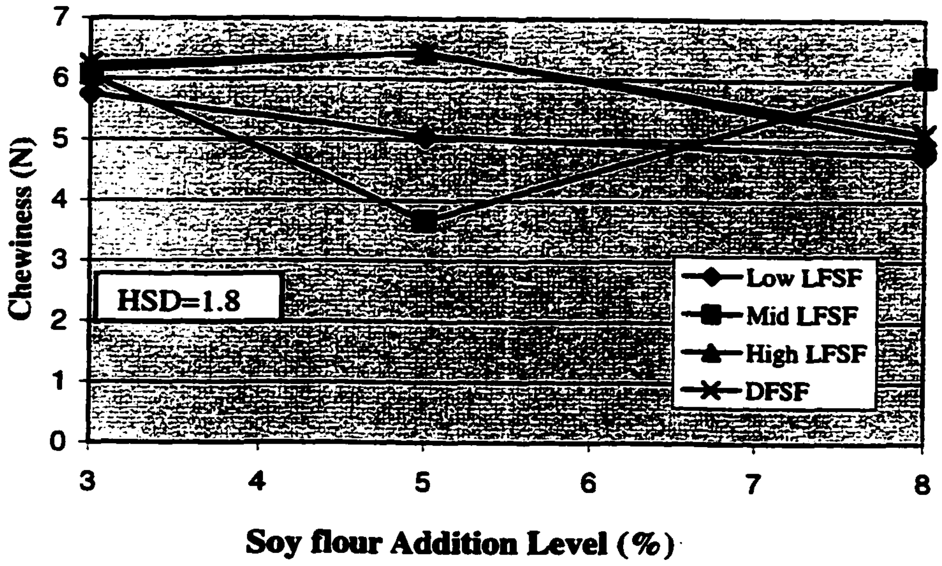


Figure 7. Moisture Levels of Doughnuts made with 3, 5, and 8% Soy flour Addition

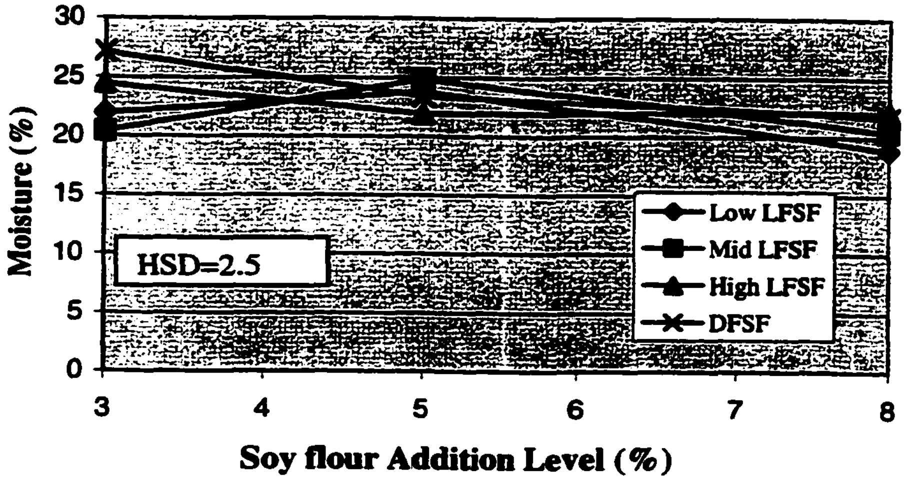
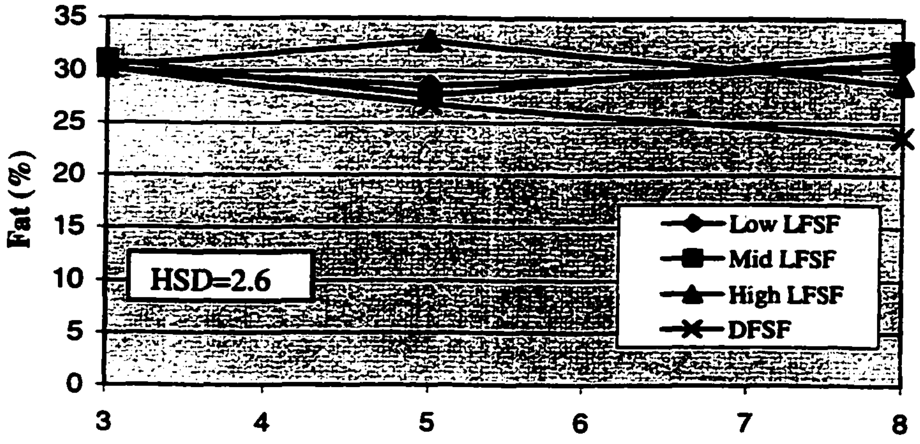


Figure 8. Fat Levels of Doughnuts made with 3, 5, and 8% Soy flour Additions



Soy flour Addition Level (%)

Figure 9. Protein Levels of Doughnuts made with 3, 5, and 8% Soy flour Additions

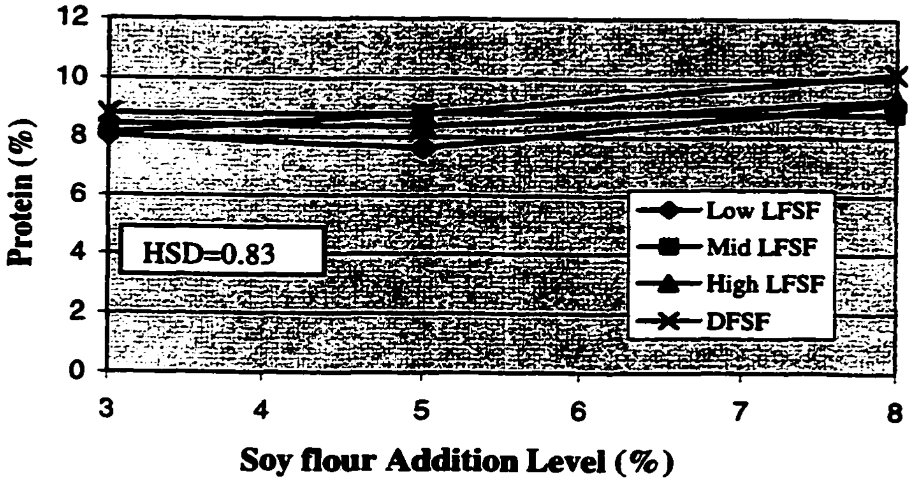


Figure 10. Percentage Fat Absorption for Soy flour Addition in a Cake Doughnut Formulation

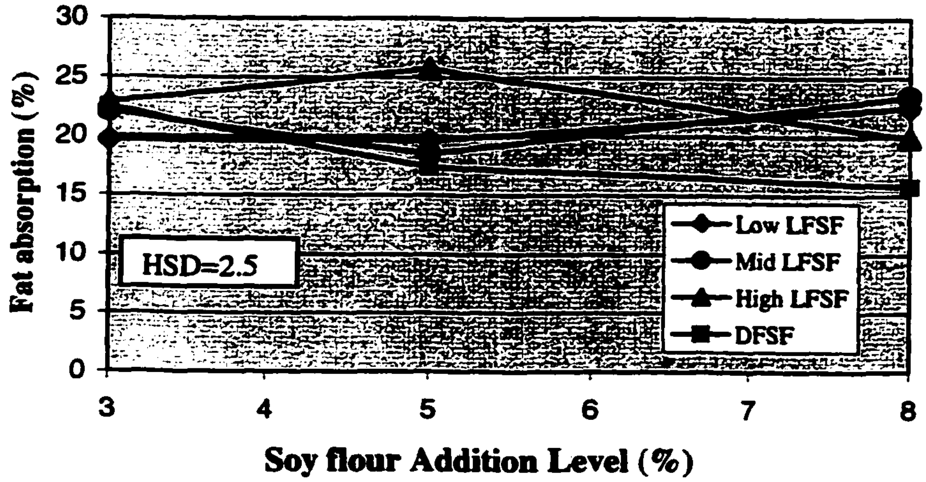


Table 3. Sensory Evaluation Means of Doughnuts made with 3, 5 and 8% LFSF and DFSF Soy flour Additions[a]

Flour[b]	Level	Oiliness	Darkness	Tenderness	Moistness	Soy Flavor
Low LFSF	3	5.2^{abc}	8.3^{bc}	9.8^{ab}	7.4^{ab}	1.7
Low LFSF	5	4.4^{a}	5.2^{a}	9.9^{ab}	6.6^{a}	1.5
Low LFSF	8	6.8^{abc}	10.1^{c}	10.9^{ab}	8.7^{bc}	1.2
Mid LFSF	3	6.2^{abc}	7.6^{abc}	10.8^{ab}	8.7^{bc}	1.5
Mid LFSF	5	7.5^{c}	6.6^{ab}	11.4^{b}	10.0^{bc}	1.3
Mid LFSF	8	6.4^{abc}	9.9^{c}	10.4^{ab}	8.1^{ab}	1.5
High LFSF	3	4.9^{ab}	10.0^{c}	9.6^{a}	8.1^{ab}	1.9
High LFSF	5	5.8^{abc}	8.7^{bc}	10.5^{ab}	8.2^{ab}	1.9
High LFSF	8	7.7^{c}	10.2^{c}	10.2^{ab}	9.0^{bc}	1.8
DFSF	3	7.1^{bc}	7.6^{abc}	10.8^{ab}	9.1^{bc}	1.7
DFSF	5	7.2^{bc}	8.4^{bc}	10.4^{ab}	8.5^{bc}	1.3
DFSF	8	5.7^{abc}	8.6^{bc}	9.6^{a}	7.3^{ab}	0.9
HSD		2.5	2.7	1.7	1.7	NS

[a] Values in vertical columns followed by the same letters are not significantly different at the $P<0.05$ level; Table 6 represents interaction means for oiliness, darkness, tenderness, moistness, and soy flavor; Values based on 15-cm line scale, 0 cm= no amount of attribute present, 15 cm=intense amount of attribute present.

[b] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6.

Table 4. Main Effect Means for Sensory Evaluation Response to Gummines[a]

Flour[c]	Gumminess
Low	8.2[ab]
Mid	9.0[b]
High	7.8[a]
DFSF	9.0[b]
HSD	1.1

[a] Values in same vertical column followed by the same letter are not significantly different at the $P<0.05$ level.
[b] Values based on 15-cm line scale, 0 cm=no amount of attribute present, 15 cm= intense amount of attribute present.
[c] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6

CHAPTER 5: FUNCTIONAL PROPERTIES OF EXTRUDED-EXPELLED SOYBEAN FLOURS FROM VALUE-ENHANCED SOYBEAN VARIETIES

A paper to be submitted to the *Journal of American Oil Chemists' Society*

A.A. Heywood[1], D.J. Myers[2], T.B. Bailey[3] and L.A. Johnson[4]

ABSTRACT: The functional properties (protein solubility, emulsification characteristics, foaming characteristics, water- and fat-binding capacities) of extruded-expelled (EE) soy flours originating from six varieties of value-enhanced soybeans (high sucrose, high cysteine, low linolenic, low saturated fatty acids, high oleic, and lipoxygenase-null) and two commodity soybeans were determined. All soy flours varied in protein dispersibility index (PDI) and residual oil (RO), with PDI ranging from 32 to 50% and RO ranging from 7.0 to 11.7%. Protein solubility data indicate a trend of lower solubility near the isoelectric region and higher solubility at both low and high pH levels. No significant differences were found for WHC or FBC. Water-holding capacity ranged from 3.7 g water/g protein to 4.2 g water/g protein while FBC ranged from 1.9 g oil/g protein to 2.2 g oil/g protein. Only the high oleic soy flour had significantly lower EC compared with the commodity soybeans and high cysteine soy flour. Emulsification activity and stability data did not significantly differ. In general, the PDI and RO levels of EE soy flours originating from value-enhanced and

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commodity soybeans have the greatest influence on the degree of protein functionality. The value enhancement of soybeans did not have a deleterious effect on functional properties.

INTRODUCTION

Soybeans are traditionally processed by solvent extraction methods. Solvent extraction processing plants involve large capital investment and the use of a hazardous solvent (i.e. hexane). One alternative to this processing method is the use of an extrusion-expelling (EE) method. This technology results in soybean meal containing approximately 7% oil (1). Extrusion-expelling processing is gaining in popularity due to low capital investment, ease of running EE equipment, and the ability to process identity-preserved (IP) soybeans.

Identity-preserved soybeans require special processing procedures. First and foremost, IP soybeans cannot be combined with any commodity-type soybean or any other IP soybean. Thus, separation must be provided at every step of processing (initial storage, processing, final product storage, and shipping). In order to maintain IP, the equipment used for the transportation, processing and storing of these soybeans must be thoroughly cleaned and inspected between uses. Documentation stating that the IP soybeans have been handled properly must follow each step of processing.

The IP soybean category title is general in nature and includes any soybeans that must be separated from both non-IP and other IP soybeans. Identity-preserved soybeans include soybeans that are organically produced, specialty soybeans produced with specific end-use target (i.e. soybeans intended for tofu production), and value-enhanced. Value-enhanced soybeans are either genetically modified or traditionally bred to modify a specific trait. Although the acreage of IP soybeans is growing, at this point in time the acreage is not nearly as large as that of commodity-type soybeans. For this reason, many large soybean processors (as defined by processors that run more than 150 T/day of soybeans) find it financially difficult, logistically challenging and generally not efficient to use these IP soybeans as starting materials.

There is a limited amount of published work on functionality as it is related to value-enhanced soybeans. There is no available literature, however, on utilizing EE processing on value-enhanced soybeans and the resultant functional properties. In the present study, the EE processing system was used to obtain low-fat soybean meal from six different value-enhanced soybean varieties. The value-enhanced varieties included high sucrose, low linolenic, lipoxygenase-null, high oleic, low saturated fat and high cysteine. Compositional data show: high sucrose soybeans have increased amounts of sucrose (6.7% sucrose) in addition to decreased amounts of stachyose (0.3% stachyose) (2); low linolenic soybeans have a decreased amount of the unsaturated fatty acid linolenic (3.1% linolenic acid); lipoxygenase-null soybeans have all three lipoxygenase isozymes removed; high oleic soybeans have increased amounts of the fatty acid, oleic acid (79.2% oleic acid); and low saturated fatty acids soybeans have decreased amounts of all saturated fatty acids (8.4% total saturated fatty acids) (3) all compared to commodity soybean varieties. Finally high cysteine soybeans have increased amounts of the amino acid cysteine in the 7S fraction of the protein. This increase is equivalent to 5 residues of cysteine per mole of 7S protein (4). Additionally, two commodity soybeans were processed using the EE system and served as controls. The objective of this study was to investigate the functional properties (including protein solubility, emulsification characteristics, foaming characteristics and water-holding and fat-binding capacities) of extruded-expelled soy flours originating from different value-enhanced soybean varieties.

EXPERIMENTAL PROCEDURES

Soybean varieties. Table 1 shows the soybean varieties used in this study, along with the variety abbreviation that will be used throughout this paper, and the trait that has been altered. In addition to the value-enhanced soybean varieties, two commodity soybeans were included as controls. Soybeans were obtained from various sources. Commodity soybeans (non value-enhanced) were obtained from West Central Cooperative (Ralston, IA) and Steine Seed Company (Adel, IA). These were two different commodity soybean varieties. Optimum Quality Grains (Des Moines, IA) provided high oleic, low saturated fatty acids, high sucrose, and low linolenic acid soybeans. An experimental high cysteine soybean line was obtained from the U.S. Department of Agriculture, Agricultural Research Service at North Carolina State University (Raleigh, NC). Finally, lipoxygenase-null soybeans were provided by the Committee for Agricultural Development, Iowa State University (Ames, IA).

Processing of soy flour. Soybean processing took place at Iowa Soy Specialties (Vinton, IA) using their EE equipment. Processing was followed as outlined in Wang and Johnson (3). Low-fat soybean meal was taken to the Iowa State University Center for Crop Utilization Research Center (CCUR) and processed into low-fat soy flour (LFSF) (100 mesh) using a pin mill (Bauermeister, Inc., Memphis, TN). Two replications were produced of each soybean variety.

Soy flour composition. Protein dispersibility index (PDI) was determined by an outside laboratory (Woodson-Tenant, Des Moines, IA) using AOCS method Ba 10-65 (5). Residual oil (RO) was determined by the Goldfisch extraction procedure (AACC 30-25) (6). Crude protein content was determined using a nitrogen analyzer (Perkin Elmer Corp., Norwalk, CT) and methods as described by AOAC (4.2.08) (7).

Solubility. A sample (250 mg) was dispersed in 25 mL of distilled water and placed into a 50-mL centrifuge tube. This solution was adjusted to the appropriate pH with 1 N HCl or 1 N NaOH, shaken at 120 rpm at 25°C and centrifuged at 16,000 rpm for 30 min. This supernatant was then filtered through Whatman No.1 paper, and nitrogen was determined on 10 mL of the filtered supernatant following the standard Kjeldahl procedure (8). Protein solubility was calculated using the following:

$$\text{Protein Solubility (\%)}: \quad \frac{\text{Supernatant protein concentration (mg/ml)} \times 25}{\text{Sample wt (mg)} \times [\text{sample protein content}/100]} \times 100 \qquad [1]$$

Emulsification capacity (EC). A modified procedure of McWatters and Holmes (9) was used. A 2% protein suspension (25 mL) at 25°C was placed in a 500-mL plastic beaker. The suspension was continuously blended with a hand-held mixer at high speed (approximately 12,000 rpm) with soybean oil (Hy-Vee brand, West Des Moines, IA) at a flow rate of 0.5 g/sec. This mixture was continuously blended until the inversion point (water-in-oil) was observed. Emulsification capacity was determined as the maximum amount of oil emulsified on a per gram protein basis.

Emulsification activity index (EAI) and stability index (ESI). A 2% protein suspension (25 mL) at 25°C was blended with 7 mL of soybean oil for 1 min using a Waring Blender outfitted with a micro-container (110-mL size, Fisher Scientific, Pittsburgh, PA) at low speed. This emulsion was immediately diluted with 0.1% SDS at a 500X dilution factor, and the absorbance was measured at 500 nm. The diluted emulsion was incubated at 95°C in a water bath. The absorbance of the emulsion was measured at time zero and at 10 min. Emulsifying activity index and emulsifying stability index were calculated using the absorbance as measured at time zero (A_0) and at 10 min (A_{10}). Calculations as defined by Pearce and Kinsella (10) were used to calculate EAI and ESI.

$$\mathrm{EAI}\ (\mathrm{m^2/g}) = 2T/\Phi C \qquad [2]$$

C=weight of protein per unit volume of aqueous phase before emulsion is formed; T=2.303 *A/l* (A=absorbance, *l*=pathlength of cuvette);

Φ=C-A-E(B-C)/C-A + (B-C) ((1+E)D_0/D_s-E) where A=mass of beaker, B=mass of beaker plus emulsion; C=mass of beaker plus dry matter; D_0=density of oil; D_s=density of protein solution and E=concentration of solutes (mass per unit mass of solvent).

$$\mathrm{ESI\ (min)} = A_0 \times \Delta t / \Delta A \qquad [3]$$

Δt=10 min and $\Delta A = A_0 - A_{10}$.

Foaming capacity (FC) and foaming stability (FS). A 0.5% protein suspension (80 mL) at 25°C was added to a glass column with a fritted glass disk (medium pore size) on the bottom. Nitrogen gas was purged through the column at a flow rate of 100 ml/min. Foaming capacity and foaming stability were calculated based on the equations described by Sorgentini et al. (11).

$$FC = V_f\ (\mathrm{ml}) \,/\, f_r\ (\mathrm{ml/min}) \times t_f\ (\mathrm{min}) \qquad [4]$$

V_f=fixed volume of foam, 150 ml; f_r=flow rate of N_2 gas, 100 ml/min; t_f=time to reach fixed foam volume.

$$FS = 1 \,/\, V_{max} \times t_{1/2}\ (\mathrm{ml}^{-1}\mathrm{min}^{-1}) \qquad [5]$$

V_{max}=volume of liquid incorporated in foam at V_f; $t_{1/2}$=time to drain half of liquid incorporated into foam.

Water-holding capacity (WHC). Modified methods of Lin and Zayas (12) were used to determine WHC. Low-fat soy flour (5 g) was weighed and dispersed into 95 mL of distilled water and mixed with a magnetic stir bar for 20 min at 25°C. Three 50-mL centrifuge tubes were filled with the flour-water solution and centrifuged at approximately 1080 g for 30 min. After the supernatant was disposed of, the WHC was calculated as the difference in weight of the hydrated flour and the weight of the original flour. Water-holding capacity was expressed as gram of water per gram of protein.

Fat-binding capacity (FBC). Fat-binding capacity was determined by stirring a 5% soy flour solution with 50 mL of corn oil (Hy-Vee Brand, West Des Moines, IA) for 30 min and allowing this mixture to sit for 30 min at room temperature (25°C). The mixture was then placed into two-50-mL centrifuge tubes and centrifuged for 30 min at approximately 1080 g. After the excess oil was disposed, the FBC was calculated as the weight of the residue divided by the original weight (12). Fat-binding capacity was expressed as grams of oil per gram of protein.

Data analysis. All functionality testing followed a randomized complete block design. The General Linear Model (GLM) procedure was used to determine treatment effects for all functionality tests. TUKEY was used for multiple comparisons. Significance for all analyses was determined at the $p<0.05$ level. Statistical evaluation was carried out using SAS statistical software (SAS Institute, Inc., version 8.0, Cary, NC, 1999).

RESULTS AND DISCUSSION

Soy flour composition. Table 2 shows the PDI, RO and protein composition for the soy flours utilized in this study. During the processing of all soy flours, the configuration of the EE equipment was not altered. Thus, the variation in the PDI and RO levels were a direct result of how the soybean performed in the EE process. Residual oil tended to be lower at lower PDI levels. This was due to a greater exposure to heat and shear in the extruder with lower PDI samples thus allowing greater degree of cell disruption. Protein contents were relatively consistent, with the exception of Wc LFSF.

Solubility. Solubility curves for soy flours are shown in Fig. 1. Soy protein was least soluble at the protein's isoelectric region (pH 4.2-4.6) (13) and increased on either side of this point. All soybean varieties followed this general trend. At pH 8.0, some of the soybean varieties show greater solubility than others. For example, Lox-null LFSF and St LFSF are more soluble than the LLL LFSF and Ls LFSF. Lox-null LFSF and St LFSF have PDI levels of 50 and 49, respectively, while LLL LFSF and Ls LFSF have PDI levels of 33 and 32, respectively.

Emulsification capacity, activity and stability. Emulsification capacity for Ho LFSF was significantly reduced when compared with Wc LFSF, St LFSF and Hc LFSF (Fig. 2). Emulsification capacity is most affected by PDI, protein conformation and surface hydrophobicity (14). Due to the lack of hydrophobicity studies in this present work, it can only be hypothesized that the more RO a sample contains, the greater the hydrophobicity of that sample. High oleic LFSF and high cysteine LFSF have are similar in PDI level, but Ho LFSF has less RO, and thus Ho LFSF has a lower EC. In this instance, the effect that RO has on the functionality of this protein outweighs that of PDI.

Emulsification activity and stability results show a reduced EAI for Wc LFSF compared with the rest of the treatments (Table 3). High cysteine LFSF showed a much lower ESI compared with the rest of the treatments. Emulsification activity index is a measure of fat globule size. The larger the size of oil globule, the more unstable an emulsion will be. Emulsification activity and stability are affected by protein conformation. At the oil/water interface, proteins act as surface-active agent. If a protein is unable to perform as a surface-active agent, then the emulsification activity will be increased (larger fat globule size). If the protein can act at the interface but cannot realign to form a stable, flexible film, however, then emulsification stability will be reduced. Thus, in this study we see that Wc LFSF maintains a smaller fat globule size and thus is able to maintain the emulsion. On the other hand, Hc LFSF has a comparative EAI to WC LFSF, yet it is unable to fully develop a stable, flexible film at the interface and thus stabilize the emulsion. One possible reason for this is the additional cysteine content in the HC soybean. These cysteine molecules are capable of making the protein conformation very rigid and thus inflexible.

Foaming capacity and stability. No statistically significant differences were found for FC, but differences were found for FS (Table 4). Foaming capacity was not affected any of the value-enhanced traits, with the exception of the Hc soybean. Similar to ES, this increase in cysteine allows for an increased amount of intra- and intermolecular disulfide bonds, which stabilize the protein and does not allow for easy unfolding at the air-water interface for foam formation. The lower the FS value, the more stable the foam is. The results show that LLL LFSF resulted in a less stable foam than Hc LFSF. Thus, Hc LFSF is unable to form large amounts of foam; however the foam that is produced is stable to leakage and breakdown. The difference here is that at the air/water interface of foams, proteins undergo a much more rigorous denaturation. For this reason, given opportunity, protein from Hc LFSF denatures and reconforms with the capacity of forming strong, flexible films.

Fat-binding and water-holding capacities. There were no significant differences in FBC and WHC (Table 5) of the different soybean varieties. There is a slight increase in WHC for the

Hc LFSF. This again may be attributed to the increased cysteine content; cysteine is a polar amino acid. Fat-binding capacity is not related to the residual oil levels in this study. Earlier work done on LFSF resulted in the conclusion that increased amounts of RO would provide for increased amounts of fat-binding and decreased amounts of water-holding (15).

In general, LFSF from value-enhanced and commodity soybeans do not significantly differ from one another in protein solubility, WHC, FBC, emulsification and foaming characteristics. The LFSF used in the present study fell into the mid to high PDI/RO ranges as defined in an earlier study (15). This previous study concluded that LFSF falling within the 42/8 and 66/11 PDI/RO range (RO, dry weight basis) had similar functional properties when compared with each other and DFSF. Protein dispersibility index and RO levels influence the functional properties of a LFSF more than the variety of soybean utilized.

ACKNOWLEDGMENTS

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Table 1. Soybean Varieties used, their Abbreviations and Trait Alteration

Soybean variety	Abbreviation	Trait altered
Low linolenic	LLL	Reduced unsaturated fatty acid, linolenic
High sucrose	Hs	Reduced oligosaccharide, increased sucrose
Low saturated	Ls	Reduced amounts of saturated fatty acids
High oleic	Ho	Increased fatty acid, oleic
High cysteine	Hc	Increased amino acid, cysteine
Lipoxygenase-null	Lox-null	Elimination of three lipoxygenase isozymes
Commodity	Wc	NA
Commodity	St	NA

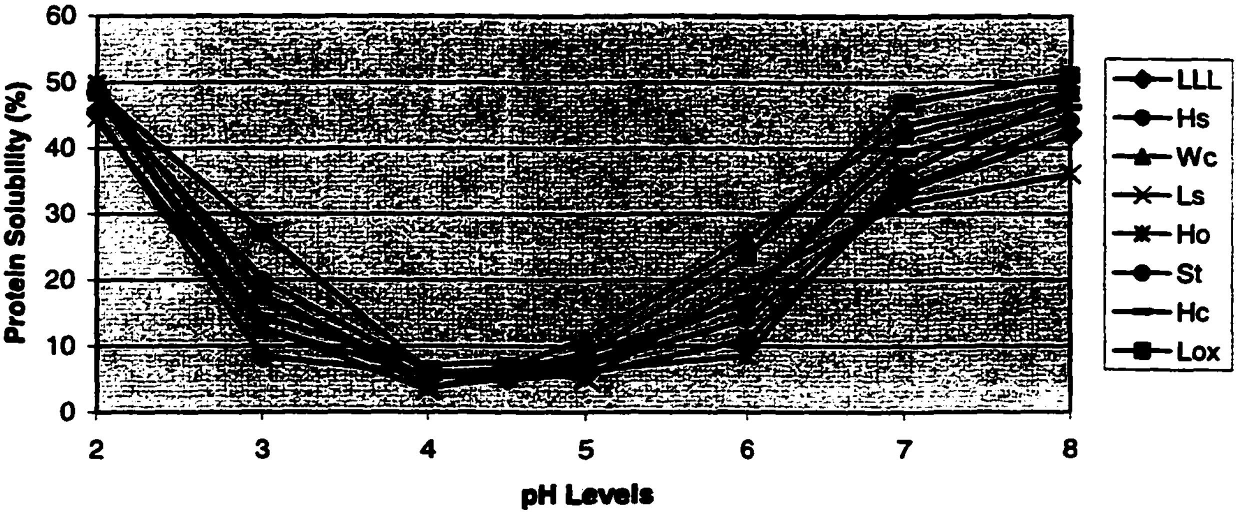
Table 2. Composition of Soy Flours

Soybean variety[a]	PDI (%)	RO (%)	Crude protein[b] (%)
LLL	32.2	7.7	52.1
Hs	35.5	7.0	52.4
Ls	32.0	7.1	51.8
Ho	45.2	7.5	51.8
Hc	42.7	9.0	51.2
Lox-null	49.5	11.7	52.6
Wc	41.2	11.0	47.4
St	48.7	10.5	50.1

[a] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

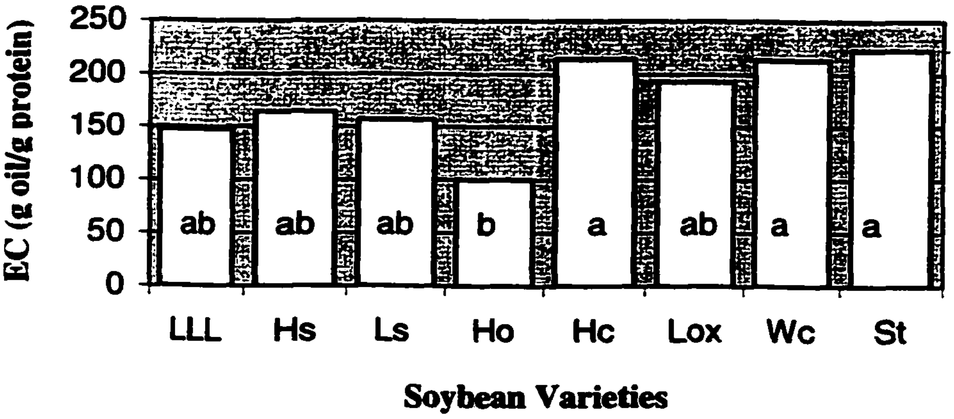
[b] Dry moisture basis.

Figure 1. Protein Solubility Curves for EE Soy Flours[a]



[a] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

Figure 2. Emulsification Capacity for Value-Enhanced Soy Flours and Commercial Soy Flours[a, b]



[a] Soybean varieties with the same letter on bar are not significantly different at the $P<0.05$ level.
[b] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

Table 3. Emulsification Activity (EAI) and Stability (ESI) for Soy flour from Value-Enhanced Soybeans and Commodity Soybeans

Soybean Variety[a]	EAI (m^2/g protein)	ESI (min)
LLL	17.5	22.1
Hs	15.7	22.7
Ls	16.6	18.8
Ho	18.0	23.9
Hc	15.6	19.0
Lox-null	14.0	18.9
Wc	15.7	19.7
St	14.4	18.3
	NS	NS

[a] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

Table 4. Foaming Capacity (FC) and Stability (FS)[a]

Soybean variety	FC[b] (mL of foam/ mL of N_2 x min)	FS (mL^{-1} min^{-1})
LLL	2.15	1.04^{b}
Hs	2.57	0.71^{ab}
Ls	2.39	0.70^{ab}
Ho	2.25	0.75^{ab}
Hc	1.98	0.24^{a}
Lox-null	2.20	0.45^{ab}
Wc	2.39	0.41^{ab}
St	2.26	0.39^{ab}
	NS	

[a] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

[b] Fc=foaming capacity; FS=foaming stability

Table 5. Fat-Binding (FBC) and Water-Holding Capacities (WHC)[a]

Soybean variety	FBC[b] (g oil/g protein)	WHC[b] (g water/g protein)
LLL	2.1	3.9
Hs	2.0	3.8
Ls	2.2	3.7
Ho	2.2	3.9
Hc	2.0	4.2
Lox-null	2.0	3.7
Wc	2.1	4.1
St	1.9	3.7
	NS	NS

[a] Soybean varieties: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; and Wc and St=commodity.

[b] FBC=fat binding capacity; WHC=water holding capacity.

CHAPTER 6: CHEMICAL, PHYSICAL, AND SENSORY CHARACTERISTICS OF EXTENDED BEEF PATTIES WITH TSP FROM EXTRUDED-EXPELLED SOYBEAN FLOUR FROM VALUE-ENHANCED SOYBEAN VARIETIES

A paper to be submitted to the *Journal of American Oil Chemists' Society*

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ABSTRACT: Texturized soy protein (TSP) originating from six different varieties of value-enhanced soybeans and two varieties of commodity soybeans was incorporated at a rehydrated level of 30% into beef patties. The value-enhanced varieties utilized included high cysteine (Hc), low linolenic (LLL), lipoxygenase triple-null (Lox-null), high sucrose (Hs), low saturated fat (Ls) and high oleic (Ho) along with two commodity soybeans (Wc and St). Sensory evaluation results for soy flavor indicated that the patties containing TSP had a significantly increased amount of soy flavor versus the all-beef control patty. Significant differences were found between some of the TSP-extended patties for tenderness and cohesiveness when compared to the all-beef patties. Physical measurements showed significant differences between some of the TSP-extended patties for hardness and springiness versus the all-beef control. Outside color measurements for TSP-extended patties were significantly lighter versus the all-beef control patties. Only patties made with St TSP were significantly lighter in color on the interior versus the all-beef control patty. Chemical analyses and cooking parameters showed no adverse effect with inclusion of TSP into ground

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beef patties at the 30% level. Adding 30% rehydrated TSP produced from extruded-expelled, value-enhanced soybeans to ground beef patties containing 20% fat did not negatively alter specific quality and sensory attributes.

INTRODUCTION

Adding soy protein to meat has been in practice since the early 1900s (1). Soy protein is added to meat products most commonly in the form of texturized soy protein (TSP), in order to maintain the meat like texture of the product (2). The primary reasons for adding TSP to meat products are to reduce manufacturing costs (3) and to reduce consumers' dietary fat intake. Consumers want these less-expensive, healthier alternatives, yet desire a product that is equal in quality to similar all-animal-protein products. Bowers and Engler (4) and Drake, *et al.* (5) found that the addition of TSP did not impart negative quality or sensory attributes to a meat product with the exception of soy flavor. However, there is debate on the effect of added TSP in ground beef and the potential impact it has on decreased meat flavor or increased soy flavor, Cross *et al.* (6), Twigg and Kotula (7) and Liu *et al.* (8) found that adding TSP to ground beef neither added soy flavor nor decreased meat flavor.

Value-enhanced soybeans include soybeans that have been altered either through genetic enhancement or through traditional plant breeding. Value-enhanced soybeans include soybeans that have altered fatty acid compositions, soybeans with altered protein contents or soybeans that have enzymes removed. Although value-enhanced soybean technology is not new, there has been a limited amount of work published on the characterization of functional properties or utilization of these value-enhanced soybeans and their processed products (i.e., flour, protein and oil) in food products.

Extrusion-expelling (EE) technology is gaining in popularity due to decreased capital investment, ease of using equipment, and the economic feasibility of processing value-enhanced soybeans. Low-fat soybean meal and crude soybean oil are the products obtained by using this processing technique. There is a limited amount of literature that focuses on using the low-fat meal, in any form (i.e., grits, flour, TSP), in a food product. In the present study, six varieties of value-enhanced soybeans plus two commodity varieties were processed using EE technology and further extruded to form TSP. A rehydrated form of this TSP was then incorporated at a 30% replacement level into ground beef. The objective of this research was to determine if the TSP manufactured from the value-enhanced soybeans had any effect on specific quality and sensory characteristics of the ground beef.

EXPERIMENTAL PROCEDURES

Raw materials. Coarse ground beef (mixture of fat and beef trimmings) was obtained from the Iowa State University Meat Laboratory (Ames, IA). Meat was formulated with 30% rehydrated TSP (1:2.6 TSP to water) in order to produce a typical fast food-style ground beef patty. During processing, fat levels were determined by using an Anyl Ray Fat Analyzer (model 316-4A, Kartridge Pak Co., Davenport, IA) standardized for low (20%) and high (50%) fat. Coarse ground beef was first ground through a 0.93-cm (3/8 inch) plate and then combined with rehydrated TSP. This mixture was blended in a ribbon mixer for 3 min and then ground through a 0.32-cm (1/8 in) plate. Patties with a target weight of 113 g were formed using a mechanical patty maker (Hollymatic Supermodel 54, Countryside, IL). Patties, interweaved with waxed patty paper, were blast frozen at -30°C and stored at -18°C until needed for further analysis.

Low-fat soy flour (LFSF) was produced at Iowa Soy Specialties (Vinton, IA) using six different value-enhanced soybean varieties and two commodity soybean varieties. These value-enhanced varieties are described in detail in an earlier study (9). An Insta-Pro 2500 dry extruder (Triple 'F' Feeds/Insta-Pro, Des Moines, IA) in combination with an Insta-Pro 1500 screw-press were used to produce the low-fat soy meal. This meal was then taken to the Iowa State University Center for Crops Utilization Research Center (CCUR) and ground to 100-mesh flour on a pin mill. Two replicates were produced for each of the eight treatments. Texturized soy protein was produced using a co-rotating lab-scale Leistritz Micro-18 (American Leistritz Corp., Somerville, NJ) twin-screw extruder, following methods as outlined by Crowe (10).

Cooking protocol. Patties were cooked on a household griddle at 162°C, 3.5 min on one side and 2 min on the other, to an internal final temperature of 71°C. For sensory evaluation, patties were cooked, covered with aluminum foil and placed in a pre-warmed oven set at 93°C for no more than 10 min before serving. For all other determinations, patties were cooked and allowed to cool to room temperature. Two replications of TSP-extended patties were produced for each variety.

Chemical analysis. All proximate analyses (moisture, protein and fat) were evaluated following standard AOAC methods (39.1.02, 39.1.16, 39.1.08) (11), respectively. Moisture and fat analyses were carried out in triplicate for each replication. Protein was measured in duplicate for each replication.

Water-holding (WHC) and bulk density (BD) measurements. Water-holding capacity and bulk density measurements were taken on the TSP prior to inclusion into ground beef patties. For water-holding capacity, 30 g of TSP was measured in a 400-mL beaker, and 150 mL of 4°C water was added. This sample was held in a refrigerator for 1 hr. The contents from the beaker were poured onto a 20-mesh screen and allowed to drain for 3 min. The screen was then weighed, and the water-holding capacity was determined using the following formula:

$$\text{Water-holding capacity} = \frac{(\text{TSP hydrated weight} - \text{TSP dry weight})}{(\text{TSP dry weight})} \qquad [1]$$

Bulk density measurements were taken by adding dry TSP to a 100-mL graduated cylinder in 20 mL increments. When the 20-mL level was reached, the cylinder was tapped against the laboratory bench surface 20 times. When 100 mL was reached, the cylinder was weighed. Bulk density was recorded in g/cc. The average of 12 readings were taken for each TSP variety.

Cooking parameters. Cooking yields, fat retention and moisture retention determinations were calculated using the following formulas (12):

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight (g)}}{\text{Raw weight (g)}} \times 100 \qquad [2]$$

$$\text{Fat retention (\%)} = \frac{\text{Cooked weight (g)} \times \text{Fat in cooked patty (\%)}}{\text{Raw weight (g)} \times \text{Fat in raw patty (\%)}} \times 100 \qquad [3]$$

$$\text{Moisture retention (\%)} = \frac{\text{Cooking yield (\%)} \times \text{Moisture in cooked patties (\%)}}{100} \qquad [4]$$

Cooking yields, fat retention and moisture retention were determined in triplicate for each replication.

Texture profile analysis (TPA). Texture profile analysis was carried out using a texture analyzer (model TA-XT2, Texture Technologies Corp., Scarsdale, NY). A 40-mm aluminum anvil was used with a compression rate of 80% and a test speed of 3.3 mm/sec. The attributes of hardness, springiness and cohesiveness were determined. Hardness was defined as the peak force at the first compression and is the "force necessary to attain a given deformation." Springiness was calculated as the proportion of compression distance recovered between the first and second compressions and is the "rate at which a deformed material goes back to its undeformed condition following removal of the deforming force." Finally, cohesiveness is the area under the 1st curve divided by the area under the 2nd curve and is a measure of the "strength of the internal bonds" (13, 14). Sample preparation included cutting 2x2x2-cm cubes from the center of the patty and placing it in the middle of the platform. Two samples were taken from each of three patties per treatment replication.

Color measurements. A Hunter Lab Spectrocolorimeter (Model LS5100, Reston, VA) was used to determine the color of patties. The Hunter spectrocolorimeter was standardized using a white tile (No. LS 14318, L=92.32, a=-0.74, b=-0.40) and a black tile. The standard observer was set at 10°, the light source was set at D65 and a 5.08-cm port size was used with a 4.45-cm view used. Transparent plastic wrap was placed over the viewing port for protection. Patties were placed directly on the plastic wrap. For exterior color, each whole patty was placed on the port, and color was measured on three distinct areas per side. For interior color, each patty was cut longitudinally, and color was measured in three distinct areas. Two patties per replication were measured.

Color analysis followed a split-plot design. Variety was used as the whole plot treatment while either inside or outside color measurement was used as the subplot treatments.

Sensory evaluation. A 12-member trained panel was used for sensory evaluation. Panel members were recruited from students and staff in the Food Sciences Building at Iowa State University. All panelists were age 20-42. Six males and six females participated in this sensory panel. Panelists were trained in two 1-hr sessions, training for soy flavor, tenderness, cohesiveness, chewiness and juiciness present in ground beef. A descriptive analysis test was used with panelists evaluating five attributes which they were trained for. Before participating, panelists were informed of the origin of the TSP (from extruded-expelled, value-enhanced soybeans) and allowed to withdraw from participation without facing any penalties. At the conclusion of each panel, panelists were offered small food treats (i.e., candy bars, fruit, cake, etc.) as compensation for their participation.

Sensory evaluations were conducted in the Sensory Evaluation area located in the Food Sciences Building on the campus of Iowa State University. Panel sessions were conducted for 6 days, three times per week for 2 weeks. At each session, panelists were presented three samples. Each session took place under red light in order to mask any potential color differences among samples.

Serving size was two pieces of one-fourth of the patty. These samples were presented warm in a glass petri dish. Warmed samples did not sit for more than 10 min in a pre-warmed oven. Each sample was assigned a random three-digit number. Presentation of samples was randomized between panelists, following a randomized incomplete block design.

Statistical analysis. Chemical and physical determinations were made following a randomized complete block design. Sensory evaluation analysis followed a randomized complete block design to maintain consistency between the production of raw material and sensory evaluation. All data were subjected to General Linear Modeling (GLM) using SAS statistical software (SAS Institute, Inc., Cary, NC 1999). TUKEY was used for multiple comparisons, and P-values were determined using least square means. Significance was determined at the $P< 0.05$ level for all data analyses. Dunnett's t-test (a comparison between each treatment versus the control) was ran on all analyses, and significance was determined at the $P<0.05$ level.

RESULTS AND DISCUSSION

Proximate analysis. There was a large variation in protein dispersibility index (PDI) and residual oil (RO) values for all treatments, with a range of 32.0 to 49.5 for PDI and a range of 7.0 to 11.7 for RO (Table 1). Protein contents all fell within the range of 47.4 to 52.6%. All of the treatments had acceptable PDI/RO to make satisfactory TSP following guidelines found in an earlier study by Crowe (10). The BD and WHC were measured on the TSP product. Bulk density and water-holding capacity were negatively correlated (R= -0.68) but were not correlated to the PDI or RO of the initial soy flour.

Chemical analysis. Cooked moisture levels ranged from 51.56 to 55.00% (Table 2). These cooked moisture levels fall within the range of other published cooked moisture values (8, 12, 15, 16). Cooked fat levels of all patties had little variation, with a range of 16.45 to 17.92%. The protein contents of the cooked patties were also very consistent, with little deviation from 21%.

Cooking parameters and texture measurements. Although there were no statistical differences, moisture retentions, fat retentions, and cooking yields were higher in TSP-extended patties compared to the all-beef control (Table 3). Texturized soy protein binds excess amounts of water and fat.

Texture profile analysis shows the addition of TSP increased the hardness of the ground beef patty. Cohesiveness scores show generally increased levels in the TSP-extended patty; again no statistically significant differences were observed. TSP-extended beef patties were less springy compared to the all-beef control. These observations verify the results of Crowe (10) that state, "...texture measurements of samples in the TSP-extended ground beef system were similar to those measured in the [all-beef control]."

Color measurements. Surface 'L' color measurements on all TSP-extended patties were greater than the all-beef control (Table 4). These higher 'L' color measurements indicated TSP inclusion with ground beef made a lighter colored product. Surface and interior 'a' and 'b' values showed no differences. Inside color measurements were much more consistent between all treatments.

Sensory evaluation. Panelists detected more soy flavor in all TSP-extended patties when compared to the control (Table 5). Soy flavor did not deviate significantly between varieties, however. Tenderness scores for TSP-extended patties made with TSP from LLL, Lst, Ho and St showed increased tenderness compared with the control, all-beef patty. Finally, cohesiveness scores for patties made with LLL TSP and Ho TSP were significantly lower compared with the control. Chewiness and juiciness scores show no significant differences between TSP-extended patties and the control. Instrumental analyses showed some differences between TSP-extended patties and all-beef control, human subjects detected alternative differences that were not necessarily the differences detected by instrumentation.

Adding TSP to beef patties from LFSF from different value-enhanced and commodity soybean varieties did not negatively affect certain sensory and quality characteristics. Soy flavor, however, was impacted by the addition of TSP into beef patties by an increased intensity detected by human subjects. Soy flavor was not affected by the variety of soybean utilized to make LFSF; all TSP-extended patties had increased soy flavor when compared with all-beef patty. Observing the sensory scores for soy flavor, the degree of soy flavor was mild.

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Table 1. Composition of EE Soy Flours[a] and Bulk Density, Water-Holding Capacity for TSP [a]

Treatment [b]	PDI[c] (%)	RO (%)	Protein (%)	BD[d] (g/cc)	WHC (g water/g protein)
LLL	32.2	7.7	52.1	0.32^{a}	2.96^{ab}
Hs	35.5	7.0	52.4	0.29^{a}	3.29^{b}
Ls	32.0	7.1	51.8	0.31^{a}	2.94^{ab}
Ho	45.2	7.5	51.8	0.34^{ab}	2.73^{ab}
Hc	42.7	9.0	51.2	0.30^{a}	2.96^{ab}
Lox-null	49.5	11.7	52.6	0.30^{a}	2.96^{ab}
Wc	41.2	11.0	47.4	0.41^{b}	2.41^{a}
St	48.7	10.5	50.1	0.30^{a}	2.97^{ab}

[a] Values in same vertical column followed by the same letter are not significantly different at the $P<0.05$ level.

[b] Treatment names: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; Wc and St=commodity soybeans.

[c] PDI=protein dispersibility index, RO=residual oil: PDI, RO and protein measured on soy flour before production of TSP.

[d] BD=bulk density; WHC=water-holding capacity: both measured on TSP.

Table 2. Chemical Compositions of Cooked Patties[a]

Treatment[b]	Moisture (%)	Fat (%)	Protein (%)
Control	54.19	17.53	21.80
LLL	52.82	17.15	20.93
Hs	53.39	17.34	21.38
Ls	55.00	16.45	21.74
Ho	53.85	17.39	21.55
Hc	53.36	17.02	20.00
Lox-null	53.88	17.44	21.77
Wc	51.56	17.12	20.89
St	54.16	17.92	20.75
	NS	NS	NS

[a] All measurements on as-is basis.

[b] Treatment names: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; Wc and St=commodity soybeans.

Table 3. Cooking Characteristics of TSP-Extended Ground Beef Patties [a]

	Cooking Parameters			TPA		
Treatment[b]	MR[c] (%)	FR (%)	Cooking Yield (%)	Hardness (g)	Cohesiveness (g)	Springiness (cm)
Control	34.7	60.7	65.85	50.04	31.49	0.94
LLL	36.1	60.7	68.31	53.98^{a}	31.18	0.90^{a}
Hs	37.0	64.0	67.88	67.10^{az}	37.22	0.86^{az}
Ls	36.0	58.3	66.09	60.65^{a}	35.30	0.89^{a}
Ho	36.7	61.6	68.66	54.48^{a}	29.43	0.88^{az}
Hc	37.4	67.2	69.67	68.26^{az}	37.96	0.87^{az}
Lox-null	35.4	60.9	65.50	66.42^{az}	36.79	0.87^{az}
Wc	34.9	59.1	65.59	52.76^{a}	30.27	0.87^{az}
St	36.4	64.7	68.00	67.70^{az}	40.18	0.87^{az}
	NS	NS	NS		NS	

[a] Values in same vertical column followed by same letter are not significantly different at the $P<0.05$ level.

[b] Treatment names: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; Wc and St=commodity soybeans.

[c] MR= moisture retention; FR= fat retention.

[z] Denotes a significant difference between this sample and all-beef control patty.

Table 4. Color Measurements on Cooked Patties [a]

Treatment[b]	Outside			Inside		
	L	a	b	L	a	b
Control	32.38	5.55	9.40	51.99	4.74	11.03
LLL	40.75[az]	5.16	8.60	53.94[a]	4.66	11.42
Hs	42.76[az]	5.22	9.42	53.01[a]	4.75	11.24
Ls	40.11[az]	5.04	8.33	53.01[a]	4.75	11.24
Ho	41.52[az]	5.00	8.72	54.34[a]	4.29	11.31
Hc	39.20[az]	5.21	8.43	53.06[a]	4.40	10.87
Lox-null	41.31[az]	5.16	8.50	52.93[a]	4.68	11.21
Wc	41.22[az]	4.90	9.67	51.65[a]	4.50	11.54
St	44.68[az]	4.91	8.09	55.18[az]	4.70	11.05
		NS	NS		NS	NS

[a] Values in same vertical column followed by same letter are not significantly different at the $P<0.05$ level.

[b] Treatment names: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; Wc and St=commodity soybeans.

[z] Denotes a significant difference between this sample and all-beef control.

Table 5. Sensory Analysis of TSP-Extended Ground Beef Patties [a]

Treatment	Soy flavor	Chewiness[c]	Tenderness	Cohesiveness	Juiciness[c]
Control	2.4	7.9	6.6	8.0	6.2
LLL	4.4[az]	7.2	8.0[az]	6.7[az]	6.2
Hs	4.9[az]	7.6	8.1[az]	7.7[a]	7.2
Ls	5.1[az]	8.1	7.8[az]	7.7[a]	6.6
Ho	4.7[az]	8.2	8.6[az]	6.4[az]	7.0
Hc	5.0[az]	7.4	7.6[a]	8.1[a]	7.4
Lox-null	5.6[az]	7.6	7.9[a]	7.2[a]	6.1
Wc	5.8[az]	7.7	7.6[a]	7.7[a]	6.2
St	5.6[az]	7.3	8.0[az]	6.9[a]	6.5
		NS			NS

[a] Values followed by the same letter are not significantly different at the $P<0.05$ level.

[b] Treatment names: LLL=low linolenic; Hs=high sucrose; Ls=low saturated fatty acids; Ho=high oleic; Hc=high cysteine; Lox-null=lipoxygenase-null; Wc and St=commodity soybeans.

[c] Chewiness and juiciness scores had no significant differences.

[z] Denotes a significant difference between this sample and all-beef control patty.

CHAPTER 7. GENERAL CONCLUSIONS

General conclusions

The research problem that the present work intended to resolve focused on the lack of published work investigating LFSM products manufactured using EE process technology. The underlying objective of this research work was to develop a foundation of knowledge, regarding functional properties and utilization potential of products from EE technology. The work published in this dissertation successfully meets the objective and fills the void of the aforementioned research problem.

Chapter three of this dissertation is a study of the functional properties of LFSF with varying levels of PDI and RO. From this work it was learned that the functional properties of the LFSF depend not only on the PDI level, but also the RO level. When LFSF is produced the hydrophobicity may be increased. This alteration in the hydrophobicity was reflected in functional properties, such as WHC, FBC and foaming. Mid PDI/RO LFSF, high PDI/RO LFSF and DFSF may be more functional ingredients in food systems then low PDI/RO LFSF. Finally, mid LFSF and high LFSF perform equivalent to one another in functional characteristics.

Chapter four takes the knowledge gained in chapter three and applies it to a food product. Cake doughnuts were produced using three of the LFSF from the functionality study. These LFSF and one DFSF were added to a standard cake doughnut formula at three levels, 3, 5, and 8% (flour weight basis). Low-fat soybean flour did not follow a consistent trend regarding decreased fat absorption. One explanation as to why the LFSF did not follow a consistent trend was that there is a protein-lipid complex found in LFSF, which was unable to form the same protein barrier that has been implicated as the mechanism for the reduction of fat uptake in cake doughnut formulations with DFSF. If a food processor requires an ingredient to reduce fat absorption in the production of cake doughnuts, DFSF is a better soy protein ingredient. However, panelists detected very little difference in soy flavor between the DFSF and all LFSF at all levels. Additional sensory attributes, such as gumminess and tenderness, were rated the same or less than the DFSF doughnuts when doughnuts were made from LFSF. These results indicate that in regards to certain quality and sensory attributes, LFSF produces an equivalent product as those doughnuts made with DFSF.

Chapter five investigated the functional properties of LFSF from EE technology when the LFSF used was produced from different value-enhanced soybean varieties. Functional characterization shows few differences between LFSF from value-enhanced varieties and LFSF from commodity varieties. Differences were observed between the commodity LFSF leading to the conclusion that differences in functional characteristics may be influenced more by factors, such as growing conditions, storage conditions, etc., than by varietal differences.

Chapter six investigated the utilization of this LFSF in the form of a texturized product in a ground meat system. Very few differences were detected between TSP-enhanced beef patties, however, differences were observed when compared to the all-beef control patty. These differences were indicative that added TSP to beef patties increased levels of soy flavor, slightly increased in cooking yields, fat retention and moisture retention due to the nature of the TSP and textural changes resulting from incorporation of a non-meat ingredient.

Recommendations for future work

As previously mentioned, this dissertation provides a foundation of knowledge to be referred to and built upon by future researchers. A detailed study of the effect of PDI and RO on the hydrophobicity of the protein is needed. In the manuscripts included herein, hypotheses are drawn that relate to such work; without more experiments designed to provide more definitive results, these hypotheses will not be confirmed. In addition to hydrophobicity studies, investigation into the potential that "lipoprotein-like" material is formed can be investigated using different methodology than was utilized in this study. Functionality studies that are more detailed and basic in nature versus the application-based work included here is needed.

Future work involving soy flour produced using value-enhanced soybeans as a starting material is of the utmost importance. One soybean in particular is of interest, the high cysteine soybean. Researchers that developed this soybean variety are not certain where the additional cysteine is found: on the interior or exterior of the protein. Detailed investigation on this soybean may uncover unique functional characteristics that may be extremely useful in food processing. Another value-enhanced soybean that requires additional investigation is the high sucrose. Although this soybean did not show any extraordinary functionality results, when this soybean was processed, the low-fat meal had a sweet taste. This sweet taste, in addition to a decreased stachyose content, may allow for a broader spectrum of usage of soy protein in food products.

Future work covering food applications, specifically a batter system, should focus on developing a mechanism for the results that were observed in this study. One particular question that requires an answer is "What is occurring in the batter of cake doughnuts containing LFSF?" The results of LFSF in this work do not follow trends that have been seen in work with other soy flours. This leads to the belief that something is occurring in the production of these cake doughnuts that differentiates them from other cake doughnuts with added soy flour.

Food applications work regarding TSP-extended ground meat work requires the use of a different system. The lack of differences observed in the TSP-extended patties indicates that it is necessary to formulate a meat analogue (100% TSP) in order to see varietal differences.

APPENDIX

Table 1. Weight, Height, and Width Data for Doughnuts with 3, 5, and 8% Soy Flour Additions[a]

Flour[b]	Level	Weight (g)	Height (cm)	Width (cm)
Low LFSF	3	55.6^{abc}	2.9^{a}	2.8
Low LFSF	5	54.1^{abc}	2.8^{a}	2.6
Low LFSF	8	54.0^{abc}	3.0^{ab}	2.8
Mid LFSF	3	59.1^{c}	3.4^{ab}	2.9
Mid LFSF	5	58.0^{bc}	3.2^{ab}	2.9
Mid LFSF	8	51.4^{ab}	2.9^{a}	2.8
High LFSF	3	56.3^{abc}	3.0^{ab}	2.9
High LFSF	5	56.9^{abc}	3.5^{b}	3.0
High LFSF	8	58.6^{c}	3.2^{ab}	2.9
DFSF	3	54.8^{abc}	3.0^{ab}	2.7
DFSF	5	52.8^{abc}	3.0^{ab}	2.9
DFSF	8	50.5^{a}	2.9^{a}	2.6
				NS

[a] Values in same vertical column followed by same letter are not significantly different at the $P<0.05$ level.

[b] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6.

Table 2. Texture Profile Analysis Results on Doughnuts made with 3, 5 and 8% Soy Flour Additions [a]

Flour[c]	Level	Hardness (Newtons)	Cohesiveness[b]	Gumminess (Newtons)	Chewiness (Nxcm)	Springiness (cm)
Low LFSF	3	9.60^{bcd}	0.64^{abc}	6.20^{bc}	5.75^{b}	0.938
Low LFSF	5	10.05^{cd}	0.56^{ab}	5.74^{bc}	5.05^{ab}	0.919
Low LFSF	8	9.02^{abc}	0.56^{a}	5.06^{ab}	4.77^{ab}	0.933
Mid LFSF	3	9.81^{cd}	0.65^{bc}	6.34^{bc}	6.06^{b}	0.053
Mid LFSF	5	7.15^{a}	0.57^{ab}	4.06^{a}	3.66^{a}	0.903
Mid LFSF	8	11.00^{cd}	0.60^{abc}	6.01^{bc}	6.06^{b}	0.039
High LFSF	3	9.67^{bcd}	0.67^{c}	6.44^{bc}	6.14^{b}	0.969
High LFSF	5	11.28^{d}	0.56^{a}	6.96^{c}	6.44^{b}	0.045
High LFSF	8	7.81^{ab}	0.65^{bc}	5.08^{ab}	4.98^{ab}	0.919
DFSF	3	9.60^{bcd}	0.66^{c}	6.42^{bc}	6.23^{b}	0.961
DFSF	5	9.67^{bcd}	0.65^{bc}	6.35^{bc}	6.42^{b}	0.989
DFSF	8	9.03^{abc}	0.61^{abc}	6.58^{bc}	5.12^{ab}	0.940
						NS

[a] Values in same vertical column followed by different letters are not significant at the $P<0.05$ level.
[b] Cohesiveness is a dimensionless measure.
[c] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6.

Table 3. Moisture, Fat, and Protein of Doughnuts made with Soy Flour added at 3, 5, and 8% Addition Levels[a]

Flour[b]	Level	Moisture (%)	Fat (%)[c]	Protein (%)
Low LFSF	3	21.9^{bc}	30.2^{cd}	8.1^{ab}
Low LFSF	5	23.8^{cde}	28.5^{bc}	7.6^{a}
Low LFSF	8	18.9^{a}	30.7^{cde}	9.2^{cd}
Mid LFSF	3	20.4^{ab}	31.0^{cde}	8.1^{ab}
Mid LFSF	5	24.9^{defg}	29.6^{b}	8.8^{bcd}
Mid LFSF	8	20.4^{ab}	31.9^{def}	8.8^{bcd}
High LFSF	3	24.5^{def}	30.4^{cde}	8.4^{bc}
High LFSF	5	21.9^{bc}	32.8^{ef}	8.4^{ab}
High LFSF	8	22.1^{bcd}	28.7^{bc}	9.3^{d}
DFSF	3	27.2^{defg}	30.2^{cd}	8.8^{bcd}
DFSF	5	23.4^{cd}	26.8^{b}	8.8^{bcd}
DFSF	8	21.7^{bc}	23.6^{a}	10.2^{e}

[a] Values in same vertical column followed by the same letter are not significantly different at the $P<0.05$ level.

[b] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, high LFSF 67.8/11.8, and DFSF 73/0.6.

[c] Fat percent represents total fat of doughnut product.

Table 4. Fat Absorption for Doughnuts made with 3, 5, and 8% Soy Flour Additions[a]

Flour[b]	Level	Fab[c] (%)
Low LFSF	3	22.3[ef]
Low LFSF	5	19.6[bcd]
Low LFSF	8	22.5[f]
Mid LFSF	3	22.1[def]
Mid LFSF	5	18.5[bc]
Mid LFSF	8	23.4[fg]
High LFSF	3	22.9[f]
High LFSF	5	25.7[g]
High LFSF	8	19.9[cde]
DFSF	3	22.5[f]
DFSF	5	17.4[ab]
DFSF	8	15.8[a]

[a] Values in same vertical column followed by the same letter are not significantly different at the $P<0.05$ level.

[b] PDI/RO levels for flours: low LFSF 18.2/6.5, mid LFSF 44.9/7.1, High LFSF 67.8/11.8, and DFSF 73/0.6.

[c] Fab=fat absorption.