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Utilization Of Okara To Produce Extruded Cereal Products

By

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ABSTRACT

THE UTILIZATION OF OKARA TO PRODUCE EXTRUDED CEREAL PRODUCTS

By

Vincent E.A. Rinaldi

Okara is the residue that remains after soymilk and/or tofu production. In North America, okara is either used as animal feed, fertilizer, or landfill. Okara has been shown to have promising nutritional attributes, however, a high moisture content and susceptibility to putrefaction have limited its use. The purpose of this study was to use wet okara to produce and enrich extruded cereal products, and to study the effects on the dietary fiber and isoflavone contents due to extrusion. Wet okara was combined with soft wheat flour to produce two formulations and extruded using four screw configuration/temperature profile conditions. Various physicochemical properties, dietary fiber by enzymatic-gravimetric method, and isoflavone content by high performance liquid chromatography were analyzed. Physicochemical analyses showed varied results. Combining okara with soft wheat flour resulted in increased protein, dietary fiber, and isoflavone contents compared to soft wheat flour alone. Extrusion of the formulations resulted in decreased insoluble fiber and increased soluble fiber content of extrudates. Extrusion decreased the total detectable isoflavones and altered the distribution of the six detected isoflavones.

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CHAPTER I

INTRODUCTION

Soybeans are an ancient crop that reportedly originated in Eastern Asia and only recently made their way to the United States as a major food crop (Waggle and Kolar 1979). In 1995, the United States produced approximately 58.56 million metric tons of soybeans (ASA 1995). The U.S. is the single largest producer of soybeans followed by Brazil, Argentina, and China (Kluis 1997).

Soybeans provide a major nutritional protein source for many people. This is of particular concern for the immediate future since it is estimated that by the year 2000 the world population will exceed six billion (Salunkhe and Kadam 1984). Soybeans have many distinctive functional and nutritional properties (Lo 1989). The most notable attributes of soybeans are their oil and protein contents, however they are also a good source of fiber and isoflavones. The soybean represents an inexpensive source of high quality protein, which may be particularly important for developing countries (Messina 1995). Isoflavones are phytochemicals and have been speculated to have anticarcinogenic effects. If this proves true, this places particular emphasis on soy because isoflavones have limited distribution in nature (Messina 1995). Evidence has also mounted that increasing consumption of a high quality source of dietary fiber not only can improve digestion and the elimination process, but also can aid in the treatment and prevention of diet-related disorders such as obesity, cardiovascular disease, and diabetes (Lo 1989).

Throughout their existence, soybeans have been utilized to make many food products. Soyfoods are typically divided into two categories: non-fermented (soy flour, soymilk, tofu, and okara) and fermented (tempeh, miso, and soy sauce) (Golbitz 1995). Two popular products in the U.S. are tofu and soymilk. The production of each of these results in a byproduct called okara. In the U.S., okara is typically discarded, but may also be used as animal feed or fertilizer. In Japan, however, it is used as a food source (e.g., okara tempeh). In any country, discarding okara as waste is potentially an environmental problem because okara is highly susceptible to putrefaction. Okara has been shown to have nutritional attributes and finding convenient ways to incorporate it into food products are needed. This would also help to eliminate possible pollution problems and to add economic value to okara. Extrusion could provide a convenient and low cost way to incorporate the okara into food products.

Okara reportedly contains about 20% vegetable protein (on a dry basis) and large amounts of dietary fiber (Watanabe and Kishi 1984). It has been shown that soybeans and their products, tofu and soymilk, contain isoflavones (Dwyer et al 1994), as does their byproduct okara (Wang and Murphy 1996). In light of the attributes of okara, it is a suitable candidate for nutritional enrichment for cereal-based products. Okara could increase the protein, dietary fiber, and isoflavone contents of these products.

The objectives of this Master's thesis research are:

1) To utilize wet okara to produce extruded cereal products.

2) To enrich extruded cereal products with okara.

3) To study the effect on isoflavone and dietary fiber contents of okara-enriched extruded cereal products due to extrusion.

The following document includes a Literature Review (Chapter II), Materials and Methods (Chapter III), Results and Discussion (Chapter IV), Conclusions (Chapter V), Recommendations for Future Research (Chapter VI), and Appendices.

CHAPTER II

LITERATURE REVIEW

2.1 Soybean Seed Structure and Composition

There are three species of soybeans: *Glycine ussuriensis*, *Glycine max*, and *Glycine gracilis*. Of the three species, *Glycine max* is the most commonly grown. There are many varieties that are grown and they typically vary in color, size, and shape, but not in general structure. The soybean consists of three main components: the hull, the cotyledon, and the hypocotyl, which constitute 8%, 90%, and 2%, respectively, of the soybean (Waggle and Kolar 1979).

The cotyledon contains the major portion of proteins and lipids (Wolf 1996). The fiber portions are concentrated in the hull and cotyledon (Lo 1989). The isoflavones seem to be concentrated in the soybean hypocotyl with low to moderate amounts in the cotyledon (Wang and Murphy 1994). Hughes and Murphy (1983) reported that the protein contents among ten varieties of soybeans ranged between 39.4 - 44.1%. A large portion, 80 - 90%, of the protein content is made up of the glycinin, β-conglycinin, and γ-conglycinin storage proteins (Garcia et al 1997). Oil, which makes up approximately 20% of the total soybean, is comprised of 88.1% neutral lipids, 9.8% phospholipids, and 1.6% glycosides (Salunkhe et al 1982). The main components of these lipids are oleic and linoleic fatty acids (Mahadvappa and Raina 1978). Carbohydrates make up 33 - 35% of the soybean (Lucas and Riaz

1978) and consist mainly of polysaccharides and indigestible fiber (Weber et al 1993; Metelongo et al 1993).

2.2 Soymilk and Tofu Production

Soyfoods are typically divided into two categories: fermented and non-fermented. Traditional non-fermented soyfoods include fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk, soymilk products, tofu, and okara (Golbitz 1995). Two of these products in particular have found their way into mainstream Western culture; they are soymilk and tofu.

Soymilk is relatively simple to produce and is made by both small cottage industries and large scale producers. According to Chen (1989), small scale production requires a minimum of five steps, while large scale production requires as many as eleven steps. Both large and small scale productions, however, follow the same general steps. They first begin with cleaned whole soybeans which are typically dehulled first. The beans are then soaked, typically in hot potable water, to inactivate enzymes and soften the beans. If the beans are not soaked in hot water, they are blanched after soaking in order to inactivate enzymes. The beans are then ground with either hot or cold water using a stainless steel vertical or horizontal grinder. The next step is to filter the slurry using a decanter centrifuge. The solids, or "okara", are removed and the liquid portion is cooked, deodorized to remove volatile components, flavored, homogenized, pasteurized, and packaged (Ang et al 1985; Chen 1989; Golbitz 1995). Chen (1989) has also reported that many of the previous steps may be combined in various ways to produce the best quality soymilk possible. Ang et al (1985) reported the typical composition of soymilk to be 1.9% protein, 0.66% fat, 0.10% ash, and 13.9% total solids.

Schaefer and Love (1992) define tofu as a gel-like precipitate obtained by adding a calcium or magnesium salt to heated soymilk. There are many types of tofu, but in North America the refrigerated type or aseptic type are most popular (Murphy et al 1997). According to Watanabe and Kishi (1984), the process of manufacturing regular tofu consists of twelve steps. Clean, whole soybeans are first soaked in water for seven to fifteen hours. The soaked beans are drained and ground. After grinding, 10 kg of water for every kg of soybeans is added to the slurry and heated. A defoaming agent is added to the slurry and the slurry is brought to a boil. Boiling continues for five minutes and then the mixture is filtered. To the filtrate, which is soy milk, a coagulant (usually calcium sulfate) is added and this mixture is stirred to disperse the coagulant. This mixture is allowed to settle, while the residue from the filtrate, which is okara, is usually discarded. The coagulated soymilk consists of curds and whey. The whey is drained off and discarded, while the curds are wrapped in cloth and immediately formed into blocks. These blocks are then soaked in cold water; the result is the final tofu product (Smith et al 1960; Schaefer and Love 1992). The typical composition of tofu is 6.0% protein, 3.5% fat, 1.9% carbohydrate, 0.6% ash, and 88.0% water (Smith et al 1960).

2.3 Okara and its Composition

Okara is the residue or soy pulp that remains after the production of soymilk and tofu. Although typically considered a waste product in the U.S., okara has been shown to have many promising attributes. It has a typical composition that ranges from 25.4% - 29.75% protein, 9.3% - 12.85% fat, 3.0% - 3.6% ash, 3.8% - 5.3% carbohydrate, and 52.8% - 58.1% total dietary fiber (40.0% - 43.6% insoluble fiber and 12.6% - 14.6 % soluble fiber), on a dry weight basis (Kronenberg et al 1984; van der Riet et al 1989; Khare et al 1995). Wang and Murphy (1996) reported that okara recovered after soymilk production contained a small amount of isoflavones (25.9mg/717g okara, dry weight basis). Hackler et al (1963) reported that the Protein Efficiency Ratio (PER) of okara was 2.71; in their study this was second only to milk casein. This indicates that the protein in okara is of high quality. Despite the obvious attributes, okara has seen only limited use as a food component for human consumption.

2.4 Utilization of Okara

Every pound (454g) of dry soybeans made into tofu or soymilk generates about 1.1 pounds (499g or 2.5 cups) of firmly packed okara (approximately a 1:1 ratio) (Shurtleff and Ayogi 1979). Though nutritionally sound, okara has found only little use as a human food source in the U.S.. This is mainly due to the high moisture content (greater than 80%) of okara and the cost associated with drying. Furthermore, wet okara is highly susceptible to putrefaction (Noguchi and Isobe 1989). These problems have rendered the use of okara in the U.S. mainly to animal feed and fertilizer. Okara's use as human food in the U.S. has been limited to the cottage industries whose small scale enables them to immediately incorporate okara into baked goods or meat products. To date in the U.S., no large scale use for okara has been found, thus a novel process to utilize okara is needed.

2.5 Dietary Fiber

In 1976, Trowell et al defined dietary fiber as the sum of lignin and plant polysaccharides that are not digested by endogenous secretions of the mammalian digestive tract. This remains the most widely accepted definition of dietary fiber. Currently, however, there is support to expand this definition to include oligosaccharides that are resistant to hydrolysis by human alimentary enzymes (Lee and Prosky 1995). In general, dietary fiber is composed of structural materials of plant cell walls (including cellulose and non-cellulosic polysaccharides such as hemicelluloses and pectic substances) and non-cellulosic polysaccharides (such as gums and mucilages) (Southgate 1995). Soy fiber is a mixture of the cellulosic and non-cellulosic structural components of the cell wall (Slavin 1991). The major fractions are non-cellulosic and consist of acidic polysaccharides, arabinogalactan, and arabinan chains; 11.0% are cellulosic components (Lo 1989). Acidic polysaccharides are highly branched with a D-galacturonic and D-galactose backbone interspersed with rhamnose, arabinose, xylose, and fructose; other non-cellulosic polysaccharides are galactomannans and xylan (Aspinall et al 1967).

2.5.1 Health Benefits of Dietary Fiber

Dietary fiber has been shown to be associated with many positive health benefits. Sacks (1993) stated that fiber-rich foods may confer protection against cardiovascular disease. Commonly, dietary fiber is termed either soluble or insoluble, based on its ability to solubilize in water. Soluble fiber is typically associated with serum cholesterol lowering and changes in glycemic response (Chen et al 1981; Ikeda 1993; Anderson et al 1994; Zhang et al 1994), while insoluble fiber is primarily thought to affect the function of the large bowel by decreasing transit time (Englyst et al 1995). Soy fiber, such as that from okara may provide a readily available source of dietary fiber for use in a variety of food products. Okara provides a source that has a good distribution of both soluble and insoluble fibers, 12.6% - 14.6% and 40.0% - 43.6%, respectively (Kronenberg et al 1984), thus providing the health benefits of both fiber types.

2.5.2 Effects of Extrusion on Dietary Fiber

Extrusion is a popular processing technique that is used to make a variety of products, most notably cereal and snack foods. Extrusion exposes the raw materials to a number of mechanical forces including heat, compression, shear, and swelling (Aoe et al 1989). The combination of these forces is likely to disorganize the structure of these raw materials resulting in changes in the materials.

In general, extrusion has a tendency to increase the soluble fiber fraction with subsequent decreases in the insoluble fiber fractions, as the severity of the extrusion conditions increases (Robertson and Eastwood 19881; Bjorck et al 1984;

Anderson and Clydesdale 1986; Caprez et al 1986; Siljestrom et al 1986; Ralet et al 1990; Wang et al 1993; Qian and Ding 1996). These observations may be a result of disruption of covalent or non-covalent bonds in the carbohydrate and protein moieties, leading to smaller soluble fragments (Wang et al 1993). It may also be caused by the degradation of the amorphous regions of the fiber and the compression of the crystalline regions (Qian and Ding 1996).

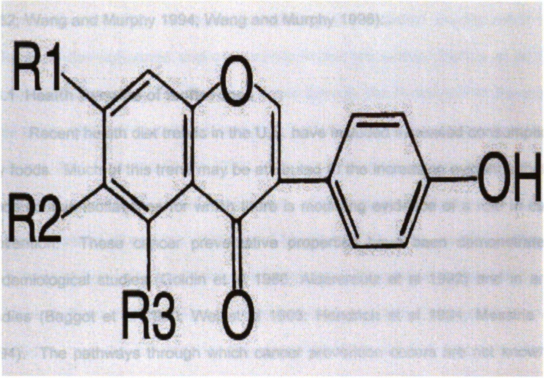
In terms of effects of extrusion on total dietary fiber, Bjorck et al (1984), Ostergard et al (1989), and Lintas et al (1995) observed slight increases in total dietary fiber. This effect is most likely due to the retrogradation of amylose forming an enzyme-resistant starch upon cooling of the product (Englyst et al 1995). In contrast, Wang et al (1993) observed slight decreases in total dietary fiber and suggested the decreases were due to the disruption of covalent or non-covalent bonds in the carbohydrate and protein moieties. On the other hand, Siljestrom et al (1986) and Schweitzer and Reimann (1986) reported no changes in total dietary fiber content. This broad spectrum of observations can most likely be attributed to inherent differences in native composition of fiber from different sources, and to differences in extrusion conditions used.

2.5.3 Determination of Dietary Fiber

Dietary fiber is typically determined by one of two methods: ezymatic-gravimetric or chemical. The enzymatic-gravimetric method, described by Lee et al (1992), is used to determine total, insoluble, and soluble dietary fiber content in food. The chemical method, described by Quigley and Englyst (1994), is used to determine the constituent sugars in the non-starch polysaccharides which make up dietary fiber. The enzymatic-gravimetric method accounts for enzyme-resistant starch content; on the other hand, the chemical method does not account for enzyme-resistant starch. The inclusion of enzyme-resistant starch in the total dietary fiber content is a source of debate, however according to Lee et al (1992) the most widely accepted definition of dietary fiber includes enzyme-resistant starch.

2.6 Isoflavones

Phytoestrogens, compounds with weak estrogenic activity such as isoflavones, coumestans, resorcyclic acid lactones, and lignans, are found in several dozen plants eaten by humans, including soybeans and soybean products (Dweyer et al 1994). Soybeans contain three types of isoflavones (daidzein, genistein, and glycitein) and each exists in four chemical forms: as the aglycones, the glucosides, the acetylglucosides (formed during processing by heat), and the malonylglucosides (Figure 2.1) (Kudou et al 1991). Tsukamoto et al (1995) reported that the majority of isoflavones found in soybeans are the glucosides genistin and daidzin, with the malonylglucosides being the primary conjugates. Anderson and Wolf (1995) found 98.0% of the isoflavones present in soybeans to be in the glucoside form and 2.0% to be in the aglycone form. Earlier studies showed the distribution of the isoflavones to be 64.0% genistin, 23.0% daidzin, and 13.0% glycitin (Naim et al 1974). It has been shown that different soybean varieties had similar isoflavone isomer distributions but the total concentrations appeared to fluctuate due to genetics, crop year, and growth location (Wang and Murphy 1994). In addition, different soy



Aglycones	**R1**	**R2**	**R3**
Genistein	OH	H	OH
Daidzein	OH	H	H

Glucosides	**R1**	**R2**	**R3**
Genistin	O-glucosyl	H	OH
Acetyl Genistin	O-glucosyl-6-$OCOCH_3$	H	OH
Malonyl Genistin	O-glucosyl-6-$OCOCH_2COOH$	H	OH
Daidzin	O-glucosyl	H	H
Acetyl Daidzin	O-glucosyl-6-$OCOCH_3$	H	H
Malonyl Daidzin	O-glucosyl-6-$OCOCH_2COOH$	H	H

Figure 2.1 Structures of Isoflavone Isomers in Soybeans.

products contained different levels of the isoflavones and their distributions were affected by particular processing techniques utilized to produce the product (Murphy 1982; Wang and Murphy 1994; Wang and Murphy 1996).

2.6.1 Health Benefits of Isoflavones

Recent health diet trends in the U.S. have included increased consumption of soy foods. Much of this trend may be attributed to the increasing evidence that soy foods contain isoflavones for which there is mounting evidence of a role in cancer prevention. These cancer preventative properties have been demonstrated in epidemiological studies (Goldin et al 1986; Aldercreutz et al 1992) and in animal studies (Baggot et al 1990; Wei et al 1993; Hendrich et al 1994; Messina et al 1994). The pathways through which cancer prevention occurs are not known but may be due to the ability of isoflavones to act as estrogen receptors (Peterson and Barnes 1991) and as free radical scavenging antioxidants (Wei et al 1995). The amount of soy isoflavones that humans would need to consume in order to provide an anticarcinogenic dose is approximately 1.5 - 2.0 mg (kg body weight)$^{-1}$day $^{-1}$ (Heindrich et al 1994). Isoflavones have also been linked to reduction in LDL cholesterol (Potter 1995) as well as the slowing down of atherosclerotic disease (Wilcox and Blumenthal 1995).

2.6.2 Effects of Processing on Isoflavones

Most soyfoods undergo some type of processing, many of which involve heat and/or fermentation. Wang and Murphy (1996) demonstrated through a mass balance study that isoflavones were not destroyed by processing but the amount and distribution of types of isoflavones in relation to the raw material was affected by the type of processing. Products that undergo fermentation usually result in an increase in the aglycones and a decrease in the glucosides (Lampe et al 1994; Wang and Murphy 1996). This may occur through the hydrolysis of the malonyl isoflavones into the glucosides and then into the aglycones by glucosidases in soybeans (Matsuura and Obata 1993). Products that undergo heat treatment, most commonly result in a partial conversion of the malonyl glucosides into their respective acetyl glucosides (Wang and Murphy 1994). The malonyl isoflavones have been shown to be heat labile and unstable at temperatures exceeding 100°C (Pratt and Birac 1979). This conversion of the malonyl glucosides to acetyl glucosides may be caused by the decarboxylation of the malonylglucosides. Knowing the isoflavone content and distribution in soy products and byproducts, and the changes they undergo during processing would be valuable information for researchers pursuing the health benefits of isoflavones in processed foods.

2.6.3 Isoflavone Determination

Chromatographic methods have been used as early as 1974 for the quantification of isoflavones. Niam et al (1974) utilized gas-liquid chromatography to quantify the content of daidzein and genistein in soybeans. West et al (1978) developed a high-performance liquid chromatography (HPLC) method to quantify genistein in soybeans. High-performance liquid chromatography methodology can directly analyze free and conjugated forms of isoflavones without derivitization

(Wang et al 1990), unlike gas-liquid chromatography which requires derivitization prior to analysis. Since 1978 various HPLC methods have been developed for the determination of isoflavones in soybeans and soybean products (West et al 1978; Eldridge 1982; Murphy 1982; Wang and Murphy 1994). Murphy (1981) investigated various solvents for the extraction of isoflavones and coumestrol and found that acetonitrile with water or hydrochloric acid was superior to all other extraction solvents.

2.7 Soy Proteins

Hughes and Murphy (1983) reported that the protein contents among 10 varieties of soybeans ranged between 39.4% - 44.1%. It was shown that the proteins were globular and heterogeneous, and were partitionable into four centrifugal components with sedimentation coefficients of 2S, 7S, 11S, and 15S (Wolf and Briggs 1956). These components range in molecular weights from 8,000 to 480,000 Da (Wolf 1982). The 7S fraction is composed of mainly 7S globulin or conglycinin and the 11S fraction is composed entirely of 11S globulin or glycinin. The globulins, conglycinin and glycinin account for approximately 80% - 90% of the proteins in soybeans (Kinsella 1984; Garcia et al 1997). The globulins are water extractable proteins and can be precipitated out in acidic conditions (pH of 4.5 - 4.8) (Fukushima 1991). Conglycinin is composed of three different subunits (α, β, and γ subunits) that range in molecular weight from 44,000 to 59,000 Da, and glycinin contains seven different acidic subunits (A_1 - A_7) and four basic subunits (B_1 - B_4)

which range in molecular weight from 37,000 to 45,000 Da and 19,000 to 22,000 Da, respectively (Brooks and Morr 1985).

2.7.1 Amino Acid Compositions of Proteins in Soybeans and Okara

The nutritional value of proteins depends on amino acid composition. Soybeans contain all nine essential amino acids required by humans (Garcia et al 1997). Moreover, it has been shown that the amino acid content in soybeans exceeds the requirements for both children and adults (Steinke 1992; Lucas and Raiz 1995). Hackler et al (1963) reported that the nutritional value of protein found in okara was higher than in other soybean fractions. Wang and Cavins (1989) showed that okara had an essential to total amino acid ratio that was comparable to soymilk and tofu (Table 2.1). The amino acid composition of okara further proves its value as a food source.

2.7.2 Processing Effects on Amino Acids

Hackler and Stillings (1967) reported that heat processing temperatures of 121°C resulted in decreases in cystine and tryptophan. Van Buren (1964) showed that spray drying temperatures between 143°C and 316°C caused a decline in lysine content. On the other hand, Khaleque and Wallace (1975) showed that processing soymilk at temperatures between 98°C and 115°C resulted in no significant changes in the amino acid composition. Jeunink and Cheftel (1979) reported that some amino acid contents (methionine, asparagine, glutamine, and histidine) were decreased and the cystine content increased after extrusion processing of soy

Table 2.1 Average Amino Acid Composition[1] (g / 16g Nitrogen)

Amino Acid	Soybeans	Okara	Soymilk	Tofu
Aspartic Acid	12.61	11.63	11.91	11.70
Threonine	4.11	4.42	4.01	4.00
Serine	5.74	5.47	5.19	5.32
Glutamic Acid	19.76	17.71	19.61	19.26
Proline	5.53	5.66	5.33	5.47
Glycine	4.46	4.61	4.16	4.14
Alanine	4.49	4.36	4.14	4.11
Valine	3.73	5.28	4.88	4.99
Cystine	0.78	trace	0.03	trace
Methionine	1.34	1.67	1.59	1.43
Isoleucine	3.46	4.50	4.66	4.85
Leucine	7.90	8.31	7.94	8.32
Tyrosine	3.90	374	3.91	3.99
Phenylalanine	4.85	5.20	5.15	5.41
Lysine	6.19	6.36	6.08	6.41
Histidine	2.60	3.07	2.64	2.64
Arginine	8.64	8.61	8.65	8.52
Essential / Total	0.36	0.39	0.38	0.39

[1] (Wang and Cavins 1989)

protein, however none of these changes were statistically significant. Changes in the solubility of protein fractions and the amino acid composition of these fractions after processing occur due to the involvement of particular amino acids in isopeptide and/or disulfide crosslinking reactions (Stanley 1989). The amino acids of importance to isopeptide crosslink formation are asparagine, aspartic acid, glutamine, glutamic acid, histidine, lysine, and methionine; the sulfur-containing amino acids, such as cysteine and methionine, are of importance to disulfide crosslink formation (Stanley 1989). On the other hand, an increase in amino acid contents of protein fractions after processing may be due to the unfolding of proteins enabling more of the amino acid groups to become available to react with test reagent (Kwok and Niranjan 1995). What all of this may suggest is that spatial relationships may be the controlling factor in the resulting effects on amino acids due to processing (Stanley 1989).

2.8 Extrusion

Extrusion is a process that involves forcing a material to flow under a variety of controlled conditions and then to pass through a shaped hole or slot at a predetermined rate (Dziezak 1989). Extrusion is used to produce diverse cereal-based products such as ready-to-eat cereals, snack foods, baked goods, and pet foods (Clark 1986).

Extruders are typically put into two categories: single-screw extruders and twin-screw extruders (Huber 1991). This discussion will be limited to twin-screw extruders, which are commonly used for production of cereal-based products. Twin-screw extruders, which contain two screws in the barrel of the extruder, are further categorized by the type of screw configuration (conjugated or nonconjugated), the relative direction of rotation (co-rotating or counter-rotating), and the degree of intermeshing of the screws (intermeshing or non-intermeshing) (Harper 1989).

The following discussion will center on conjugated, co-rotating, and intermeshing twin screw extruders. Conjugated refers to screws whose flights have the same shape and dimension, and tightly fit the channels of the other screw with minimum clearance (Martelli 1983). This is also referred to as self-wiping. Co-rotating means the two screws rotate in the same direction. Intermeshing refers to screws in which the distance between their centers is less than twice their outside radius, therefore one screw engages with the other (Martelli 1983). The conjugated and intermeshing screw design allows the material in the barrel of the extruder to be conveyed forward with a positive pumping action, this being the basic difference between single- and twin-screw extruders.

Several variables such as screw configuration, screw speed, material feed rate, and temperature of the various heating zones of the barrel affect the extrusion function, product quality, and product characteristics (Huber 1991). These variables generate two effects: heating and shear. Upon heating and shearing cereal-based materials, the macromolecules in food ingredients lose their native, organized tertiary structure and form a continuous viscous dough (Harper 1986). The flow of the material within the extruder screws aligns the large molecules in the direction of the flow which exposes bonding sites and may lead to crosslinking which results in a reformed structure (Harper 1986).

2.8.1 Screws and Paddles, Shear and Heat

Extruder screws are made up of a series of modular screw elements that can be assembled in different configurations. The two basic types of elements used to build screw configurations are feed screws, which can be either twin- or single- lead, and kneading elements, which are also referred to as paddles and possess multiple lobes. Twin-lead screws have two leads or flights which are 180° from each other. The flight is a helical rib which wraps around the screw element. In contrast, single-lead screw elements have only one lead. Twin-lead screw elements are used to convey material while single-lead elements help to build pressure in the barrel of the extruder directly before the die exit.

Kneading elements are typically bi-lobed, and may be visualized as having two blunt points on opposite ends. The kneading elements or paddles usually occupy about a quarter of the length of the feed screws, and these sections are considered areas where the majority of mechanical shear takes place. Mechanical shear, in this thesis, refers to the physical disruption of material. The kneading elements can be offset by 30°, 60°, or 90° with regard to the prior kneading element and create significant shearing action (Harper 1986). The 30° offset is considered to cause the least amount of mechanical shear and the greatest amount of material conveyance; the 90° offset is considered to cause the greatest amount of mechanical shear and the least amount of material conveyance. Kneading elements can also be offset clockwise (forward) which causes forward conveyance of material or counterclockwise (reverse) which causes backflow of material. Backflow of material results in increased residence time of the material in the barrel and subjects the material in the barrel of the extruder to the effects of shear and heat for a longer period of time.

The number of kneading sections in a screw configuration, the offset degree of the kneading elements and their forward or reverse orientation are primarily what defines the overall degree of shear provided by the screw configuration. Shear is partially responsible for physical and molecular disruption of the native state of the raw material (Cumming et al 1972; Hager 1984; Harper 1986; Dahl and Villota 1991). Shear also results in the generation of mechanical energy which is dissipated as heat (Harper 1986). Heat is also added externally to the barrel of the extruder by either electricity or steam. Extrusion processing is unique from other heat processes in that the material is also subject to shear (Wang et al 1993).

Conditions in the barrel of the extruder are considered more severe as heat and shear increase, thus the more kneading elements, the greater the effects of shear and heat on the material in the barrel of the extruder. The combination of heat and shear have been shown to be responsible for decreasing viscosity (Meuser and Wiedman 1989), increasing starch gelatinization (Bhattacharya and Choundhurry 1994), affecting the solubility and bonding forces of proteins (Cumming et al 1972; Hager 1984; Dahl and Villota 1991; Ferreira-Prudencia and Areas 1993), and increasing the total and soluble fiber fractions (Bjorck et al 1984; Caprez et al 1986; Wang et al 1993). The thermomechanical treatment that causes these changes can be characterized by the determination of the specific mechanical energy (Brent et al 1997), which is an indication of abusive conditions in the barrel of the extruder.

2.8.2 Determination of Physical Characteristics of Extruded Products

Twin-screw extrusion is one of the most poplar processes used by the food industry to produce expanded snack foods, ready-to-eat cereals, and pet food (Chinnaswamy et al 1989). The microstructural properties of extruded products depends on the influence of physical forces on chemical components (Stanley 1986). Various analyses have been used to determine the physical characteristics that make up the microstructure of extruded products.

Radial expansion ratio has been used to determine the degree of expansion of a product in comparison to the size of the exit die orifice (Moore et al 1990; Lue et al 1991; Jin et al 1995). Bulk density values have been used to determine the density of the products in relation to a specific volume (Hsieh et al 1991; Lue et al 1991; Jin et al 1995). High bulk density values are typically related to samples with low radial expansion. Breaking strength has been used to determine how much force is required to break extruded products. Jin et al (1995) related samples with low expansion ratios to samples with high breaking strength values. Water absorption and water solubility indices have been used to determine the ability of extruded products to absorb water and to measure the amount of water soluble material present in extruded products (Ralet et al 1990; Jin et al 1995), respectively. The heat and shear from the extrusion process impact the water absorption and water solubility indices. Rapid visco-analysis has also been used to determine the pasting properties of extruded products (Ryu et al 1993; Whalen et al 1997). Pasting properties are related to starch and the effects extrusion may have on starch. It has also been shown that sucrose, shortening, non-fat dry milk, sodium bicarbonate, fiber, protein, and fat affect the pasting properties of wheat flour (Ryu et al 1993; Whalen et al 1997).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Wet okara was obtained from American Soy Products Inc. (Saline, MI). The okara was derived from the production of soymilk and was obtained immediately after the soymilk production. The okara was placed in air-tight five gallon containers (previously sterilized with a 10% chlorine bleach solution) and stored in a freezer (-10°C) until use.

Soft wheat flour (characteristics listed in APPENDIX I) was obtained from the King Milling Co. (Lowell, MI). Upon receiving, the four 50 pound sacks of flour were combined in an air-tight bin and then stored in the freezer (-10°C) until needed.

3.2 Methods

3.2.1 Preparation of Formulations

Wet okara and soft wheat flour were combined in different proportions in order to develop two formulations, designated A and B (Table 3.1). The wet okara and flour were weighed in the appropriate proportions for the respective formulation and combined in a large mixing bowl in a temperature (22°C) and humidity (45%) controlled room. The materials were mixed at low speed for three minutes with a Hobart A-200 mixer (The Hobart MFG. Co., Troy, OH). The materials were mixed in such a manner, to develop a formulation that had a particulate flow-like consistency.

Table 3.1 Proportions by Weight of Okara and Soft Wheat Flour Used for Formulations A and B

Formulation	Okara (%)	Soft Wheat Flour (%)
A[1]	33.33	66.67
B[2]	40.00	60.00

[1] Composition of A = 11.0% okara (d.b.), 33.0% water, and 56.0% flour (d.b.).
[2] Composition of B = 14.0% okara (d.b.), 37.5% water, and 48.5% flour (d.b.).

The particulate flow-like consistency was imperative in order to continuously deliver a consistent amount of material to the barrel of the extruder. The materials were mixed immediately prior to the extrusion process.

3.2.2 Extrusion Equipment

Extrusion was performed using an APV Baker MP19TC-25 (APV Baker, Grand Rapids, MI) co-rotating and intermeshing twin-screw extruder with a 2kW motor. The barrel diameter of this particular extruder was 19 mm and had a length to diameter ratio (L:D) of 25:1. The barrel was a clam-shell style and had flighted shafts that supported a modular screw system allowing the operator to design unique screw configurations. There were five independently heated zones, designated from feed port to die exit as zone 1 (Z1) through zone 5 (Z5). Electricity provided the source of heat for the extrusion process. The particulate ingredients were fed into the barrel of the extruder via a K-Tron K2M (K-Tron Corp., Pittman, NJ) twin-screw volumetric feeder. Liquid ingredients could be fed into the barrel of the extruder via a Brook Crompton E2 Metripump (Brook Crompton, Hudders Field, England). The die used for this study consisted of a single circular orifice with a 3 mm diameter. A variable speed cutter, designed and built at Michigan State University, was used to cut the extrudate to lengths of approximately 5 mm.

3.2.3 Extrusion Parameters

Two screw configurations were used for this study and were designated low shear (LS) and high shear (HS) (Table 3.2). The LS configuration contained fewer

Table 3.2 Screw Elements[1] Used for Low Shear and High Shear Configurations

Low Shear Configuration (LS)	High Shear Configuration (HS)
8 D Twin Lead Feed Screws	8 D Twin Lead Feed Screws
7x30° Forward Kneading Elements	7x30° Forward Kneading Elements
8 D Twin Lead Feed Screws	4 D Twin Lead Feed Screws
3x60° Forward Kneading Elements	4x60° Forward Kneading Elements
3x30° Reverse Kneading Elements	4x30° Reverse Kneading Elements
2 D Single Lead Feed Screws	2 D Twin Lead Feed Screws
4x60° Forward Kneading Elements	6x60° Forward Kneading Elements
3x30° Reverse Kneading Elements	4x30° Reverse Kneading Elements
2 D Single Lead Feed Screws	1 D Single Lead Feed Screws
	7x90° Kneading Elements
	2 D Single Lead Feed Screws

[1] One kneading element = .25 D; One D = 19 mm

kneading element sections with more benign offsets than the HS configuration, thus the HS configuration was considered more abusive. Two temperature profiles were used for this study and were designated low temperature (LT) and high temperature (HT) (Table 3.3). The comparatively higher temperatures of zones 3-5 for the HT profile were considered more abusive than those delivered by the LT profile.

The two screw configurations and two temperature profiles were combined to form four different extrusion conditions. The four conditions, in order of least abusive to most abusive, were: low shear/low temperature (LS/LT), low shear/high temperature (LS/HT), high shear/low temperature (HS/LT), and high shear/high temperature (HS/HT).

3.2.4 Extrusion Process

The two formulations A and B were extruded in two replicates at each of the four possible extrusion conditions. The existing water in the okara was the only source of moisture for the formulations; no water was injected in the barrel of the extruder for the extrusion process of either of the two formulations. The feed rate for formulation A was 1.95 kg/hr and for formulation B was 3.71 kg/hr. The volumetric feeder was set at 1000 rpm for the delivery of the two formulations. The screw speed for the barrel of the extruder was held constant at 100 rpm (500 rpm was maximum for this extruder) for all extrusion work.

A flour/water sample was extruded in two replicates using the HS/HT condition only. The flour/water sample (37.5% moisture) was extruded using the

Table 3.3 Temperature of the Five Heating Zones Used for the Low Temperature (LT) and High Temperature (HT) Profiles

Temperature Profile	Zone 1 (°C)	Zone 2 (°C)	Zone 3 (°C)	Zone 4 (°C)	Zone 5 (°C)
LT	40	60	125	135	145
HT	40	60	130	155	170

traditional extrusion process of injecting water into the barrel of the extruder. The flour feed rate was 3.0 kg/hr (the volumetric feed rate was set at 925 rpm). The screw speed was held constant at 100 rpm. Water was injected into the barrel of the extruder at a rate of 0.5 kg/hr; the liquid pump piston stroke was set at 11%.

Approximately two kg of extrudate was collected for each sample extruded. The extrudates were placed on a table in a temperature (22°C) and humidity (45%) controlled room until they could be placed in a forced-air oven. The extrudates were allowed to dry overnight at 45°C in the forced-air oven. After drying, one kg of each extrudate was ground using a Udy cyclone mill (Udy Corp., Fort Collins, CO) with a 1.00 mm mesh screen. Ground extrudate was placed in air-tight ziplock plastic bags and stored in the freezer (-10°C) until analyzed. The remaining one kg of each extrudate was left unground, placed in air-tight ziplock plastic bags, and stored in the freezer (-10°C) until analyzed.

3.2.5 Proximate Analysis of Samples

Proximate analysis of samples was performed on non-extruded okara, flour, formulations A and B, extruded flour/water sample at the HS/HT condition , and extruded formulations A and B for all conditions. Proximate analysis included moisture, protein, ash, and fat analyses. Moisture, micro-Kjeldahl protein, and ash analysis were determined utilizing AACC approved methods (1990).

Total fat analysis was performed utilizing the Tecator Soxtec HT 1043 extraction unit (Tecator, Hoganas, Sweden). The procedure used for fat analysis was described by Foster et al (1992) with the following modifications: the samples were not weighed into extraction thimbles and mixed with sand, but were weighed onto 11.0 cm diameter Whatman No.1 filterpaper (Whatman International LTD., Maidstone, England) and placed into the extraction thimbles.

3.2.6 Radial Expansion Ratio, Bulk Density, and Breaking Strength

Radial expansion ratios of the dried extrudates were determined by measuring the cross sectional diameter of the unground extrudate divided by the die orifice opening (3.0 mm). The cross sectional diameter of the extrudate was measured with Verneir calipers (Glogau and Co., Chicago, IL).

Bulk density has been defined as the weight of unground extrudate per unit volume. Bulk density was determined by weighing pieces of extrudate after placing them into a 100 ml graduated cylinder. The bottom of the cylinder was gently tapped on a laboratory bench until there was no further reduction in sample volume.

Breaking strength has been defined as the maximum breaking force (N) required to compress a sample of unground extrudate a given distance, divided by the mass (g) of the sample. Breaking strength was measured utilizing a TA-XT2 Texture Analyser (Stable Micro Systems, Surry, England) with a 0.5 inch diameter stainless steel ball as the compression implement. Extrudate pieces from a sample were placed into a cylinder with a 15 mm diameter opening and a 10 cm depth. Sample weight was recorded and the sample then crushed a distance of 10 mm at a rate of 1 mm/second. The peak force in Newtons (N) was recorded by Texture Expert Software (Stable Micro Systems, Surry, England).

3.2.7 Water Absorption Index and Water Solubility Index

Water absorption index (WAI) has been defined as the weight of gel obtained per gram of dry ground sample. Water absorption index was performed according to AACC method 56-20 (1990).

Water solubility index (WSI) was defined by Jin et al (1995) as the percentage of dry matter recovered after the supernatant from WAI analysis is evaporated. The supernatant from the WAI analysis was decanted into a preweighed plastic weighing boat and dried overnight in a forced-air oven at 45°C. The calculation for WSI is as follows:

WSI (%) = [weight of dried supernatant / sample dry weight] x 100

3.2.8 Analysis of Pasting Properties

Pasting properties for non-extruded flour, non-extruded formulations A and B, extruded flour/water sample at the HS/HT condition, and extruded formulations A and B for all extrusion conditions were measured using the Rapid Visco-Analyser (RVA) (Newport Scientific Pty. LTD., NSW, Australia). Five grams of each sample (14% moisture basis) and 25 ml (plus moisture adjustment) of distilled water were placed in an aluminum sample can as previously described by Deffenbaugh and Walker (1989). A no.8 rubber stopper was inverted and placed over the opening of the sample can in order to allow the sample can to be inverted ten times immediately after the water was added to the sample. Inverting the sample helps to prevent clumping and incomplete wetting of the material in the sample can. The walls of the sample can were then wiped down with the stirring paddle blades and placed into the RVA for analysis. The pasting profile used (Table 3.4) was described by Ryu et al (1993). Four pasting parameters were measured: Peak Viscosity, Breakdown, Setback, and Final Viscosity.

The term viscosity refers to the measurement of resistance on the RVA motor and is expressed in rapid visco units (RVU). Peak viscosity is defined as the maximum viscosity recorded by the RVA from the time the analysis begins until immediately after the holding temperature is reached. Peak viscosities that occur prior to the addition of heat may be considered a "cold paste"; peak viscosities occurring after the holding temperature has been reached may be considered a "hot paste" (Newport Scientific RVA Operation Manual 1995). Breakdown is defined as the ability of a sample to withstand mechanical shear delivered by the RVA as the holding temperature and stirring rate are held constant for a given period of time (Newport Scientific RVA Operation Manual 1995). The setback value demonstrates the degree to which retrogradation of starch occurs as the sample slurry is cooled (Tipples 1980). The final viscosity is the viscosity of the sample recorded at the end of the pasting profile.

3.2.9 Specific Mechanical Energy

Specific mechanical energy (SME) was determined on all extruded products. The SME is defined as the mechanical energy required to obtain 1 kg of extrudate. The SME was calculated using the following equation described by Brent et al (1997) :

SME = [screw rpm (run)/screw rpm (rated)]x [% torque/100] x [motor power/feed rate]

Table 3.4 Pasting Profile[1] Used for RVA Analysis

Time (min.)	Temperature (°C)	Stirring Rate (rpm)
0 - 2	50	160
2 - 4	50 - 95	160
4 - 10	95	160
10 - 18	95 - 50	160

[1] Ryu et al (1993)

3.2.10 Dietary Fiber Analysis

The determination of dietary fiber followed the AOAC method 991.43 as described by Lee et al (1992). The amounts of total dietary fiber (TDF) and insoluble dietary fiber (IDF) were directly determined by analysis, while the soluble dietary fiber (SDF) was indirectly determined by the difference (TDF - IDF = SDF). For the determination of both TDF and IDF contents, duplicate samples were weighed for each analysis. The fiber residue from one of the duplicate samples was used to determine ash content and the fiber residue from the other duplicate sample was used to determine protein content.

Two 1.000 g ± .005 g samples (sample wt.1 and sample wt.2) were used for each analysis. The samples were suspended in a 2-(N-Morpholino)ethanesulfonicacid-Tris(hydroxymethyl)aminomethane (MES-TRIS) buffer (pH 8.2 at 24°C). This was followed by three consecutive enzymatic digestion steps using α-amylase, protease, and glucosidase, sequentially. The digestion steps were performed for both TDF and IDF samples. To determine IDF, samples were filtered through a filtering crucible with a fritted disk (40μm - 60μm) immediately after enzymatic digestion. To determine TDF, 230 ml of 95% ethanol was added to samples after enzymatic digestion and allowed to sit for one hour. This is the defining step for TDF because the addition of ethanol allows the SDF fraction to precipitate out of the solution. After the precipitation period, the sample was filtered in the same manner as the IDF sample. After filtering, all samples were dried overnight in a forced-air oven at 130°C. The dried residues (residue wt.1 and residue wt.2) were weighed and then one of the duplicate samples was ashed at

525°C and the other was analyzed for protein content using AACC method 46-13 (AACC 1993). Blanks consisting of reagents only were also analyzed. Both TDF and IDF were determined with the following calculation:

$$\{(A - B - C - D) / E\} \times 100$$

A = (residue wt. 1 + residue wt. 2) / 2
B = protein wt.
C = ash wt.
D = blank wt.
E = (sample wt. 1 + sample wt. 2) / 2

3.2.11 Analysis of Isoflavones

Isoflavone analysis was based on the procedure developed by Wang and Murphy (1994). One modification was that methanol was used as the mobile phase instead of acetonitrile. The extraction of isoflavones for this study was based on a 1.000 g dried sample of okara or equivalent such that when the formulated samples were analyzed, the sample size was adjusted to equal 1.000 g of okara. The weighed sample was first wet with 15 ml of 0.1 N HCl, after which 20 ml of absolute methanol was added. The sample was then sonicated for 20 minutes and the volume brought to 50 ml with absolute methanol. A 1.0 ml sample was drawn from the 50 ml volume and centrifuged for five minutes at 10,000 x g. The supernatant (extract) was then ready for analysis.

The isoflavone extracts were analyzed by High Performance Liquid Chromatography (HPLC) with a Dionex AGP-1 Gradient Pump and Dionex EDM-2

Eluent Degas Module (Dionex Corp., Sunnyvale, CA), a Waters 486 Tunable Absorbance Detector (Waters Corp., Milford, MA), and a Microsorb MV column (5μ, 100Å, C_{18}) (Rainin Instrument Co., Woburn, MA). Samples were injected by a Spectrasystem AS 3000 autoinjector (Spectrasystem, Freemont, CA) onto a 20μl loop. Sample data were collected and analyzed with a Peaksimple Chromatography Data System (Peaksimple, Las Vegas , NV). A linear gradient (Table 3.5) of two solvents was used: solvent A was 89.9% water, 10% methanol, and 0.1% acetic acid, solvent B was 99.9% methanol, and 0.1% acetic acid. Detection of isoflavones occurs at 254 to 264 nm, and for this study the detector was set at 260 nm. The total run time for each analysis was 48 minutes.

Six isoflavone peaks were identified, they were daidzin, genistin, malonyl daidzin, malonyl genistin, acetyl genistin, and genistein (in order of elution). The area under each peak was measured and μg isoflavone / g okara was calculated as follows:

1.) area under peak / conversion factor = μg of isoflavone (20 μl injection)$^{-1}$

Conversion factors are (unit of area (μg of isoflavone)$^{-1}$):

Daidzin = 3333.04

Genistin = 4380.7

Malonyl Daidzin = 2966.952

Malonyl Genistin = 4039.9

Acetyl Genistin = 4039.9

Genistein = 7100

2.) μg(20μl)$^{-1}$x 50,000μl (wt. okara equivalent)$^{-1}$ = μg isoflavone (g okara)$^{-1}$

Table 3.5 Linear Gradient Program Used for HPLC Analysis of Isoflavones

Time (min.)	Solvent A (%)	Solvent B (%)
0.0	80	20
2.0	70	30
28.0	30	70
30.0	15	85
35.0	80	20
48.0	80	20

3.3 Statistical Analysis

All statistical analyses were performed with the Statistical Analysis System (SAS Institute Inc., Cary, NC) under the General Linear Model procedure. One-way and two-way analysis of variance (ANOVA) was performed and Fishers least significant difference (LSD) was used for multiple mean comparison.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Proximate Analysis of Non-Extruded and Extruded Samples

Proximate analysis data for all of the non-extruded materials, extruded flour/water sample at the high shear/high temperature condition, and extruded formulations A and B for all conditions are listed in Table 4.1. The data for the two formulations (A and B) reflect the combination of okara and flour in different ratios. The moisture contents of the extruded samples listed were determined after oven drying and grinding. The wide variation in moisture content may be a result of differences in the length of time the extrudate samples were exposed to ambient conditions during sample grinding. The moisture variation may also be due to different amounts of enzyme-susceptible starch (gelatinized) in the samples. Wang et al (1993) reported a higher percentage of enzyme-susceptible starch increased water absorption. The extruded samples varied slightly in protein and ash contents but showed no general trends compared to their respective non-extruded sample. The protein, ash, and fat contents for all of the extruded samples varied slightly among extrusion conditions but resulted in no general trends. The fat content of all samples appeared to decrease slightly after extrusion. Mercier (1980) suggested that monoglycerides and free fatty acids can form complexes with amylose during extrusion cooking. The formation of these

Table 4.1 Proximate Analysis[1] Data for Non-Extruded and Extruded Samples

Extrusion Conditions[2]	% Moisture	% Protein	% Ash	% Fat
Okara				
Non-Extruded	77.65	24.21	4.06	9.83
Flour				
Non-Extruded	10.57	8.54	0.56	1.03
Flour/Water(HS/HT)	3.55	8.53	0.57	0.97
Formulation A				
Non-Extruded	33.00	9.75	0.96	1.59
LS/LT	7.78	9.63	0.98	1.56
LS/HT	6.42	9.76	0.98	1.54
HS/LT	5.95	9.82	0.98	1.55
HS/HT	1.87	9.81	0.98	1.53
Formulation B				
Non-Extruded	37.50	10.44	1.07	1.80
LS/LT	6.48	10.42	1.10	1.74
LS/HT	7.13	10.39	1.09	1.74
HS/LT	5.16	10.39	1.08	1.76
HS/HT	5.09	10.38	1.07	1.75

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

complexes may have made extraction of fat more difficult. Wang et al (1993) also reported that fat content in whole wheat flour decreased after extrusion and attributed the decrease to formation of complexes with amylose.

4.2 Physicochemical Analysis of Non-Extruded and Extruded Samples

4.2.1 Radial Expansion Ratio, Bulk Density, and Breaking Strength

Data for the radial expansion ratio, bulk density, and breaking strength for extruded flour/water at the high shear/high temperature condition, and formulation A and B extruded at all conditions are listed in Table 4.2. The expansion of extruded products is generally attributed to starch content and type. Increasing the content of certain ingredients has been demonstrated to affect the radial expansion of extruded products. For example, increasing fiber content has been demonstrated to decrease the radial expansion ratio (Moore et al 1990; Lue et al 1991; Jin et al 1995). Decrease in the radial expansion ratio as fiber content was increased was also observed in the present study. The extruded flour/water sample had the lowest fiber content and the greatest radial expansion ratio, followed by formulation A and then by formulation B which had the greatest fiber content and in general the lowest radial expansion ratio. Jin et al (1995) suggested that increasing fiber content caused thickening of the cell walls and decreased air cell size in the microstructure of the extrudate, resulting in decreased radial expansion. There was also a decreasing trend in the radial expansion ratio as extrusion conditions became increasingly more severe for formulations A and B. This may be due to the breaking down of components into

Table 4.2 Radial Expansion Ratio, Bulk Density, and Breaking Strength for Extruded Samples

Extrusion Conditions[1]	Radial Expansion Ratio	Bulk Density (g/100ml)	Breaking Strength (N/g)
Flour/Water			
HS/HT	1.57	48.85	33.33
Formulation A			
LS/LT	1.25	51.15	38.46
LS/HT	1.07	55.65	39.61
HS/LT	1.00	58.30	42.54
HS/HT	0.98	59.40	45.15
Formulation B			
LS/LT	1.00	55.60	40.09
LS/HT	0.96	57.40	44.28
HS/LT	0.95	61.30	45.86
HS/HT	0.93	63.10	47.24

[1] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

smaller particles, interfering with bubble expansion, reducing the extensibility of cell walls and causing premature rupture of steam cells in the extrudate microstructure resulting in decreased radial expansion (Guy 1985).

The bulk density values increased as radial expansion decreased (Table 4.2). This is due to the increased density of the extrudate microstructure as the radial expansion decreases. Increased bulk density as radial expansion decreased was also observed by other researchers (Hsieh et al 1991; Lue et al 1991; Jin et al 1995).

Jin et al (1995) demonstrated that breaking strength of extruded materials is related to radial expansion. The results from the present study showed that breaking strength increased as radial expansion ratios decreased (Table 4.2). The increase in breaking strength is presumed to be due to the thickening of the cell walls and decrease in air cell size. This may provide increased strength to the extrudate microstructure and increased resistance to fracture.

4.2.2 Water Absorption Index and Water Solubility Index

Water absorption index (WAI) and water solubility index (WSI) for okara, non-extruded flour, non-extruded formulations A and B, extruded flour/water at the high shear/high temperature condition, and extruded formulations A and B for all conditions are listed in Table 4.3. Water absorption and water solubility indices were increased for all samples after extrusion. The WAI was most likely increased after the extrusion process due to the pregelatinization of starch and

Table 4.3 Water Absorption Index (WAI) and Water Solubility Index (WAI) for Non-Extruded and Extruded Products

Extrusion Conditions[1]	WAI	WSI (%)
Okara		
Non-Extruded	4.74	7.33
Flour		
Non-Extruded	1.91	4.84
Flour/Water (HS/HT)	7.64	8.25
Formulation A		
Non-Extruded	2.09	5.83
LS/LT	6.54	9.20
LS/HT	6.90	9.63
HS/LT	6.70	9.56
HS/HT	7.55	9.91
Formulation B		
Non-Extruded	2.13	6.12
LS/LT	5.48	7.21
LS/HT	6.23	9.07
HS/LT	6.72	8.47
HS/HT	6.72	8.50

[1] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

an increased ability of fiber to absorb water compared to the respective non-extruded sample. Pregelatinzed starch can absorb more water at ambient temperature than raw intact starch and the extrusion process may break fiber into smaller particles that have a greater surface area and thus are able to absorb more water. The increased WSI contents were likely caused by increased amounts of small soluble particles as a result of heat and shear from the extrusion process, as described by Jin et al (1995) after extruding corn meal at various extrusion conditions.

The WAI for the extruded samples of both formulations A and B had increasing trends as extrusion conditions became more severe. The increase in WAI as extrusion conditions become more severe is contradictory to the findings of other researchers who found WAI decreased with increased severity of extrusion conditions (Jin et al 1995; Qian and Ding 1996). On the other hand, Ralet et al (1990) and Wang et al (1993) found WAI of wheat bran to exhibit an increasing trend as extrusion conditions became more severe, as observed in the present study. Ralet et al (1990) proposed that interstices in fiber are emptied of soluble material during extrusion and can quickly fill with water. Wang et al (1993) proposed that a higher percentage of enzyme-susceptible (gelatinized) starch in extruded samples contributed to higher water absorption. Variation in extrusion conditions, types of raw materials, and combinations of raw materials may account for the differences in WAI values observed in various studies. The high fiber content of okara (53.25 % d.b.) may have been the major factor in the WAI values observed in the present study, both prior to and after extrusion.

Water solubility indices increased as severity of extrusion conditions increased for both formulations A and B. This trend was observed by other researchers (Ralet et al 1990; Jin et al 1995). The increase in soluble components, indicated by the WSI values in the present study, were a result of the increase in temperature and mechanical shear as extrusion conditions became more severe. The soluble particles may be derived from the soluble starch component, smaller protein molecules, and other soluble components, most likely soluble fiber of the okara.

4.2.3 Pasting Properties

Pasting properties obtained from the Rapid Visco-Analyser (RVA) for non-extruded flour, formulations A and B, and the extruded flour/water sample at the high shear/high temperature condition are listed in Table 4.4. Decreasing trends were observed for peak viscosity, breakdown, setback, and final viscosity as fiber content increased in non-extruded formulation A to formulation B. Viscosity increases are mainly attributed to swelled starch granules coming into contact with each other (Rasper 1980). Continued heating causes starch granules to rupture and leach out soluble amylose and amylopectin; the peak viscosity is the equilibrium point at which swelling of starch and leaching out of soluble starch components occur at equal rates (Rasper 1980). The decreasing trend in peak viscosity coinciding with increasing fiber contents of the non-extruded samples may be due to competition for water between starch and fiber components making less water available for starch swelling and causing peak viscosity to

Table 4.4 Rapid Visco-Analyser Data[1] for Non-Extruded Formulations A and B, Non-Extruded Flour, and Extruded Flour/Water at the HS/HT[2] Condition

Sample	Peak Viscosity	Breakdown	Setback	Final Viscosity
Formulation A	431	282	214	361
Formulation B	401	273	102	319
Flour	489	372	236	403
Flour/Water(HS/HT)	482	451	46	77

[1] All data expressed in Rapid Visco Units (RVU).

[2] HS/HT = high shear/high temperature extrusion condition.

decrease. Ryu et al (1993) observed decreased peak viscosity in wheat flour based samples when various baking ingredients that competed with starch for water were added. A proportionally decreased starch content as fiber content was increased may also have contributed to the decreasing trend in peak viscosity in the present study.

Breakdown represents the ease with which a sample can be broken down by mechanical shear while constant temperature and stirring are maintained. Small breakdown values represent more resistance to mechanical shear. The decreasing trend in breakdown (Table 4.4) as fiber content increased in the non-extruded samples may be due to the decreased starch contents and/or the resistance to mechanical shear under the RVA conditions. The setback and final viscosity values represent the reformation of the starch matrix as the sample is cooled and is referred to as retrogradation. As retrogradation occurs, the reformation of polymer-polymer bonds produce an increase in viscosity. There was a decreasing trend for both setback and final viscosity values (Table 4.4) as fiber content increased in the non-extruded samples of formulations A and B.

Figure 4.1 depicts viscograms of non-extruded flour and the extruded flour/water sample at the high shear/high temperature condition. There is a distinct shift of the peak to the left for the extruded sample as compared to the non-extruded flour sample. This was observed for all extruded samples compared to their respective non-extruded samples. The shift in the peak is probably due to the pregelatinization of starch in the extruded samples which enables starch granules to imbibe water and swell without the addition of heat.

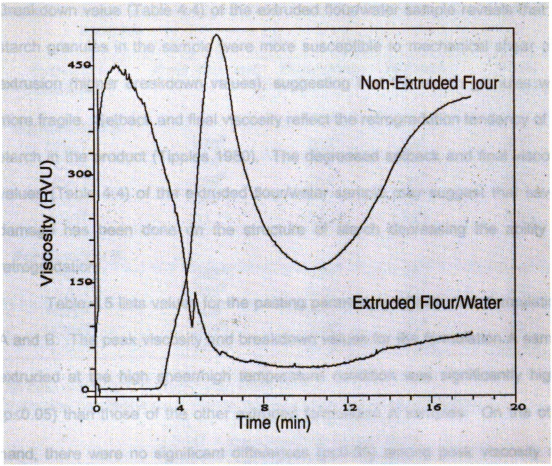


Figure 4.1 Rapid Viscograms for Non-Extruded Flour Sample and Extruded Flour/Water Sample at the High Shear/ High Temperature Condition.

Breakdown value (Table 4.4) of the extruded flour/water sample reveals that the starch granules in the sample were more susceptible to mechanical shear after extrusion (higher breakdown values), suggesting that the starch granules were more fragile. Setback and final viscosity reflect the retrogradation tendency of the starch in the product (Tipples 1980). The decreased setback and final viscosity values (Table 4.4) of the extruded flour/water sample may suggest that severe damage has been done on the structure of starch decreasing the ability for retrogradation.

Table 4.5 lists values for the pasting parameters of extruded formulations A and B. The peak viscosity and breakdown values for the formulation A sample extruded at the high shear/high temperature condition was significantly higher ($p \leq 0.05$) than those of the other extruded formulation A samples. On the other hand, there were no significant differences ($p \leq 0.05$) among peak viscosity and breakdown values of formulation B, except for the sample extruded at the low shear/low temperature condition, which was significantly higher. The peak viscosities for formulation B had a decreasing trend as extrusion conditions became more severe. The setback and final viscosity values for the extruded formulation A samples varied in their significant differences, however, no significant differences were observed among the extruded formulation B samples, regardless of the extrusion conditions.

The results for the RVA study of the extruded formulations are difficult, at best, to attribute to any one component of the two formulations in this study. Rapid visco-analyser results of extruded products may be affected by starch,

Table 4.5 Rapid Visco-Analyser Data[1] for Extruded Formulations A and B Samples

Extrusion Conditions[2]	Peak Viscosity	Breakdown	Setback	Final Viscosity
Formulation A				
LS/LT	270^{B}	232^{B}	53^{A}	90^{A}
LS/HT	315^{B}	284^{B}	45^{B}	77^{C}
HS/LT	289^{B}	254^{B}	54^{A}	89^{AB}
HS/HT	449^{A}	417^{A}	50^{AB}	82^{BC}
LSD[3]	111.31	112.92	5.88	8.05
Formulation B				
LS/LT	383^{A}	324^{A}	65^{A}	109^{A}
LS/HT	285^{B}	246^{B}	53^{A}	92^{A}
HS/LT	304^{B}	266^{AB}	58^{A}	97^{A}
HS/HT	254^{B}	219^{B}	51^{A}	86^{A}
LSD[3]	54.89	68.29	32.48	25.25

[1] All data expressed in Rapid Visc Units (RVU)

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

[3] Least Significant Difference: samples sharing the same letter within a column for Formulation A or Formulation B are not significantly different at $\alpha=.05$

starch composition, starch-lipid interactions, starch-protein matrix, and soluble fiber components (Whalen et al 1997). These various effects become increasingly complicated when taking into account not only the possible interactions of starch, protein, lipids, and fiber, but also the difference in the sources of these components (soft wheat flour and okara). Gomez and Aquilera (1984) proposed a model of starch degradation during extrusion in which starch exists in three main states (raw, gelatinized, and dextrinized) and three intermediate states (mechanically damaged, free polymers, and oligosacharides). They observed decreased peak viscosity on amylograms as the content of dextrinized starch increased in extruded corn starch samples. Ryu et al (1993) demonstrated that adding shortening powder in extruded wheat flour products decreased both peak viscosity and breakdown values on rapid viscograms. They suggested that lipid-amylose complexes caused inhibition of starch hydration resulting in decreased swelling of starch granules decreasing the viscosity and susceptibility to mechanical shear. Whalen et al (1997) suggested that starch-protein matrix formation in extruded corn flour resulted in increased resistance to breakdown and high setback values in rapid viscograms. It is presumed that fiber, and soluble fiber in particular, can result in decreased peak viscosity due to competition with starch for water. Determining what particular interactions may have occurred for both formulations A and B and their effects on the rapid viscograms were beyond the scope of the present study.

4.2.4 Specific Mechanical Energy

The specific mechanical energy (SME) for the extruded formulations A and B and the extruded flour/water sample at the high shear/high temperature condition are listed in Table 4.6. The SME was higher overall for formulation A than for formulation B. This was expected due to the lower moisture content and greater proportion of flour in formulation A than formulation B resulting in a higher apparent viscosity for formulation A. The higher viscosity material required more energy to turn the screws in the extruder and this was reflected in higher SME values. Martelli (1993) determined that SME increased with increasing dough mass viscosity. The SME decreased as temperature increased from the low to the high temperature profile for both screw configurations and for both formulations A and B. The decreased SME can be attributed to a decrease in the viscosity of the material in the barrel of the extruder as the temperature was increased. Weidmann (1990) observed a similar effect with SME values decreasing when barrel temperature was increased during the extrusion of wheat flour.

Changing screw configuration from low to high shear was expected to increase SME due to the slowing down of the forward flow of material in the barrel of the extruder and the increasing energy required to turn the screws of the extruder, however, the opposite effect was observed in the present study. The unexpected decrease may have been a result of the fiber in the okara. The high shear configuration may have broken the fiber into smaller fragments, increasing the surface area of the fiber and its lubricating effect, and decreasing the torque

Table 4.6 Torque, Feed Rate, and Specific Mechanical Energy (SME) Data for Extruded Formulations A and B and Extruded Flour/Water at the HS/HT Condition

Sample	Extrusion Conditions[1]	Torque (%)	Feed Rate (kg/hr)	SME (kW h/kg)
Formulation A	LS/LT	42.5	1.95	0.0876
Formulation A	LS/HT	37.5	1.95	0.0773
Formulation A	HS/LT	40	1.95	0.0824
Formulation A	HS/HT	32.5	1.95	0.0670
Formulation B	LS/LT	35	3.71	0.0378
Formulation B	LS/HT	32.5	3.71	0.0351
Formulation B	HS/LT	35	3.71	0.0378
Formulation B	HS/HT	30	3.71	0.0324
Flour/Water	HS/HT	30	3.00	0.0399

[1] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

values required. The SME calculation is based on percent torque values and the motor speed (Brent et al 1997); if torque decreases while the screw speed and material feed rate are maintained constant, the SME will decrease. The torque value for extruded flour/water at the high shear/high temperature condition did not differ from that of formulation B and was only 2.5% lower than formulation A extruded at the same conditions, however, the SME for the flour/water sample was not similar to SME values for either formulation A or B. This is due to the feed rate of the flour/water sample which was 3.0 kg/hr compared to 1.95 kg/hr for formulation A and 3.71 kg/hr for formulation B.

4.3 Dietary Fiber Contents in Non-Extruded and Extruded Samples

The total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents of the non-extruded samples are listed in Table 4.7. The TDF contents of non-extruded formulations A and B increased 247 percent and 262 percent, respectively, compared to TDF contents of non-extruded flour, as a result of the combination of okara and flour. This demonstrates that okara can be used to enrich the TDF content of soft wheat flour. The IDF contents of the two formulations increased, as expected, due to the combination of okara and flour. Formulation A resulted in a slight increase in SDF content due to the combination of okara with flour, however formulation B which was also expected to increase in SDF content actually decreased slightly. The decrease in SDF content for formulation B was within the acceptable range of variation for

Table 4.7 Insoluble, Soluble, and Total Dietary Fiber Contents[1] of Non-Extruded Okara, Formulations A and B, and Comparison Between Non-Extruded Flour and Extruded Flour/Water at the HS/HT[2] Condition

Sample	% Insoluble	% Soluble	% Total
Okara	43.27	9.98	53.25
Formulation A	7.66	2.08	9.74
Formulation B	8.84	1.46	10.30
Flour	2.22^{A}	1.71^{A}	3.93^{A}
Flour/Water (HS/HT)	1.38^{B}	2.13^{B}	3.50^{B}
LSD[3]	0.49	0.35	0.32

[1] All data expressed on a dry basis

[2] Extrusion condition used (screw configuration/temperature profile):
HS/HT = high shear/high temperature

[3] Least Significant Difference: samples sharing the same letter within a column are not significantly different at α=.05

the dietary fiber assay (personal communication, Bennink, M.R., Michigan State University).

The TDF, IDF, and SDF contents were significantly different ($p \leq 0.05$) for the flour/water sample after extrusion at the high shear/high temperature condition (Table 4.7). Wang et al (1993) also observed decreased TDF and IDF contents and increased SDF contents after extrusion of whole wheat.

Table 4.8 lists the TDF, IDF, and SDF contents for non-extruded and extruded formulations A and B. The TDF content for the formulation A sample extruded at the low shear/low temperature condition was significantly lower ($p \leq 0.05$) than that of the non-extruded formulation A sample. This was the only extruded sample to significantly differ from the non-extruded formulation A sample. Based on the premise that the low shear/low temperature condition is the most benign of the extrusion conditions used in the present study and the least likely candidate to cause a decrease in TDF content, the decreased TDF content may be attributed to variation in sampling. The TDF content for the formulation B sample extruded at the high shear/low temperature condition was the only one significantly different (higher; $p \leq 0.05$) than that of the non-extruded formulation B sample. Englyst et al (1995) suggested the increase in TDF contents of starchy foods, such as those containing white flour, after heat processing is due to the formation of enzyme-resistant starch which takes place during the cooling of the product and is a result of retrogradation.

The IDF contents of the extruded products of both formulations A and B decreased for all extrusion conditions compared to their respective non-extruded

Table 4.8 Insoluble, Soluble, and Total Dietary Fiber Contents[1] for Non-Extruded and Extruded[2] Formulations A and B

Sample	% Insoluble	% Soluble	% Total
Formulation A			
Non-Extruded	7.66^{A}	2.08^{B}	9.74^{A}
LS/LT	6.42^{B}	2.82^{BA}	9.24^{B}
LS/HT	6.63^{B}	3.16^{A}	9.79^{A}
HS/LT	5.81^{C}	3.69^{A}	9.50^{AB}
HS/HT	5.71^{C}	3.72^{A}	9.43^{AB}
LSD[3]	0.56	0.98	0.48
Formulation B			
Non-Extruded	8.84^{A}	1.46^{D}	10.30^{B}
LS/LT	8.50^{A}	2.22^{C}	10.72^{B}
LS/HT	7.31^{B}	3.19^{AB}	10.50^{B}
HS/LT	8.31^{A}	3.09^{B}	11.40^{A}
HS/HT	6.72^{C}	3.64^{A}	10.36^{B}
LSD[3]	0.56	0.48	0.43

[1] All data expressed on a dry basis

[2] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

[3] Least Significant Difference (LSD): samples sharing the same letter within a column for Formulation A or Formulation B are not significantly different at α=.05

formulation. The IDF contents were decreased significantly ($p \leq 0.05$) at all extrusion conditions for formulation A, however, only the formulation B samples extruded at the low shear/high temperature and high shear/high temperature conditions resulted in significant decreases ($p \leq 0.05$). Changing the temperature profile from low to high temperature had a significant effect ($p=0.0007$) on the IDF contents for the extruded formulation B samples; on the other hand, changing screw configuration from low to high shear had a significant effect ($p=0.0086$) on the IDF content of the extruded formulation A samples. It appears that the extrusion conditions required to affect the IDF contents are formulation dependent.

The SDF contents for extruded formulation A and B samples increased significantly ($p \leq 0.05$) for all extrusion conditions compared to their respective non-extruded formulations with the exception of the formulation A sample extruded at the low shear/low temperature condition. The nonsignificant increase for the low shear/low temperature extruded formulation A sample may have been significant if the TDF content for this sample were not significantly decreased. No one extrusion parameter had a significant effect on the SDF content for extruded formulation A samples, however, both temperature and screw configuration had independent but significant effects ($p=0.0067$ and $p=0.0104$, respectively) on the SDF contents of formulation B.

In the present study, the IDF contents decreased and the SDF contents increased after extrusion for flour/water and formulations A and B as compared to their respective non-extruded samples. The IDF contents for formulations A and

B tended to decrease as extrusion conditions became more severe; on the other hand, the SDF contents tended to increase. Other researchers reported similar findings (Robertson and Eastwood 1981; Bjorck et al 1984; Anderson and Clydesdale 1986; Caprez et al 1986; Siljestrom et al 1986; Ralet et al 1990; Wang et al 1993; Qian and Ding 1996). Wang et al (1993) suggested that the disruption of covalent and non-covalent bonds in the carbohydrate and protein moieties, leading to smaller soluble components results in increased SDF and decreased IDF contents during extrusion. Recently Qian and Ding (1996) suggested that decreased IDF and increased SDF contents after extrusion are a result of the degradation of the amorphous regions and the compression of the crystalline regions in fiber. In general there were no significant changes in total dietary fiber contents after extrusion with the exception of the low shear/low temperature formulation A sample and the high shear/low temperature formulation B samples. Various effects on TDF contents due to extrusion have been observed. Bjorck et al (1984), Ostergard (1989), and Lintas et al (1995) observed increased TDF contents and attributed them to formation of enzyme-resistant starch. Siljestrom et al (1986) and Schweitzer and Reimann (1986) reported no changes in TDF contents after extrusion. Wang et al (1993) observed decreased TDF contents after extrusion and attributed this to components being broken down into smaller soluble components. The variation in results observed for TDF contents after extrusion are most likely due to the inherent differences in the native compositions of fibers from different sources and to variations in the extrusion conditions used.

4.4 Isoflavone Contents in Non-Extruded and Extruded Samples

There were no detectable levels of isoflavones in the non-extruded flour and the extruded flour/water sample at the high shear/high temperature extrusion condition (data not shown), based on spectroscopy at 260 nm. Six isoflavone compounds were identified in okara and the non-extruded and extruded samples of formulations A and B, confirming that okara can be used to enrich the isoflavone contents of soft wheat flour. The six isoflavone compounds were, in order of elution: daidzin, genisitin, malonyl daidzin, malonyl genistin, acetyl genistin, and genistein (Figure 4.2). The levels of detected isoflavones and total detectable isoflavones for non-extruded samples of formulations A and B are listed in Table 4.9.

The glucoside, malonyl, and acetyl forms of daidzin and genistin combined represent 90-98 percent of the total detectable isoflavones in okara and the non-extruded formulations A and B. Wang and Murphy (1994) and Anderson and Wolf (1995) also found greater than 90 percent of the total detectable isoflavones to be a combination of the glucoside, malonyl, and acetyl isoflavones in soy and soy products. Malonyl daidzin and malonyl genistin were the most abundant of the isoflavone compounds in okara and the non-extruded samples of formulations A and B. There was little difference in the levels of total detectable isoflavones among the samples. The two formulations exhibited changes in the distribution of the isoflavones in comparison to the okara sample. Non-extruded samples of formulations A and B had decreased levels of daidzin, genistin, malonyl daidzin, and malonyl genistin and increased acetyl genistin and genistein levels as

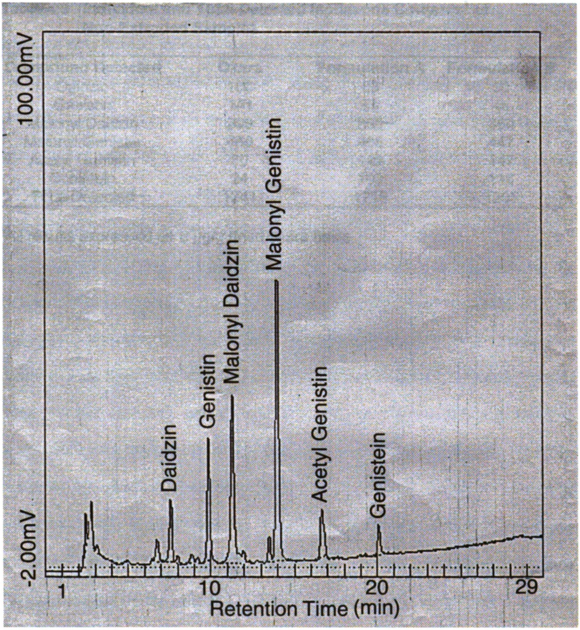


Figure 4.2 HPLC Chromatogram for Okara Depicting Detected Isoflavones in Order of Elution.

Table 4.9 Individual and Total Detected Isoflavone Contents[1] of Non-Extruded Samples

Compound Detected	Okara	Formulation A	Formulation B
Daidzin	108	83	90
Genistin	141	65	45
Malonyl Daidzin	399	360	360
Malonyl Genistin	489	465	447
Acetyl Genistin	80	142	147
Genistein	24	103	116
Total Detected	1241	1219	1205

[1] All results expressed on a μg/g dried okara basis

compared to the okara sample. A possible explanation for the redistribution of isoflavones in the two formulations may be an interaction between okara and flour and/or fermentation of the formulations during mixing that may have caused the conversion of the glucoside and malonyl isoflavones to the acetyl and aglycone isoflavones via a hydrolysis reaction with glucosidases already present in the formulations. Wang and Murphy (1994) observed the occurrence of similar distributional changes in fermented soy products.

Tables 4.10 and 4.11 list levels of the six detectable isoflavones and the total detectable isoflavones for the non-extruded and extruded formulations A and B samples, respectively. Total detectable isoflavone levels for both formulations A and B decreased after extrusion for all extrusion conditions compared to their respective non-extruded formulation. The total detectable levels of isoflavones in formulation A samples extruded at the low shear/high temperature and the high shear/low temperature conditions decreased significantly ($p \leq 0.05$). The total detectable levels of isoflavones in formulation B samples extruded at the low shear/high temperature and high shear/high temperature conditions were decreased significantly ($p \leq 0.05$). The decreased total detectable isoflavone levels after extrusion may be due to degradation of the isoflavone compounds or conversion to non-detected isoflavone compounds as a result of the heat and shear generated from the extrusion process.

The glucoside, malonyl, and acetyl forms of the isoflavones represent 96-98 percent of the total detected isoflavones after extrusion of the formulation A and B samples. This is in agreement with other findings (Wang and Murphy

Table 4.10 Individual and Total Detected Isoflavone Contents[1] for Non-Extruded and Extruded[2] Formulation A Samples

Compound Detected	Non-Extruded	LS/LT	LS/HT	HS/LT	HS/HT	LSD[3]
Daidzin	83^{D}	171^{BC}	198^{B}	155^{C}	261^{A}	34.112
Genistin	66^{C}	177^{B}	202^{B}	163^{B}	298^{A}	86.373
Malonyl Daidzin	360^{A}	302^{B}	145^{C}	263^{B}	131^{C}	47.357
Malonyl Genistin	466^{A}	327^{B}	150^{D}	267^{C}	146^{D}	56.774
Acetyl Genistin	142^{B}	121^{B}	238^{A}	119^{B}	212^{A}	36.850
Genistein	104^{A}	36^{B}	36^{B}	32^{B}	29^{B}	17.585
Total Detected	1219^{A}	1133^{AB}	963^{C}	1002^{B}	1072^{AB}	151.490

[1] All results expressed on a μg/g dried okara basis

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

[3] Least Significant Difference (LSD): samples sharing the same letter in a row are not significantly different at α=.05

Table 4.11 Individual and Total Detected Isoflavone Contents[1] for Non-Extruded and Extruded[2] Formulation B Samples

Compound Detected	Non-Extruded	LS/LT	LS/HT	HS/LT	HS/HT	LSD[3]
Daidzin	90^{B}	153^{A}	201^{A}	192^{A}	198^{A}	47.829
Genistin	45^{B}	188^{A}	180^{A}	214^{A}	226^{A}	116.86
Malonyl Daidzin	361^{A}	335^{A}	155^{C}	273^{B}	138^{C}	63.499
Malonyl Genistin	448^{A}	358^{B}	181^{C}	313^{B}	155^{C}	57.709
Acetyl Genistin	147^{A}	104^{B}	181^{A}	104^{B}	180^{A}	41.814
Genistein	116^{A}	36^{B}	35^{B}	32^{B}	40^{B}	27.398
Total Detected	1205^{A}	1175^{A}	935^{B}	1133^{A}	967^{B}	84.039

[1] All results expressed on a μg/g dried okara basis

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

[3] Least Significant Difference (LSD): samples sharing the same letter in a row are not significantly different at α=.05

1994; Anderson and Wolf 1995). Extrusion of formulations A and B resulted in distributional changes in the detected isoflavone compounds compared to their respective non-extruded formulation. Distributional changes among isoflavone compounds after processing have been reported (Pratt and Birac 1970; Matsuura and Obata 1993: Lampe et al 1994; Wang and Murphy 1994; Wang and Murphy 1996). The glucosides, daidzin and genistin, significantly increased ($p \leq 0.05$) after extrusion for both formulations A and B regardless of the extrusion conditions. Wang and Murphy (1984) also observed increased glucoside isoflavone contents in soy protein isolate compared to intact soybeans from the same source. A possible cause for this change could be a loss of the malonyl group resulting in the respective glucoside form of the isoflavone. The malonyl group may have been cleaved off as a result of heat and/or shear generated by the extrusion process.

Malonyl daidzin and malonyl genistin contents decreased after extrusion under all extrusion conditions for both formulations A and B. Decreases in malonyl daidzin and malonyl genistin contents were significant ($p \leq 0.05$) at all extrusion conditions except for the malonyl daidzin content of formulation B extruded at the low shear/low temperature condition. The largest decreases in both malonyl daidzin and malonyl genistin occurred at the high temperature extrusion conditions for both formulations. The decreased malonyl genistin contents at the high temperature conditions were offset by significantly increased ($p \leq 0.05$) acetyl genistin contents for formulation A and non-significant increases for formulation B after extrusion. Farmakalidis and Murphy (1985) reported that decreased malonyl isoflavone contents were offset by increased acetyl isoflavone contents after toasting defatted soy flakes. In addition, Wang and Murphy (1984) proposed that malonyl isoflavones may be converted into their respective acetyl isoflavones via the decarboxylation of the malonyl isoflavone. Malonyl isoflavones are heat labile and unstable at temperatures exceeding 100°C (Pratt and Birac 1979). Temperatures used in the present study exceeded 100°C during extrusion using both the low and high temperature profiles and may explain the decreased malonyl daidzin and malonyl genistin contents in the extruded formulation A and B samples.

The aglycone, genistein, was significantly decreased ($p \leq 0.05$) after extrusion regardless of extrusion conditions for both formulations A and B. No literature was found to support decreased genistein contents after extrusion processing, however, it is presumed that genistein is either degraded or converted to forms of isoflavones nondetectable at 260 nm.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two formulations of wet okara and soft wheat flour (A and B) were extruded at four screw configuration/temperature profile conditions. Extrusion conditions affected the physical characteristics of the extrudates. Radial expansion ratio decreased, and bulk density and breaking strength values increased as extrusion conditions became more severe for both formulations A and B. Increasing trends were observed for both water absorption index and water solubility index for both formulations as severity of extrusion conditions increased. Pasting parameters for extruded products showed the complexity of the formulations. Peak viscosity and breakdown values resulted in an increasing trend for formulation A as the extrusion conditions became more severe; on the other hand, formulation B (which had proportionally more okara and less flour by weight) resulted in an opposite trend. Low setback and final viscosity values were observed for all extruded samples demonstrating that the starch granules in the samples had sustained irreparable damage. Specific mechanical energy values tended to decrease as extrusion conditions became more severe for both formulations A and B.

Combining okara with soft wheat flour resulted in increased protein, dietary fiber, and isoflavone contents compared to soft wheat flour alone. The extrusion process had little effect on the protein contents of the extrudates of formulations

A and B. Variable effects were observed for the total dietary fiber contents of the two formulations' extrudates. The extrusion process consistently decreased the insoluble dietary fiber content and increased the soluble dietary fiber content of both formulations as the extrusion conditions became more severe. Total detectable isoflavone contents were decreased at all extrusion conditions compared to their respective non-extruded formulation. Changing the extrusion temperature profile from low to high resulted in decreased malonyl daidzin, malonyl genistin, and genistein contents, while acetyl genistin content increased for both formulations A and B.

The overall results demonstrate that by utilizing a novel twin screw extrusion process to cook, sterilize, and remove the major portion of water, wet okara could successfully be used to make extruded products. Changing the extrusion conditions resulted in varied physical characteristics for the extruded products of the two formulations. Combining wet okara with soft wheat flour enriched the protein, dietary fiber, and isoflavone contents. The extrusion process had little effect on the protein content of the extrudates, however, dietary fiber and isoflavone contents were affected. Extrusion decreased insoluble dietary fiber and increased soluble dietary fiber contents. The extrusion process decreased total detectable isoflavone contents and altered the distribution of the six detectable isoflavones.

CHAPTER VI

SUGGESTIONS FOR FUTURE RESEARCH

Wet okara was successfully used to produce extruded products and enrich the protein, dietary fiber, and isoflavone contents of soft wheat flour, making it a desirable component for use in commercially extruded products. Commercial formulations, however, contain other ingredients such as sugar and shortening that not only complicate the extrusion process, but can have adverse effects on extrudate color, texture, and flavor. In order for the use of wet okara to be considered by product manufacturers, it is necessary to develop a study that demonstrates wet okara can be used in a scaled-up manufacturing process to successfully produce commercially acceptable extruded products.

The first phase of the study would be to develop formulations utilizing not only wet okara, but also sugar and shortening at levels used in commercial formulations, scale up the extrusion process from laboratory to pilot scale, and extrude these formulations under various conditions. A second phase of the study would include analyzing the physicochemical properties of the extruded products and comparing those properties to the phyicochemical properties of commercial products. This phase would also include microscopy analysis of the extrudates in order to study the microstructure. The microstructure is largely responsible for the textural characteristics of extruded products. The last phase of this study would be a sensory evaluation of the extruded products made with the wet okara in order to determine the consumer acceptability of the texture, flavor, and color of these products.

Utilizing wet okara in a commercial extrusion process would add value to this currently valueless by-product in North America. Furthermore, utilizing okara in commercial extruded products would provide consumers with a source of dietary fiber and isoflavones.

APPENDICES

APPENDIX I

Flour Characteristics Determined by [1]Farinograph Constant Flour Weight Method

Flour Weight (g)	Consistency (BU)	Water Absorption (%)	Arrival Time (minutes)	Stability (minutes)	Mixing Tolerance Index (BU)
48.08	510	50.80	1.00	1.00	90

[1] Flour weight for the farinograph constant flour weight method was adjusted for a 14% moisture basis. Consistency and Mixing Tolerance Index are expressed in Brabender Units (BU).

APPENDIX II

Raw Data[1] for Moisture Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Moisture 1 (%)	Moisture 2 (%)	Moisture 3 (%)
Okara			
Non-Extruded	77.77	77.66	77.57
Flour			
Non-Extruded	10.84	10.72	10.15
Flour/Water Rep 1(HS/HT)	3.93	4.17	4.14
Flour/Water Rep 2(HS/HT)	3.02	3.04	2.99
Formulation A			
Non-Extruded	32.50	34.19	32.23
LS/LT Rep 1	8.24	8.50	8.48
LS/LT Rep 2	6.98	7.24	7.21
LS/HT Rep 1	5.47	5.95	5.87
LS/HT Rep 2	7.08	7.03	7.13
HS/LT Rep 1	7.10	7.61	7.68
HS/LT Rep 2	4.27	4.37	4.64
HS/HT Rep 1	1.97	1.98	1.96
HS/HT Rep 2	1.78	1.77	1.75
Formulation B			
Non-Extruded	37.81	38.91	35.78
LS/LT Rep 1	6.70	6.97	7.12
LS/LT Rep 2	5.85	6.09	6.11
LS/HT Rep 1	6.27	6.67	6.76
LS/HT Rep 2	8.02	7.46	7.59
HS/LT Rep 1	4.24	4.21	4.26
HS/LT Rep 2	5.89	6.10	6.24
HS/HT Rep 1	4.52	5.16	5.03
HS/HT Rep 2	4.86	5.15	5.19

[1] All data for extruded samples were determined after the samples were dried and ground.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX III

Raw Data[1] for Protein Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Protein 1 (%)	Protein 2 (%)	Protein 3 (%)
Okara			
Non-Extruded	24.28	23.66	24.28
Flour			
Non-Extruded	8.56	8.74	8.32
Flour/Water Rep 1(HS/HT)	8.66	8.55	-
Flour/Water Rep 2(HS/HT)	8.68	8.19	-
Formulation A			
Non-Extruded	9.77	9.77	9.70
LS/LT Rep 1	9.68	9.61	-
LS/LT Rep 2	9.67	9.53	-
LS/HT Rep 1	9.73	9.80	-
LS/HT Rep 2	9.45	10.04	-
HS/LT Rep 1	10.01	9.70	-
HS/LT Rep 2	9.76	9.80	-
HS/HT Rep 1	10.10	9.72	-
HS/HT Rep 2	9.54	9.86	-
Formulation B			
Non-Extruded	10.50	10.46	10.35
LS/LT Rep 1	10.08	10.62	-
LS/LT Rep 2	10.26	10.69	-
LS/HT Rep 1	10.09	10.51	-
LS/HT Rep 2	10.70	10.25	-
HS/LT Rep 1	10.60	10.44	-
HS/LT Rep 2	10.35	10.16	-
HS/HT Rep 1	10.22	10.52	-
HS/HT Rep 2	10.44	10.34	-

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX IV

Raw Data[1] for Ash Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Ash 1 (%)	Ash 2 (%)
Okara		
Non-Extruded	4.04	4.07
Flour		
Non-Extruded	0.56	0.55
Flour/Water Rep 1(HS/HT)	0.57	0.56
Flour/Water Rep 2(HS/HT)	0.56	0.57
Formulation A		
Non-Extruded	0.96	0.96
LS/LT Rep 1	0.97	0.96
LS/LT Rep 2	0.98	0.97
LS/HT Rep 1	0.97	0.98
LS/HT Rep 2	0.98	0.97
HS/LT Rep 1	0.98	0.97
HS/LT Rep 2	0.97	0.98
HS/HT Rep 1	0.97	0.98
HS/HT Rep 2	0.97	0.98
Formulation B		
Non-Extruded	1.05	1.08
LS/LT Rep 1	1.10	1.09
LS/LT Rep 2	1.10	1.09
LS/HT Rep 1	1.10	1.08
LS/HT Rep 2	1.08	1.09
HS/LT Rep 1	1.08	1.08
HS/LT Rep 2	1.08	1.08
HS/HT Rep 1	1.06	1.09
HS/HT Rep 2	1.06	1.06

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX V

Raw Data[1] for Fat Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Fat 1 (%)	Fat 2 (%)	Fat 3 (%)
Okara			
Non-Extruded	9.90	9.73	9.87
Flour			
Non-Extruded	1.04	1.00	1.06
Flour/Water Rep 1(HS/HT)	0.99	0.96	1.03
Flour/Water Rep 2(HS/HT)	0.97	0.96	0.92
Formulation A			
Non-Extruded	1.60	1.56	1.61
LS/LT Rep 1	1.51	1.58	1.58
LS/LT Rep 2	1.54	1.61	1.58
LS/HT Rep 1	1.49	1.55	1.58
LS/HT Rep 2	1.54	1.49	1.58
HS/LT Rep 1	1.50	1.55	1.58
HS/LT Rep 2	1.57	1.50	1.58
HS/HT Rep 1	1.49	1.57	1.55
HS/HT Rep 2	1.55	1.54	1.48
Formulation B			
Non-Extruded	1.80	1.77	1.84
LS/LT Rep 1	1.71	1.73	1.76
LS/LT Rep 2	1.75	1.78	1.73
LS/HT Rep 1	1.70	1.77	1.73
LS/HT Rep 2	1.79	1.77	1.69
HS/LT Rep 1	1.71	1.77	1.80
HS/LT Rep 2	1.76	1.72	1.80
HS/HT Rep 1	1.74	1.78	1.69
HS/HT Rep 2	1.70	1.79	1.77

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX VI

Raw Data[1] for Water Absorption Index (WAI) Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	WAI 1	WAI 2
Okara		
Non-Extruded	4.74	4.74
Flour		
Non-Extruded	1.90	1.91
Flour/Water Rep 1(HS/HT)	7.62	7.48
Flour/Water Rep 2(HS/HT)	7.73	7.71
Formulation A		
Non-Extruded	2.08	2.09
LS/LT Rep 1	6.55	6.60
LS/LT Rep 2	6.52	6.50
LS/HT Rep 1	6.95	6.93
LS/HT Rep 2	6.86	6.86
HS/LT Rep 1	6.30	6.65
HS/LT Rep 2	7.14	6.72
HS/HT Rep 1	7.60	7.38
HS/HT Rep 2	7.65	7.58
Formulation B		
Non-Extruded	2.12	2.14
LS/LT Rep 1	5.39	5.43
LS/LT Rep 2	5.53	5.55
LS/HT Rep 1	6.35	6.24
LS/HT Rep 2	6.23	6.11
HS/LT Rep 1	6.87	6.73
HS/LT Rep 2	6.66	6.62
HS/HT Rep 1	6.87	6.71
HS/HT Rep 2	6.62	6.67

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX VII

Raw Data[1] for Water Solubility Index (WSI) Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	WSI 1 (%)	WSI 2 (%)
Okara		
Non-Extruded	7.49	7.16
Flour		
Non-Extruded	4.80	4.88
Flour/Water Rep 1(HS/HT)	8.35	8.33
Flour/Water Rep 2(HS/HT)	8.35	7.98
Formulation A		
Non-Extruded	5.83	5.83
LS/LT Rep 1	9.40	9.15
LS/LT Rep 2	9.21	9.03
LS/HT Rep 1	10.09	9.75
LS/HT Rep 2	9.36	9.33
HS/LT Rep 1	8.21	8.75
HS/LT Rep 2	10.39	10.89
HS/HT Rep 1	9.36	9.92
HS/HT Rep 2	10.15	10.21
Formulation B		
Non-Extruded	6.12	6.11
LS/LT Rep 1	6.83	6.97
LS/LT Rep 2	7.46	7.57
LS/HT Rep 1	9.13	9.02
LS/HT Rep 2	8.85	9.26
HS/LT Rep 1	9.23	8.88
HS/LT Rep 2	8.05	7.73
HS/HT Rep 1	10.06	9.59
HS/HT Rep 2	8.25	8.10

[1] All data expressed on a dry basis.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX VIII

Raw Data for the Radial Expansion Ratio[1] Analysis of the Extruded Samples

Extrusion Conditions[2]	1	2	3	4	5	6	7	8	9	10	Avg	Ratio
Flour												
HS/HT Rep 1	4.6	4.5	4.6	4.8	4.8	4.7	4.5	4.8	4.8	4.7	4.68	1.56
HS/HT Rep 2	4.7	4.7	4.8	4.5	4.8	4.8	4.9	4.7	4.5	4.6	4.70	1.57
Formulation A												
LS/LT Rep 1	4.2	4.0	3.8	4.1	3.7	3.8	3.5	3.8	3.5	3.5	3.79	1.26
LS/LT Rep 2	3.7	3.9	3.6	3.8	4.1	3.7	3.9	3.6	3.5	3.5	3.73	1.24
LS/HT Rep 1	3.0	3.2	3.4	3.4	3.0	3.5	3.1	3.2	3.2	3.2	3.22	1.07
LS/HT Rep 2	3.3	3.3	3.4	3.0	3.1	3.2	3.4	3.3	3.2	3.0	3.22	1.07
HS/LT Rep 1	2.9	3.1	3.0	2.9	3.1	3.2	3.3	3.0	2.8	3.1	3.04	1.01
HS/LT Rep 2	3.0	3.0	3.1	2.9	2.8	3.1	3.0	2.9	3.2	2.9	2.99	1.00
HS/HT Rep 1	3.0	2.7	2.8	3.1	3.2	3.2	3.1	2.8	3.0	2.9	2.98	0.99
HS/HT Rep 2	2.9	2.9	3.0	2.8	2.9	3.1	2.7	2.8	2.9	3.0	2.90	0.97
Formulation B												
LS/LT Rep 1	3.1	2.9	2.8	3.0	3.1	3.2	2.8	2.9	3.0	3.0	2.98	0.99
LS/LT Rep 2	3.1	2.9	2.9	3.0	2.9	2.9	3.0	3.0	2.9	2.8	2.94	0.98
LS/HT Rep 1	3.0	3.1	2.7	2.9	3.0	3.0	3.0	2.7	2.8	2.9	2.91	0.97
LS/HT Rep 2	2.6	3.0	2.9	3.1	2.8	3.0	2.9	2.8	2.8	2.7	2.86	0.95
HS/LT Rep 1	2.9	3.0	3.0	2.8	2.7	2.7	2.9	2.6	2.9	2.9	2.84	0.95
HS/LT Rep 2	2.8	2.6	3.0	2.9	2.9	2.8	2.9	2.6	2.9	2.8	2.82	0.94
HS/HT Rep 1	2.7	2.7	2.8	2.7	3.0	2.9	2.7	2.8	2.8	2.9	2.80	0.93
HS/HT Rep 2	2.6	2.8	2.8	2.8	2.7	2.8	2.8	2.9	2.7	2.8	2.77	0.92

[1] Radial Expansion Ratio was determined by averaging ten diametrical measurements (mm) of the extruded samples and then dividing by the diameter of the die opening (3 mm).

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX IX

Raw Data for Bulk Density (BD) Analysis of the Extruded Samples

Extrusion Conditions[1]	BD 1 (g/100ml)	BD 2 (g/100ml)	BD 3 (g/100ml)
Flour			
Flour/Water Rep 1(HS/HT)	48.6	48.2	48.3
Flour/Water Rep 2(HS/HT)	49.7	49.3	49.0
Formulation A			
LS/LT Rep 1	52.5	52.7	53.5
LS/LT Rep 2	49.7	49.9	49.4
LS/HT Rep 1	55.4	55.9	55.5
LS/HT Rep 2	55.4	56.0	55.7
HS/LT Rep 1	56.8	56.9	57.4
HS/LT Rep 2	57.0	57.7	57.2
HS/HT Rep 1	59.5	59.9	59.3
HS/HT Rep 2	58.9	59.3	59.3
Formulation B			
LS/LT Rep 1	55.6	56.3	55.7
LS/LT Rep 2	55.0	55.3	55.5
LS/HT Rep 1	56.2	56.0	55.8
LS/HT Rep 2	58.8	59.0	58.7
HS/LT Rep 1	60.9	61.2	60.8
HS/LT Rep 2	61.8	61.5	61.4
HS/HT Rep 1	63.8	64.2	63.6
HS/HT Rep 2	62.5	62.3	62.0

[1] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX X

Raw Data for Breaking Strength[2] (BS) Analysis of the Extruded Samples

Extrusion Conditions[2]	BS 1 (N)	BS 2 (N)	BS 3 (N)	BS 4 (N)	BS 5 (N)	SW[3] (g)	Avg BS (N/g)
Flour							
Flour/Water Rep 1	193.88	203.40	193.84	207.03	225.52	6.90	29.67
Flour/Water Rep 2	234.10	256.89	266.75	250.74	230.45	6.70	36.98
Formulation A							
LS/LT Rep 1	299.04	261.79	268.05	258.44	230.41	7.20	36.60
LS/LT Rep 2	293.98	301.01	274.85	312.36	289.26	7.30	40.31
LS/HT Rep 1	310.65	316.60	267.75	276.43	321.15	7.50	39.80
LS/HT Rep 2	336.43	302.39	297.02	277.92	264.30	7.50	39.42
HS/LT Rep 1	343.77	306.07	291.22	354.37	346.01	7.80	42.09
HS/LT Rep 2	329.01	352.93	304.42	377.79	352.49	8.00	42.99
HS/HT Rep 1	427.28	397.22	385.79	443.33	381.65	8.50	47.89
HS/HT Rep 2	400.36	343.91	341.59	387.81	371.57	8.70	42.42
Formulation B							
LS/LT Rep 1	340.79	362.44	350.44	344.47	365.10	8.50	41.49
LS/LT Rep 2	291.97	330.01	349.77	344.76	317.97	8.30	38.70
LS/HT Rep 1	406.99	445.44	420.36	426.08	380.87	8.90	46.74
LS/HT Rep 2	378.94	383.89	383.38	341.45	352.49	8.80	41.82
HS/LT Rep 1	457.96	399.76	437.96	416.48	394.95	8.90	46.74
HS/LT Rep 2	403.84	386.52	388.03	395.54	427.50	8.90	44.98
HS/HT Rep 1	420.08	462.46	402.15	417.25	486.44	9.20	47.57
HS/HT Rep 2	396.50	419.44	416.66	422.53	408.51	8.80	46.90

[1] Breaking strength was determined by averaging five breaking strength values (N) and then dividing by the sample weight (g).

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

[3] SW = Sample weight used to determine breaking strength

APPENDIX XI

Raw Data for Viscosity Properties[1] of the Extruded Samples

Extrusion Conditions[2]	Peak Viscosity		Breakdown		Setback		Final Viscosity	
Flour								
Non-Extruded	490	489	320	323	236	235	405	401
Flour/Water Rep 1(HS/HT)	443	451	410	420	45	47	77	78
Flour/Water Rep 2(HS/HT)	523	513	491	483	46	47	77	77
Formulation A								
Non-Extruded	434	428	281	271	213	193	360	320
LS/LT Rep 1	256	259	218	221	52	52	89	89
LS/LT Rep 2	277	288	240	251	54	54	90	91
LS/HT Rep 1	288	294	255	261	44	45	77	79
LS/HT Rep 2	339	343	308	312	46	45	76	76
HS/LT Rep 1	305	299	269	263	55	56	91	92
HS/LT Rep 2	273	280	239	247	53	52	86	85
HS/HT Rep 1	505	590	472	459	47	48	80	79
HS/HT Rep 2	409	393	376	362	52	53	85	84
Formulation B								
Non-Extruded	398	402	271	275	193	191	320	319
LS/LT Rep 1	401	394	352	346	70	71	118	119
LS/LT Rep 2	343	393	304	293	59	59	98	98
LS/HT Rep 1	292	297	246	255	48	48	85	84
LS/HT Rep 2	276	275	235	233	58	59	98	99
HS/LT Rep 1	283	284	245	248	57	59	95	95
HS/LT Rep 2	326	322	287	283	58	60	97	98
HS/HT Rep 1	249	244	214	209	51	51	87	86
HS/HT Rep 2	261	264	226	229	50	50	86	85

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[1] Viscosity properties were determined using the Rapid Visco-Analyser and were expressed in Rapid Visco Units (RVU); each test was run in duplicate.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX XII

Raw Data for Screw Speed, Torque, Feed Rate, and Specific Mechanical Energy (SME) for the Extruded Samples

Extrusion Conditions[1]	Screw Speed (rpm)	Torque (%)	Feed Rate (kg/hr)	SME (kW h/kg)
Flour				
Flour/Water Rep 1(HS/HT)	100	30	3.0	0.0399
Flour/Water Rep 2(HS/HT)	100	30	3.0	0.0399
Formulation A				
LS/LT Rep 1	100	40	1.95	0.0824
LS/LT Rep 2	100	45	1.95	0.0927
LS/HT Rep 1	100	35	1.95	0.0721
LS/HT Rep 2	100	40	1.95	0.0824
HS/LT Rep 1	100	40	1.95	0.0824
HS/LT Rep 2	100	40	1.95	0.0824
HS/HT Rep 1	100	30	1.95	0.0721
HS/HT Rep 2	100	35	1.95	0.0618
Formulation B				
LS/LT Rep 1	100	30	3.71	0.0324
LS/LT Rep 2	100	40	3.71	0.0432
LS/HT Rep1	100	30	3.71	0.0324
LS/HT Rep 2	100	35	3.71	0.0378
HS/LT Rep 1	100	35	3.71	0.0324
HS/LT Rep 2	100	35	3.71	0.0378
HS/HT Rep 1	100	30	3.71	0.0324
HS/HT Rep 2	100	30	3.71	0.0324

[1] Extrusion conditions used (screw configuration/temperature profile):
LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX XIII

Raw Data for Insoluble, Soluble, and Total Dietary Fiber[1] Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Insoluble (%)		Soluble (%)		Total (%)	
Okara						
Non-Extruded	43.24	43.30	10.06	9.89	53.30	53.19
Flour						
Non-Extruded	2.00	2.44	1.75	1.67	3.75	4.11
Flour/Water Rep 1(HS/HT)	1.38	1.23	2.11	2.30	3.49	3.53
Flour/Water Rep 2(HS/HT)	1.59	1.31	1.90	2.18	3.49	3.49
Formulation A						
Non-Extruded	7.73	7.59	1.86	2.29	9.59	9.88
LS/LT Rep 1	6.51	6.70	2.49	2.37	9.00	9.07
LS/LT Rep 2	6.31	6.13	3.06	3.37	9.37	9.50
LS/HT Rep 1	6.57	6.99	3.15	2.59	9.72	9.58
LS/HT Rep 2	6.52	6.44	3.41	3.47	9.93	9.91
HS/LT Rep 1	5.99	5.89	3.50	3.47	9.49	9.36
HS/LT Rep 2	5.60	5.73	3.93	3.87	9.53	9.60
HS/HT Rep 1	5.83	5.88	3.60	3.46	9.43	9.34
HS/HT Rep 2	5.61	5.50	3.78	4.04	9.39	9.54
Formulation B						
Non-Extruded	8.66	9.02	1.48	1.43	10.14	10.45
LS/LT Rep 1	8.47	8.47	2.33	2.30	10.80	10.77
LS/LT Rep 2	8.53	8.50	2.14	2.11	10.67	10.61
LS/HT Rep 1	7.44	7.56	3.11	2.98	10.55	10.54
LS/HT Rep 2	7.13	7.11	3.27	3.37	10.40	10.48
HS/LT Rep 1	8.23	8.30	2.95	2.93	11.18	11.23
HS/LT Rep 2	8.29	8.39	3.35	3.14	11.64	11.53
HS/HT Rep 1	6.96	6.91	3.44	3.47	10.40	10.38
HS/HT Rep 2	6.48	6.52	3.76	3.90	10.24	10.42

[1] All data expressed on a dry basis; all samples were run in duplicate.

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

APPENDIX XIV

Raw Data for Isoflavone[1] Analysis of the Non-Extruded and Extruded Samples

Extrusion Conditions[2]	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6	
Okara												
Non-Extruded	108	109	142	140	397	402	483	494	81	78	24	25
Formulation A												
Non-Extruded	82	84	68	63	358	362	462	469	142	142	104	103
LS/LT Rep 1	169	162	166	162	294	290	333	330	121	118	36	35
LS/LT Rep 2	182	170	193	186	313	308	326	318	123	122	36	36
LS/HT Rep 1	199	206	214	216	172	168	182	175	215	222	43	42
LS/HT Rep 2	190	197	191	184	116	122	122	121	256	257	67	64
HS/LT Rep 1	138	131	186	175	265	258	263	264	121	117	36	38
HS/LT Rep 2	169	178	208	200	273	246	278	262	121	116	33	35
HS/HT Rep 1	258	270	272	268	141	141	167	163	198	202	27	26
HS/HT Rep 2	264	151	336	316	120	124	124	131	223	223	22	21
Formulation B												
Non-Extruded	90	90	42	47	360	361	444	451	146	148	115	117
LS/LT Rep 1	144	133	165	192	302	317	348	347	121	117	48	47
LS/LT Rep 2	164	171	200	195	355	371	362	375	90	88	24	23
LS/HT Rep 1	178	184	182	179	175	167	215	211	167	168	42	40
LS/HT Rep 2	226	215	243	256	141	136	144	153	191	197	27	28
HS/LT Rep 1	191	192	222	218	277	282	316	317	118	114	38	37
HS/LT Rep 2	189	194	208	210	284	270	317	303	90	93	26	25
HS/HT Rep 1	177	184	217	220	157	161	165	160	190	190	46	45
HS/HT Rep 2	210	220	235	233	118	115	144	148	171	168	32	33

[1] All data expressed on a dry basis and units are μg isoflavone/g dry okara; all samples were run in duplicate. Peaks are (in order of elution):

Peak 1 = Genistin
Peak 2 = Daidzin
Peak 3 = Malonyl Daidzin
Peak 4 = Malonyl Genistin
Peak 5 = Acetyl Genistin
Peak 6 = Genistein

[2] Extrusion conditions used (screw configuration/temperature profile):

LS/LT = low shear/low temperature
LS/HT = low shear/high temperature
HS/LT = high shear/low temperature
HS/HT = high shear/high temperature

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