

CHARACTERIZATION OF EDIBLE BEAN FLOURS: PROPERTIES AND  
FUNCTIONALITY

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**DOCTOR OF PHILOSOPHY**

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## ABSTRACT

Consumption of pulses is considered part of a healthy diet. Therefore, the opportunity exists for development of new pulse-based ingredients. However, a better understanding of their properties is necessary. The compositional and functional properties will vary depending on the bean type, their physical form (pre-cooked, raw flour, starch or protein fractionates) and growing location.

In this study, edible bean flours (pinto, navy, black and small red) were subjected to extrusion cooking to produce snacks and texturized high-protein flour. The extrudates were studied to determine the effect of extrusion on the physical, physicochemical, chemical, sensory, and digestibility properties. Texturized high-protein flour was used in a bread formulation study. Finally, a preliminary study of location effect on production of grassy compounds, e.g. hexanal and hexanol, in pinto beans was conducted to determine importance of growing environment on flavor development during storage. The results of these studies showed that bean flours generally had excellent extrusion properties (good expansion and texture). However, pre-cooked flours had much lower expansion and textural integrity compared to raw bean flours and starch fractionates. Nutritional content (protein, total starch, fiber and ash) of flours were generally retained after extrusion. Lipids and resistant starch (RS) however were significantly reduced. Significant reduction in RS resulted in snacks having high glycemic index. Extrudates had 20% lower raffinose content suggesting reduced potential for flatulence after extrusion processing. Sensory evaluation of pinto, navy and black bean snacks indicated good overall acceptability. Pinto bean high-starch fraction differed in composition and functionality (viscosity and thermal properties) compared to its raw whole flour and extruded form. Adding 5% texturized pinto bean

protein to bread increased its lysine content by 50%; without significantly affecting bread quality.

A significant statistical interaction between growing location and storage time on hexanol and hexanal concentrations was observed for pinto beans grown in Forest River, Johnstown and Hatton North Dakota.

Results presented in Dissertation will allow pulse-manufacturers to better understand the potential for beans as a food ingredient, and their respective applications. These may include use in breads and other baked products, extruded puffed snacks, pasta, and soups. The use of beans can improve nutritional quality and provide unique functionality to food systems.

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## **DEDICATION**

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## CHAPTER 1. GENERAL INTRODUCTION

This dissertation research provides food manufacturers with important information that they will need to develop and utilize edible bean flours as food ingredients. The soybean processing industry has been very successful. This is due to the marketing of soybean as a highly nutritious alternative to meat proteins (Kuan et al 2012). Similar health benefits however can be derived from the consumption of pulses such as dry edible beans, without the allergen problem that soybean has (Geil 1994). Soybean contains proteins that causes severe allergic reactions within minutes to hours after consumption, and is among the top eight food allergens (Cordle 2004). Processing of dry edible beans into value-added products is therefore a viable alternative for individuals affected by allergic reactions from soy products. Furthermore, availability of new plant based high-protein foods will broaden the range of healthy choices for the consumer. Edible beans (pinto, navy, black and red) were studied in this research since North Dakota is the number one producer in the United States (USDA Economic Research Service, 2011). A better understanding of the properties of dry edible beans and their potential food manufacturing applications will be a significant boon to the state's agricultural-based economy.

Edible beans are pulse crops belonging to the family Leguminosae (or Fabaceae); the third largest family of flowering plants. It includes over 600 genera and 20000 species (Doyle 2004). The genus to which edible beans belong is *Phaseolus*. The *Phaseolus* genus has 150-200 species which includes *Phaseolus vulgaris*; the major cultivated specie, also referred to as the common bean. The common bean includes hundreds of varieties and over 14000 cultivars (Nwokolo, 1996). Common usage of names for edible beans is typically based on names of market classes. Major commercial market classes in the US include black, navy, light red kidney, dark red kidney, cranberry, great northern, pinto, yellow eye, small red, white kidney, pink and

soldier beans. Major bean classes grown in North Dakota are pinto, navy and black beans (USDA Economic Research Service, 2011). North Dakota is the leading state in edible bean production in the US. The short growing season (< 100 days) of beans make them suitable for cultivation in the Upper Midwest and Great Lakes region. Planting is done in early June, and harvest from mid to end of September (Kelly 2010).

The common bean can be described as an annual herbaceous plant. There are two types; a short bush-erect type (25-30 cm high) and a twining-pole type (2-3 m or longer). The plant flowers about 4 weeks after sowing. Flowers may have basic colors of white, pink, purple or may be variegated. Pods change from slender and flat to cylindrical as they mature. They grow up to 10-20 cm long and 1.0-1.5 cm wide and also come in different colors including green, yellow, black and purple. Maturation takes place as early as 2 weeks after flowering. Seeds may be 6-8 per pod and weigh from 10-30 g per 100 seeds in small white navy to 50-70 g per 100 seeds in kidney beans. Seed color varies from red, white, tan and black, to mottled (Christou 1992; Nwokolo, 1996).

The high nutrient density of pulses allows them to be a very good source of starch, dietary fiber and protein. Total carbohydrates in pulse flours range from 49 - 68 % (dry weight basis). Total starch range from 13-49% of carbohydrate dry weight. Starch in pulses consists of mainly C-type polymorphs, which is a mixture of both A and B-type polymorphs. A-type starch polymorphs has six closely packed hexagonal crystals with eight water molecules per unit, and B-type polymorphs has a more open hexagonal packing with 36 water molecules per unit (Chibbar et al 2010). Starch granule size vary (oval, round, elliptical, irregular) with width of 5-55  $\mu\text{m}$  and length 5-70  $\mu\text{m}$  (Hoover et al 2010). Amylose contents in pulse flours range from 24-88% (Ratnayake et al 2001). High amylose is associated with high resistant starch in pulses (10-



20%) (Chibbar et al 2010). Dietary fiber in pulses range from 8-27.5%, which includes soluble fiber (3.3 -13.8%) (Guillon and Champ 2002). Crude fiber content was reported at 1.2-13.5% (Reddy et al 1984). Protein content in pulses (pea, chickpea, bean and lentil) ranges from 17-30% (Sathe et al 1984). Major proteins present are globulins (legumin and vicilin) and albumins (enzymatic proteins, protease, inhibitors and lectins) (Chibbar et al 2010). On a dry weight basis, beans contain 45-70% globulins and 10-30% albumins (Sathe 2002). Sutivisedsak et al (2011) reported lipid content in beans (kidney, great northern and pinto) to be up to 2% triacylglycerides, with fatty acids being mainly polyunsaturated in the form of linolenic acid (41.7-46%) and linoleic acid (24.1-33.4%).

Several health benefits have been attributed to the consumption of cooked beans. Lowering the risk of diabetes, colon cancer, obesity and heart disease has been linked to the slow digestibility of beans (Reyes-Moreno and Paredes-López 1993). Soluble fibers, including resistant starch, in pulses are anaerobically fermented by bacteria for energy in the large intestine, producing short chain fatty acids (SCFA) such as lactic, propionic, butyric and valeric acids. They travel to the liver via the intestinal walls, where they are metabolized (BeMiller and Huber, 2008). SCFAs stimulate the growth of useful gut bacteria; inhibit the growth of pathogens; lower gut pH; stimulate colon blood flow; improve colon muscular contraction; improve nutrient flow, increase fecal weight, prevent constipation, and lower production of toxic substances (Sajilata et al 2006). Butyric acid enhances the growth of normal colon cells and inhibits the growth of malignant ones, promotes DNA repair and retards growth of tumor cells (Bird et al 2000).

Increased water salvage in children and adults suffering from chronic diarrhea has been reported after treatment with resistant starch (Ramakrishna et al 2000; Raghupathy et al 2006).

Lopez et al (2001) reported an increase in mineral absorption from resistant starch intake. They explained that this was likely due to reduced colonic pH, which increased mineral solubility. Furthermore, SCFAs were reported to enhance colon tissue growth, resulting in an increase in absorption area (Lopez et al 2001).

Consumption of foods high in resistant starch can also help in controlling diabetes, which is a growing problem in the US. The Centers for Disease Control (CDC), reported that approximately 25.8 million people in the United States were affected with diabetes (CDC 2011). This represented 8.3% of the US population. Among US residents aged 65 and older, 10.9 million or 26.9% had diabetes in 2010. The CDC (2011) reported diabetes as the leading cause of kidney failure, non-traumatic limb amputation and blindness in the US. The disease was also a major cause of heart disease and stroke and was the seventh leading cause of death in the US (CDC 2011).

The ability of high resistant starch foods such as edible beans to reduce risks of diabetes and improve weight management is due to slower glucose release to the blood compared to foods that digest more rapidly (Raben et al 1994; Reader et al 1997). Therefore, high resistant starch foods provide fewer calories. Pawlak et al (2004) reported decrease in total body fat and percentage adiposity in resistant-starch fed rats. Furthermore, resistant starch increases fecal energy wastage, increase satiety hormone production, and inhibits fat accumulation; thereby, improving its weight lowering capacity (Behall and Howe 1995; Higgins et al 2004; Zhou et al 2006; Keenan et al 2006). Additionally, resistant starch lowers cholesterol and reduces gallstone formation (Malhotra 1968; Han et al 2003).

Greater consumption of edible beans also addresses the problem of gluten intolerance, since they can be used as a source of protein in gluten-free products. The National Digestive

Diseases Information Clearinghouse reported that more than 2 million people in the US suffer from celiac disease (Anonymous, 2010). This is a condition that is induced by gluten proteins in individuals that are genetically susceptible (Broeck, et al 2009). The disease damages the small intestine and retards absorption of nutrients.

Although there are many health benefits associated with pulse consumption, the amount consumed in the US is low (16.1g/day per capita) compared to other countries (Barampama and Simard 1993). There may be several factors contributing to this. In wealthier economies more consumers can afford to purchase meat as their main source of protein. In contrast, developing countries consume more pulses because they provide a cheap source of protein and other nutrients (Bouis 2007). Secondly, sensory and digestibility properties of beans may limit their consumer acceptance. The presence of flatulence factors such as raffinose is associated with ‘bloating’ stomachs resulting in discomfort after consumption. Pulses are sometimes described as having an unacceptable “beany” or “grassy” flavor (Sessa 1979). Therefore sensory and digestibility properties may be among the primary reasons for the low consumer acceptance regardless of the health benefits. This also could hinder usage of pulse-based ingredients from edible beans.

Several researchers have studied the effect of pulse flours in several food applications such as breads, crackers, granola bars, pasta and extruded snacks (Maurer et al 2005; Anton et al 2009; Han et al 2010; Nagi et al 2012). However, their application has been limited. Presently, opportunities for processing of edible beans are largely concentrated to canning. About 90% of navy beans and kidney beans processed in the US are processed as canned foods (Kelly 2010). The research in this dissertation will help to expand application possibilities for edible beans by utilizing extrusion technology as a processing tool to improve sensory, digestibility and

functional properties. Extrusion processing involves cooking the flour under high pressure using steam and water. Cooking causes gelatinization and other physical and physio-chemical and chemical changes in the extruded materials (Faraj et al 2004; Rehman and Shah 2005).

Degradation of macromolecules during extrusion is a function of temperature, feed moisture and screw speed (Davidson et al 1984). As the product leaves the extruder it is expanded due to differences between the high internal pressure of the extruder and atmospheric pressure. The extruder type may be single or twin, but twin screw types are more common. Typical twin extruder parameters include feed moisture, 11-35%; product temperature, 80-200°C; and screw speed, 200-500 rpm. Feed moisture of 22% and temperature of 160°C have been found to be suitable for extruded bean flours (Anton et al 2009; Balandran-Quintana et al 1998).

Using extrusion technology, the properties of bean flours were studied to determine its effect on various properties. The research provides critical information for manufacturing applications. Precooked bean flours were first studied to determine the effect of extrusion speeds on their properties. This was done primarily to determine the approximate range of screw speeds that would be best suited for bean flour extrusion. In addition, this study helped to determine how extrusion impacts in vitro digestibility of starch (Chapter 1). Raw edible beans (not precooked) were then extruded using conditions established in Chapter 1 as a basis for extruder settings. Physiochemical properties were studied to determine the effect of extrusion on the raw flour (Chapter 2). After studying the effect of extrusion on whole bean flours (precooked and raw) in the previous chapters, the effect of extrusion on bean fractions (high starch and high protein fractions) were studied to determine the effect on their properties and their potential applications, including bread making (Chapters 3 and 4). It was hypothesized that the properties observed would be different based on the original form of the bean flour (precooked, raw or

fraction), and that these differences would create opportunities for unique applications in food manufacturing. Since shelf life of food ingredients is important to food manufacturers, a final study was done to determine effect of storage on hexanal and hexanol content in beans produced over time. Hexanal and hexanol are aromatic compounds associated with unacceptable off-taste in beans. In the study, growing location and storage time were used as the two independent variables to determine their effect on hexanal and hexanol development. The results of this final study provides food manufacturers with information on expected changes in aroma development in bean flours during storage; and provides preliminary data to breeders on the potential effect of growing location on flavor development during storage. It was hypothesized that because different growing locations provides different growing conditions, that this could significantly influence aroma production during storage.

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## **CHAPTER 2. <sup>1</sup>EFFECTS OF EXTRUDER SCREW SPEEDS ON PHYSICAL PROPERTIES AND IN VITRO STARCH HYDROLYSIS OF PRECOOKED PINTO, NAVY, RED, AND BLACK BEAN EXTRUDATES**

### **2.1. Abstract**

Precooked pinto, navy, red, and black bean flours were extruded at different screw speeds (320, 380, and 440 rpm) with a twin-screw extruder. Effect of speed on physical properties and in vitro starch hydrolysis was investigated. Increasing screw speeds reduced water activity, expansion index, and texture. Extrudates could not be obtained from pinto bean flour at 440 rpm because of the high shear effect. Water absorption index and water solubility index were not significantly affected by screw speed but were significantly higher than for unextruded precooked flour. A significant change in color was observed in navy beans, characterized by increasing *b* values on the Hunter color scale. Resistant starch ranged from 3.65 to 4.83% db and was not significantly affected by screw speed. Glycemic index of all extrudates was high, ranging from 81.3 to 86.9.

### **2.2. Introduction**

Beans are highly nutritious. They are a significant source of protein, complex carbohydrates, resistant starch (RS), minerals, and fiber (Reyes-Moreno and Paredes-López 1993; Rehman et al 2001). Beans are classified as low glycemic index (GI) foods because the starch is digested slowly into glucose (Foster-Powell and Miller 1995). Low-GI foods help to lower postprandial glucose response and therefore assist in diabetes management. Other health

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<sup>1</sup> Chapter based on published manuscript: Simons, C. W., Hall, C., III and Tulbek, M. 2012. Effects of extruder screw speeds on physical properties and in vitro starch hydrolysis of precooked pinto, navy, red, and black bean extrudates. *Cereal Chem.* 89:176-181.

benefits reported include reduced risk of obesity, coronary heart disease, and colon cancer (Raben et al 1994; Salmeron et al 1997; Bird et al 2000; McMillan-Price et al 2006; Brand-Miller et al 2007). These benefits have been associated with high levels of slowly digestible starch and RS, which cause slow digestion in the small intestine and fermentation in the large intestine, respectively (Englyst et al 1992). RS acts as a prebiotic in the large intestine for gut bacteria growth. This prebiotic behavior results in an increased production of short-chain fatty acids, improvement in mineral absorption, and general colon health (Coudray et al 1997; Topping et al 2003).

In spite of the health benefits of beans that have been presented, bean consumption in the United States is low (16.1 g/day per capita) (Barampama and Simard 1993). To increase bean consumption, extrusion technology has been used to produce snack foods because of the excellent expansion and functional properties of bean starch (Skierkowski et al 1990; Gujska and Khan 1991; Borejszo and Khan 1992; Czarnecki et al 1993; Martin- Cabrejas et al 1999; Abd El-Hady and Habiba 2003; Rocha- Guzman et al 2008; Ruiz-Ruiz et al 2008). A large body of research examining the effect of extrusion on RS in cereal-grain products has been reported. However, inconsistent and conflicting results were presented. Some researchers have indicated an increase in RS after extrusion, whereas others have reported a reduction or no formation of RS. Kim et al (2006) did not find any significant correlations between RS formation and screw speed. Unlu and Faller (1998) reported that production of high levels of RS in corn starch (up to 38.4%) was possible when high moisture was applied (up to 67%) during extrusion. A negative relationship between formation of RS, in the RS3 form, and screw speed was also observed (Unlu and Faller 1998). RS3 refers to RS developed as a result of retrograded amylose crystals (Englyst and Cummings 1987). Ruiz-Ruiz et al (2008) indicated that lower screw speed

increased residence time in the extruder and thereby increased RS formation. Faraj et al (2004) reported very low RS in cereal grain-based foods (0–0.6%) and found that RS3 generally decreased during extrusion. They postulated that the RS3 reduction could result from the conditions of high shear, causing depolymerization of amylose into molecules with a degree of polymerization <26. Very low amounts of RS3 were formed in barley flours studied, even under extreme conditions of low screw speed (60 rpm) and high moisture content (up to 40%) (Faraj et al 2004). They concluded that increasing RS in extruded products was only feasible by incorporating other ingredients and optimizing storage temperature conditions. Because these studies were conducted with different raw materials, at different screw speeds, moistures, and temperatures, it was difficult to ascertain how extrusion would affect physical properties and digestibility of precooked pinto, navy, black, and red beans. Therefore, the objective of this study was to determine the effect of extruder screw speed on physical characteristics and RS composition of extrudates. This research will provide useful information on the effect of extrusion on precooked beans and will give guidance on the selection of a screw speed in processing.

### **2.3. Materials and Methods**

Precooked VegeFull cooked ground beans were purchased from Archer Daniels Midland Company (Enderlin, ND). Products included pinto (PCN-192001), black (PCN-292001), small red (PCN-392001), and navy (PCN – 492001). Megazyme RS and total starch (TS) kits were purchased from Megazyme International (Bray, Ireland). White bread was purchased from a local grocery and used as a standard for comparison of starch hydrolysis curves.

### 2.3.1. Proximate Analysis of Pre-Cooked Flour

Proximate analysis was done on precooked flour (Table 2.1). Moisture was determined based on AOAC method 925.10 (AOAC 1990). Protein analysis was determined based on AACC International Approved Method 46-30.01 (2010). Triplicate measurement was conducted with a nitrogen combustion analyzer (Leco FP-528, St. Joseph, MI).

Lipid analysis was based on method adapted from AOCS methods Af 3-53, Am 2-93, and Aa 4-38 (AOCS 1998). Round-bottom flasks (250 mL) were preweighed. Bean flour samples (5 g) were weighed and collected in a folded 15 cm filter paper (415, qualitative, VWR International, Radnor, PA) and transferred to a thimble holder and soxhlet unit (combo mantle, Glas-Col, Terre Haute, IN). Lipids were then extracted with hexane for 16 hr and cooled to room temperature. Hexane was evaporated from extracted lipids with a rotary evaporator (Rotavapor RE-111, Büchi, Flawil, Switzerland) for 10 min at 58°C and high vacuum. Flasks were then reweighed and the weight difference used to calculate percentage of total lipids extracted. Ash content was determined following AACCI Approved Method 08-01.01 (AACCI 2010).

**Table 2.1. Proximate analysis of commercial pre-cooked bean flours**

	Black	Navy	Pinto	Red
Moisture (%)	6.3 ± 0.3	5.2 ± 0.3	6.1 ± 0.4	5.8 ± 0.1
Protein (% d.b.)	22.6 ± 0.1	23.6 ± 0.0	22 ± 0.0	21.4 ± 0.0
Total Starch (% d.b.)	38.8 ± 1.3	37.5 ± 0.2	40.0 ± 1.8	38.4 ± 0.6
Total Lipids (% d.b.)	1.9 ± 0.1	2.4 ± 0.0	1.8 ± 0.1	1.9 ± 0.1
Ash (% d.b.)	3.4 ± 0.1	3.1 ± 0.1	3.6 ± 0.2	3.2 ± 0.0

### 2.3.2. Extrusion Conditions

Extrusion at each treatment level (320, 380, and 440 rpm) was conducted twice on each of two separate extrusion days (run 1 and run 2). Extrusion was done with a corotating, fully intermeshing, self-wiping Wenger TX52 twin-screw extruder (Wenger Manufacturing, Sabetha,

KS). Moisture was adjusted to 22% by metering water and steam into the extruder barrel at rates of 7.02 and 5.88 kg/hr, respectively. Moisture adjustments were determined based on calculation following equation 2.1 (*personal communication*, Gordon Huber, 2010).

$$S_p + W_p + S_e + W_e = \frac{F_d \times (M_f - M_e)}{M_e - 1} \quad (2.1)$$

$(S_p + W_p + S_e + W_e) = WR$ , where WR is the total amount of moisture required to achieve the target moisture content in the extruder. WR does not take into consideration moisture or vapor loss from the preconditioner or extruder sometimes referred to as “flash off.”  $M_f$  is the moisture of the feed;  $M_e$  is the moisture out/in the extruder;  $F_d$  is the dry feed rate (kg/hr);  $S_p$  is the steam injected into the preconditioner (kg/hr);  $S_e$  is the steam injected into the extruder (kg/hr);  $W_p$  is the water added to the preconditioner (kg/hr); and  $W_e$  is the water added to the extruder (kg/hr). Moisture definitions are expressed as wet basis in decimal percent or fraction. Bean flours were fed from a feed hopper at a rate of 64.2 kg/hr. The extruder had 52 mm diameter screws and a barrel length-to diameter ratio of 25.5:1. The barrel had six temperature zones from the feed to die section. Temperatures were maintained at approximately 45°C (zone 1), 70°C (zones 2–5), and 90°C (zone 6). Configuration of screws from feed to die end consisted of six conveying, one shear lock forward, three conveying, one shear lock forward, three conveying, one shear lock forward, two conveying, one shear lock forward, one shear lock backward, and one cone screw. A circular die with a 3 mm diameter was used. The cutting knife had two blades and was positioned off-center at the end of the die. Flours were extruded on two separate extrusion days, using screw speeds of 320, 380, and 440 rpm. Extrudates were collected within 5 min after a steady state was reached in the extruder. Large plastic bags were used to collect the extrudates after they had cooled to room temperature on trays. Bags were left open to prevent condensation

during additional cooling. The bags were sealed and stored at 4°C until ready for analysis.

Specific mechanical energy (SME), a measure of work done on the feed, was calculated from equation 2.2 for extrusion at 320 and 380 rpm and equation 2.3 for extrusion at 440 rpm runs (*personal communications*, Brian Prattner, Wenger Manufacturing, 2011).

$$SME = \frac{L_e \times \frac{N_a}{N_b} \times P_e}{m} \quad (2.2)$$

$$SME = \frac{L_e \times P_e}{m} \quad (2.3)$$

$N_e$  is the extruder screw speed;  $L_e$  is the extruder drive motor load;  $N_a$  is the actual screw speed in revolutions per minute;  $N_b$  is the base screw speed (402 rpm);  $P_e$  is the rated power of extruder drive motor (22.4 kW); and  $m$  is the mass flow rate (kg/hr).

### 2.3.3. Sample Preparation

For analyses requiring milled samples, extrudates of runs 1 and 2 were randomly sampled from storage bags and ground separately in a Brinkmann Retsch centrifugal mill (Retsch, Haan, Germany) with a 1 mm sieve. Individual samples were mixed to ensure homogeneity. Each treatment replicate was stored in a sealed ziplock plastic bag and kept at 4°C until completion of analysis. Commercial white bread used for the control in starch hydrolysis assays was freeze-dried and milled in a similar manner as the extrudates. Analyses in this study were completed within a two month period following extrusion.

### 2.3.4. Physical and Functional Properties

Water activity was determined with an AquaLab water activity meter (AquaLab 3TE, Decagon Devices, Pullman, WA). Measurements were repeated for each treatment replicate for a total of four measurements ( $n = 4$ ). Expansion index (EI) of extrudates was determined based on



the procedure by Baik et al (2004). Ten pieces of extrudates within each treatment replicate ( $n = 20$ ) were randomly selected, and diameters were measured with a caliper. EI was reported as the average diameter of extrudates divided by the diameter of the extruder die. A C-Cell imaging instrument (Calibre Control International, Warrington, U.K.) fitted with a 75 mm lens (magnification factor of 1 pixel = 0.025 mm) was used to determine cell cross-sectional area and structure of cells. Cross-sectional cuts of extrudates were made with a scalpel blade. The sample was then blown with a quick blast of air to create a clean, dust-free surface. Samples were then placed in the imaging device for analysis. Two images of each extrusion run were taken ( $n = 4$ ). A hardness test on whole extrudates was conducted with a texture analyzer with a three-point bend assembly probe (CT3 texture analyzer, Brookfield, Middleboro, MA). The instrument was set at test speed, 0.5 mm/sec; target value, 5 mm; trigger load, 7.0 g; and data acquisition rate, 100 points/sec. Ten pieces of extrudates within each treatment replicate ( $n = 20$ ) were randomly selected and were compressed to determine the peak force (g) needed to break each sample. Color was determined on milled samples with a Minolta colorimeter to determine  $L$ ,  $a$ , and  $b$  values. Readings were replicated for each extrusion run ( $n = 4$ ). Water absorption index (WAI) and water solubility index (WSI) were determined in replicate ( $n = 4$ ), based on a procedure by Anderson (1982). Milled samples ( $<212 \mu\text{m}$ , 2.5 g) were added to centrifuge tubes containing magnetic stir bars. Distilled water (30 mL) was added, and the tubes were sealed and vigorously agitated to break lumps. Tubes were placed on a magnetic stirrer, mixed for 30 min, and then centrifuged at 3,000 rpm. The supernatant was decanted and the container weighed. The weight of sediment was determined by difference. The supernatant collected was placed in a crucible and dried on a hot plate to remove liquid and to determine the amount of dissolved solids. WAI and WSI were calculated from equations 2.4 and 2.5, respectively:

$$WAI = \frac{\text{weight of the wet sediment (g)}}{\text{initial weight of the dry flour (g)}} \quad (2.4)$$

$$WSI(\%) = \frac{\text{weight of the solids in supernatant (g)}}{\text{initial weight of the dry flour (g)}} \times 100 \quad (2.5)$$

### 2.3.5. Starch Composition and Digestibility

Triplicate and duplicate analysis of RS and TS, respectively, was conducted with Megazyme RS and TS kits and reported as percentage dry basis. Estimated GI (eGI) was determined in duplicate by collecting 100 mg of bean flours in 14 mL test tubes. Aqueous ethanol (80% v/v, 5 mL) was added and the tube incubated at 80 - 85°C for 5 min to remove glucose and maltodextrins. The contents were mixed and another 5 mL of 80% v/v aqueous ethanol added. Tubes were centrifuged for 10 min at 3,000 rpm and the supernatant discarded. Samples were resuspended in 10 mL of 80% v/v aqueous ethanol, stirred on a vortex mixer, and centrifuged again. The supernatant was discarded. The residue was digested with amylase and amyloglucosidase from the Megazyme RS test kit. Makeup of the enzyme solution was as described in the RS procedure. Extruded bean flours, unextruded precooked flours, and white bread control samples were placed in a shaking water bath (200 strokes/min) and digested for 30, 60, 90, 120, 150, and 180 min at 37°C. The reaction was terminated at each interval with 4 mL of 99% v/v ethanol. Glucose released was determined by reacting glucose with glucose oxidase–peroxidase reagent in the Megazyme RS kit, followed by spectrophotometric measurement at 510 nm. Hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve by the corresponding area under the white bread control. The eGI was then calculated from equation 2.6 (Chung et al 2008):

$$eGI = 8.198 + 0.862HI \quad (2.6)$$

### **2.3.6. Statistical Analysis**

Data was analyzed with statistical analysis software (SAS Institute, Cary, NC) based on a completely randomized experimental design. The two extrusion runs were considered separate experimental units. Analysis was done on each replication of experimental unit. The number of replications for each analysis is presented in the description of each method. An analysis of variance (ANOVA) and means comparison following a least significant difference procedure were applied to establish differences. Differences were significant at  $P < 0.05$ . The  $P$ -value is the probability that the null hypotheses is true, i.e. there is no difference in the data set being compared. In the analysis, the  $P$ -value was compared with the  $\alpha$ -level (0.05). An  $\alpha$ -level of 0.05 means that there is a 5% chance that the data obtained is by random chance and that there is a 95% chance that it was based on a significant difference. Therefore when  $P$ -value was less than 0.05, we rejected the null hypothesis and said that there was a significant difference.

## **2.4. Results and Discussion**

### **2.4.1. Specific Mechanical Energy (SME)**

Based on equations 2 and 3, SME used during extrusion operation were 0.07, 0.09, and 0.10 kW·hr/kg for extrusion at 320, 380, and 440 rpm, respectively. This was not unexpected since the high extrusion rates would be expected to have higher SME.

### **2.4.2. Physical and Functional Properties**

All bean types except pinto beans at 440 rpm were successfully extruded. The high shearing effect at 440 rpm is likely the reason for the failure of the pinto bean flour to form an extrudate. Significant differences were observed in cross-sectional slice area of black and navy bean extrudates at 320 and 440 rpm (Table 2.2), which also corresponded to differences in

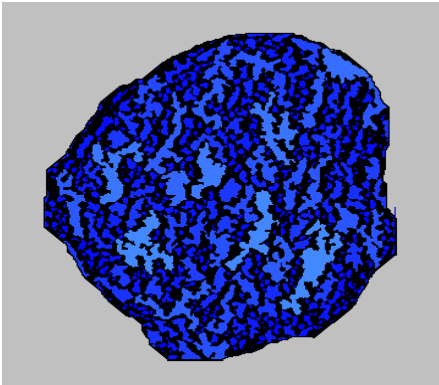
observed EI. In contrast, no significant difference in cross-sectional area was observed in red and pinto bean extrudates, although differences were observed in EI. Analysis of cell-structure parameters (i.e. number of cells per unit area, area of cells, cell diameter, cell volume, wall thickness, number of holes, area of holes, total volume of holes, coarse/fine clustering, average cell elongation, and cell alignment) were not statistically different (Figure 2.1). This lack of statistical difference could be due to the low number of replicates within experimental units ( $n = 4$ ). The C-Cell imaging instrument can be used as a rapid method for analyzing cell structures of extrudates; however, high variability of cell structure requires higher number of replications to increase statistical power. Cell diameter and number of cells per unit ( $\text{mm}^2$ ) of maize snack decreased as extrusion screw speeds (250, 300, and 350 rpm) increased (Pike 2006).

**Table 2.2. Effect of extruder speed on physical properties and digestibility of black, navy, pinto and red bean extrudates**

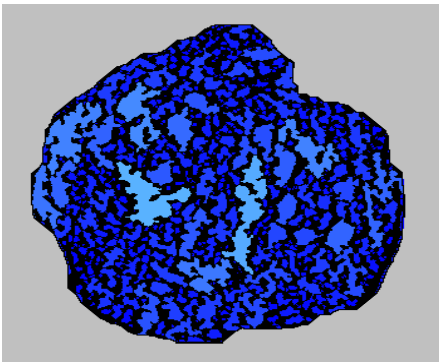
Speed	$a_w$	Slice Area (mm <sup>2</sup> )	EI	Hardness(g)	TS (%)	RS (%)	eGI
<b>Black Bean</b>							
320 rpm	0.25 ± 0.04 <sup>a</sup>	43.5a	2.27 ± 0.23 <sup>a</sup>	725 ± 256.9 <sup>a</sup>	40.6 ± 3.8 <sup>a</sup>	4.06 ± 0.32 <sup>a</sup>	84.2 ± 3.9 <sup>a</sup>
380 rpm	0.22 ± 0.00 <sup>ab</sup>	35.5ab	2.24 ± 0.15 <sup>a</sup>	353 ± 131.2 <sup>b</sup>	40.8 ± 3.0 <sup>a</sup>	3.89 ± 0.21 <sup>a</sup>	84.2 ± 2.7 <sup>a</sup>
440 rpm	0.20 ± 0.00 <sup>b</sup>	28.3b	1.92 ± 0.20 <sup>b</sup>	213 ± 126.5 <sup>c</sup>	40.7 ± 1.9 <sup>a</sup>	3.67 ± 0.32 <sup>a</sup>	83.3 ± 1.6 <sup>a</sup>
PCF	*		*	*	38.8 ± 1.3 <sup>a</sup>	5.02 ± 0.16 <sup>a</sup>	86.9 ± 2.7 <sup>a</sup>
<b>Navy Bean</b>							
320 rpm	0.22 ± 0.00 <sup>a</sup>	60.5a	2.66 ± 0.26 <sup>a</sup>	769.2 ± 272 <sup>a</sup>	41.4 ± 2.9 <sup>a</sup>	4.46 ± 0.47 <sup>a</sup>	82.1 ± 1.9 <sup>a</sup>
380 rpm	0.21 ± 0.00 <sup>a</sup>	44.5b	2.49 ± 0.12 <sup>b</sup>	813.5 ± 190.8 <sup>a</sup>	40.2 ± 1.9 <sup>b</sup>	4.23 ± 0.46 <sup>a</sup>	85.3 ± 1.7 <sup>b</sup>
440 rpm	0.21 ± 0.00 <sup>a</sup>	40.3b	2.53 ± 0.13 <sup>b</sup>	678.8 ± 156.9 <sup>a</sup>	40.5 ± 3.0 <sup>b</sup>	4.21 ± 0.34 <sup>a</sup>	84.6 ± 1.7 <sup>a</sup>
PCF	*		*	*	37.5 ± 0.2 <sup>b</sup>	5.27 ± 0.66 <sup>b</sup>	88.9 ± 1.0 <sup>c</sup>
<b>Pinto Bean</b>							
320 rpm	0.21 ± 0.00 <sup>a</sup>	41.8a	2.50 ± 0.23 <sup>a</sup>	742.9 ± 252.0 <sup>a</sup>	41.3 ± 3.4 <sup>a</sup>	4.59 ± 0.30 <sup>a</sup>	81.3 ± 3.3 <sup>a</sup>
380 rpm	0.19 ± 0.00 <sup>b</sup>	39.5a	2.21 ± 0.23 <sup>b</sup>	338.4 ± 208.3 <sup>b</sup>	39.7 ± 1.2 <sup>a</sup>	4.67 ± 0.16 <sup>a</sup>	85.0 ± 2.1 <sup>a</sup>
PCF	*		*	*	40.0 ± 1.8 <sup>a</sup>	5.78 ± 0.53 <sup>b</sup>	82.9 ± 1.9 <sup>a</sup>
<b>Red Bean</b>							
320 rpm	0.22 ± 0.01 <sup>a</sup>	46.0a	2.50 ± 0.38 <sup>a</sup>	963.4 ± 442.0 <sup>a</sup>	42.0 ± 3.1 <sup>a</sup>	4.48 ± 0.49 <sup>a</sup>	83.2 ± 4.0 <sup>a</sup>
380 rpm	0.21 ± 0.01 <sup>a</sup>	46.8a	2.19 ± 0.24 <sup>b</sup>	612.8 ± 223.6 <sup>b</sup>	40.5 ± 2.0 <sup>ab</sup>	4.83 ± 0.37 <sup>a</sup>	86.8 ± 1.7 <sup>a</sup>
440 rpm	0.20 ± 0.01 <sup>b</sup>	42.5a	2.36 ± 0.21 <sup>ab</sup>	431.9 ± 199.8 <sup>b</sup>	40.1 ± 1.9 <sup>ab</sup>	4.73 ± 0.23 <sup>a</sup>	85.7 ± 3.0 <sup>a</sup>
PCF	*		*	*	38.4 ± 0.6 <sup>bc</sup>	5.40 ± 0.25 <sup>a</sup>	84.4 ± 1.1 <sup>a</sup>

\*Data not collected. EI = Expansion Index; TS = Total Starch; RS = Resistant Starch and eGI = Estimated Glycemic Index; PCF = Precooked flour. Letters show statistical differences between extrusion speeds under each bean type. Bean types were not compared. Means followed by different letters within each bean type are significantly different at  $p < 0.05$  according to mean separation test using LSD procedure.

**A**



**B**



**C**

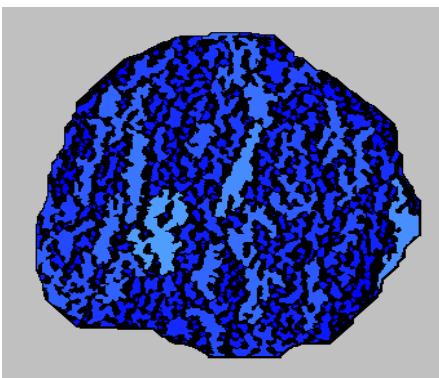


Figure 2.1. Example of cross-sectional C-Cell image showing distribution of cells in navy beans at 320 rpm (A) 380 rpm (B) and 440 rpm (C)

EI decreased significantly as extrusion rate increased above 320 rpm (Table 2.2). However, differences in EI were not always significant between 380 and 440 rpm. Li et al (2005) also observed a reduction in EI in soybean–corn extrudates with increasing extruder speed. Reduction in EI was associated with increased damage to the starch matrix (Balandrán-Quintana et al 1998). Hardness values decreased significantly as screw speed increased, except for the navy bean extrudates (Table 2.2). The high shear effect in this study may have severely degraded the starch matrix, reducing its capacity to expand and producing a weaker and more fragile structure.

Water activity decreased significantly as screw speed increased, except for navy bean extrudates. This trend could correlate to a loss of water content during extrusion as screw speeds increase. Li et al (2005) reported that water content of extrudates made from corn/soybean blends decreased as screw speeds increased. There was no significant change in water activity in navy beans, possibly due to structural characteristics of the flour. For example, hardness values in navy beans extruded at different speeds did not significantly change, while hardness in the other bean extrudates did.

*L* and *a* color values were not significantly different in bean extrudates at different speeds. The *b* values in navy bean extrudates significantly increased at each extrusion rate (Table 2.3), indicating increased yellowness. The effect of extrusion on color of precooked flour in all beans, however, was significant. *L* values significantly decreased, indicating increased darkening resulting from extrusion. The *a* and *b* values also increased, indicating higher red and yellow intensities, respectively.

WSI and WAI were not significantly affected by extruder screw speed (Table 2.4), except for black beans, which showed a significant difference in WAI at 320 rpm (3.8) versus 440 rpm

(3.2). Seker and Hanna (2005) reported that WAI and WSI were not significantly affected by screw speed ( $P > 0.05$ ) in modified maize starch/soy protein mixtures extruded at varying screw speeds (60–180 rpm). WSI is correlated to molecular degradation. The lack of statistical difference in WSI between screw speeds indicated that although textural differences were evident, there were no significant differences in molecular changes across the screw speeds (Tang and Ding 1994; Balandrán-Quintana et al 1998). It was evident, however, that there were significant molecular changes in the precooked flour during extrusion, as WSI significantly increased for all beans. Extrusion increased breakdown of the bean matrix, such as starch depolymerization, resulting in more soluble particulates in the supernatant (Balandrán- Quintana et al 1998). The effect on WAI during extrusion was similar. Precooked beans had significantly lower WAI compared with extruded beans. Typically, extrusion results in gelatinization of starch, causing increased water-holding properties (Martin-Cabrejas et al 1999). Because the bean flours were already gelatinized, further cooking at higher speeds did not increase water-holding properties.



**Table 2.3. Effect of extruder speed on color of black bean extrudates**

	Color values		
	l	a	b
<b>Black Bean</b>			
320 rpm	65.5 ± 3.5 <sup>a</sup>	6.8 ± 0.8 <sup>a</sup>	14.0 ± 0.8 <sup>a</sup>
380 rpm	67.3 ± 1.2 <sup>a</sup>	6.7 ± 0.4 <sup>a</sup>	14.5 ± 0.8 <sup>a</sup>
440 rpm	68.1 ± 0.5 <sup>a</sup>	6.7 ± 0.1 <sup>a</sup>	14.9 ± 0.7 <sup>a</sup>
<b>PCF</b>	74.1 ± 0.5 <sup>b</sup>	3.1 ± 0.0 <sup>b</sup>	7.4 ± 0.1 <sup>b</sup>
<b>Navy Bean</b>			
320 rpm	70.9 ± 2.8 <sup>a</sup>	6.1 ± 0.6 <sup>a</sup>	18.9 ± 0.2 <sup>a</sup>
380 rpm	71.2 ± 2.5 <sup>a</sup>	6.0 ± 0.6 <sup>a</sup>	19.9 ± 0.2 <sup>b</sup>
440 rpm	72.0 ± 1.3 <sup>a</sup>	5.8 ± 0.4 <sup>a</sup>	20.4 ± 0.1 <sup>c</sup>
<b>PCF</b>	87.5 ± 0.0 <sup>b</sup>	0.5 ± 0.0 <sup>b</sup>	8.9 ± 0.1 <sup>d</sup>
<b>Pinto Bean</b>			
320 rpm	69.1 ± 1.2 <sup>a</sup>	7.0 ± 0.5 <sup>a</sup>	14.4 ± 1.9 <sup>a</sup>
380 rpm	70.2 ± 3.3 <sup>a</sup>	6.9 ± 0.9 <sup>a</sup>	17.2 ± 1.2 <sup>a</sup>
<b>PCF</b>	81.5 ± 0.0 <sup>b</sup>	2.6 ± 0.1 <sup>b</sup>	10.0 ± 0.2 <sup>b</sup>
<b>Red Bean</b>			
320 rpm	69.7 ± 4.2 <sup>a</sup>	7.0 ± 0.9 <sup>a</sup>	15.8 ± 2.0 <sup>a</sup>
380 rpm	70.3 ± 1.8 <sup>a</sup>	7.0 ± 0.3 <sup>a</sup>	16.0 ± 1.5 <sup>a</sup>
440 rpm	71.2 ± 2.5 <sup>a</sup>	6.8 ± 1.1 <sup>a</sup>	15.4 ± 0.5 <sup>a</sup>
<b>PCF</b>	80.1 ± 0.0 <sup>b</sup>	3.6 ± 0.0 <sup>b</sup>	8.6 ± 0.0 <sup>b</sup>

Hunter L\*, a\*, b\* color scale where L\* = brightness; a\* = greenness (-)/redness (+); and b\* = blueness (-)/yellowness (+). Letters show statistical differences between extrusion speeds under each bean type. Bean types were not compared. Means followed by different letters within each bean type are significantly different at p < 0.05 according to mean separation test using LSD procedure. PCF = Precooked flour.

**Table 2.4. Effect of extruder speed on water solubility and water absorbance index of bean extrudates**

<b>Speed</b>	<b>Black</b>	<b>Navy</b>	<b>Pinto</b>	<b>Red</b>
<b>Water solubility index</b>				
320	27.8 ± 3.1 <sup>a</sup>	31.9 ± 2.0 <sup>a</sup>	29.6 ± 3.1 <sup>a</sup>	31.7 ± 4.4 <sup>a</sup>
380	35.9 ± 2.7 <sup>a</sup>	30.1 ± 1.1 <sup>a</sup>	33.1 ± 6.2 <sup>a</sup>	31.4 ± 2.6 <sup>a</sup>
440	30.4 ± 7.9 <sup>a</sup>	32.7 ± 2.0 <sup>a</sup>	*	33.0 ± 2.8 <sup>a</sup>
PCF	15.9 ± 0.04 <sup>b</sup>	13.9 ± 0.11 <sup>b</sup>	16.5 ± 0.11 <sup>b</sup>	17.3 ± 0.13 <sup>b</sup>
<b>Water absorbance index</b>				
320	3.8 ± 0.2 <sup>a</sup>	3.7 ± 0.1 <sup>a</sup>	3.9 ± 0.0 <sup>a</sup>	3.7 ± 0.1 <sup>a</sup>
380	3.6 ± 0.3 <sup>ab</sup>	3.6 ± 0.2 <sup>a</sup>	3.6 ± 0.3 <sup>a</sup>	3.8 ± 0.1 <sup>a</sup>
440	3.2 ± 0.4 <sup>b</sup>	3.7 ± 0.1 <sup>a</sup>	*	3.8 ± 0.2 <sup>a</sup>
PCF	3.4 ± 0.03 <sup>ab</sup>	3.5 ± 0.01 <sup>b</sup>	3.2 ± 0.01 <sup>b</sup>	3.2 ± 0.01 <sup>b</sup>

\*Data not collected. PCF = Precooked flour. Means followed by different letters are significantly different at  $p < 0.05$  according to mean separation test using LSD procedure.

### 2.4.3. Starch Composition and Digestibility

TS was not significantly different among bean flours except for navy beans (Table 2.2). There was no significant reduction of TS even at 440 rpm. RS did not change significantly with changes in extrusion rates (Table 2.2). This result supports those of other researchers that extrusion results in a decrease or does not have any effect on RS (Unlu and Faller 1998; Faraj et al 2004; Kim et al 2006). RS in precooked flour ranged between 5.02 and 5.78% and was significantly higher in pinto and navy beans compared with their extrudates. eGI in both precooked flour and extruded beans was high in terms of GI classification, which is traditionally classified as low ( $\leq 55$ ), medium (56–69), and high ( $\geq 70$ ) (Miller et al 2006). High eGI indicates a high rate of in vitro digestibility (Ruiz-Ruiz et al 2008). Figure 2.2 shows that starches in precooked flour and extrudates were completely hydrolyzed within the first 30 min of digestion. Rate of hydrolysis; however, was much lower (up to 13%) than the white bread control.

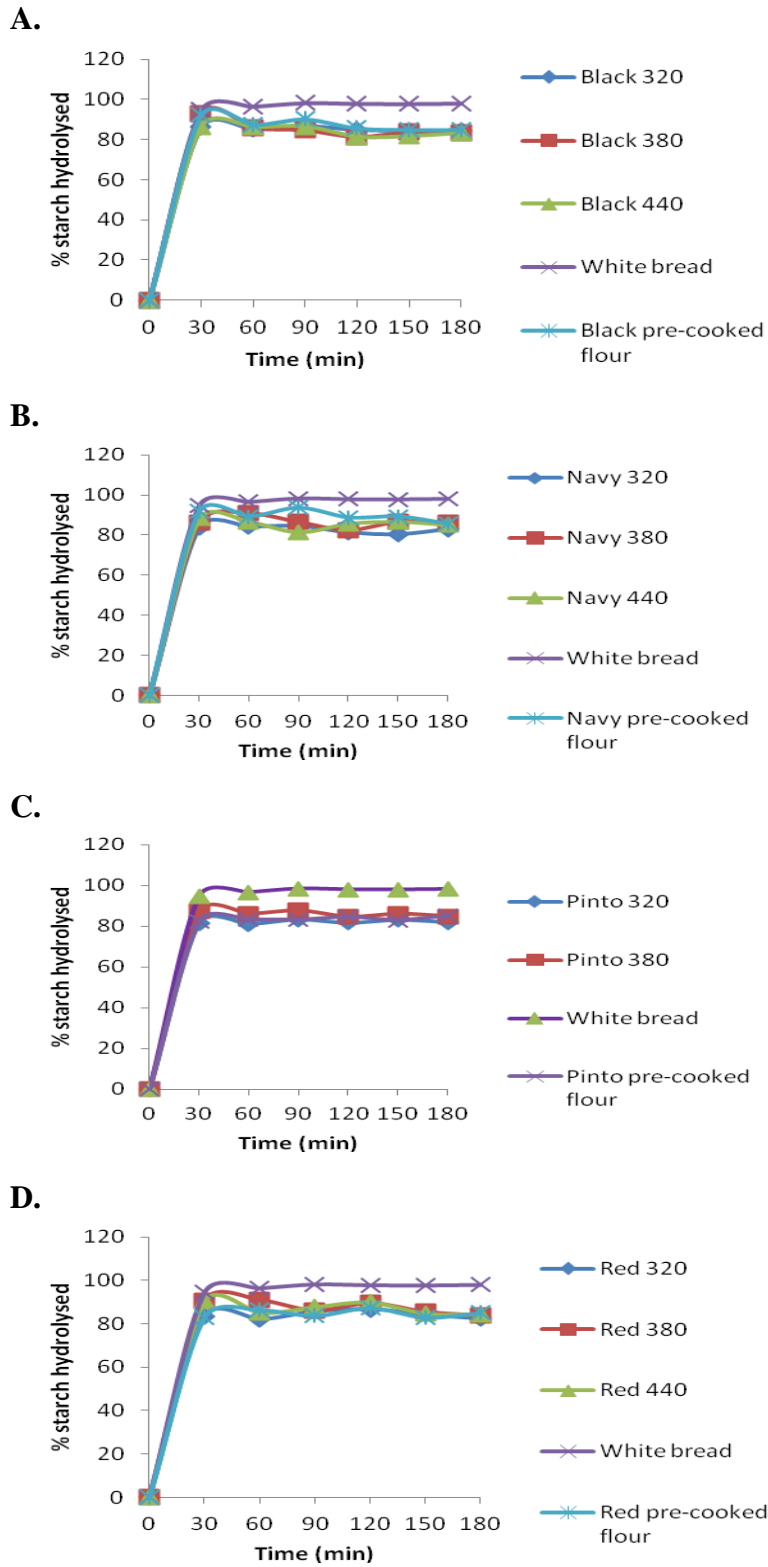


Figure 2.2. Starch hydrolysis curves of black (B), navy (B), pinto (C) and red (D) bean flours

Although RS in precooked flour was in some cases higher than extrudates, as in pinto and navy beans (Table 2.2), no significant reduction in eGI was observed. Therefore, extrusion generally did not offer any advantage in slowing the digestion of starch in the extrudates compared to its pre-cooked form.

## **2.5. Conclusions**

A screw speed of 440 rpm was too high to form extrudates using pinto bean flours. However black, navy, and red precooked flours were all successfully extruded at screw speeds ranging between 320 and 440 rpm. Increased screw speed reduced water activity in precooked black, pinto, and red beans but had no effect on navy beans within this range. Increasing extrusion speed had a negative effect on EI and texture. RS was reduced at higher screw speeds, but not significantly. The amount of RS present was not enough to produce a low-eGI product. Further studies are needed to investigate processing conditions (screw speed, temperature, and moisture combinations) to increase formation of RS while still maintaining good texture and expansion.

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## **CHAPTER 3. CHARACTERIZATION AND ACCEPTABILITY OF EXTRUDED PINTO, NAVY AND BLACK BEANS**

### **3.1. Abstract**

Sensory properties and consumer acceptance of edible bean snacks will play a significant role in their marketing and sales in the United States. In this study, dry pinto, navy, and black beans were milled using a hammer mill. Milled samples were extruded using a twin screw Wenger TX 52 extruder. Unflavored extruded snacks were evaluated by untrained panelists using a hedonic likeness scale for appearance, flavor, texture and overall acceptability. Raw flour and extrudates were characterized for total protein, total starch, resistant starch, crude fiber, total hexane extractable lipids, fatty acids, phytic acid and raffinose contents. Sensory results indicated that all beans met or exceeded the minimum requirement for acceptability. Overall acceptability of navy and pinto beans were not significantly different while acceptability of black bean snacks were significantly lower. Total protein in extrudates ranged from 19.8% (pinto) to 21.7% (navy) and was significantly different among the three beans. Total starch ranged from 39.8% - 40.6% and was not significantly different. Resistant starch, total extractable lipids and raffinose contents were significantly reduced by extrusion. Extrusion did not have any significant effect on crude fiber and phytic acid contents. Fatty acids present in beans were palmitate, stearate, oleate, linoleate and linolenate. Total amount of unsaturated fatty acids to saturated fatty acids in samples was approximately 4:1.

### **3.2. Introduction**

Pulse ingredients including its flours, fibers, starch and protein fractions, is a burgeoning industry. Fueling this growth is the realization of their health benefits and the need for consumers to maintain health and fitness. Pulses have been shown to reduce the risk of diabetes, obesity,

cardiovascular disease and colon cancer (Venn and Mann 2004; Noethlings et al 2008; Trinidad et al 2010; Hutchins et al 2012; Jenkins et al 2012). These benefits are very important for the US since among its population, many of these diseases have become prevalent. For example, colon cancer is the second leading type of cancer in the US. In 2009, 136,717 people were diagnosed with the disease, and of these, 51,848 died (CDC 2013a). The diabetic population in the US stands at 25.8 million people or 8.3% of the US population (CDC 2011). In terms of obesity, more than a third of the US population (35.7%) is obese leading to obesity-related conditions such as heart disease, stroke and type-2 diabetes (CDC 2013b). Also contributing to growing interests in pulse ingredients is the rise in celiac disease, where presently 0.7% (1:141) of the US population is affected. Patients with this disease cannot safely consume gluten (Briani et al 2008). Therefore, diets with pulse-based ingredients provide a viable option. Market for gluten-free foods and beverages grew annually by 30% between 2006 and 2010 and is projected to continue on this trend in the future (Sloan 2012).

Research that has been done on extrusion of pulses either as 100% pulse flour or as an inclusion with cereals, confirms that pulse flours have good extrusion properties and are capable of producing healthy snack alternatives (Campoy et al 1984; Gujska and Khan 1990; Borejszo and Khan 1992; Balandran-Quintana et al 1998; Anton et al 2009; Berrios et al 2004; Gujska and Khan 2002; Lazou et al 2010; Kelkar et al 2012; Simons et al 2012). A major hurdle for expansion of the pulse ingredient industry however is producing quality products that have acceptable organoleptic properties. Pulses are known to produce grassy and ‘beany’ off-flavors due to lipoxygenase catalyzed lipid oxidation (Sessa 1979).

Few studies have been completed on the sensory properties of extruded pulse snacks or ingredients. Campoy et al (1984) prepared extruded snacks from blends of sesame meal and

pinto beans or chickpea. A sensory panel of untrained Latin Americans rated the products as acceptable. Nyombaire et al (2008) extruded whole dry light red kidney beans and used the extrudates as ingredient to make a porridge consisting of extruded flour, sugar and water. Panelists evaluated the porridge on a hedonic nine-point scale for color, texture, flavor and overall quality. The panelists rated the acceptability for the porridge for each attribute above six. Pawar et al (2009) made ready-to-eat extruded snacks from different blends of rice, corn and legume malt and reported that extruded snacks with legume malt had better sensory quality attributes than snacks without malted legume flour. Recently, Siddiq et al (2013) used low temperature (85°C) extrusion and steam cooking to process navy and pinto bean flours for gluten-free cookies. Sensory tests were conducted on the cookies using a nine-point hedonic scale for flavor, texture and overall acceptability. Cookies from extruded flours had higher scores than the steam cooked flours. Overall, 94 of the 107 panelists could not detect a beany flavor Siddiq et al (2013).

These studies demonstrate excellent potential for pulse flours to make extruded snack food and ingredients. However, research comparing sensory qualities of bean snacks made from pinto, navy and black beans has not been reported. These three market classes are of significant economic value to the US market since they represent the leading market classes produced and exported. As the world's sixth leading dry bean producer, the US is positioned to be a leader in processing and exporting of value-added pulse-based food ingredients from pinto, navy and black beans (USDA 2012). The objective of this study was to determine physicochemical properties and sensory acceptability of extruded pinto, navy and black beans.

### **3.3. Materials and Methods**

#### **3.3.1. Extrusion Conditions**

Cleaned pinto, navy and black beans were purchased from distribution warehouses in North Dakota during the 2010 crop year, and milled using a FitzMill Comminutor (Sint-Niklaas, Belgium) fitted with a stainless steel screen with 0.5 mm circular openings. Milled bean flours were extruded on two separate days on a co-rotating, fully intermeshing, self-wiping TX 52 Wenger extruder (Wenger Manufacturing, Sabetha, KS). The extruder had 52 mm diameter screws and a barrel length to diameter ratio of 25.5:1. Moisture was adjusted from 11.5% to 13% by injecting water into the extruder barrel. Screw speed was set at 320 rpm. Bean flours were fed from a feed hopper at a rate of 58.8 kg/hr. The barrel had six temperature zones from feeding to die section. Temperatures were set at 40°C (zones 1 - 4), and 80°C (zones 5 - 6). Configuration of screws from feed to die end consisted of 9 conveying, 3 shear lock forward, 1 conveying, 3 shear lock forward, 1 conveying, 3 shear lock forward, 1 conveying, 3 shear lock forward, 1 conveying, 3 shear lock forward, 1 conveying and 1 cone conveyor. A rectangular die (7.9 mm x 1.6 mm) was used to form extrudates into bits resembling rice-shaped puffs. Extrudates were collected on meshed metal trays within 5 min after a steady state was reached, and then cooled on cooling racks. After cooling, extrudates were transferred to large plastic storage bags and kept in a refrigerator at 4°C until ready for analysis. Specific mechanical energy (SME) used during extrusion was 91 W.h/kg (See Chapter 1 for calculations).

#### **3.3.2. Physical Properties of Extrudates**

Expansion index (EI) of extrudates was determined based on the procedure of Baik et al (2004). Ten pieces of extrudates from each extrusion run ( $n = 20$ ) were randomly selected, and diameters were measured with a caliper. EI was the average diameter of extrudates divided by

the diameter of the extruder die. Hardness of extrudates was determined using a CT3 texture analyzer (Brookfield, Middleboro, MA) equip with a 4.5 cm x 4.5 cm x 5.7 cm Ottawa cell probe assembly with a grid base plate. Peak force (g) was determined by placing exactly ten grams of sample in the cell and compressing the sample with a 4.3 cm x 4.3 cm plunger. The instrument settings included target value, 5 mm; hold time, 0 seconds; trigger load, 10 g; test speed, 5 mm/s; return speed, 4.5 mm/s; and data acquisition rate, 100 points/s.

### **3.3.3. Sensory Evaluation**

Sensory evaluation of extrudates were based on a hedonic test involving 40 untrained adult participants including students, staff and faculty at North Dakota State University. On the day of evaluation, approximately five grams of navy, pinto and black bean snacks from extrusion runs 1 and 2 were placed into separate 20 ml plastic cups labeled with random three-digit numbers. The samples were then presented to the panelists in random order to be rated based on appearance, flavor, texture and overall acceptability on a seven-point hedonic scale (7- like extremely; 6 – like moderately; 5 – like slightly; 4 – neither like nor dislike; 3 – dislike slightly; 2 – dislike moderately; 1 – dislike extremely). A rating of four was considered the minimum score for acceptability. In addition to scoring, panelists were also allowed to add comments on the evaluation sheets. The taste testing room was brightly lit with soft white fluorescent lighting. Participates were briefed not to interact with each other during tasting.

### **3.3.4. Chemical Composition of Extrudates**

Moisture analysis was based on AOAC oven drying Method 925.10 (AOAC 1990). Total protein was determined in duplicate using a Dumatherm Nitrogen/Protein Analyzer (Northamptonshire, UK). Triplicate bean flour samples of 50 milligrams were weighed in tin

foils, packed air-tight, and then placed into empty sample chambers in the autosampler. The sample was then combusted in the presence of oxygen at 1000°C to oxidize N to N-oxides. The N-oxides then reacted with copper reduction catalyst to form elemental Nitrogen (N<sub>2</sub>), which was detected on a thermal conductivity detector. A conversion factor of 6.25 was used to determine protein in samples.

Resistant starch was determined in triplicate using a resistant starch test kit obtained from Megazyme International (Bray, Ireland). Total starch was the sum of resistant starch and non-resistant starch obtained from the resistant starch procedure. Crude fiber determination followed AOAC Method 962.09 (1982) and was determined in duplicate.

Total lipids were determined in duplicate by extracting lipids with hexane on a Soxtherm Rapid Extraction System (C. Gerhardt GmbH & Co., Germany) and then measuring extracted lipids gravimetrically. Briefly, samples (8 g) were placed in folded envelopes made from 15 cm filter paper (415, qualitative, VWR International, Radnor, PA). One gram of finely ground sand was added to the sample to prevent aggregation, and the envelope placed inside a thimble. The thimble was covered with cotton balls and saturated with hexane. Thimbles were afterwards transferred to Soxtherm glassware and extraction carried out with hexane (140 ml) for 3.5 hours. Glasswares containing the samples and extracts were then removed from the envelopes, and the samples redistributed using a ceramic mortar and pestle. Samples were returned to the envelope and extracted in hexane (130 ml) for another 3.5 hours. After extraction, hexane in the Soxtherm glassware was gently evaporated from oil by heating at approximately 25°C and exposing to a continuous flow of nitrogen. The crude oil thus obtained was weighed to determine percentage oil extracted.

The extracted lipids were further evaluated to determine fatty acids present. Duplicate samples of 50 mg of the extracted oil were transferred to test tubes and 5 ml of 2% H<sub>2</sub>SO<sub>4</sub> (v/v) in methanol containing 200 mg/L of C17:0 was added. The tubes were then capped and the samples vortexed to ensure that all the oil was mixed into the solvent. The tubes were then placed in a Mult-Heater unit at 110 °C for one hour with vortexing every 15 minutes and at the end of the hour. Fatty acids were then separated by first adding 3 mL of hexane, and then adding 3 mL of deionized water; vortexing the tubes between each addition. The tubes were then allowed to stand for 10 minutes to promote phase separation. A Pasteur pipette was used to draw off part of the upper layer (hexane + oil), and the sample deposited into GC vials.

Fatty acids were separated using a Hewlett Packard HP 5890 Series Gas Chromatograph equipped with FID detector and Supelco SP-2380 column (Bellefonte, PA, USA); 30 m x 0.25 mm internal diameter and 0.20 µm film thickness. Helium was used as carrier gas with column head pressure set at 15 psi. Temperature of oven was initially at 150 °C, which was held for 5 minutes. Temperature was then ramped to 180 °C at a rate of 10°C per minute and held for 12 minutes. The injector and detector temperatures were set at 200 °C and 270 °C, respectively. Fatty acid methyl ester (FAME) peaks were identified based on comparison with retention times of reference standards.

Phytic acid and raffinose contents were determined in duplicate using Megazyme Phytic Acid (Phytate)/Total Phosphorus assay procedure and Megazyme Raffinose/Galactose assay procedure, respectively (Megazyme International, Bray, Ireland).

### **3.3.5. Statistical Analysis**

Bean flours were extruded on separate days, representing two different experimental units. Extrudates of separate runs were then evaluated and data reported as mean of both runs.

Analysis of sensory data was based on a completely randomized block design (RCBD) with panelists representing 40 randomized blocks. Physical and chemical analysis was based on a completely randomized design (CRD). Statistical analysis systems software (SAS Institute, Cary NC) was used to conduct the analysis of variance (ANOVA) and means comparison using least significant difference procedure. Differences were significant at  $P < 0.05$ .

### 3.4. Results and Discussion

#### 3.4.1. Physical Properties of Extrudates

Expansion index of bean extrudates ranged from 3.2 to 3.5 and were not significantly different (Table 3.1). This expansion was much higher than observed in a previous study evaluating expansion of pre-cooked pinto, navy and black bean flours under similar processing conditions (Simons et al 2012). In that study, expansion index ranged from 2.27 to 2.66. Higher expansion observed in this study suggests greater capacity for expansion using raw bean flours compared to pre-cooked bean flours. Hardness of navy bean extrudates (Table 3.1) was significantly higher than pinto and black bean extrudates. This could be due to stronger component interactions of navy bean flour during extrusion. Stability in hardness of extrudates processed at increasing screw speeds, using pre-cooked bean flours suggested greater ability to resist shear during extrusion compared to pinto, black and red beans (Simons et al 2012).

**Table 3.1. Texture (hardness) and expansion index (EI) of navy, black and pinto bean extrudates**

<b>Bean</b>	<b>Hardness (g)</b>	<b>EI</b>
Navy	4564 ± 1332a	3.5 ± 0.52a
Black	3921 ± 984b	3.2 ± 0.59a
Pinto	3367 ± 442b	3.5 ± 0.52a

EI = expansion index. Values within the same column followed by the same letter are not significantly different at  $P < 0.05$ .



### 3.4.2. Sensory Evaluation

Sensory evaluation of the bean extrudates indicated that significant differences existed between the bean extrudates (Figure 3.1). There were significant differences in appearance of all three bean snacks. Navy beans had the highest likeness rating (6.1) compared to pinto beans (4.9) and black beans (3.4). Low acceptance rating of black beans indicates that appearance was not acceptable. Although bean snacks were 100% bean with no added flavor ingredients, they all exceeded the minimum flavor value for acceptability (Figure 3.1). There were no significant differences in flavor acceptability among bean extrudates. Evidence of ‘beany’ aftertaste was noted on panelist’s comments as an undesirable flavor they observed during tasting. Nyombaire et al (2011) conducted sensory evaluation on extruded red kidney beans and reported that no ‘beany’ flavor was identified. They used much higher cooking temperatures (120 °C and 130 °C), which could be key in modifying and eliminating off-flavors. Texture of navy and pinto beans were rated just over five (liked slightly) with no significant difference, while black beans were rated significantly less (4.7). This rating was not reflected in differences observed based on instrument analysis (Table 3.1). Some panelists commented that the extrudates had a tendency to stick to their palate during chewing. There was no further analysis however to differentiate levels of stickiness. Overall acceptability of bean snacks exceeded the minimum requirement for acceptability in navy and pinto extrudates, while black beans met the minimum acceptable level. This supported results of Camoy et al (1984) and Nyombaire et al (2008) who reported above-minimum acceptability of extrudates containing pulses, based on responses of untrained panelists.

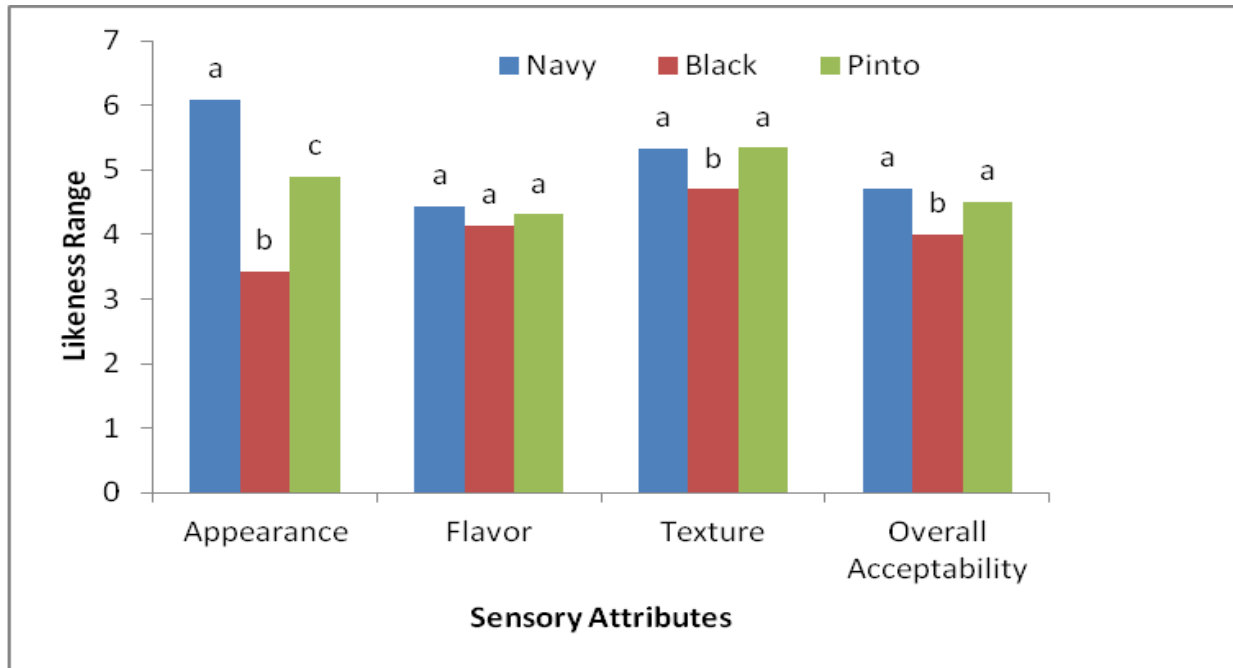


Figure 3.1. Acceptability of extruded navy, black and pinto beans. Bars with the same letters within the same sensory attribute are not significantly different at  $P < 0.05$

### 3.4.3. Chemical Composition of Extrudates

Similar to sensory results, differences in bean composition was observed (Table 3.2).

Total protein was significantly different between beans; with navy beans having the highest (21.7%) and black beans having the lowest (19.5%). Protein percentage was generally not affected by extrusion (Table 3.2).

Total starch in the raw bean flours ranged from 44.6% to 48.6% and was not significantly different between bean types. Significant reduction in total starch was observed after extrusion however. The reason for this observation was not clear. Several possible reasons include starch degradation and lipid-starch complexes.

**Table 3.2. Effect of extrusion on chemical composition (d.b.) of navy, black and pinto beans**

<b>Bean</b>	<b>Protein (%)</b>	<b>Total Starch (%)</b>	<b>Resistant Starch (%)</b>	<b>Crude Fiber (%)</b>	<b>Total Lipids (%)</b>	<b>Phytic Acid (g/100g)</b>	<b>Raffinose (g/100g)</b>
<i>Raw bean flour</i>							
Navy	21.7 ± 0.20a	44.6 ± 1.22a	38.7 ± 1.38a	4.1 ± 0.12ab	2.0 ± 0.06a	1.6 ± 0.09a	1.9 ± 0.20a
Black	20.9 ± 0.10b	44.1 ± 0.90a	37.9 ± 0.73a	3.9 ± 0.32abc	1.7 ± 0.27a	1.4 ± 0.11b	2.1 ± 0.22a
Pinto	19.5 ± 0.06c	48.6 ± 0.09a	42.4 ± 0.19b	3.7 ± 0.02c	1.9 ± 0.02a	1.2 ± 0.04c	1.8 ± 0.13a
<i>Extrudates</i>							
Navy	21.7 ± 0.21a	40.6 ± 4.29b	0.5 ± 0.11c	4.2 ± 0.11a	0.3 ± 0.06b	1.4 ± 0.18a 1.5 ±	1.5 ± 0.24b
Black	21.1 ± 0.09b	39.8 ± 3.28b	0.9 ± 0.13d	3.8 ± 0.11bc	0.3 ± 0.03b	0.11ab	1.6 ± 0.15 b
Pinto	19.8 ± 0.16d	40.6 ± 3.70b	1.0 ± 0.14e	4.0 ± 0.07abc	0.3 ± 0.05b	1.2 ± 0.08c	1.5 ± 0.19b

Values within the same column followed by the same letter are not significantly different at  $P < 0.05$ .

Total lipids extracted from raw beans ranged from 1.72 - 2.04% and were not significantly different. Extractable lipids from extrudates was approximately 0.3%; a reduction of approximately 84.0 % in recoverable lipids. Several researchers have shown that lipids form complexes during extrusion such as amylose-lipid and protein-lipid complexes, making them less extractable (Colonna and Mercier 1983; Schweizer and Reimann 1986; Izzo and Ho 1989; Bhatnagar and Hanna 1994). Reduction of available lipids for lipid oxidation is expected to have significant implications with respect to improving shelf life of pulse flour ingredients. Five fatty acids were identified in raw beans (Table 3.3). They included palmitate (15.8 - 19.4%), stearate (2.7 - 3.3%), oleate (12.9 - 16.5%), linoleate (32.1 - 33.1%) and linolenate (29.5 - 34.5%). Samples had approximately four times more unsaturated fatty acids compared to saturated fatty acids. Sutivisedsak et al (2011) reported fatty acids in black and pinto beans to contain palmitate (10.7 and 12.7%), stearate (1.7 and 1.8%), oleate (5.9 and 9.3%), linoleate (31.1 and 32.1%) and linolenate (41.7 and 43.3%), respectively. Total unsaturated fatty acids in black and pinto beans were reported as 85.2% and 83.7%, respectively, with a ratio of saturated to unsaturated fatty acids being approximately 1:6 (Sutivisedsak et al 2011).

**Table 3.3. Fatty acid composition (% d.b.) of raw navy, black and pinto bean flour**

<b>Fatty acid</b>	<b>Navy</b>	<b>Black</b>	<b>Pinto</b>
Palmitate (C16:0)	19.4 ± 0.36	18.1 ± 1.32 ab	15.8 ± 0.35 b
Stearate (C18:0)	2.7 ± 0.09	3.3 ± 0.75 a	2.8 ± 0.44 a
Oleate (C18:1)	15.0 ± 0.20 a	16.5 ± 3.81 a	12.9 ± 1.68 a
Linoleate(C18:2)	32.1 ± 0.12 a	32.7 ± 2.22a	33.1 ± 0.91 a
Linolenate (C18:3)	34.5 ± 0.76 a	29.5 ± 3.66 a	31.9 ± 0.86 a
Σ Saturated fatty acids <sup>a</sup>	18.4 ± 0.45 a	21.4 ± 2.06 a	22.2 ± 0.08 a
Σ Unsaturated fatty acids <sup>b</sup>	81.6 ± 0.45 a	78.6 ± 2.06a	77.8 ± 0.08 a

<sup>a</sup> C16:0 + C18:0; <sup>b</sup> C18:1 + C18:2 + C18:3. Values within the same row followed by the same letter are not significantly different at  $P < 0.05$ .

Extrusion did not have any significant effect on crude fiber. Therefore extrusion processing is not expected to affect the benefits derived from insoluble fiber in the extruded beans. Resistant starch in raw bean flours was very high; with pinto bean flours having the highest level (42.4%). Resistant starch was almost completely eliminated after extrusion (Table 3.2). The low moisture content in the extrusion process may have contributed to the observed reduction in the amount of resistant starch. Lower moisture content could lead to more aggressive treatment of the material. Since water acts as a lubricant in the extruder, less water will result in greater friction and starch damage during the process.

Phytic acid was not significantly reduced after extrusion (Table 3.2). Other studies have shown that extrusion processing only achieved partial reduction of phytic acid. Batista et al (2010) observed partial degradation of phytic acid by up to 26 % when hard-to-cook common beans were processed at a high temperature and moisture of 150°C and 20 %, respectively. Abd-El-Hady and Habiba (2003) reported that soaking of seeds prior to milling and extrusion was effective in reducing phytic acid levels. In addition, higher extrusion temperatures (180 °C) were more effective in reducing phytic acid than lower extrusion temperatures (140 °C). A similar trend was observed when feed moisture increased from 18 % to 22 %. Nergiz and Gokgoz (2007) also observed phytic acid reduction during soaking in a number of dry bean varieties. They observed up to 57 % reduction by pre-soaking beans. Absence of significant reduction of phytic acid in pinto, navy and black bean extrudates in this study could be due to a combination of the low temperature and moisture used, and the absence of a pre-soaking step.

Raffinose content was not significantly different among beans, but was significantly reduced by extrusion by up to 22%. Raffinose is an oligosaccharide associated with flatulence. Reducing raffinose therefore, is important in lowering discomfort due to bean consumption.

Berrios et al (2010) observed significant raffinose reduction in dry pea and lentil flour by 48% and 82% respectively after extrusion. This high reduction could be due to the high temperature (160 °C) and extrusion screw speed (500 rpm) in the study. These conditions likely resulted in more extensive disruption and degradation of carbohydrates in the material, including raffinose. Borejszo and Khan (1992) also observed a reduction in raffinose content by up to 47% after extruding pinto bean starch fraction and reported increasing reduction of raffinose as extrusion temperature increased from 110 °C to 163 °C. They concluded that extrusion was effective in reducing flatulence-causing factors in beans.

### **3.5. Conclusions**

Overall extruded pinto, navy and black beans extrudates had acceptable sensory properties. There was no difference in overall acceptability between navy and pinto bean extrudates, although navy beans had a much more acceptable appearance. Snacks from black beans had the lowest acceptability and will therefore pose the greatest consumer acceptability challenge; especially related to appearance. Additional innovation will be needed to enhance appearance for consumer acceptance. Grassy-after taste as noted by some panelists will be a concern for all beans. Steps in processing will need to be taken to reduce development or retention of grassy flavors by adjusting processing conditions, for example pre-conditioning material in the extruder, and or processing at higher temperatures.

Bean extrudates using processing conditions in this study are not expected to have low glycemic index due to the very low resistant starch in the final product. Processing adjustments in moisture, and screw configuration will need to be done to reduce aggressiveness of shear and excessive starch damage. However, compromising aggressiveness will likely affect ability of the process to remove off-flavors adequately. The reduction of raffinose content will result in

production of bean snacks with lower capacity to produce flatulence. Phytic acid in extrudates however will remain unchanged based on processing conditions in this study. Further studies need to be done to assess beany flavors in extruded snacks and to develop formulations and process conditions to minimize and eliminate them.

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## **CHAPTER 4. PROPERTIES OF PINTO BEANS AIR-CLASSIFIED HIGH STARCH FRACTION AND ITS EXTRUDATES**

### **4.1. Abstract**

Pinto beans were milled and air classified in a commercial mill to obtain a high starch fraction, and then extruded on a twin screw extruder. Properties of non-extruded high starch fraction (NE-HSF) and extruded high starch fraction (E-HSF) were compared with whole pinto flour (WPF). Mean extrudate expansion index and hardness were 3.3 and 1949 g, respectively. Composition (d.b.) of WPF was 4% ash, 1.6% extractable lipid (EL), 22% protein and 46.4 % total starch (TS), where 6.5% was soluble starch (SS) and 39.9% resistant starch (RS). Significant differences in composition of NE-HSF were observed in ash (2.8%), EL (1.1%), protein (13.3%), RS (39.9%), SS (7.9%) and TS (56%). Composition of E-HSF was significantly different from the other two flours with 0.0% RS, 55.7% SS and 0.1% EL. Brightness (L\*) of NE-HSF was the highest (87.5) compared to WPF (81.5) and E-HSF (77). Water solubility index (WSI) of NE-HSF was 14.1% compared to 24.7% in WPF and 32.7% in E-HSF. Water absorption index of NE-HSF and E-HSF were significantly lower than WPF. Thermal properties of NE-HSF indicated that it underwent lower transition temperatures than WPF. Pasting properties of WPF, NE-HSF and E-HSF were significantly different. Differences in composition and functional properties of extruded and non-extruded starch fraction provide support for the use of air-classification fractionation methods, and extrusion technology to produce pulse-based ingredients with unique food applications.

## 4.2. Introduction

A pulse-rich diet offers several health benefits, all of which have the potential to drive new product development innovation in pulse-based ingredients. Characteristics of pulses associated with health benefits include their low glycemic index, high protein, significant amounts of minerals and vitamins, high dietary fiber, low fat and antioxidants. The combination of these features reduce the risk of certain diseases (e.g. diabetes, obesity, colon cancer and cardio-vascular disease) when they are regularly consumed (Tharanathan and Mahadevamma 2003; Brennan 2005; Brand-Miller et al 2007; Finley et al 2007; Rochfort and Panozzo 2007; Campos-Vega et al 2010; Tosh and Yada 2010). Health benefits derived from pulses are associated with specific food components. These components can be isolated and reintroduced into food formulations for a more targeted approach in mitigating disease risks and producing desired food functionality. Pulse starches for example, can be added as a food ingredient to lower glycemic index due to its slow digestibility (Jenkins et al 2012). Nevertheless, established commercial food applications for pulse starches have been limited. Hoover et al (2010) attributed their limitations to undesirable characteristics such as high gelatinization transition temperatures and high syneresis.

Air-classification is a method used to fractionate raw materials into starch and protein fractions. It is faster and much more economical compared to wet fractionation methods (Sathe 2002). Wet fractionation involves suspending dehulled whole pulse flour in slurry and solubilizing proteins using an alkali. The slurry is then centrifuged and the supernatant discarded. The precipitate, or high starch fraction, is finally neutralized, washed and dried (Gujska et al 1994; Wani et al 2010). Air-classification does not require water or chemicals; reducing the potential for microbial contamination and the need for effluent disposal. The process can handle a much larger volume of material at a time. The procedure entails milling the material to very

fine flour and then aspirating in a pneumatic system. Separation is achieved based on differences in specific gravity. Fine particles (low specific gravity) are collected as a high protein fraction and coarse particles (high specific gravity), as a high starch fraction (Tyler et al 1981; Hoover et al 2010).

This study will provide pulse processors with more comprehensive and relevant information on properties of air-classified and extruded pinto bean high starch fraction compared to previous studies. Previous studies were limited to the use of laboratory scale fractionation and extrusion equipment (Skierkowski et al 1990; Gujska and Khan 1991a; Gujska and Khan 1991b; Borejszo and Khan 1992). Although providing relevant information, data may not adequately represent expected outcomes when processing is done at a commercial scale. Therefore in this study, a commercial high-volume air-classifier and high capacity extruder (200 kg/hr) with potential for reliable scale up was utilized. Pinto bean was used as a model since it is the leading bean market class produced in the US.

The objective of this study was to characterize and determine differences in properties of pinto bean flour in the form of whole flour, raw high starch fraction, and extruded high starch fraction. Differences in properties will provide direction for new pulse-based ingredient applications.

### **4.3. Materials and Methods**

#### **4.3.1. Air-Classification**

Pinto beans were purchased from a commercial warehouse (Kelley Bean Co., Hatton ND). Beans were then pin-milled and fractionated in a commercial mill (Particle Control, Albertville MN). The flour obtained consisted of 88% 44  $\mu\text{m}$  particles after pin milling. Air-

classification resulted in 80% of the material collected as coarse (starch fraction) and 20% as fines (protein fraction).

#### **4.3.2. Extrusion Conditions**

Extrusion of the starch fraction was completed at the Northern Crops Institute (Fargo, ND) using a co-rotating twin screw extruder (Wenger TX-52, Sabetha, KS). The extruder had two 52 mm diameter screws, and barrel length-to-diameter ratio of 25.5:1. The barrel had six temperature zones, from feeding to die section, and set at 40 °C, 60 °C, 60 °C, 60 °C, 60 °C and 100 °C. Configuration of screws from feed to die end consisted of twelve conveying, one shear lock reverse, two conveying, one cut flight, one shear lock reverse, one conveying, one cut flight, one shear lock forward, two conveying and one cone screw. Screw speed of extruder was 300 rpm. The die was circular with a 4.7 mm diameter. Cutting knife was positioned off-center at the end of the die. The flour was fed from the feed hopper at a rate of 64.2 kg/hr. The moisture of the raw flour was adjusted to 16% by metering water into the extruder barrel. Specific mechanical (SME) was 78 W.hr/kg based on calculation method by Simons et al (2012). Extrudates were collected on meshed metal trays after 5 minutes of steady state extrusion was achieved. Trays were then conveyed through an impingement oven (Lincoln, Middlesex, Great Britain) at 163 °C for 4 minutes to dry. Trays were then cooled on metal racks. Cooled samples were stored in large plastic bags, sealed and stored at 4 °C until ready for analysis. Extrudates were milled using a centrifugal mill with a 0.5 mm sieve (Retsch, Haan, Germany) before compositional and functional property analysis.



### 4.3.3. Physical Properties

Hardness of extrudates was determined using a texture analyzer with a three-point bend assembly probe (CT3 Texture Analyzer, Brookfield, Middleboro, MA). The instrument was set at a test speed of 5 mm/sec; 8 mm target value, 30 g trigger load; and 100 points/sec data acquisition rate. Five pieces of extrudates within each extrusion replication ( $n = 10$ ) were randomly selected and tested to determine the peak force (g). Expansion index (EI) of extrudates was determined based on the procedure by Baik et al (2004). Ten pieces of extrudates within two extrusion replications ( $n = 20$ ) were randomly selected, and diameters measured with a caliper. EI was reported as the average diameter of extrudates divided by the diameter of the extruder die. Color was determined in triplicate using a Minolta colorimeter and reported as L, a and b values.

### 4.3.4. Chemical Composition

Moisture was determined based on AOAC method 925.10 (AOAC 1990). Water activity was determined in duplicate using an AquaLab water activity meter (AquaLab 3TE, Decagon Devices, Pullman, WA). Protein content was determined in triplicate using a nitrogen combustion analyzer (Leco FP-528, St. Joseph, MI), based on AACCI Method 46-30 (AACCI 2010). A factor of 6.25 was used to convert N to protein.

Amino acid content was determined in duplicate based on method adapted from Ozols (1990), and Cooper et al (2000). Ten mg of bean flour samples were weighed in duplicate and transferred to hydrolysis tube with 200  $\mu$ l 6N HCl / 1% phenol. Samples were hydrolyzed at 110 °C for 24 hr to release amino acids. Samples were then dried and dissolved in pickering Na buffer (Mountain View, California) with 40 nmol/mL norleucine added as an internal standard. Final buffer volume was 15 ml, from which 50  $\mu$ l was injected into an automatic amino acid

analyzer (AAA) (HITACHI L-8800, Japan) with transgenomic ion-exchange column. An amino acid standard solution for protein hydrolysate (Sigma-Aldrich A-9906, St. Louis Missouri) was used to determine response factors and to calibrate the AAA for all amino acids before analysis. Tryptophan and Methionine were not reported since these amino acids are typically destroyed during hydrolysis.

Resistant starch and soluble starch were determined in triplicate using Megazyme resistant starch assay procedure (Megazyme, International (Bray, Ireland). Total starch was the sum of resistant starch and soluble starch.

Lipid analysis was completed in triplicate based on method adapted from AOCS methods Af 3-53, Am 2-93, and Aa 4-38 (AOCS 1998). Bean flour samples (5 g) were weighed and collected in a folded 15 cm filter paper (415, qualitative, VWR International, Radnor, PA) and transferred to a thimble holder. Lipids were extracted with hexane for 12 hr with a soxhlet apparatus (combo mantle, Glas-Col, Terre Haute, IN). Hexane was evaporated using a rotary evaporator (Rotavapor RE-111, Büchi, Flawil, Switzerland) at 60 °C and vacuum at 25 in Hg. The lipid obtained was gravimetrically determined.

The fatty acid profile was determined on extracted lipids. Lipids were methylated on 50 mg of extracted lipids from two separate lipid extractions. Five ml of 2% H<sub>2</sub>SO<sub>4</sub> (v/v) in methanol was then added. The sample was mixed by vortexing, capped tightly and heated at 110 °C for 1 hr; vortexing every 15 min. Tubes were then cooled to room temperature and 3 ml hexane and 3 ml water added; vortexing between addition of each. Samples were allowed to stand for 10 minutes to separate. The top layer was removed and transferred to GC vials. Fatty acids were separated using a Hewlett Packard HP 5890 Series gas chromatograph equipped with FID detector and Supelco SP-2380 column (Bellefonte, PA, USA; 30 m x 0.25 mm internal

diameter and 0.20  $\mu\text{m}$  film thickness). Helium was used as carrier gas with column head pressure set at 15 psi. Temperature of oven was initially at 150  $^{\circ}\text{C}$ , which was held for 5 minutes. Temperature was then ramped to 180  $^{\circ}\text{C}$  at a rate of 10  $^{\circ}\text{C}$  per minute and held for 12 minutes. The injector and detector temperatures were set at 200  $^{\circ}\text{C}$  and 270  $^{\circ}\text{C}$ , respectively. Fatty acid methyl ester (FAME) peaks were identified based on comparison with retention times of reference standards (GLC Reference STD 603 and 94, Nu-Check Prep Inc., MN).

Phytic acid content was determined in triplicate following Megazyme phytic (phytate)/total phosphorus assay procedure (Megazyme International, Bray, Ireland). Exactly 1 g of pinto bean flour sample was weighed in triplicate and placed in a 75 mL glass beaker. Twenty ml of hydrochloric acid (0.66 M) was then added and the beaker covered with foil and stirred on a magnetic stir plate for 12 hours at room temperature. One ml of the extract was then transferred to a 1.5 mL microfuge tube and centrifuged at 13,000 rpm for 10 min. Exactly 0.5 mL of the resulting extract supernatant was immediately transferred to a fresh 1.5 mL microfuge tube and neutralized by addition of 0.5 mL of sodium hydroxide solution (0.75 M). The neutralized sample was then used in an enzymatic dephosphorylation reaction procedure where phytase and alkaline phosphatase enzymes were used to convert phytic acid in the extract, to myo-inositol and inorganic phosphate (Pi). The Pi was then reacted with ammonium molybdate (5 % w/v) to form 12-molybdophosphoric acid (12-MPA). 12-MPA was then reacted with ascorbic acid (10 % w/v)/  $\text{H}_2\text{SO}_4$  (1M) to form molybdenum blue. The amount of molybdenum blue was proportional to Pi present, and was measured spectrophotometrically by increase of absorbance at 655 nm. Pi was quantified as phosphorus from a calibration curve generated using standard known concentrations. Phytic acid was calculated using equation 4.1.

$$c = \frac{M \times 20 \times F}{10000 \times 1.0 \times v} \times \Delta A_{\text{phosphorus}} \quad (4.1)$$

where  $c$  = concentration of phytic acid (g/100g);  $M$  = mean value of phosphorus standards [ $\mu\text{g}/\Delta A_{\text{phosphorus}}$ ];  $20$  = original sample extract volume [mL];  $F$  = dilution factor (55.6);  $\Delta A$  = absorbance change of sample;  $10,000$  = conversion from  $\mu\text{g/g}$  to  $\text{g}/100 \text{ g}$ ;  $1.0$  = weight of original sample material [g];  $v$  = sample volume used in the colorimetric determination step (1 ml).

Total dietary fiber was determined in duplicate following Megazyme dietary fiber assay procedure (Megazyme International, Bray, Ireland). Duplicate samples (1 g) of pinto bean flours were collected into 400 ml tall-form beakers. Forty ml of MES-TRIS blend buffer solution (pH 8.2) was then added and stirred on a magnetic stir plate until the flour was completely dispersed in solution. Exactly 50  $\mu\text{L}$  heat-stable  $\alpha$ -amylase solution was then added while stirring at low speed. The beaker was then covered with aluminum foil squares and placed in a shaking water bath (100 strokes/minute) at 95 °C for 35 min, and allowed to incubate under continuous agitation. The samples were then removed and cooled to 60 °C. The foil cover was removed and ring around beaker along with any gel at the bottom dislodge using a spatula. The sides of the beaker and spatula were then rinsed using 10 ml distilled water. The temperature of the water bath was adjusted to 60 °C. Protease solution (100  $\mu\text{L}$ ) was added to each beaker and the beakers covered again with aluminum foil covers. The samples were again incubated in the shaking water bath at 60 °C, with continuous agitation for 30 min. Samples were then removed and 5 ml of 0.561 N HCl solution added to adjust pH to 4.1- 4.8. Amyloglucosidase solution (200  $\mu\text{l}$ ) was then added and the samples incubated as before in shaking water bath at 60 °C for 30 min.

Dietary fiber was precipitated by adding to the samples, 225 ml of 95% ethanol preheated to 60 °C. Beakers were left to sit at room temperature for 1 hr to allow enough time for complete

precipitation of residue. Precipitates were then filtered by pouring into crucibles (Corning No. 36060 Büchner, fritted disk, Pyrex 60 mL, pore size, coarse, ASTM 40-60 µm) containing celite, and applying suction. After completion of transfer, residues were washed twice with successive 15 mL portions of 78% ethanol, 95% ethanol, and acetone. Crucibles with residue were then dried overnight in 103°C oven. The crucibles were then cooled in a desiccator for approximately 1 hr and then weighed. Weight of residue was determined by subtracting weight of crucible and celite. One extracted residue from each duplicate was analyzed for protein and the second for ash. Protein was determined using nitrogen combustion analyzer as described earlier. Ash content was determined in triplicate using AACCI Method 08-01.01 (AACCI 2010). With each assay, two blanks were run along with samples to measure any contribution from reagents to residue.

The percentage of dietary fiber was determined using equations 4.2 and 4.3.

$$\text{Dietary Fiber (\%)} = \frac{\frac{R1 + R2}{2} - p - A - B}{\frac{M1 + M2}{2}} \times 100 \quad (4.2)$$

where R1 = residue weight 1 from M1; R2 = residue weight 2 from M2; M1 = sample weight 1; M2 = sample weight 2; A = ash weight from R1; p = protein weight from R2; and B = blank

$$B = \frac{BR1 + BR2}{2} - BP - BA \quad (4.3)$$

where BR = blank residue; BP = blank protein from BR1; BA = blank ash from BR2.

#### **4.3.5. Functional Properties**

Water absorption index (WAI) and water solubility index (WSI) were determined in triplicate based on method by Anderson (1982). Samples were agitated to pass through 212  $\mu\text{m}$  sieve. Exactly 2.5 g was collected and 30 ml of distilled water added. The mixture was stirred at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 min. The supernatant was collected and the remaining gel weighed. WAI was gram gel/gram dry sample. To determine WSI, the supernatant was evaporated and the dry solids remaining weighed. WSI was calculated as percentage dry solid / 2.5 g sample.

Thermal characteristics of bean flours was determined in triplicate using a Perkin-Elmer Differential Scanning Calorimeter, DSC-7 using the method modified by Ovando-Martínez et al (2011). Flours (3.5 mg) were weighed in DSC aluminum pans and deionized water (8  $\mu\text{l}$ ) added. The pans were hermetically sealed and left to sit at room temperature overnight before analysis. Samples were heated from 20  $^{\circ}\text{C}$  to 120  $^{\circ}\text{C}$  at a rate of 10  $^{\circ}\text{C}/\text{min}$ . An empty aluminum pan was used as the reference.

Pasting properties were determined in triplicate using a Newport Scientific Rapid Visco-Analyzer (RVA) based on approved AACCI Method 76-21.01 (AACCI 2010). Four grams of sample (14% moisture basis) was added to 25 ml deionized water in an RVA canister and agitated to break up lumps. Slurries were held at 50  $^{\circ}\text{C}$  for 1 min and then heated 95  $^{\circ}\text{C}$  at a rate of 12  $^{\circ}\text{C}/\text{min}$ . The temperature was kept at 95  $^{\circ}\text{C}$  for 2 min and 12 seconds. The slurry was then cooled to 50  $^{\circ}\text{C}$  and held for 2 min while rotating at 160 rpm.

#### **4.3.6. Statistical Analysis**

Pinto high starch fraction was extruded on two separate days. Extrudates of the two extrusions runs were characterized based on a completely randomized (CRD) design. Statistical

analysis systems software (SAS Institute, Cary NC) was used to analyze data. An analysis of variance (ANOVA) and means comparison following a least significant difference procedure were used to determine differences. Differences were considered to be significant when  $P < 0.05$ .

## **4.4. Results and Discussion**

### **4.4.1. Physical Properties**

The extrudates resembled corn puffs except that the color was brown (Figure 4.1). Mean expansion and mean hardness values were 3.3 and 1968 g, respectively. These values were greater than expansion and hardness observed in previous study using similar conditions and pre-cooked pinto flour (Simons et al 2012). Extrudates from pre-cooked pinto flour had expansion and mean hardness values of 2.5 and 743 g, respectively. The higher starch composition in the pulse starch fraction relative to protein likely contributed to the higher expansion and hardness compared to the extrudates from precooked flour. Presence of protein can retard expansion during extrusion (Faubion et al 1982; Peri et al 1983). Furthermore, greater degradation in amylopectin in precooked flour could be the reason for lower expansion since breakdown of amylopectin reduces expansion in extruded starch material (Meshram et al 2009).

Color change in flours from WPF to E-HSF generally trended towards a reduction in  $L^*$  value (darker lightness intensity), increase in redness ( $a^*$ ) and reduction in yellowness ( $b^*$ ) on the Hunter color scale. The color tended to be brown (Figure 4.1).



Figure 4.1. Extruded pinto bean obtained from high starch fraction. Ruler unit is in inches

#### **4.4.2. Chemical Composition**

Differences in chemical and functional properties of WPF, NE-HSF and E-HSF were observed (Table 4.1). Moisture after air classification was significantly reduced, possibly due to heat generated and increased surface area during pin-milling. An additional significant loss in moisture was observed after extrusion. This corresponded to a relatively higher water activity in WPF (0.43) compared to NE-HSF (0.27). Higher water activity in E-HSF (0.31), despite having the lowest moisture (7.1%), indicated decreased water binding. However, all water activity levels fell within a range where lipid oxidation rate is expected to be lowest based on moisture sorption isotherm (Labuza and Altunakar 2007).



**Table 4.1. Chemical and functional properties of whole pinto flour, non-extruded high starch fraction and extruded high starch fraction**

<b>Bean properties</b>	<b>WPF</b>	<b>NE-HSF</b>	<b>E-HSF</b>
Moisture	10.0 ± 0.15a	9.5 ± 0.10b	7.1 ± 0.05c
Protein (% db)	22.2 ± 0.06a	13.3 ± 0.1b	13.5 ± 0.66b
Resistant starch (% db)	39.9 ± 2.54a	48.1 ± 1.85b	0.0 ± 0.00c
Soluble starch (% db)	6.5 ± 1.91a	7.9 ± 1.48a	55.7 ± 0.88b
Total starch (% db)	46.4 ± 3.08a	56.0 ± 4.05b	55.7 ± 2.78b
Lipids (% db)	1.64 ± 0.02a	1.14 ± 0.07b	0.14 ± 0.02c
Ash (% db)	4.03 ± 0.06a	2.75 ± 0.03b	2.67 ± 0.06b
Phytic acid (g/100g)	8.30 ± 0.36a	3.73 ± 1.36b	4.43 ± 0.58b
Total dietary fiber (% db)	17.43 ± 0.50a	18.13 ± 1.20a	16.28 ± 1.08a
WAI (g/g)	2.92 ± 0.04 a	2.69 ± 0.03a	4.39 ± 0.21b
WSI (%)	24.66 ± 0.53a	14.1 ± 0.10b	32.67 ± 2.36c
Color			
L*	81.5 ± 0.01a	87.5 ± 0.02b	77.0 ± 0.06c
a*	2.6 ± 0.06a	0.50 ± 0.02b	3.3 ± 0.03c
b*	10.0 ± 0.15a	8.9 ± 0.07b	8.0 ± 0.06c

WPF = whole pinto flour; NE-HSF = non extruded high starch fraction; E-HSF = extruded high starch fraction; L\* = brightness; a\* = greenness (-)/redness (+); and b\* = blueness (-)/yellowness (+). Values within the same row followed by the same letter are not significantly different at P < 0.05.

Protein content in pinto flour was 22.2% and was significantly reduced to 13.3% in the NE-HSF after air-classification. This was lower than the 15.5% protein content in HSF reported by Gujska and Khan (1991b). This may be due to differences in separation method. The commercial air-classification equipment used in this research may have achieved higher protein separation efficiency. Total protein reduction corresponded to significant reduction in eight essential amino acids measured (Table 4.2) including lysine. However, compared to non-extruded fraction (NE-HSF), extrudates had higher concentrations of essential amino acids, except for lysine and arginine, which were not significantly different.

**Table 4.2. Essential amino acids in whole pinto bean flour, non-extruded high starch fraction and extruded high starch fraction**

Essential Amino acids	<sup>a</sup> Wheat	WPF	NE-HSF	E-HSF
	mg/100g flour			
Threonine	388.6 ± 0.28a	1048.9 ± 0.49d	628 ± 1.2b	647.48 ± 2.58c
Valine	591.2 ± 3.11a	1223.4 ± 1.7d	713.2 ± 1.48b	741.6 ± 5.16b
Isoleucine	508.5 ± 1.34a	1108 ± 3.75d	641.6 ± 2.55b	662.9 ± 8.94c
Leucine	998.3 ± 4.88a	1989 ± 5.73d	1147.2 ± 5.94b	1184.5 ± 3.54c
Phenylalanine	723.5 ± 5.09a	1418.5 ± 5.73d	810.6 ± 5.59b	837.3 ± 1.56c
Histidine	314.3 ± 0.28a	709.3 ± 2.05d	421.1 ± 0.78b	434.8 ± 8.10c
Lysine	309.3 ± 6.29a	1742 ± 3.89c	1042.2 ± 19.45b	992.4 ± 43.52b
Arginine	530.7 ± 8.13a	1457.1 ± 4.95c	848.2 ± 1.84b	838.2 ± 29.63b

WPF = whole pinto flour; NE-HSF = non extruded high starch fraction; E-HSF = extruded high starch fraction. <sup>a</sup>Wheat flour containing 13.5% protein. Values within the same row followed by the same letter are not significantly different at P < 0.05.

General increase in amino acids after extrusion could be associated with increased digestibility of proteins after extrusion (Abd El-Hady et al 2003). This may have resulted in greater amino acid digestion during the hydrolysis step in the amino acid assay. Although amino acids were significantly reduced after air classification and extrusion compared to WPF, they were still significantly higher compared to high-protein wheat flour containing 13.5 % protein, which was used as a standard for comparison. Lysine content in air-classified pinto beans for example was 237 % higher than in the wheat flour. Pinto high starch fraction containing small amounts of protein can therefore be used in cereal-based foods to improve amino acid profile. Aguilera et al (1984) also reported improvement in amino acid profile when extruded snacks were made from a blend of air-classified HSF of raw beans and corn.

Total starch increased from 46.4 % in WPF to 56.0 % after air-classification. Gujska and Khan (1991b) reported 64.7% total starch after air classification. Starch content was not significantly different after extrusion. Resistant starch in WPF and NE-HSF was 39.9 % and 48.1

%, respectively. This was close to the 41.6 % resistant starch in native pinto bean starch reported by Simsek et al (2012) and 45.4 % reported by Ambigaipalan (2011). After extrusion, resistant starch was completely destroyed resulting in 100 % digestion of starch after 16 hr of digestion with  $\alpha$ -amylase and amyloglucosidase at 37 °C. This indicated that the extrusion process failed to produce and retain retrograded starch (RS3), which is the main resistant starch typically present after cooking. Faraj et al (2004) indicated that increasing resistant starch in extruded products was difficult and only feasible by adding other ingredients and manipulating storage temperatures after extrusion. Based on previous study (Simons et al 2012), resistant starch (RS3) present in the raw material prior to extrusion can be retained by up to 80%. Haralampu (1999) also supports this finding; showing that up to 100% of a commercial RS3 ingredient survived the extrusion process. The key therefore in producing extrudates with high resistant starch is to either add resistant starch as an ingredient or using precooked flours that have been optimized for high retrogradation. However, precooked flours could significantly reduce expansion (Simons et al 2012).

Total hexane extractable lipids in WPF, NE-HSF and E-HSF were 1.64 %, 1.14 %, and 0.14 %, respectively, and were all significantly different. Total lipids in NE-HSF (1.14 %) was similar to the amount (1.04%) reported by Gujska and Khan (1991a). Reduction of lipids in NE-HSF was caused by the partial removal of proteins during air-classification. Lipids are concentrated in the protein fraction of legumes (Sahasrabudhe et al 1981). The further reduction observed by extrusion was likely caused by binding of lipids with other components like proteins and amylose (Colonna and Mercier 1983; Izzo and Ho 1989; Bhatnagar and Hanna 1994). Significant reduction in total lipids, including unsaturated fatty acids (Table 4.3), will help to reduce potential lipid oxidation of unsaturated fatty acids. Fatty acids present in pinto beans

included linoleate, linolenate, oleate, palmitate and stearate. Sutivisedsak et al (2011) also found these to be the major fatty acids in pinto beans.

**Table 4.3. Fatty acids in whole pinto bean flour, non-extruded high starch fraction and extruded high starch fraction**

Fatty acid	Fatty Acid Concentration (mg/g)		
	WPF	NE-HSF	E-HSF
Palmitate	2.07 ± 0.20a	1.73 ± 0.08b	0.22 ± 0.01c
Stearate	0.27 ± 0.02a	0.22 ± 0.03b	0.08 ± 0.02c
Oleate	1.15 ± 0.06a	0.80 ± 0.05b	0.12 ± 0.02c
Vaccenate	0.22 ± 0.00a	0.17 ± 0.01b	0.02 ± 0.01c
Linoleate	5.62 ± 0.24a	4.26 ± 0.22b	0.45 ± 0.04c
Linolenate	7.07 ± 0.12a	4.22 ± 0.37b	0.52 ± 0.01c

WPF = whole pinto flour; NE-HSF = non extruded high starch fraction; E-HSF = extruded high starch fraction. Values within the same row followed by the same letter are not significantly different at  $P < 0.05$ .

Ash content following air-classification and extrusion was significantly reduced from 4.03% in WPF to 2.75% in NE-HSF. Gujska and Khan (1991a) reported 2.7% ash in pinto air-classified HSF. The reduction in ash content corresponded to a reduction in phytic acid from 8.3 g/100 g in WPF to 4.43 g/100 g in E-HSF. Since phytic acid binds minerals, the mechanism for reduction of ash content could be binding with phytic acid and subsequent removal of phytic acid with protein fraction, where phytic acid is highly concentrated (Poel et al 1990). Another mechanism could be the presence of mineral-binding proteins, which are removed during air-classification.

Total dietary fiber percentage in WPF was 17.43% and was not significantly different from percentages found in air classified and extruded samples (Table 4.1). Gujska and Khan (1991a) reported 14.46% total dietary fiber in pinto air-classified HSF. Hence, despite fractionation, the starch fraction still had a high percentage of fiber. This may be due to collection of fiber with starch fraction as reported by Vose et al (1976). While total dietary fiber

in the air classified fraction was higher than the whole pinto flour, the difference was not significant; possibly due to the high variability in the data. Lack of significant difference in total dietary fiber between extruded and non-extruded starch fraction indicated stability in total fiber content during extrusion. However, further research needs to be done to determine changes in distribution of soluble and insoluble fibers.

#### **4.4.3. Functional Properties**

WAI in WPF (2.92) and NE-HSF (2.69) were not significantly different (Table 4.1); however, there was a significant increase after extrusion (4.39). Whalen (1999) reported that WAI increased due to swelling of highly degraded starch. Therefore, starch damage during pin milling step was not large enough to alter water absorption, but was significantly changed during extrusion. WSI (Table 4.1) decreased from 24.7% in WPF to 14.1% in NE-HSF, but increased to 32.7% after extrusion. Reduction in WSI after air-classification was expected since native starch is insoluble in cold water. Increased WSI after extrusion was likely due to starch depolymerization (Baladrán-Quintana et al 1998).

The thermal properties of WPF and NE-HSF were significantly different, while there was no DSC endotherm for E-HSF (Table 4.4). Ozcan and Jackson (2005) also reported the absence of DCS endotherm in extruded corn starch compared to native corn starch. Onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), and conclusion temperature ( $T_c$ ) were significantly reduced after air-classification. Lower transition temperatures suggest that less heat is required to melt starch crystals in pinto high starch fraction. Therefore, there is a potential for lower energy requirement for extrusion cooking of high starch fraction versus extruding whole pinto flour. Further research should be done to determine if the difference would be significant.

**Table 4.4. Thermal properties of whole pinto bean flour and non-extruded high starch fraction**

Thermal Property	Treatment		
	WPF	NE-HSF	E-HSF
T <sub>o</sub> (°C)	74.4 ± 0.54a	72.7 ± 0.41b	Nd
T <sub>p</sub> (°C)	81.2 ± 0.1a	79.4 ± 0.08b	Nd
T <sub>c</sub> (°C)	89.1 ± 0.80a	86.0 ± 1.22b	Nd
ΔH (J/g)	3.5 ± 0.57a	6.1 ± 2.24a	Nd
T <sub>c</sub> -T <sub>o</sub> (°C)	14.7 ± 1.10a	13.3 ± 1.63a	Nd

WPF = whole pinto flour; NE-HSF = non extruded high starch fraction; E-HSF = extruded high starch fraction. Nd = none detected; T<sub>o</sub> = onset temperature; T<sub>p</sub> = peak temperature; T<sub>c</sub> = conclusion temperature, ΔH - gelatinization enthalpy; T<sub>c</sub>-T<sub>o</sub> = gelatinization temperature range. Values within the same row followed by the same letter are not significantly different at P < 0.05.

Pasting properties of WPF, NE-HSF and E-HSF also varied (Table 4.5 and Figure 4.2).

Peak time and pasting temperature in NE-HSF was significantly lower than in WPF, indicating that NE-HSF will gelatinize quicker and at a lower temperature. This corresponded to lower T<sub>o</sub> and T<sub>p</sub> in NE-HSF (Table 4.4). All viscosity measurements (breakdown, trough, setback and final) in E-HSF were significantly lower than viscosities in WPF and NE-HSF. Ozan and Jackson (2005) also reported much lower viscosity values in extruded corn starch compared to native starch. These differences clearly indicated different functionality and potential uses for the flours.

**Table 4.5. Pasting properties of whole pinto bean flour, non-extruded high starch fraction and extruded high starch fraction**

Pasting Property	Treatment		
	WPF	NE-HSF	E-HSF
Peak Time (min)	6.8 ± 0.20c	5.2 ± 0.04b	1.6 ± 0.5a
Pasting Temperature (°C)	82.5 ± 0.13c	80.9 ± 0.05b	52.6 ± 4.2a
Peak (cP)	2165.0 ± 59.8b	3966.0 ± 36.2c	265.7 ± 22.8a
Breakdown (cP)	120.3 ± 14.5a	861.3 ± 8.7c	165.3 ± 13.3b
Trough (cP)	2044.7 ± 46.3b	3104.7 ± 38.8c	100.3 ± 20.1a
Setback (cP)	1373 ± 82.1b	3148 ± 52.7c	126.7 ± 24.7a
Final (cP)	3417.7 ± 64.8b	6252.7 ± 91.4c	227.0 ± 18.0a

WPF = whole pinto flour; NE-HSF = non extruded high starch fraction; E-HSF = extruded high starch fraction. Values within the same row followed by the same letter are not significantly different at P < 0.05.

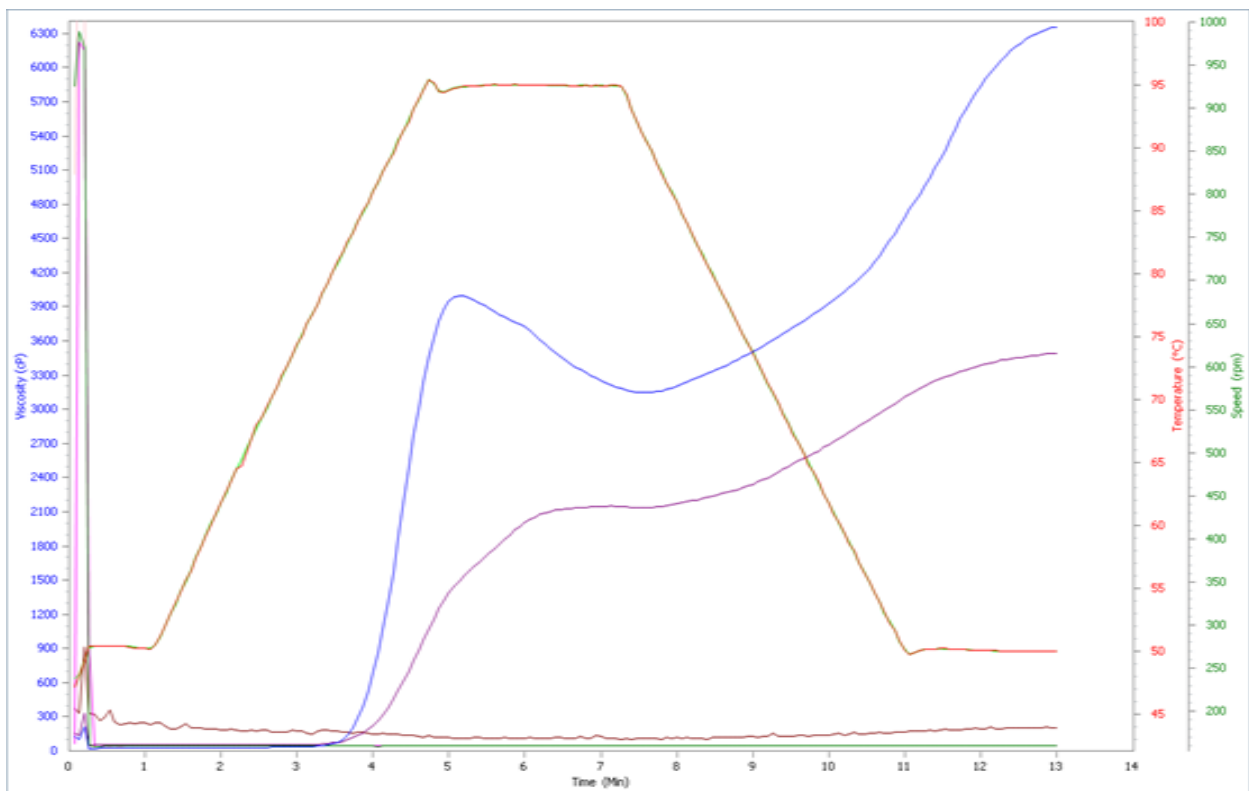


Figure 4.2. Pasting properties of whole pinto bean flour ( — ), non-extruded high ( — ) starch fraction and extruded high starch fraction ( — )

#### **4.5. Conclusions**

Extrusion of pinto bean high starch fraction produced extrudates with high expansion and firm texture. The extrudate was high in lysine and dietary fiber and low in fat. Addition to cereals will therefore result in production of snacks that are healthier. Improvement in sensory qualities compared to snacks made from whole pinto flour is predicted due to significantly low lipid content. WPF, NE-HSF and E-HSF have different functional properties that will result in different applications. For example, high viscosity of NE-HSF could be utilized in formulations where low caloric value and low glycemic index is required. It could also be added to foods to increase viscosity. For example in gluten free pasta, it could help to improve firmness, reduce cooking loss and surface stickiness. High water absorption, high solubility and low viscosity of E-HSF may have applications in bread formulations to increase water absorption and bread weight without negatively affecting staling rates. High solubility could also have practical use in dry soup mixes. The combination of high water solubility, high water absorption and high fiber also makes E-HSF a good ingredient for addition to minced meat formulations to help retain moisture and improve fiber content.

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## **CHAPTER 5. TEXTURIZED PINTO BEAN PROTEIN FORTIFICATION IN STRAIGHT DOUGH BREAD FORMULATION**

### **5.1. Abstract**

Pinto beans were milled and then air-classified to obtain a raw high protein fraction (RHPF) followed by extrusion to texturize the protein fraction. The texturized high protein fraction (THPF) was then milled to obtain flour, and combined with wheat flour at 5%, 10%, and 15% levels to make bread. Air-classification process produced flour with high concentration of lipids and phytic acid in the protein-rich fraction. However, extrusion significantly reduced both lipids and phytic acid. Total protein and lysine contents in composite flours increased significantly as THPF levels increased in composite flour. Bread with 5% pinto protein had 48% more lysine than the 100% wheat flour (control). Protein texturization from extrusion helped to maintain dough strength by reducing mixing tolerance index (MTI), maintaining dough stability and increasing departure time on Farinograph. Bread loaf volume was significantly reduced above 5% THPF addition. THPF increased water absorption causing an increase in bread weights by up to 6%. Overall, loaf quality deteriorated at 10% and 15% THPF levels while bread with 5% THPF was not significantly different from the control.

### **5.2. Introduction**

Pulses and cereal grains complement each other nutritionally since pulses are high in lysine and low in sulfur-containing amino acids such as methionine, cysteine and tryptophan while cereals are low in lysine but have abundant amounts of sulfur-containing amino acids (Duranti 2006). Pulse protein fractions have been obtained using both wet fractionation and air-classification methods (Lorimer et al 1991; Czarnecki et al 1993; Sathe 2002; Fenn et al 2010; and Schutyser and Goot 2011). Wet fractionation can produce higher protein extraction levels.

However, the process is more time consuming, complex, expensive and may increase the risk of contamination due to use of water. Air classification on the other hand produces a lower protein yield but is faster and less expensive. It involves pin-milling followed by air separation of pulse flour based on density. Finer, less dense materials are collected as a high protein fraction while coarser and more dense material is collected as a high starch fraction (Sathe 2002).

Both whole pulse flour and protein fractions have been added to bread to improve nutritional quality. Despite nutritional improvements however, overall physical characteristics of bread is typically compromised due to gluten dilution (Fleming and Sosulski 1977, 1978; Deshpande et al 1983; Silaula et al 1989; Lorimer et al 1991; Fenn et al 2010). This problem can be addressed by texturization of pulse proteins using extrusion. Texturization imparts structural changes in the protein matrix; resulting in strong protein-protein association from disulfide bonds and non-covalent interactions (Ke Shun and Fu-Hung 2007, 2008). These associations deliver a fibrous texture to pulse proteins; making them suitable for use as meat analogs. The structural changes could produce unique functionality in bread that is not typically observed when pulses are not texturized. The new networks formed could help to strengthen the dough, allowing addition of pulse flours at higher concentrations without breakdown. So far, no work has been reported on the effects of texturized high-protein pulse flours in bread. Therefore, the objectives of this research were to enhance protein quality of white pan bread using texturized pinto bean flour and determine the effects of this protein source on bread quality.

### **5.3. Materials and Methods**

#### **5.3.1. Fractionation and Extrusion Processing**

Dried pinto beans were purchased from Kelley Bean Co. (Hatton ND) and then pin-milled and air-classified at Particle Control Inc. (Albertville MN) utilizing a proprietary method.

After milling, the flour consisted of 88% 44 micron particles. The flour was air-classified into two streams; a high starch stream (80%) and a high protein stream (20%). The high protein stream was collected and stored at room temperature until ready for extrusion. Period between air-classification and extrusion was approximately three weeks. Extrusion was done using a co-rotating twin screw extruder (Wenger TX-52, Sabetha, KS). The flour was fed from a feed hopper at a rate of 34.2 kg/hr. The extruder had 52 mm diameter screws and barrel length-to-diameter ratio of 25.5:1. The barrel had six temperature zones, from feeding to die section, and set at 40, 40, 70, 70, 60 and 60 °C, respectively. Configuration of screws from feed to die end consisted of four conveying, one shear lock backward, two conveying one shear lock backward, two conveying, one interrupted flight, one shear lock forward, one conveying, one interrupted flight, one shear lock forward, one shear lock backward, two conveying, one shear lock forward, one conveying, one shear lock forward and one cone screw. Screw speed of extruder was 250 rpm. The die had a square shaped opening with dimensions 13 mm x 13 mm. The cutting knife had two blades and was positioned off-center at the end of the die. The moisture of the raw flour was raised from 12% to 40% by addition of water and steam during cooking. The specific mechanical (SME); a measure of work done on the feed, was 0.06 kw. hr kg<sup>-1</sup>, calculated based on Equation 1.2 (Personal Communications, Brian Prattner, Wenger Manufacturing, 2011).

Extruded beans were dried in an impingement oven (Lincoln, Middlesex, Great Britain) at 150 °C for 3 minutes and then placed on racks overnight to cool. Final moisture of extrudates was 7.4%. Samples were collected in plastic bags and stored in a refrigerator at 4 °C until ready for further analysis and bread making. For characterization and bread making, dried extrudates were milled using a centrifugal mill with a 0.5 mm sieve (Retsch, Haan, Germany).



Three 1 kg batches of composite flours were made consisting of white flour from wheat (Dakota Miller's Choice, ND State Mill, Grand Forks ND) and 5%, 10% and 15% texturized high protein fraction (THPF) flour, respectively. Bread made from 100% wheat flour was used as the control.

### **5.3.2. Chemical Properties**

Protein was determined in triplicate using a nitrogen combustion analyzer (Leco FP-528, St. Joseph, MI), and was completed according to AACC Method 46-30.01 (AACCI 2010). A factor of 6.25 was used to convert N to protein. Total starch (TS) was determined in triplicate using Megazyme TS analysis procedure (Megazyme International, Bray, Ireland).

Amino acid content was determined based on method adapted from Moore et al (1958), Ozols (1990) and Cooper et al (2000). Ten mg of bean flour samples were weighed in duplicate and transferred to hydrolysis tube with 200  $\mu$ l 6N HCl/1% phenol. Samples were hydrolyzed at 110  $^{\circ}$ C for 24 hr to release amino acids. Samples were then dried and dissolved in pickering sodium buffer (Mountain View, California) with 40 nmol/mL norleucine added as the internal standard. Final buffer volume was 15 ml, from which 50  $\mu$ l was injected into an automatic amino acid analyzer (AAA) (HITACHI L-8800, Japan) with transgenomic ion-exchange column. An amino acid standard solution for protein hydrolysate (Sigma-Aldrich A-9906, St. Louis Missouri) was used to determine response factors and to calibrate the AAA for all amino acids before analysis. Tryptophan and Methionine were not reported since these amino acids are destroyed during hydrolysis.

Total hexane extractable lipids was determined in triplicate based on method adapted from AOCS methods Af 3-53, Am 2-93, and Aa 4-38 (AOCS 1998). Bean flour samples (5 g) were weighed and collected in a folded 15 cm filter paper (415, qualitative, VWR International,

Radnor, PA) and transferred to a thimble holder. Lipids were extracted with hexane for 12 hr with a soxhlet apparatus (Combo mantle, Glas-Col, Terre Haute, IN). Hexane was evaporated using a rotary evaporator (Rotavapor RE-111, Büchi, Flawil, Switzerland) at 60 °C under high vacuum. The weight of the oil remaining in the flask was recorded and used to calculate the percentage oil in the total weight of the sample.

Ash was determined in triplicate following AACC Method 08-01.01 (AACCI 2010). Phytic acid analysis was done in triplicate following phytic (phytate)/total phosphorus analysis procedure (Megazyme International, Bray, Ireland).

### **5.3.3. Water Absorption and Water Solubility Index**

Water absorption index (WAI) and water solubility index (WSI) were determined in triplicate based on procedure by Anderson (1982). Milled samples (< 212 µm; 2.5 g) were added to centrifuge tubes containing magnetic stir bars. Distilled water (30 ml) was added and the tubes were sealed and vigorously agitated to break lumps. Tubes were placed on a magnetic stirrer and mixed for 30 minutes, and then centrifuged at 3000 rpm. The supernatant was decanted and the container weighed. The weight of sediment was determined by difference. The supernatants were collected in 100 ml beakers and dehydrated for 12 hr to remove liquid and to determine the amount of dissolved solids. WAI and WSI were then calculated using equations 1.4 and 1.5, respectively (Chapter 1).

### **5.3.4. Dough, Baking and Bread Evaluation**

Prior to bread baking, composite flours were evaluated to determine rheological dough properties following AACCI Farinograph Method 54-21.02 (2010) and AACCI Extensograph

Method 54-10.01 (AACCI 2010). Farinograph measurements were determined in triplicate and extensograph measurements in quadruplet.

Breads were baked on two separate days. On each of these days, one control and three replicates of bread at each treatment level (5%, 10% and 15%) were made. Baking experiments followed AACCI Method 10-09.01 (AACCI 2010). Mixing time was determined as the time taken for full dough development based on visual observation. At full development, the dough was soft, smooth, and highly extensible with silky appearance. Bread volume was determined based on AACCI Method 10-05.01 (AACCI 2010). Specific volume was calculated by dividing the volume of individual loaves by their weight. Crumb color was determined using a Minolta colorimeter to determine L\*, a\*, and b\* values on the Hunter scale. Color of the crust was based on a subjective color evaluation chart ranging from one to ten; where ten was the darkest color.

### **5.3.5. Statistical Analysis**

Data replications (as indicated under each description of analysis) were analyzed using statistical analysis systems software (SAS Institute, Cary NC) based on a completely randomized design (CRD). An analysis of variance (ANOVA) and means comparison following LSD procedure was used to establish significant differences ( $P < 0.05$ ).

## **5.4. Results and Discussion**

### **5.4.1. Chemical Properties**

Total protein decreased significantly from 44.2% in the RHPF to 39.2% in the THPF (Table 5.1). This could be explained by a reduction in nitrogen recovery during the protein assay due to strong protein-protein complex formation in the extruder. Utilization of THPF to produce composite flours resulted in significant increase in protein and all essential amino acids (Table

5.2). Total protein increased from 13.5% to 17.4% between control (0%) and 15% THPF addition levels. Lysine content increased by 48%, 89.8% and 139.3% at 5, 10 and 15% THPF addition, respectively.

**Table 5.1. Characteristics of protein fraction before (<sup>1</sup>RHPF) and after extrusion (<sup>2</sup>THPF)**

Property	<sup>3</sup> Fraction Type	
	RHPF	THPF
Moisture (% db)	11.6 <sup>a</sup>	7.4 <sup>b</sup>
Total protein (% db)	44.2 <sup>a</sup>	39.2 <sup>b</sup>
Total starch (% db)	1.3 <sup>a</sup>	2.1 <sup>a</sup>
Total lipids (% db)	3.2 <sup>a</sup>	1.2 <sup>b</sup>
Ash (% db)	6.5 <sup>a</sup>	6.7 <sup>b</sup>
Phytic Acid (g/100g)	21.8 <sup>a</sup>	19.37 <sup>b</sup>
WAI	3.6 <sup>a</sup>	3.1 <sup>b</sup>
WSI (%)	39.6 <sup>a</sup>	30.9 <sup>b</sup>

<sup>1</sup>RHPF = raw high protein fraction. <sup>2</sup>THPF = texturized high protein fraction. <sup>3</sup>Values with different superscripts indicates significant (P < 0.05) difference between raw and texturized fractions (i. e. row).

Total starch contents in RHPF and THPF was 1.3% and 2.1%, respectively (Table 5.1), and were not significantly different. Czarnecki et al (1993) also reported low starch content in protein fraction of air-classified pinto beans (2.5%). The low starch reported could be an underestimation as the high level of phytic acid in the protein fraction may interfere with starch digestion by  $\alpha$ -amylase in the starch assay. Yoon et al (1983) reported that phytic acid can retard  $\alpha$ -amylase activity by binding calcium.

#### **5.4.2. Water Absorption and Water Solubility Index**

WSI was significantly reduced from 39.6% in RHPF to 30.9% in THPF, supporting observations that extrusion processing reduces protein solubility (Gujska and Khan 1991). WAI was also significantly reduced from 3.6 in RHPF to 3.1 in THPF. Texturization therefore did not

improve water binding property; however, increased levels of THPF increased percentage water absorption in composite flours.

**Table 5.2. Effect of <sup>1</sup>THPF addition on total protein and essential amino acids (EAA) in composite flour**

	<sup>2</sup> Treatment			
	Control	5%	10%	15%
Total protein (%)	13.5 <sup>a</sup>	15.0 <sup>b</sup>	16.1 <sup>c</sup>	17.4 <sup>d</sup>
EAA(mg/100g)				
Threonine	388.6 <sup>a</sup>	468.0 <sup>b</sup>	533.3 <sup>c</sup>	617.3 <sup>d</sup>
Valine	591.2 <sup>a</sup>	680.0 <sup>b</sup>	746.0 <sup>c</sup>	811.8 <sup>d</sup>
Isoleucine	508.6 <sup>a</sup>	590.0 <sup>b</sup>	650.8 <sup>c</sup>	721.2 <sup>d</sup>
Leucine	998.3 <sup>a</sup>	1144.0 <sup>b</sup>	1254.7 <sup>c</sup>	1391.3 <sup>d</sup>
Phenylalanine	723.5 <sup>a</sup>	828.8 <sup>b</sup>	913.8 <sup>c</sup>	1005.8 <sup>d</sup>
Histidine	314.3 <sup>a</sup>	367.8 <sup>a</sup>	432.7 <sup>b</sup>	458.3 <sup>c</sup>
Lysine	309.3 <sup>a</sup>	458.0 <sup>b</sup>	587.0 <sup>c</sup>	740.1 <sup>d</sup>
Arginine	530.7 <sup>a</sup>	641.2 <sup>b</sup>	738.3 <sup>c</sup>	844.6 <sup>d</sup>

<sup>1</sup>THPF = texturized high protein fraction. <sup>2</sup>Values with different superscripts indicates significant ( $P < 0.05$ ) difference in the data between treatments (i. e. row).

A total hexane extractable lipid in RHPF (Table 5.1) was 3.2% compared to 1.2 % in THPF. This data supported work by several researchers who reported that lipids form complexes during extrusion making them more difficult to extract (Colonna and Mercier 1983; Schweizer and Reimann 1986; Izzo and Ho 1989; Bhatnagar and Hanna 1994). Reduction in total lipids may help reduce the rate of rancidity caused by lipid oxidation.

Ash content in RHPF and THPF (Table 5.1) was high; 6.5% and 6.7%, respectively, and were significantly different. Czarnecki et al (1993) also reported high ash content in air-classified pinto beans (7.1%). High ash content is likely due to the presence of high levels of phytic acid in air-classified pinto flour resulting in mineral binding (Jenab and Thompson, 1998). Phytic acid content in RHPF was 21.8 g/100g and was significantly reduced to 19.4 g/100g in THPF.

### 5.4.3. Dough, Baking and Bread Evaluation

Mixing time of dough (Table 5.3) was significantly increased with addition of more THPF. This corresponded to an increase in arrival time at higher THPF addition (Table 5.4). Therefore, as THPF increased, dough took a longer time to reach a standard viscosity of 500 Brabender Units (BU) on the Farinograph. Loaf weight was significantly increased from 131.0 g in control to 139.2 g at the 15% THPF level. This corresponded to the increased water absorption observed as THPF increased (Table 5.4). Loaf volume and specific volume at 5% THPF was not significantly different from control, but decreased at 10% and 15% THPF levels. These results were consistent with results observed by Kasprzak and Rzedzicki (2012). They reported a reduction in bread volume and an increase in bread yield with addition of grasspea wholemeal to traditional white bread formulation. Fenn et al (2010) reported that legume protein addition to wheat flour reduced specific volume with significant reduction observed at 5% addition.

**Table 5.3. Effect of <sup>1</sup>THPF addition on mixing time, loaf weight, volume, density and color**

	<sup>2</sup> Treatment			
	Control	5%	10%	15%
Mixing Time (s)	210 <sup>a</sup>	240 <sup>b</sup>	255 <sup>c</sup>	300 <sup>d</sup>
Loaf weight (g)	131.0 <sup>a</sup>	133.5 <sup>b</sup>	136.7 <sup>c</sup>	139.2 <sup>d</sup>
Loaf volume (cc)	931.7 <sup>c</sup>	904.4 <sup>c</sup>	808.9 <sup>b</sup>	710 <sup>a</sup>
Specific volume (cc/g)	7.1 <sup>c</sup>	6.8 <sup>c</sup>	5.9 <sup>b</sup>	5.1 <sup>a</sup>
Crust color	9 <sup>b</sup>	9 <sup>b</sup>	10 <sup>a</sup>	10 <sup>a</sup>
<sup>3</sup> Crumb Color				
L*	79.6 <sup>b</sup>	76.3 <sup>b</sup>	73.2 <sup>ab</sup>	70.9 <sup>a</sup>
a*	-0.3 <sup>a</sup>	1.4 <sup>b</sup>	2.8 <sup>c</sup>	3.9 <sup>d</sup>
b*	15.1 <sup>a</sup>	15.3 <sup>ab</sup>	15.8 <sup>bc</sup>	16.3 <sup>c</sup>

<sup>1</sup>THPF = texturized high protein fraction. <sup>2</sup>Values with different superscripts indicates significant (P < 0.05) difference in the data between treatments (i. e. row). <sup>3</sup>L\*a\*b\* = color values based on Hunter scale where L = brightness, a = greenness (–) and redness (+), and b = blueness (–) and yellowness (+).

Peak time (Table 5.4), i.e. time taken to reach the highest point on the Farinograph curve, was not significantly different between control and bread with 5% THPF. However, the peak time was longer by approximately 2 minutes when 10% and 15% THPF was added. Lorimer et al (1991) also reported increased arrival and peak times when wheat flour was replaced with high-protein legume flours, concentrates and isolates at substitution levels of 5% and 10%. Silaula et al (1989) observed an increase in arrival time and time required for dough development with addition of dry-roasted air-classified pinto and navy beans to wheat flour at 10%, 15% and 20% levels.

**Table 5.4. Farinograph properties of dough with and without <sup>1</sup>THPF**

<b>Dough Property</b>	<b><sup>2</sup>Treatment</b>			
	<b>Control</b>	<b>5%</b>	<b>10%</b>	<b>15%</b>
Water absorption	67.2 <sup>a</sup>	70.1 <sup>b</sup>	74.2 <sup>c</sup>	76.1 <sup>d</sup>
Arrival time (min)	2.0 <sup>a</sup>	3.1 <sup>a</sup>	8.1 <sup>b</sup>	8.2 <sup>b</sup>
Peak time (min)	10.1 <sup>a</sup>	10.5 <sup>a</sup>	11.8 <sup>b</sup>	11.8 <sup>b</sup>
Stability (min)	16.8 <sup>ab</sup>	17.8 <sup>b</sup>	16.3 <sup>ab</sup>	13.8 <sup>a</sup>
MTI (BU)	40 <sup>b</sup>	36.7 <sup>b</sup>	30 <sup>a</sup>	28.3 <sup>a</sup>
Departure (min)	18 <sup>a</sup>	20.8 <sup>b</sup>	24.4 <sup>c</sup>	21.9 <sup>b</sup>

<sup>1</sup>THPF = texturized high protein fraction. MTI = mixing tolerance index. <sup>2</sup>Values with different superscripts indicates significant ( $P < 0.05$ ) difference in the data between treatments (i. e. row).

Mixing tolerance index (MTI), i.e. how much the dough will soften after a certain degree of mixing, was not significantly different between control and 5% THPF addition. However, MTI decreased at 10% and 15% levels. Stability of the dough was not significantly different across levels of THPF. Addition of THPF increased the departure time, with 10% THPF level being the longest (24.4 min) compared to control (18 min). Results of stability, MTI and departure time indicated a general resistance to over-mixing. In contrast, Deshpande et al (1983) reported reduction in stability and increase in MTI with addition of wheat-pulse composite flours containing 10%, 20% and 30% pulse flour.

Resistance to over-mixing could be due to a structural property imparted by the texturized protein. Extensograph data (Table 5.5) showed that there was a general increase in resistance at the higher THPF addition. However, extensibility tended to decrease; which may account for the drop in volume at higher THPF addition. Another contributing factor affecting volume could be changes in protein-starch complex within the dough matrix. Fleming and Sosulski (1978) showed that supplemented proteins disrupt the protein-starch complex observed in wheat flour bread. In addition, they observed small pores in the cell walls of the supplemented bread, which they suggested allowed gases to escape from the structure during baking.

**Table 5.5. Extensograph properties of dough with and without <sup>1</sup>THPF**

Indices	Proving Time (min)	Treatment <sup>2</sup>			
		Control	5%	10%	15%
Energy (cm <sup>2</sup> )	45	137.0a	130.0ab	111.8bc	103.3c
	90	186.0a	163.8b	146.0bc	129.0c
	135	176.3a	140.8bc	151.5ab	121.3c
Extensibility (mm)	45	160.5a	151.5a	145.8a	123.8b
	90	156.8a	149.3a	141.3a	115.8b
	135	148.8a	124.0ab	135.8a	108.5b
Resistance to extension (BU)	45	423.0a	469.8ab	430.8a	515.3b
	90	599.5a	594.8a	590.5a	723.0b
	135	644.0a	720.3a	661.0a	762.0a
Ratio number <sup>3</sup>	45	4.2a	3.1a	3.9a	4.8b
	90	6.1a	5.8a	5.6a	7.5a
	135	6.3a	7.9b	6.6b	8.1b

<sup>1</sup>THPF = texturized high protein fraction. <sup>2</sup>Values with different superscripts indicates significant (P < 0.05) difference in the data between treatments (i. e. row). <sup>3</sup>Ratio number = Resistance/Extensibility.

Based on visual observation, bread loaves (Figures 5.1 – 5.3) with 5% THPF were similar to the control except for coarser break and shred. Break and shred refers to the shredded or



comb-like pattern observed on one side of the bread due to loaf expansion and stretching of gluten fibers. A good break and shred should be uniform and smooth (Swanson 2004).

Coarseness in break and shred at 10% and 15% THPF level was more accentuated compared to control. This suggests that THPF imparts rigidity to the dough system, reducing ability of gluten fibers to expand smoothly and uniformly. Crust color of 5% THPF addition level was not significantly different from control but was significantly darker at 10% and 15%. Crumb color gradually lost brightness as the amount of THPF increased, and had higher intensities of red and yellow. Fenn et al (2010) also reported reduction in brightness ( $L^*$ ) as pulse substitution increased from 2% to 8%.



Figure 5.1. Comparison of control bread (far left) and breads with 5% texturized high protein fraction



Figure 5.2. Comparison of control bread (far left) and breads with 10% texturized high protein fraction



Figure 5.3. Comparison of control bread (far left) and breads with 15% texturized high protein fraction

## 5.5. Conclusion

Increased concentrations of THPF addition in composite dough system imparted dough strengthening properties resulting in stable dough, reduction of MTI and longer departure time. Optimum inclusion of texturized THPF will be between 5% and less than 10%. At 5% THPF level, lysine concentration was increased by 48% without causing any significant negative effects on quality parameters. THPF can therefore be used as an ingredient in bread to improve protein quality.

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## **CHAPTER 6. LIPOXGENASE ACTIVITY AND HEXANAL AND HEXANOL CONCENTRATION OF LARIAT PINTO BEANS GROWN AT VARIOUS LOCATIONS**

### **6.1. Abstract**

Reducing grassy flavors in edible beans can improve opportunities for their use as food ingredients. Growing environment can have a significant impact on lipoxygenase, total lipids and amount of unsaturated fatty acids synthesized in seeds. This will in turn affect grassy flavors development during processing and storage. Lipoxygenase activity, total hexane extractable lipids and fatty acid profile of pinto beans (Lariat variety) grown at three different locations in North Dakota (Hatton, Johnstown and Forest River) were evaluated. Samples were also stored four weeks to determine changes in hexanal and hexanol concentrations. Lipoxygenase activity in beans from the Hatton was significantly higher than in beans from other locations. The amount of peroxides produced was 245.08  $\mu\text{M}$  for beans from Hatton, while beans from Johnstown had the lowest (99.30  $\mu\text{M}$ ). Total lipids in beans from Hatton (1.32%) were significantly lower than beans from Johnstown and Forest River (1.48%). Linolenic acid in beans from Hatton was significantly higher (51.9%) compared to beans from Johnstown and Forest River (49.3%). There was an interaction between location and storage time, demonstrating the importance of growing location on development of aroma compounds of interest.

### **6.2. Introduction**

Pulses are highly nutritious in that they contain complex carbohydrates (i.e. dietary fibers, resistant starch, and oligosaccharides), high lysine content, important vitamins and minerals including B-vitamins, folates and iron, plus antioxidants and polyphenols (Reyes-Moreno and Paredes-López 1993; Rehman et al 2001; Han et al 2010). As a result pulse flours have been incorporated in several food formulations such as breads, crackers, granola bars, pasta and

extruded snacks to improve nutrition (D'Appolonia 1977; Tovar et al 2003, Maurer et al 2005; Anton et al 2009; Han et al 2010; Nagi et al 2012). However, the beany and grassy flavors pose a potential challenge to significant expansion in marketing and distribution of pulse ingredients. Several compounds have been found to be associated with beany and grassy flavors in pulses. These include n-hexanal, 3-cis hexenal, 2-pentyl furans, 1-penten-3-one, n-pentanol, n-hexanol, n-heptanol, 1-octen-3-ol, trans, trans 2-4 nonadienal, trans, trans 2,4 decadienal, trans-2-nonenal, trans, cis-2,4 nonadienal, butyric acid, 2-methyl butyric acid methyl ester, 2-pentyl pyridine, pentanal and acetophenone (Sessa and Rackis 1977; Hsieh et al 1982; Boatright and Crum 1997; Boatright and Lei 1999). The mechanism for development of these compounds involves the breakdown of unsaturated fatty acids catalyzed by lipoxygenase (LOX) enzyme on exposure to oxygen to form hydroperoxides, which further break down to form several compounds including aldehydes, ketones and alcohols (Sessa and Rackis 1977). Although lipids in edible beans are low, the majority of lipids are in the form of unsaturated fatty acids, which are excellent substrates for enhanced LOX activity (Sutivisedsak 2011).

While processing can help to mitigate development of off-flavors in pulse seeds, controlling flavor development factors at the crop production stage can be advantageous. These factors may include total lipids, percentage unsaturated fatty acid and LOX activity since these are related to development of off-flavors (Sessa and Rackis 1977). So far no study has investigated the effect of growing location on the development of grassy off-odor compounds in pinto beans. This study will provide bean breeders with preliminary data to identify growing locations that are conducive to improved flavor characteristics based on development of grassy indicator-compounds (hexanal and hexanol) during accelerated storage of bean flours. Hexanal and hexanol were selected since they have been identified as major volatiles in pulses flours

(McCurdy, 1980; Mtebe and Gordon 1987; Oomah et al 2007; Azarnia et al 2011). Various methods have been used to measure volatile compounds. However, solid phase microextraction (SPME) is a common method to extract volatiles since it simple, rapid and robust as a screening technique (Jelen, 2006; Oomah et al 2007). Oomah et al (2007) identified 62 volatiles in dry bean market classes (black, pinto and dark red kidney beans) including hexanal and hexanol, using this technique and reported that hexanal was the most important authentic volatile marker for dry beans due to its high concentration and low threshold value. In chapter 2, sensory panelists identified grassy and 'beany' flavor notes as negative sensory characteristics. Therefore, assessing whether the volatiles are an artifact of processing or a natural characteristic of the bean flour is important. The objective of this study was to determine if growing location of pinto beans has any significant effect on development of off-flavor compounds, using hexanal and hexanol as indicators. A limitation of this study was that only one market class, and one variety of bean was used. Also, only one growing season was studied. Therefore the data is preliminary, providing a basis to determine if further investigation is needed.

### **6.3. Materials and Methods**

Lipid hydroperoxide standard (50  $\mu$ M ethanolic solution of 13-hydroperoxy octadecadienoic acid) was purchased from Cayman Chemical Company (Ann Arbor, Michigan). 3-Methyl-2-benzothiazolinone (MBTH), 3-(dimethylamino), hemoglobin (bovine), sodium lauryl sulfate solution, tween 20, and linoleic acid analytical standard were purchased from Sigma-Aldrich (St. Louis, MO). One kg packages of Lariat variety pinto beans from three different locations in North Dakota (i.e. Johnstown, Hatton and Forest River) were obtained from the Plant Sciences Department, Dry Bean Breeding and Genetics Program at North Dakota State University. The beans were dehydrated after collection from the field to reduce moisture content

to a safe level to prevent spoilage (< 10%). All samples were obtained from the 2011 growing season. Pinto beans from Hatton were reported to have experienced higher drought conditions compared to the other two. The beans were among several varieties grown at these locations based on a randomly complete block design. Each package represented a composite of seeds grown within blocks at the three locations. To prepare samples, beans were ground to flour using a Brinkmann Retsch centrifugal mill (Retsch, Haan, Germany) with 0.5 mm sieve openings. Ground samples were collected in zip locked plastic bags and then stored in a walk-in freezer at -15°C until ready for further analysis.

### **6.3.1. Moisture and Lipid Analysis**

Moisture was determined in duplicate based on AOAC method 925.10 (AOAC 1990). Total hexane extractable lipid was determined in triplicate based on method adapted from AOCS methods Af 3-53, Am 2-93, and Aa 4-38 (AOCS 1998). Round-bottom flasks (250 mL) were pre-weighed. Bean flour samples (5 g) were added to a folded 15 cm filter paper (415, qualitative, VWR International, Radnor, PA) and transferred to a thimble holder. Lipids were extracted with hexane for 12 hr with a soxhlet apparatus (combo mantle, Glas-Col, Terre Haute, IN). Hexane was evaporated using a rotary evaporator (Rotavapor RE-111, Büchi, Flawil, Switzerland) at 60°C and high vacuum. Flasks were then reweighed to determine increase in weight. Weight difference was equivalent to weight of lipids extracted.

Fatty acid profile was determined on extracted lipids in duplicate. Lipids were methylated by collecting 50 mg of extracted lipids from two separate lipid extractions and placing in 20 ml test tubes. Five ml of 2% H<sub>2</sub>SO<sub>4</sub> in methanol was then added. The tube was vortexed and tightly capped followed by heating at 110 °C for 1 hr; vortexing every 15 min. Tubes were then cooled to room temperature and 3 ml hexane and 3 ml water added; vortexing between addition of each.



Samples were allowed to stand for 10 minutes to separate. The top layer was drawn off with a glass pipette and transferred to GC vials. Fatty acids were separated using a Hewlett Packard HP 5890 Series Gas Chromatograph equipped with FID detector and Supelco SP-2380 column (Bellefonte, PA, USA); 30 m x 0.25 mm internal diameter and 0.20  $\mu\text{m}$  film thickness. Helium was used as carrier gas with column head pressure set at 15 psi. Temperature of oven was initially at 150 °C, which was held for 5 minutes. Temperature was then ramped to 180 °C at a rate of 10 °C per minute and held for 12 minutes. The injector and detector temperatures were set at 200 °C and 270 °C, respectively. Fatty acid methyl ester (FAME) peaks were identified based on comparison with retention times of reference standards.

### **6.3.2. Lipoxygenase Analysis**

Lipoxygenase activity was determined in triplicate based on a modified procedure developed by Anthon and Barrett (2001), where the amount of hydroperoxide produced was used as an indicator of lipoxygenase activity. The principle of the assay involved reaction of linoleic acid substrate to produce 13-hydroperoxy octadecadienoic acid. This compound acted as an oxidant to cause an oxygen coupling reaction between 3-Methyl-2-benzothiazolinone and 3-(dimethylamino) benzoic acid. The coupling reaction was catalyzed by heme group in hemoglobin and produced a purple indamine dye. The dye was measured spectrophotometrically at 598 nm. All assays were done at room temperature (23 °C). To prepare for lipoxygenase activity assay, 100 g of bean flours were dried overnight at 40 °C in a convection oven (Binder Inc., Bohemia, NY) and then defatted using hexane (1:10 w/v) at room temperature for 12 hours. Hexane was then allowed to evaporate at room temperature by spreading the bean flour slurry on a Pyrex glass surface, and placing it in a fume hood. Lipoxygenase was extracted by adding 40 ml of 0.2 M sodium acetate buffer (pH 4.5) to 5 g of bean flour and mechanically stirring for 1

hour at 2 - 4 °C. The mixture was then filtered using cheesecloth followed by centrifuging at 3000 rpm for 10 min at 4 °C. The pH of the supernatant was adjusted to 6.8 using 1N NaOH. The crude extract was separated into 5 ml aliquots and frozen until ready for use. Before lipoxygenase analysis, samples were diluted by adding 15 ml distilled water to 5 ml of the extract.

Stock solutions of 10 mM MBTH was prepared by dissolving 0.22 g MBTH in 100 ml deionized water (MW = 215.07 g/mol). Stock Solution of 5 mg/mL hemoglobin was prepared by dissolving 0.5 g hemoglobin in 100 ml deionized water (MW = 68000 g/mol). Solution containing 20 mM DMAB and 100 mM phosphate buffer was prepared by dissolving 330 mg DMAB in 5 mL of 1 N HCl and diluting to about 80 mL with water. Na<sub>2</sub>HPO<sub>4</sub> (1.42 g) was then added and pH adjusted to 6.0 with HCl, and the volume brought to 100 mL with distilled water. Linoleic acid (25 mM) stock substrate solution was prepared by placing 5 ml of distilled water in a 10 ml test tube and adding 155 µl (140 mg) of linoleic acid. Tween 20 (280 mg) was then added before transferring all contents to a test tube. The test tube was vortexed followed by addition of 0.6 ml of 1N NaOH, and vortexed again. The contents were transferred to a 30 ml test tube and 14 ml distilled water added to make a total volume of 20 ml. The tube was vortexed again and the solution divided into 1ml aliquots and placed into 1 ml GC glass vials. The vials were flushed with nitrogen, capped and stored at -20 °C. Three more solutions were prepared; solution A, B and C. Solutions A and B were prepared daily. Solution A was prepared by placing 10 ml of 20 mM DMAB/phosphate buffer solution in a 30 ml test tube; adding 0.4 ml of 25 mM linoleic acid stock solution, followed by 9.6 ml of distilled water. Solution B was prepared by mixing 0.4 ml of 10 mM MBTH; 0.4 ml of 5 mg/ml hemoglobin and 19.2 ml water in a 30 ml

text tube. Solution C (1% of sodium lauryl sulfate solution) was prepared by diluting 1 g of sodium lauryl sulfate into 100 ml volume with water.

For the lipoxygenase activity assay, 10  $\mu$ l of the diluted sample was transferred to 12 x 75 mm glass tubes and 0.5 ml of solution A added. The tube was then vortexed and incubated at room temperature for 5 minutes. Solution B (0.5 ml) was added and the tube vortexed again and incubate at for another 5 minutes. To terminate the reaction, 0.5 ml of 1% sodium lauryl solution was added. Absorbance was read at 598 nm. A blank was prepared by adding 0.5 ml of solutions A, B and C to a 12 x 75 mm. A standard curve (Appendix A) was prepared by transferring solution A and B to five 12 x 75 mm glass tubes and then adding hydroperoxide standard to each test tube in the following volumes 200  $\mu$ l, 300  $\mu$ l, 400  $\mu$ l, 500  $\mu$ l and 600  $\mu$ l, respectively. The tubes were incubated at room temperature for 5 minutes and solution C added. Absorption was read immediately at 598 nm.

### **6.3.3. Accelerated Storage**

To prepare for analysis of volatiles, pinto flour samples were removed from the freezer, and approximately 10 g from each growing location collected and sealed in eight 76 mm x 178 mm transparent polyethylene sample bags (Whirl-pak, VWR International, Radnor, PA) with pull wire ends. Two bags from the eight were returned to the freezer while the remaining were stored in an incubator (Fisher Scientific Isotemp 550D, New Hampshire) maintained at 30°C. Samples from each location were incubated for two, three and four weeks. At the end of each week, duplicate samples from each location were pulled from the incubator and analyzed to quantify hexanal and hexanol concentrations. Samples that were not stored were considered week 0 for the analysis.

#### 6.3.4. Hexanal and Hexanol Analysis

Volatiles were analyzed by collecting 1.80 g of pinto flour bean samples in 4 ml GC sample vials. The vials were then heated in a boiling water bath for 15 minutes to move volatiles into the headspace. Vials were then transferred to a 60 °C water bath with sonicator (Branson 3200 Ultrasonic Corporation, Danbury CT). A SPME fiber (DVB/CAR/PDMS) (Supelco, Bellefonte PA) was then placed into the headspace of the vial for volatile adsorption for 15 minutes. Volatile separation was done by desorbing the volatile from the fiber into an Agilent 7820A gas chromatograph equipped with FID detector and HP-5 capillary column (Agilent Technologies, Santa Clara, CA) with dimensions 30 m × 320 µm ID, with 0.25 µm film thickness. Helium was used as carrier gas with inlet pressure set at 15.3 psi, split ratio 20:1, and column flow of 4ml/min. Initial temperature of the oven was 35 °C, which was held for 5 minutes, and then ramped to 80 °C at a rate of 6 °C per minute and held for one minute; and then to 250 °C at a rate of 20 °C/min and held for 1 minute. Total run time was 23 minutes. The injector and detector temperatures were set at 250 °C.

Target volatiles (hexanal and hexanol) were quantified based on standard curve created by spiking white whole wheat flour (North Dakota Mill, Grand Forks, ND) with analytical standards purchased from Sigma-Aldrich. To create standard curve for hexanol, 200 g of the whole wheat flour was placed in a 1000 ml glass jar. Exactly 5µl of hexanol was then added, and the bottle sealed quickly and shaken rigorously for three minutes. The bottle was then inverted and left to equilibrate at room temperature for three hours. Sub-samples from the spiked flour ranging from 0.25 g to 2.0 g, were collected and transferred to a 14 ml centrifuge tube, and diluted to a final weight of 10 g. The tubes were quickly closed and shaken rigorously for approximately 3 minutes to ensure thorough mixing. Duplicate samples were then transferred

immediately to GC vials for SPME extraction and GC analysis. A standard curve (Appendix B) was used to quantify the volatiles.

The procedure for creating a standard curve for hexanal (Appendix C) was similar, except that spiking was done by adding 5  $\mu$ l hexanal to 100 g wheat flour sample in a 500 ml glass bottle. In addition, the sub-samples of spiked flour were diluted to a final weight of 10 g, ranged from 1 g (maximum dilution) to 10 g (no dilution) using non-spiked flour.

### **6.3.5. Statistical Analysis**

Moisture, total hexane extractable lipids, lipoxygenase activity and percentage fatty acid data was analyzed based on a completely randomized design (CRD) using ANOVA with Proc GLM. Means were separated using the least significant difference (LSD) procedure (SAS Institute, Cary, NC). Differences were considered significant when  $P < 0.05$ . Interaction of growing location and storage time with respect to their effect on hexanal and hexanol concentrations was determined using three locations and four storage times. Samples from each location was duplicated within each week of the storage study. The data was subjected to analysis of variance with Proc Mixed (SAS Institute, Inc.) to determine if there was a significant interaction between time and location. Simple effects comparing locations within weeks and weeks within locations were obtained via the SLICE statement in Proc Mixed. The Tukey adjustment was used to control the Type I error in these follow up analyses with statistically significant differences being where  $P < 0.05$ .

## **6.4. Results and Discussion**

### **6.4.1. Moisture**

Moisture content in beans from all locations were below 10% (Table 6.1), which is good for storage since moisture content below 10% inhibits lipoxygenase activity (Mtebe and Gordon 1987). Moisture content in beans from Johnstown (8.28%) was significantly lower than in beans from Forest River (9.72%) and Hatton (9.71%).

### **6.4.2. Total Lipids and Fatty Acid Profile**

Total lipids in Hatton (1.30%) was significantly lower than beans from Johnstown and Forest River (1.48%) (Table 6.1). In general, fatty acid profiles of beans from all locations were similar (Table 6.2). However, beans from Hatton had a significantly higher percentage of linolenate (51.87%) compared to Johnstown (49.30%) and Forest River (49.34%). This observation may not be due to differences in irrigation conditions. Lee et al (2008) reported that irrigation generally has no significant influence on development of unsaturated fatty acid accumulation in soybean genotypes. Also, Bennett et al (2008) reported only subtle effects on fatty acid profiles in four soya bean cultivars. Fatty acid profiles in Table 6.2 were similar to profiles reported by Sutivisedsak (2011). Therefore the higher percentage of linolenate observed in beans from Hatton could be simply attributed to the lower percentage of total lipids observed.

**Table 6.1. Moisture, total lipids and lipoxygenase activity in Lariat pinto beans**

	<b>Johnstown</b>	<b>Forest River</b>	<b>Hatton</b>
Moisture (%)	8.3 ± 0.13a	9.7 ± 0.35b	9.7 ± 0.07b
Total hexane extractable lipids (%)	1.5 ± 0.02b	1.5 ± 0.03b	1.3 ± 0.03a
Lipoxygenase activity (µM hydroperoxide)	99.3 ± 6.06a	174.1 ± 26.11b	245.1 ± 15.34c

Numbers with the same letters within the same row are not significantly different (P<0.05).

**Table 6.2. Fatty acid profile of Lariat pinto beans**

<b>Fatty acid</b>	<b>Johnstown</b>	<b>Forest River</b>	<b>Hatton</b>
Palmitate (%)	8.2 ± 0.45ab	8.7 ± 1.28b	6.0 ± 0.55a
Stearate (%)	1.8 ± 0.11a	2.6 ± 0.10b	1.8 ± 0.31a
Oleate (%)	6.0 ± 0.01b	5.0 ± 0.06a	5.9 ± 0.08b
Vaccenate (%)	1.7 ± 0.08a	1.7 ± 0.08a	1.8 ± 0.05a
Linoleate (%)	33.0 ± 0.13a	32.6 ± 0.31a	32.7 ± 0.01a
Linolenate (%)	49.4 ± 0.30a	49.3 ± 0.84a	51.9 ± 0.84b

Numbers with the same letters within the same row are not significantly different (P<0.05).

### 6.4.3. Lipoxygenase Activity

Lipoxygenase activity was significantly different in all three growing locations (Table 6.1). Pinto beans from Hatton was the highest (285.08 µM) compared to beans from Johnstown (99.30 µM) and Forest River (174 µM). The difference could be due to the more severe drought conditions experienced at Hatton. Lipoxygenase gene expression has been reported to increase in plants exposed to stress, including water deficit (Bell et al 1991).

Edible beans with high lipoxygenase activity could be useful in bakery formulations where use of soy must be excluded. High lipoxygenase soy is used as an enzyme active ingredient to produce important functional properties in bread such as producing whiter crumb, increasing mixing tolerance index (MTI) and improving dough rheology (Emken 1978; Faubion

and Hosenev 1981). Further studies would need to be done to determine if enzyme activity in pinto beans could produce these results in bread fortified with pinto bean flour or its fractions.

Although beans from Hatton had the highest lipoxygenase activity, this did not result in greater lipid oxidation compared to beans from the other two locations. Therefore, lipoxygenase activity alone is not a good predictor of storage life. What appeared to be of greater importance in this study was the actual amount of lipids present. Hatton had the lowest percentage of total lipids which likely explains why overall concentrations of hexanal and hexanol were the lowest compared to the other locations (Figures 6.1 and 6.2).

#### **6.4.4. Location Effect on Hexanal Concentration**

There was a significant interaction between the two independent variables (location and storage time) since hexanal and hexanol concentrations behaved differently depending on each variable. This can be observed visually by the lack of parallelity among the graphs representing the three locations (Figure 6.1). Therefore the development of hexanal and hexanol during storage was being influenced by both the growing location and the storage time.

Pinto beans for Johnstown had an average of the highest average hexanal concentration from week 0 to 4 (2.43 ppm), and pinto beans from Hatton, the lowest (1.83 ppm) (Figure 6.1). Comparison between locations at each week interval indicated the following differences in hexanal concentrations that were significant: a) week 0 - hexanal higher in Johnstown compared to Forest River pinto beans; b) week 2 - hexanal higher in Johnstown compared to both Forest River and Hatton pinto beans; c) week 3 - hexanal higher in Johnstown compared to Forest River pinto beans and d) week 4 - hexanal higher in Forest River compared to Hatton pinto beans.

A comparison of week by week hexanal concentrations within each location indicated the following differences that were significant: a) hexanal in Forest River pinto beans increased



between week 0 and 2 and between week 0 and 4. There was a reduction in hexanal concentrations between week 2 and 4 and between 3 and 4; b) hexanal in Johnstown pinto beans decreased between week 0 and 2; and c) there was no difference in Hexanal concentration in Hatton pinto beans over the duration of storage. Therefore, Hatton pinto beans not only had the lowest hexanal concentration overall, but demonstrated the highest stability over time. This indicates less potential for development of grassy flavors during storage compared to Johnstown and Forest River pinto beans. This is likely due to the significantly lower percentage of total lipids present in Hatton pinto beans.

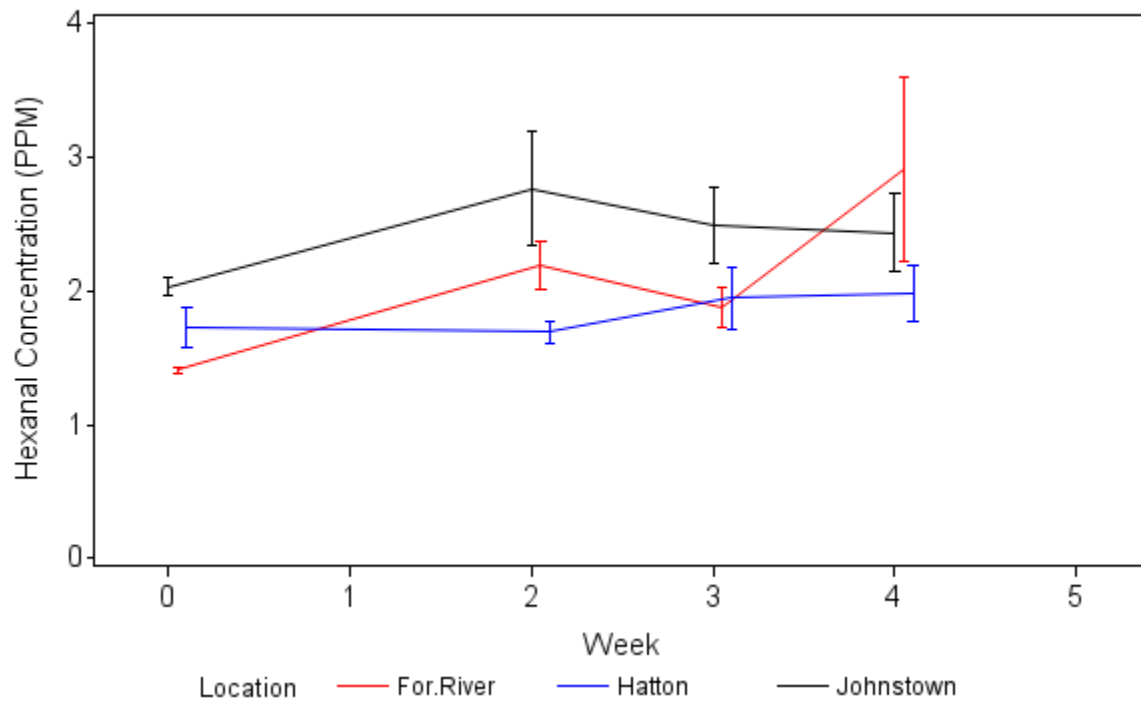


Figure 6.1. Interaction plot showing effect of location and storage time (weeks) on hexanal concentration

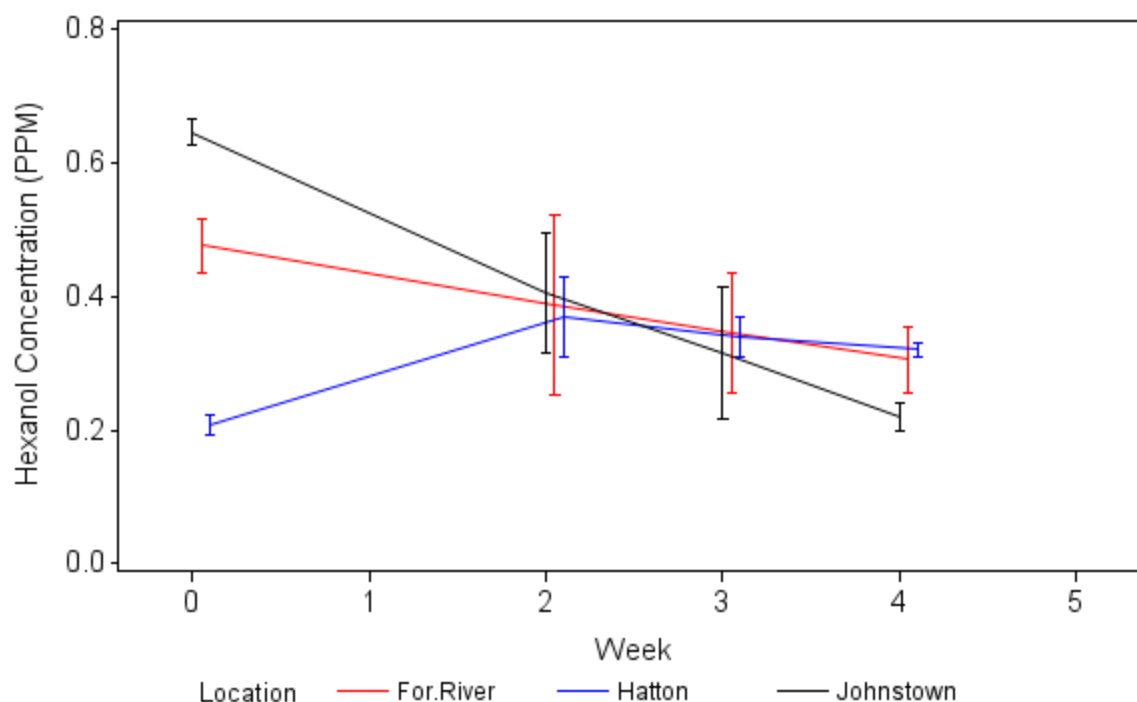


Figure 6.2. Interaction plot showing effect of location and storage time (weeks) on hexanol concentration

#### 6.4.5. Location Effect on Hexanol Concentration

Hexanol concentration data also showed a significant interaction between both location and storage time (Figure 6.2). Similar to hexanal concentrations, pinto beans from Johnstown had the highest average hexanol concentration from week 0 to 4 (0.40 ppm) and pinto beans from Hatton the lowest (0.31 ppm). Hexanol concentrations at time 0 were all significantly different, with Johnstown having the highest concentration (0.65 ppm) and Hatton the lowest (0.21 ppm) (Figure 6.2). After week 0, however, there were no significant differences in hexanol concentrations among the three locations. Comparison within locations indicate that there was a significant decrease in hexanol from week 0 to 4 in pinto beans from Forest River; and a significant decrease in hexanol from week 0 to 2 in pinto beans from Hatton. Johnstown had a significant decrease in hexanol between time 0 and all other time intervals, and between week 2

and 4. Hexanol is reported to enhance grassy flavors in pulses (Sessa and Rackis 1977), the overall reduction in hexanol could indicate potential for reduced grassy sensory experience after bean flours are stored over time.

### **6.5. Conclusions**

Concentration of hexanol and hexanal in pinto beans before and after storage can be influenced by growing location. For example, the relatively lower and stable hexanal concentration in Hatton pinto beans and its reduced hexanol concentration during storage, indicates potential longer shelf life and improved flavor. Percentage of total lipids in beans appears to be a better indicator for potential storage life of beans compared to lipoxygenase content. The results indicated that even small reduction in lipid content can result in significant differences in volatiles. Therefore, to produce beans with less potential for grassy flavor development, breeders may consider screening for beans with lower lipid contents. Even a difference of 0.18% in total lipids can potentially improve and extend shelf-quality quality. Ingredient buyers of edible beans may consider using total lipid content as an important criterion in their buying decisions. The results of this study provides a basis for a broader investigation to determine the effect of irrigation on total lipid development and its associated effect on aromatic compounds leading to grassy flavors.

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## CHAPTER 7. CONCLUSIONS

The results of this study supported the hypothesis that the properties of bean extrudates were different according to the physical form of the raw material before extrusion, and therefore end products had potential for different ingredient applications. In addition, statistical interaction between growing location and storage time proved that growing location is an important factor influencing bean flavor development.

Pre-cooked bean flours can be used to make extruded snacks and will offer advantage over the use of raw bean flours in taste; however they will produce extrudates with weaker texture and much lower expansion. Therefore, as an extruded snack they will find better application as a partial substitute in cereal based snacks than extruding alone. Raw whole bean flours can make excellent puffed snacks due to high expansion and strong texture. Grassy notes and overall acceptability however may be a concern when extruded. To minimize this, they should be used as a partial substitute as well. High-starch fractionates have the ability of standing alone as a snack food; that is 100% substitution. They will have superior expansion and texture and potentially better flavor due to its very low lipid content. Although low in protein, its extrudates will still have more lysine and other essential amino acids compared to wheat flour.

Overall, the nutritional properties of bean flours and extrudates make them suitable for incorporation in a variety of consumer products in addition to extruded snacks. These include breads, pasta, biscuits, crackers, cakes, nutrition bars and soup mixes. Their application will be different based on functional properties of the bean flour ingredient such as water absorption and water solubility index, thermal and pasting properties; and effect on dough rheology.

## CHAPTER 8. RECOMMENDATIONS FOR FURTHER RESEARCH

Several projects completed in this study points to opportunities for future research. Extrusion resulted in extrudates with high glycemic index (GI). Low GI is a unique selling attribute for consuming pulse-rich foods. The data indicated that digestibility properties of extruded snacks can be improved if the bean flour has higher amounts of the thermo-stable RS<sub>3</sub> resistant starch before extrusion. Presently, commercial pre-cooked bean flours do not have enough RS<sub>3</sub> to enable production of extrudates with low GI. A patentable pre-cooking method can be developed to significantly enhance RS<sub>3</sub> resistant starch in bean flours. Beans can be pre-cooked and treated with starch-degrading enzymes to modify starch. The resulting material can then be stored under temperature and time conditions that would increase retrogradation of amylose and optimize RS<sub>3</sub> resistant starch formation. The end product would have significantly lower GI. The effect of the product on glycemic response could then be validated using in vivo and in vitro tests.

Raw high-starch fraction of pinto beans had strong viscosity properties which could have application for use as a thickening agent and in gluten-free pasta to help reduce cooking loss and surface stickiness. The high viscosity effect could also help to lower GI in baked products due to its high starch retrogradation; typically associated with resistant starch formation. This presents a product development opportunity.

Grassy notes may be a problem in producing snack products from beans, especially for flours that have not undergone pre-cooking. Processing conditions including adding pre-conditioning step could be attempted to reduce off-flavors and optimized processing conditions for extruded beans. Flavor notes could then be evaluated using gas chromatography such as GC-

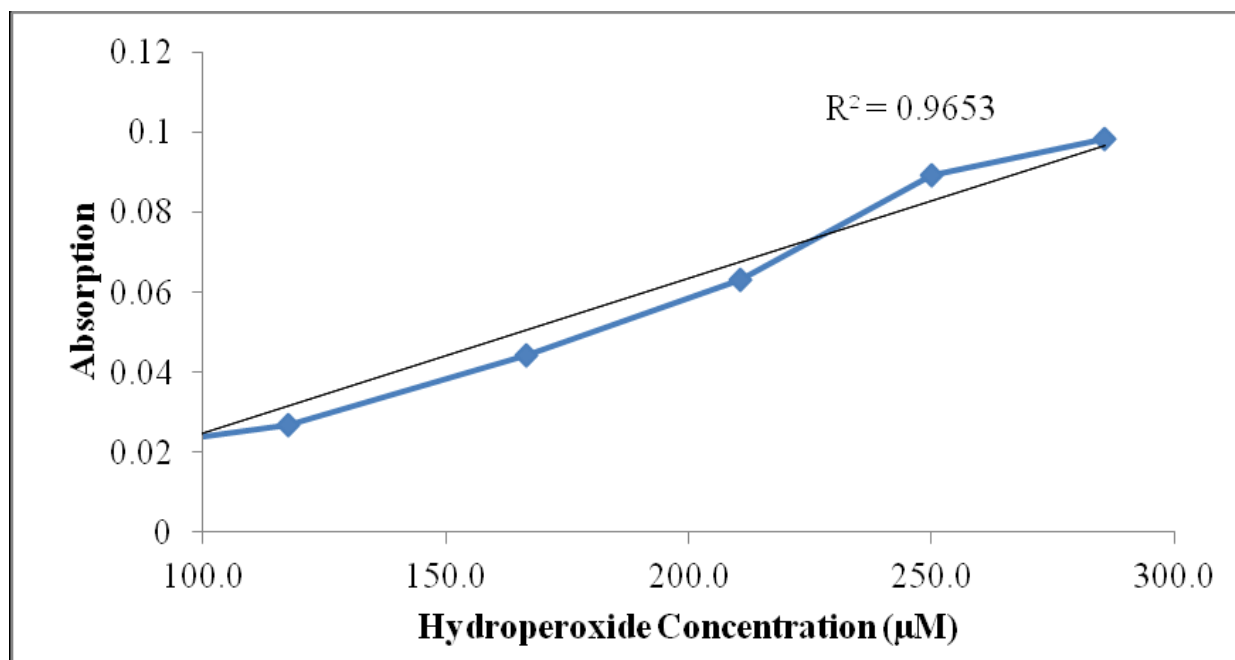


MS and GC-Olfactory, and trained sensory panelists to determine effect of processing conditions.

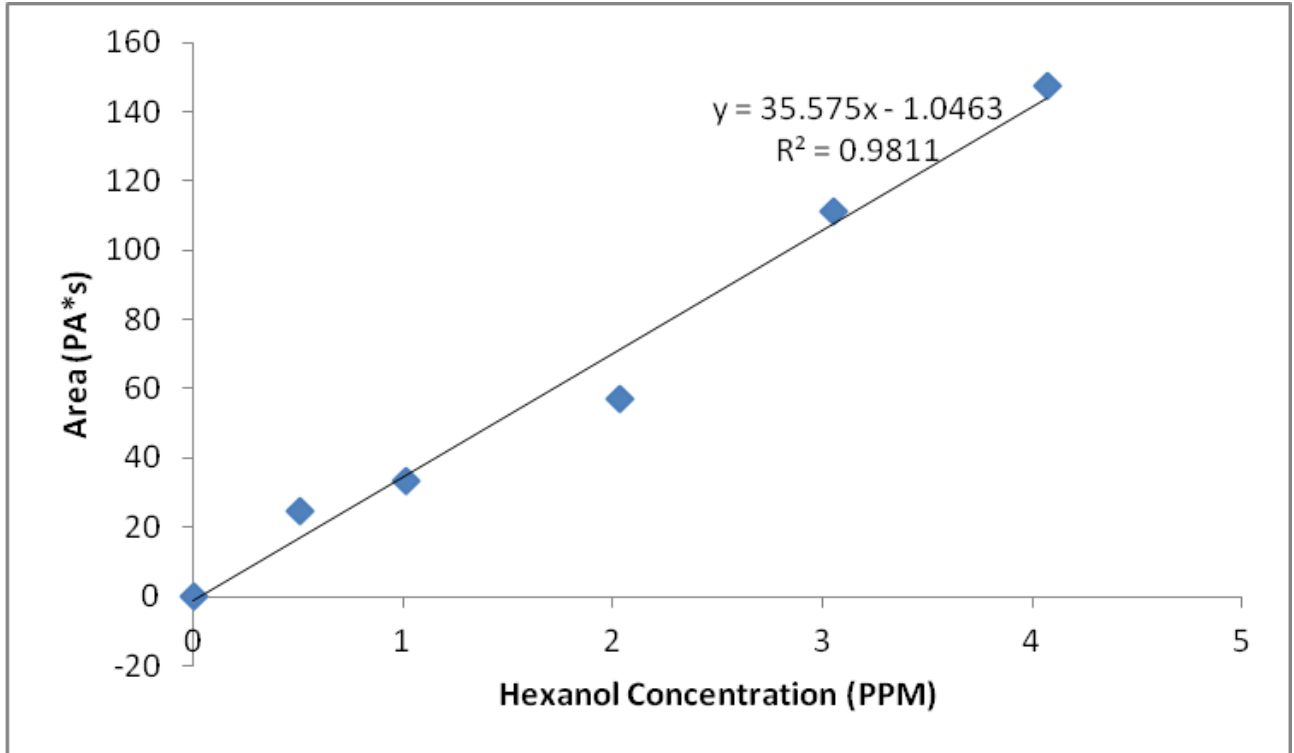
While texturized pinto protein worked well in bread, its properties would not make it a good meat analog comparable to soy. For better results a concentrate with much higher protein; for example >90% would be required to facilitate better protein-protein interactions. This will create a product with more a meaty texture that rehydrates well and has stronger structural integrity during food preparation. Therefore the air-classification method used to obtain the protein fraction in this study is not ideal for making meat analogs from edible beans. A wet fractionation method; producing a purer protein concentrate is expected to work better.

Statistical interaction between location and storage time in the final study (Chapter 5) confirmed that location significantly affected storage time. It may not be practical to select growing location for bean flavor, however differences in effects on the drought-exposed Hatton pinto beans provides a basis for a broader investigation to determine the effect of irrigation on total lipid development and its associated effect on aromatic compounds leading to grassy flavors.

## APPENDIX A. STANDARD CURVE FOR HYDROPEROXIDE CONCENTRATION



**APPENDIX B. STANDARD CURVE FOR HEXANOL**



**APPENDIX C. STANDARD CURVE FOR HEXANAL**

