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Studies on *Phaseolus vulgaris* L. Var. Great Northern Bean for Utilization in Food Processing

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STUDIES ON *PHASEOLUS VULGARIS* L. *VAR.* GREAT NORTHERN BEAN FOR ULTILIZATION IN FOOD PROCESSING

by

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Dry bean is an underutilized crop with remarkable nutritional quality. Utilization of dry beans, in the food industry, would considerably improve the nutritional intake in the population. In addition, being a readily available commodity all over the world, dry beans could make a huge economic impact on farmers and rural communities, where they are grown. To improve the use of dry beans by the food industry, much research needs to be done in order to fulfill the current limited knowledge of the functionalities of bean components and bean related product processing technologies. This research covers two critical areas with gaps in current knowledge in chemistry and functionality of dry-edible beans. The first study aimed at investigating starch isolated from five selected bean cultivars. Starch properties varied in thermal and rheological properties, based on the source/cultivar. The second study investigated dry-bean incorporation in to instant noodles, in an attempt to improve the product nutritional quality. Instant noodles were produced with selected levels of flour substitution with Great Northern bean powder. Wheat flour could be replaced, up to 25% (w/w), with Great Northern bean powder, under pilot-scale processing conditions, to obtain instant noodles with improved nutritional value, without compromising product quality. Replacing a portion of wheat flour with Great Northern bean powder significantly increased protein and fiber contents of instant noodles.

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ABBREVIATIONS

AACCI	American Association of Cereal Chemists International
ACS	American Chemical Society
AOAC	Association of Official Agricultural Chemists
BVA	Brabender visco-amylograph
CRD	Completely randomized designs
d.b	dry basis
DMSO	dimethylsulfoxide
DSC	Differential scanning calorimetry
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GADDS	General area detector diffraction system
GIA	Global Industry Analysts
GNBP	Great Northern bean powder
HPSEC	High-performance size exclusion chromatography
HRW	Hard red winter wheat
HSD	Honestly significant difference
NASS	National Agricultural Statistics Service
R.B.D	Refined bleached deodorized
RS	Resistant starch
RVA	Rapid visco analyzer
SEM	Scanning electron microscopy
T_c	Conclusion/end temperature
T_o	Onset temperature
T_p	Peak temperature

TGA	Thermogravimetric analysis
TPA	Texture profile analysis
USDA	U.S. Department of Agriculture
WAI	Water absorption Index
WINA	World Instant Noodles Association
WSI	Water solubility Index
XRD	X-ray diffraction
ΔH	Transition enthalpy (DSC)

INTRODUCTION

Dry bean is an important food source worldwide. India, Myanmar, Brazil, and China are the major producers of dry beans in the world. South America and Central America record the highest annual per capita consumption (FAOSTAT 2011). Several studies have been carried out to explore the utilization of dry beans in traditional products, such as flour, spaghetti, and crackers (Aguilera et al. 1982; Chillo et al. 2010; Han et al. 2010). However, the current commercial-scale food uses of dry beans in the United States, especially value-added products, are very limited. The presence of antinutrients in raw beans, beany flavor, and limited processing technologies have hindered the food industry from both using dry-edible beans in processing and implementing research findings in commercial operations.

The superior nutritional value of dry bean has attracted researchers to conduct studies in understanding the properties and functionalities of the commodity in order to improve the consumption. Most published studies, however, have not been translated successfully in to commercial food processing applications due to a variety of reasons, primarily lack of commercial food ingredients prepared based on dry beans. In commercial food processing, starch and protein are worth special attention for their roles in functionality of dry-edible bean as an ingredient. Starch is of special importance because; (a) its presence in high proportions compared to other components in composition, and (b) its ability to influence functional properties of dry bean-based ingredients such as powders and flours. Dry bean starch is known to be different from cereals and tuber starches in most physicochemical properties. Utilization of dry bean as a food ingredient, therefore, requires a comprehensive understanding of the functional properties of dry bean starch.

Developing value-added processing applications is an opportunity for improving the use of dry beans in the food industry worldwide. The superior nutritional value and world-wide availability of dry bean makes it an ideal candidate to improve the nutritional value of convenient food products, such as instant noodles. Several market classes (*i e.,* varieties) of dry beans are popular in the United States. However, not all of them are suitable for common food applications, due to source-specific quality characteristics.

Addition of new ingredients, to improve nutritional value of products, might alter certain critical product properties, such as texture and sensory attributes, making them markedly different from their traditional counterparts, and, sometimes, unacceptable. In selecting dry-beans for commercial food processing, light/white colored beans, such as Great Northern bean, are appropriate for most food applications. Great Northern beans also carry certain functional properties, such as thermal stability, that are suitable for food ingredient applications (Sathe and Salunkhe 1981; Wang et al. 2012). Instant noodle, a popular product with inherently low nutritional value, is consumed worldwide and the demand is expected to increase during the next decade. Improving the nutritional value of instant noodle is of importance for several reasons; (a) an advantage and would benefit the food industry, especially in making health-claims on improved commercial products, (b) consumers would be benefited by improved nutritional value, and (c) dry-bean producers would be benefited by improved utilization of the commodity for value-added processing. Great Northern bean, being a light color variety with high nutritional value, is considered an ideal ingredient source in improving the nutritional value of instant noodles. However, the insufficient knowledge on Great Northern bean physicochemical properties and functionalities has hindered its use as an ingredient in value-added food processing.

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Objectives

Overall objective:

To evaluate and characterize the properties of dry-edible bean (*Phaseolus vulgaris* L.) variety Great Northern in order to utilize it as an ingredient in potential value-added food product applications.

Specific objectives:

1. To characterize the physicochemical properties of starches isolated from five cultivars of Great Northern bean grown in Nebraska.

<u>Hypothesis:</u>

The physicochemical properties of starches from different cultivars of Great Northern beans are comparable to each other, and have appropriate functionalities for food applications.

2. To evaluate the properties of instant noodles prepared with Great Northern bean powder at selected levels of flour substitution.

<u>Hypothesis:</u>

Great Northern bean powder is suitable for the production of instant noodles with improved nutritional value.

CHAPTER 1. LITERATURE REVIEW

1.1. Background

The *Leguminosae (Fabaceae)* family is an important food source for people, feed for livestock, and other non-food applications (Graham and Vance 2003). Legumes are second only to the grasses (*i. e.,* cereals) in providing food, with more than 18,000 identified legume species currently used for food and forage (Lewis 2005). Although thousands of food legume species exist, soybean (*Glycine max*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), and common beans (*Phaseolus vulgaris*) are among the most widely produced legumes around the world. More than 7000 years ago, common beans were domesticated in tropical and subtropical areas such as Central and South America (Kaplan 1965). Common beans are annual herbaceous plants which include dry beans, green beans, shelling beans, and popping beans. Dry-edible beans, among other classes of crops (Figure 1.1) are important sources of plant-based proteins in the modern day diet. It includes most species of genus *Phaseolus* and some species of *Vigna*.

Dry beans are grown worldwide due to the crop's high environmental adaptability. According to recent statistics, India, Myanmar, Brazil, and China are the leading producers of dry beans, with an annual seed production of 4,470,000 tons, 3,721,950 tons, 3,435,370 tons, and 1,583,498 tons, respectively (FAOSTAT 2011). Influenced by the economic development, changing dietary patterns, climate and other factors, Asia contributes to approximately half of the world dry bean production (Table 1.1).

The United States contributes 3.8% of the world dry bean production (FAOSTAT 2011). Approximately 25% of the dry beans produced in the United States are exported. Additionally, U.S. ranks third among leading world dry bean exporters, behind Myanmar and China. In the U.S, the major dry bean producing states are North Dakota (32%), Michigan (17%), Nebraska (11%) and Minnesota (9%). Among all the leading classes are Pinto bean (42%), Navy bean (17%), Black bean (11%), Garbanzo bean (5%) and Great Northern bean (5%). Nebraska contributes to approximately 28% and 99% of Pinto bean and Great Northern bean annual production, respectively (USDA-AMS, 2012).

For each major market class, various cultivars have been developed and released to obtain better yield and production under regional environmental, disease, water, and stress conditions. For instance, the arid condition and limited supply of irrigation water in western Nebraska drive breeding research to select drought tolerant cultivars (Urrea et al. 2009). Pathogens, such as fungi, bacteria, and viruses, are also major threats to dry bean crops. Cultivars, such as Matterhorn and Coyne have been released to improve the disease resistance in Great Northern beans (Kelly et al. 1999; Urrea et al. 2009). These cultivars have provided alternative choices for farmers.

Dry edible bean consumption patterns vary depending on the region (Lucier et al. 2000). South America and Central America consume approximately 10kg per capita annually and this is considered much higher than other regions in the world (FAOSTAT 2009). Normally, Americans consume approximately 3kg of beans per capita per year. On a daily basis, between 1999 and 2002, only 7.9% of Americans consume beans, peas, or lentils (Mitchell et al. 2009).

Dry beans consist of 20 to 30% of protein (Hermida et al. 2006; Sathe et al. 1982a; Takayama et al. 1965). The levels of amino acids provided by dry bean protein are double than that of cereals. The amount of lysine, an essential amino acid, is 5-6 times more in dry beans compared to cereal grains. Among different varieties, Great Northern beans have been found to have higher amount of protein content (28.50%, d.b.) than other varieties (Rui et al. 2011). Relatively low fat content at 1 - 3% has been found in dry bean varieties (Sutivisedsak et al. 2011). Polyunsaturated fat consists up to 70% of the total fat content.

Starch is the main nutrient in dry bean composition, accounting for approximately 60% of total carbohydrates present in the seed (Reddy et al. 1984). Compared to common cereal starches, the digestion rate of bean starches is slow and relatively incomplete, as reported by both *in vitro* and *in vivo* studies (Hoover and Zhou 2003; Madhusudhan and Tharanathan 1995; Sandhu and Lim 2008; Tovar et al. 1992). The low digestion rate, in turn, makes beans a low glycemic food, compared to cereals (Jenkins et al. 1983). Processing operations and cooking, however, increases starch susceptibility to enzyme digestion due to loss of the granular structure during phase transition. A high degree of gelatinization allows starch to be rapidly digested. Accordingly, the processing conditions of bean-based foods influence their glycemic responses. By using a randomized, crossover, placebo-controlled experiment, Winham et al. (2007a) reported that consumption of bean spread/paste did not alter blood glucose level or insulin response when consumed with high-glycemic index food. Consumption of whole bean, mashed bean, bean powder, and bean flakes show the slow digestion of starch and absorption of glucose by the body (Dilawari et al. 1980; Jang et al. 2001; Leathwood and

Pollet 1988; Tappy et al. 1986; Torsdottir et al. 1989). Factors, such as particle size, degree of food processing, and condition of the consumer (individual health) could also determine the digestibility of dry beans. Starch structure, amylose and amylopectin polymer composition, presence of antinutrients that effect of enzyme activity, and cell structure also could influence the low glycemic response of dry beans (Bjorck et al. 1994). Several studies have suggested that cooking of legume flours increased the amount of resistant starches (Cheung and Chau 1998; Costa et al. 2006). Resistant starches in processed beans, fermented by colonic bacteria, would produce beneficial short chain fatty acids in the intestine. Compared to typical cereal grains, beans also provide notable levels of dietary fiber, vitamin B, and an array of minerals.

According to the U.S. dietary guidelines, dry beans are the only food that is included in two groups: vegetables and proteins. Bean is a valuable source of bioactive compounds and recent studies suggest that dry beans were rich in tocopherols, flavonoids, polyphenols, and phenolics (Boschin and Arnoldi 2011; Sutivisedsak et al. 2011). Some of these antioxidants, such as flavonoids, are heat resistant and could survive extreme processing conditions (Kon 1979). Health benefits of dry-edible beans have been related to those bioactive compounds (Cardador-Martinez et al. 2002; Chung et al. 2002; Ewald et al. 1999). It has been reported that legume powder consumption help coronary artery disease patients in reducing insulin demand, lipid peroxidation, and plasma homocysteine concentrations (Jang et al. 2001). The research evidence on weight control (Udani and Singh 2007) and potential disease prevention abilities, such as reducing colon carcinogenesis (Hangen and Bennink 2002), have had considerable influence on improving the utilization of the commodity in commercial food applications.

1.2. The importance of starch in food applications of dry beans

Carbohydrates, together with proteins and lipids determine the structural and textural properties of the food products. Published research on bean starch is very limited compared to cereal and tuber starches; information on the physicochemical properties and functionalities of bean starches is very scarce so that basic properties such as crystallinity of some beans starch granules is not available in the published literature. Bean starches from different growing conditions and genera are distinct in swelling factors, gelatinization temperatures, and other functionalities (Hoover and Ratnayake 2002). Therefore, an insight into the physicochemical properties of starches isolated from various cultivars within the same variety is crucial for a better understanding of bean starches.

1.2.1. Bean starch Isolation

To characterize starches, pure raw starches are often recovered by dry- or wet-milling processes. Wet-milling includes steeping/soaking, grinding, separation by filtration/centrifuge, washing, drying. Dry-milling starts the milling with dry material, and follows by sieving or air classification or other separation steps. Generally, fractionation by wet-milling results in relatively pure starch but less desired than dry-milling for most industrial applications due to economic reasons (BeMiller and Whistler 2009). For obvious reasons, wet fractionation of starch is preferred by researchers. Combining dry and wet milling methods with sonication treatment has been tested on field peas (Naguleswaran and Vasanthan 2010). Although a satisfactory recovery rate of starches was observed from wet fractionated groat flour, the purity of isolated starch was lower than starch that was isolated solely by wet fractionation.

1.2.2. Physicochemical properties of bean starch

1.2.2.1. Morphology and size distribution

The shape, size, and other morphological characteristics of starch granules vary depending on many factors; in general, the morphology of starch granules primarily depends on the botanical source, but also on environmental conditions under which the crop was grown. Regular microscopy, scanning electronic microscopy (SEM), and polarized light microscopy are commonly used to observe the shape of starch granules and birefringence characteristics. Raw dry bean starches appear to be oval, smooth, and elliptical (Gujska et al. 1994; Hoover and Ratnayake 2002).

The size distribution and shape of granules have an impact on functional properties of a given starch. Traditionally, various microscopic techniques are used as the main techniques for granule size determination. Recently, more advanced methods such as focused beam reflected analysis, and laser scattering/diffraction have been used to analyze starch granule size distribution more accurately (Ambigaipalan et al. 2011). Starch granule size distributions of certain bean varieties have been documented. Within the same variety, variations have been detected among cultivars. The size of Navy bean starch granules have been reported to be 12 - 49 µm in length and 9 - 40 µm in width by different studies (Gujska et al. 1994; Hoover and Sosulski 1985). Granule sizes of some dry bean starches reported in literature are listed in Table 1.2. Both genetic factors and analytical methods may have caused discrepancies.

Granule size is important in determining many functional properties of starch. Larger starch granules tend to be more crystalline than smaller ones. The smaller granules gelatinized at higher temperature with lower gelatinization enthalpy than larger granules (Chiotelli and Le Meste 2002). Schoch and Maywald (1968) suggested that the size and shape of starch granules may influence the viscosity patterns. For food processing, large granules are more likely to be damaged during milling than small ones. This is important because the degree of starch damage will influence flour functionalities, such as water absorption and flour dough properties (Dexter et al. 1994; Oh et al. 1985). The influence of granule size distribution on starch properties is important in food processing (Tester et al. 2004).

1.2.2.2.% Relative crystallinity of bean starch granules

Native starches are classified based on their X-ray diffraction patterns; A, B, and C. Generally, cereal starches, such as corn and wheat, exhibit A patterns; tuber starches, like potato exhibit B patterns; and legume starches have a mixture patterns A and B, which is classified as C pattern. Starch has a semi-crystalline structure. The unique diffraction pattern of each kind of starch is characterized based on variations on the scattered intensity at specific 2θ angles. X-ray diffraction is used to identify both polymorphorism and percent relative crystallinity of starch. Relative crystallinity is a proportion of crystallinity in starch granules measured against 0 and 100% references, such as amorphous starch and quartz, respectively (Tester et al. 2004). Relative crystallinities of dry bean starches are reported in literature listed in Table 1.3. The crystallinity of starch provides information about structural integrity and polymer assembly. The crystal structure and the nature of their transitions determine the end-use of a particular starch (Zobel 1988). In addition to molecular properties and nature of molecular assembly, processing and storage conditions also influence the crystalline structure of starches. For example, beans stored under nitrogen-modified atmosphere have shown lower relative crystallinity and lower heat energy requirements for gelatinization compared to those stored under normal atmospheric conditions (Rupollo et al. 2011).

1.2.2.3. Amylose/amylopectin ratio of bean starches

Starch granules are made up of primarily two polysaccharides: amylose and amylopectin. Amylose is an essentially linear polymer consisting of glucose units by α(1-4) linkage. Amylopectin is the larger branched molecule joined by both α (1-4) and α(1-6) linkages. The ratio of amylose to amylopectin varies from one starch source to another. Generally, cereal starch granules contain approximately 20% amylose (Jenkins and Donald 1995). The total amylose contents of bean starches generally range from 23 to 30% (Hoover and Ratnayake 2002). The differences in botanical sources, crop conditions, and analytical methods could influence the results. The amylose to amylopectin ratio and polymer characteristics are considered as the factors in determining starch functional properties that are important for food applications. Amylopectin chain length is related to crystal structure (Hizukuri 1985). Amylopectin is ascribed to generate the ordered crystalline structure of starch granules; amylose is considered to disrupt this structural order. The ratio of two is also a crucial factor in determinig pasting properties. High amylose content is associated with increased pasting temperature, low peak viscosity and shear thinning, and increased set-back in Rapid Visco Anaylsis (RVA) profiles (Jane et al. 1999).

1.2.2.4. Starch polymer molecular weights

Molecular weights of bean starch polymers have been reported by previous studies. Various chromatography techniques have been used in detecting the molecular weight of starches. Characterizing starch molecular weight has important implications for the food industry in utilizing starch as ingredients. Compared to cereal starches, bean starch polymers have relatively higher molecular weights, with amylose and amylopectin molecular weights of $10^5 – 10^6$ and $10^7 – 10^9$, respectively (Biliaderis et al. 1979; Rayas-Duarte and Rupnow 1993). Except for the two reports referred above, based on traditional gel filtration chromatography using Sepharose CL-2B, there is a dearth of information on molecular weights of dry bean starches in the published literature. The distributions of polymers molecular weights vary widely among varieties. High values of average molecular weights of amylose and amylopectin have been reported to positively correlate with the pasting viscosity of starches (Shibanuma et al. 1996).

1.2.3. Functional properties of bean starch

1.2.3.1. Phase transition

Starch, when heated in excess water, undergoes an ordered to a disordered structural phase transition, which is generally known as gelatinization. Gelatinization involves water absorption by starch granules, swelling, loss of ordered structure, loss of inter-molecular interactions, and dispersion of starch polymers in solution (Ratnayake and Jackson, 2008). The sequence of structural changes that take place during gelatinization has important implications on the functionality of a given starch because the structures of the starch granules depend on the source. The order-to-disorder transition process of starch granules can be characterized by a variety of techniques under shearless conditions; hot-stage microscopy with polarized light, NMR, X-ray diffraction, light scattering, SEM, and DSC/TGA (Biliaderis 1983). The morphological changes of starch granules at different stages of gelatinization have been studied in pure water and salt solutions by SEM (Hahn et al. 1977; Rockland et al. 1977). The gelatinization initiated at around 76 ˚C for water-soaked and at 85 ˚C for salt-soaked beans (2.0% sodium chloride, 1.0% sodium tripolyphosphate, 0.75% sodium bicarbonate, and 0.25% sodium carbonate) (Rockland et al. 1977). The application of DSC to analyze starch gelatinization was first reported by Stevens and Elton (1971). DSC parameters, T_o, T_p, and T_c, are influenced by many factors, such as granule crystallinity, lipid-amylose complex, and amylopectin content. In dry bean flour analysis by DSC, Chung et al. (2008) and Kaur and Singh (2007) have reported that bean flour had slightly higher T_o, T_p, and T_c values, but lower melting enthalpies than corresponding starches. This variation could be due to the matrix created by protein-starch in flour. Therefore, less amount of starch embedded in flours may require less energy to gelatinize those starches. During starch isolation process, starch may be impacted resulting different transition temperature.

The gelatinization of starch plays an important role in determining the texture of starch-based foods. Amylopectin-rich starch has relatively higher gelatinization enthalpy compared t o amylose-rich starch (Liu et al. 2009). Relatively high gelatinization temperatures enable dry bean starches to tolerate high temperature processing.

Determining the gelatinization parameters is also useful in identifying appropriate cooking conditions for dry beans. In food processing applications, the functionalities of bean starches could depend on other components such as salt, proteins, and lipids.

1.2.3.2. Pasting properties

Pasting is an important property of starch, especially for food processing applications. Pasting occurs when starch is heated over the gelatinization temperature under mechanical stress, during which the granules swell and disintegrate in excess water (1:3, starch:water). Brabender visco-amylography and Rapid Visco Analysis (RVA) are used to analyze the pasting properties of starch. Typically, in standard pasting profile analyses, viscosity of the tested sample is recorded at three different temperature stages (Figure 1.2). The reports available in the literature on the pasting properties of dry bean starches are primarily based on Brabender visco-amylography. These results are not comparable to current standard methods (*i. e.,* RVA) due to the methodological differences.

Various factors, such as botanical sources, granule size, degree of crystallinity, presence of fat and protein, and branch chain-length distribution of starch polymers, have been shown to impact on starch pasting properties (Jane et al. 1999; Jane and Chen 1992; Karim et al. 2007). Amylose and lipids have been shown inhibition to granule swelling. High amylopectin levels are known to increase granule swelling in cereal starches (Tester and Morrison 1990). Differences in these starch physicochemical properties have a major impact on pasting properties. Noticeable differences in granular swelling, amylose leaching, and other pasting properties among different bean varieties have been reported

(Ambigaipalan et al. 2011). Within a particular legume variety, cultivars have been shown to differ in pasting properties.

1.3. Utilization of dry beans in food manufacturing

The adaptability to different environments and drought tolerance make dry beans a crop that can be grown around the world. The long storage time after harvest ensures the consistent availability of dry beans throughout the year. Thus, dry beans have been served as staples or commonly consumed vegetables historically. In the Eastern parts of the world, dry beans are processed for food in a variety of ways. They are served as staples, pastry filing, vermicelli, beverages, and consumed with fresh noodles. Roasting, boiling, germination, and fermentation methods are used in various bean-based foods to enhance the nutritional quality (Reddy et al. 1982). Traditionally in the Western world, dry beans are often used in stews, chili, and canned products. In terms of commercial processed products, dry beans come in several forms including triple cleaned whole beans, canned beans, pre-cooked, and dehydrated beans.

At present, dry beans also serve as an alternative choice for vegetarians and are gaining popularity among health-conscious consumers. Due to their superior nutrient composition, beans are finding their way in to more processed food products, such as dips, snacks, sheeted, and extruded pasta. In addition, dry beans can be processed into farina without degreasing, due to the low lipid content. Nevertheless, the food processing industry is yet to exploit the full potential of the commodity, in manufacturing functional foods, which is the fastest growing industry segment in recent years. Ingredient development technologies and new product applications have not been sufficiently researched and implemented to efficiently utilize dry-edible beans by the food industry. The anticipated increase in convenience (*i. e.,* processed) foods and ingredients with high nutritional value, within next decade (Global Industry Analysts 2012), would create new market opportunities for value-added dry-edible bean products.

The utilization of a food ingredient is generally determined by its composition and in-product functionality. Value-added products made out of dry beans could enhance the nutritional value of convenient food products. The increased consumption, driven by health concerns, would benefit both farmers and the food industry. Among many market classes widely grown in the United States, Great Northern beans are preferred for food ingredient development due to light color and lack of tannins, lack of off flavors, and superior thermal stability (Chang and Satterlee 1981). The wide availability of Great Northern beans would be also important and beneficial for the food industry. Prior to exploring the food applications for Great Northern beans, a better understanding on their composition and properties is critical. The functionality of starch in dry beans primarily determines its in-product behavior in food processing. In a food product, starch acts as a thickener or binder, viscosity builder, structure former, and helps to keep the general integrity. However, there is a dearth of information about the physicochemical characteristics and functionality of Great Northern bean starches, particularly in regard to ingredient development. To fulfill the need, investigating the physicochemical properties of Great Northern bean starches is essential.

1.3.1. Value-added food applications for dry edible beans: Instant noodles

The earliest recorded evidence of noodle consumption dates back to 206BC-220AD - era of Han Dynasty, in China (Tauger 2011). Gradually, Chinese noodles were introduced to other countries in the region and rest of the world. Nowadays, various kinds of noodles are available in the supermarkets worldwide.

Based on different criteria, such as type of flour and processing method, noodles can be classified into different types. For example, Japanese Udon noodles are made of soft wheat flours; Chinese salted noodles require hard wheat flours (Hou and Kruk 1998). Compared to other noodles, instant noodle is a rapidly growing market worldwide. Additionally, under certain circumstances, such as travelling, natural disasters or other situations when convenient and high energy foods are needed, instant noodles becomes an ideal choice, because it provides relatively higher amounts of energy compared to other staples, such as rice, and produced, stored and transported more conveniently compared to products such as bread. Currently, instant noodle is solely marketed based on convenience claims, *i. e.,* 2 - 5 minutes preparation time, and cheap price compared to most other consumer foods. Despite its popularity, instant noodle is considered one of the worst food items in terms of nutritional quality. This is mainly due to its simple ingredient formulation. Traditionally, instant noodle is prepared using wheat flour, salt, carbonates, and water. Lack of sufficient quantity and quality of proteins, fiber, and minor nutrients, other than those provided by dried vegetables in seasoning and additive packets, contributes to the inherently low nutritional value of instant noodles. The notoriety of the product is also due to high fat (up to 15%, w/w) and salt (*i. e.,* sodium) contents of fried instant noodles. Therefore, there are health risks associated with long-term consumption of instant noodles (Lee et al. 2009).

To improve the nutritional quality of noodles, other nutritive ingredients have been tested (Jitpukdeebodintra and Jangwang 2009; Noda et al. 2006). Addition of soy protein could improve lysine and vitamin B contents (Sun and Song 2004). Meanwhile, the emulsifying effect of soy protein helps in lowering the oil content. In a study to improve fiber levels of instant noodles (Reungmaneepaitoon et al. 2006), three types of oat brans, at selected levels, were tested. They concluded that 10 - 15% (w/w) addition of oat bran produced acceptable quality instant noodles with 260% increase in dietary fiber level.

Dry bean flour/powder could be an ideal alternative for commercial-scale instant noodle processing, in improving nutritional value of the product. Dry-bean flours, on average, contain 23.8% protein and 23.5% (weight basis) fiber. In addition, dry-edible beans contain other important nutrients, such as iron, magnesium, and copper (CIAT 2001). Investigations on the effect of dry bean flour addition to spaghetti formulations has shown that carbohydrates were progressively slowly digestible with increasing levels of bean substitution (Infante et al. 2010).

The instant noodle manufacturing process involves making dough sheets, slitting, steaming, and frying/drying (Fu 2008; Hou 2001; Hou 2010). Different processing steps have specific purposes in noodle manufacturing. To make an uniform and non-sticky dough, the optimum water absorption of noodle flours in obtained at 35% (Park and Baik 2004). Resting the ingredient bled, prior to sheeting, allows homogenous and adequate hydration of the flour mix, and promotes gluten development, ensuring an even smooth and non-sticky dough sheet later during processing. Sheeting and compounding are aimed at developing gluten network and making flour mix hydrate evenly. Steaming gelatinizes starch and denatures protein to make and maintain structure of the product. Frying removes exterior water, then water migrates. The surface of instant noodle swells during frying. Well gelatinized starch in noodles lowers the required cooking time and gives less sticky surface (Hou 2001b).

Both processing conditions and ingredient combination determine the properties of instant noodles, resulting in unique color and springy textured strands. Various kinds of wheat flours are used in noodle manufacturing. The United States, Australia, and Canada produce hard red spring/winter wheat and soft wheat for noodle industry around the world (Kim et al. 2006). The type of flour is selected based on the type of noodle and end product properties (Vocke 1998). Two main components in wheat flours are gluten protein and starch. Gluten proteins in wheat flour are important in developing the desired textural and structural properties of the product, especially for Chinese noodles (Hou 2001). The influence of flour protein content and quality on instant noodles qualities have been well documented by Park and Baik (2004). Flour of low protein content causes instant noodles to retain more fat, during processing, compared with flour of higher protein content (Moss et al. 1987). Starch properties, including low amylose content and higher starch pasting viscosities, are preferred for noodle processing and eating quality (Zhao et al. 1998). Increasing levels of amylose content in flours lower the water binding of cooked noodles (Toyokawa et al. 1989). Low water-binding of cooked noodles results in increased firmness of noodles.

Adding dry beans into instant noodles could improve the protein content, dietary fiber, and many other nutrients of the product. Regardless of their health benefits, there are several concerns with using dry-edible beans in commercial food processing operations; (a) flatulence – presumably caused by certain carbohydrates (Murphy et al. 1972), (b) 'beany' flavor (Vara-Ubol et al. 2004), (c) insufficient formation on in-product functionality of bean flour. The first two issues have been addressed fairly successfully by commercial food ingredient manufacturers. Commercial bean flours/powders are not available in limited quantities for certain product applications.

1.4. Summary

Despite the strong evidence on the superior nutritional value of dry-edible beans and their wide availability, the lack of understanding on how to effectively use whole beans as an ingredient in commercial food processing has hindered both food manufacturers and consumers from recognizing dry beans as a high quality ingredient/food, regardless of government efforts to increase consumption to improve nutritional value of the diet. Currently, the utilization of dry beans in large scale food manufacturing operations is very limited. One major reason is the lack of knowledge of bean properties, especially the differences among varieties or market classes. To address the issue, a comprehensive understanding of dry beans is required, especially the physicochemical properties and functionalities. The structure and property relationship of starch primarily determines dry bean product applications.

Given the continuing demand for healthy and nutritional foods, the potential for expanding bean processing and utilization in large-scale food manufacturing appears to be promising. The availability of bean products, in various forms, is expected to increase. Convenient foods market, especially instant noodles, could be one of the best targets for dry bean utilization. In this regard, a comprehensive knowledge of bean based ingredients and their application in possible products need to be investigated.

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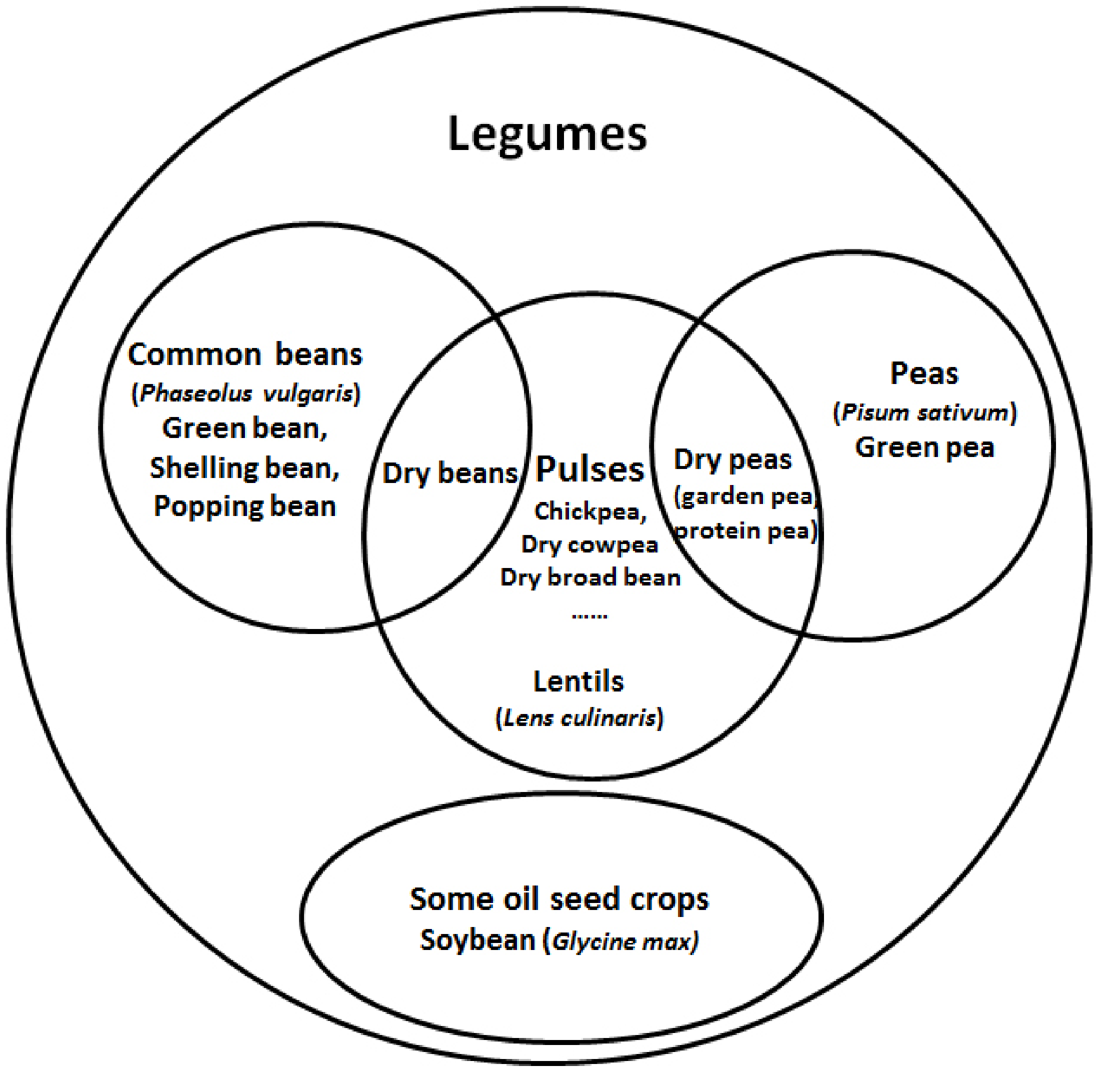

Figure 1.1 Classification of dry beans, adapted from FAOSTAT database - Primary pulses (FAOSTAT 2011).

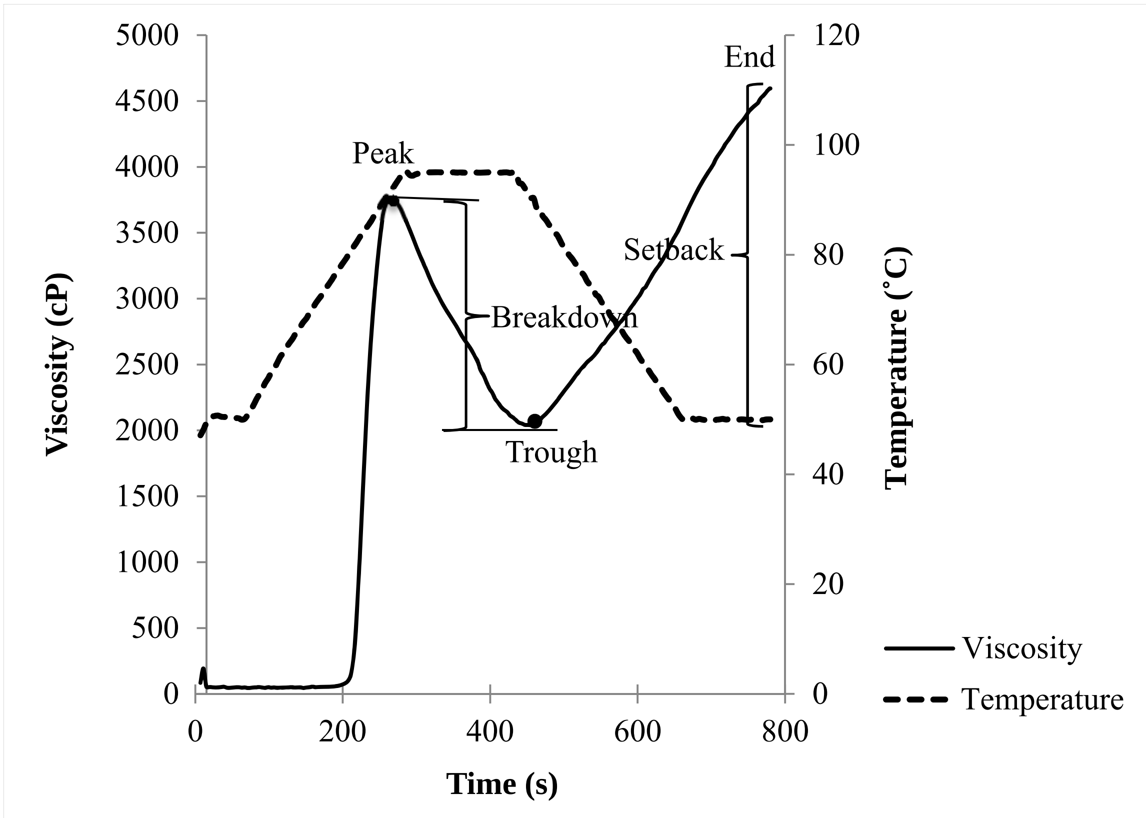

Figure 1.2 Typical RVA profile.

Table 1.1. Dry bean annual production and yield. [1]

Country	Production (tons)	Yield (kg/km^2)
World	23,250,253	79,590
India	4,470,000	44,260
Myanmar	3,721,950	130,790
Brazil	3,435,370	93,530
China	1,583,498	157,140
United States	899,610	192,310
Tanzania	675,948	91,630
Indonesia	341,097	114,800
Mexico	567,779	63,440
Canada	144,600	217,770
Kenya	577,674	55,720

[1]Data of selected countries adapted from FAOSTAT (2011).

Table 1.2. Granule sizes of dry bean starches from different cultivars of *Phaseolus vulgaris* L.

Type	Granule dimension (μm)			Reference
	Width	Length	Diameter	
Black bean	7 - 37	24 - 70		Hoover and Ratnayake (2002)
Great Northern	12 - 40	12 - 58		Sathe et al. (1982b)
Kidney bean	20 - 42	20 - 60		Hoover and Sosulski (1985)
Navy bean	9 - 32	12 - 32		Gujska et al. (1994)
Pinto bean	8 - 36	14 - 40	10 - 45	Gujska et al. (1994); Jane et al. (1994)

Table 1.3. Relative crystallinities of dry bean starches from different cultivars of *Phaseolus vulgaris* L.

Variety	% Relative crystallinity	References
Black bean	17 - 22	Hoover and Ratnayake (2002)
Kidney bean	28 - 30	Chung et al. (2008)
Navy bean	19 - 20	Hoover and Ratnayake (2002)
Pinto bean	21 - 23	Ambigaipalan et al. (2011)

CHAPTER 2. PHYSICOCHEMICAL AND THERMAL PROPERTIES OF GREAT NORTHERN BEAN STARCH

Abstract

The compositions and properties of five Great Northern bean cultivars (Beryl-R, Coyne, Gemini, Marquis, and Orion) were investigated. Starch was isolated from each cultivar by wet milling, without heat treatments. Isolated, unmodified starches were characterized for granular and molecular level properties. Smooth surfaces and essentially similar granule sizes and shapes were observed among all cultivars. Amylose contents were in the range 21.0 to 22.6%. Amylose and amylopectin molecular weights were approximately 10^5 and 10^9 Da, respectively. Typical C-type X-ray pattern was observed in all cultivars. Significant differences were observed among cultivars in % relative crystallinities, which were in the range 18.2 to 23.8%. The relative crystallinity was independent of amylose proportion and molecular weight. The five Great Northern bean cultivars differed in their thermal and rheological properties. Coyne and Gemini had low gelatinization enthalpies. In pasting profile analysis, Coyne had the lowest peak and final viscosities. Granule size, polymer proportion, and molecular weights had major influences on gelatinization and pasting properties.

2.1 Introduction

Historically, beans (especially the varieties and cultivars of *Phaseolus vulgaris* L.) have played a major role in the human diet. In dry beans, up to 60% of the total seed weight is composed of carbohydrates (Reddy and others 1984). Except for the various types of reducing and non-reducing sugars, and oligosaccharides, carbohydrates in dry beans mainly exist as starch in the seed endosperm. Consequently, starch defines most of the structural and functional properties of dry bean based foods.

Great Northern bean is a variety of *Phaseolus vulgaris* L. which is preferred in processed food applications due to its light color (*i. e.,* white to off white color seeds) and produced in various parts of the world. Being the main component in the seed composition, starch has a major influence on the in-product functionalities of Great Northern bean when used as a food ingredient (Sathe and Salunkhe 1981a). Except for few reports, based on partial characterizations (Sathe and Salunkhe 1981a; Rayas-Duarte and Rupnow 1993; Hoover and Sosulski 1985), detailed information is not available on Great Northern bean starch properties in the published literature. The functionality of both starch and whole bean-based food ingredients depend largely on starch properties, especially those related to gelatinization and pasting. It is well known that starch granular/structural, and molecular properties determine the functionalities that are important for product applications. Starch physicochemistry determines, to a greater extent compared to other components, primarily the ingredient functionalities of isolated starch, starch-rich fractions, flour, and powders prepared out of legume seeds (Sosulski and others 1985; Hoover and Sosulski 1991). Previous studies have reported inconsistent findings on certain important starch physical and chemical properties, such as amylose content and pasting properties of starch (Hoover and Sosulski 1985; Sathe and others 1981). In addition, important properties, such as polymer molecular weights and relative crystallinity of granules, were either not studied or in disagreement. As a result, the starch structural features that dictate the observed differences in functional properties of Great Northern bean starch are still largely unknown.

In food applications, starch is primarily responsible for building and maintaining the structure. It is well known that legume starches have unique structural features and polymer assembly patterns within the starch granules, compared to cereal and tuber starches that are widely used in food processing applications. Great Northern beans (and legumes, in general) are not widely used as sources of starch or flour for processed food applications, and, therefore, could be considered as an underutilized resource for food ingredients. A better understanding on Great Northern bean starch physicochemical properties will enhance our knowledge in legume starches and their structure-function relationships. In addition, such knowledge on unique properties of C-type starches, that are not widely utilized for food processing, would be of interest in widening their utilization.

In this study, our objectives were (1) to investigate and understand the granular structural and physicochemical features that influence unique functionalities, especially thermal behavior, and (2) to investigate the differences, if any, among starches isolated from the selected cultivars of Great Northern bean.

2.2 Materials and methods

2.2.1 Materials

Five Great Northern bean (a variety of *Phaseolus vulgaris* L.) cultivars; Beryl-R, Coyne, Gemini, Marquis, and Orion, grown in the 2011 crop season, were obtained from the University of Nebraska-Panhandle Research and Extension Center, Scottsbluff, Nebraska, USA. The cultivars used in this study have been developed to resist drought conditions and certain diseases (Urrea and others 2009). Cleaned whole beans were stored at -21^{o}C and thawed at room temperature for 24 hours prior to use for the experiments. All chemicals and solvents used for the experiments were of ACS certified grade.

2.2.2 Composition analysis

Proximate compositions of whole Great Northern beans were analyzed by standard AACCI (AACC International 2002) and AOAC (AOAC 2009) methods. Total starch was analyzed using YSI Select 2700 Biochemistry Analyzer (YSI Life Sciences, Yellow Springs, OH) (YSI 2000).

2.2.3 Starch isolation

Starches were isolated and purified according to previously published wet-milling procedures (Hoover and Sosulski, 1985), with minor modifications, as follows. An amount of 225g whole beans were steeped in 600ml distilled water containing 0.01% (w/v) sodium metabisulfite for approximately 20 hours at 35˚C. Seed coats were manually removed. Soaked seeds were homogenized by a Waring blender (Model 31BL91-7010, Waring Commercial, New Hartford, Connecticut, USA) for 1 minute at low speed and 2 minutes at high speed, at 30 second intervals. The homogenate was filtered using a Buchner funnel, fitted with a cheesecloth, under vacuum, using a Welch filtration vacuum system (Model 2515B-75, Welch Vacuum Technology, Inc., Monroe, LA, USA) at approximately 7.5psi vacuum pressure. The filtrate was collected and sediment was suspended in a 0.10g/L sodium hydroxide solution and kept at 5°C (refrigerator), undisturbed for starch containing sediment to precipitate. The supernatant was renewed every 12 hours, for three washing cycles. The sediment was then filtered through 74µm polyester filter (Spectra Mesh, Spectrum Laboratories, Inc. Rancho Dominguez, CA, USA) under vacuum at 7.5psi, using a Buchner funnel. The filtrate suspended in 0.1g/L sodium hydroxide solution and was left to stand undisturbed overnight at 5°C. Then, the sediment was neutralized to pH 7.0 with hydrochloric acid and filtered through 10µm polyester filter (Spectra Mesh) under 7.37psi vacuum pressure. Final filter cakes were collected and freeze dried under -50°C and 0.22mbar by bench top freeze dry system (FreeZone 4.5 Benchtop Freeze Dryer, Labconco Co., Kansas City, MO, USA) for approximately 85 hours.

2.2.4 Size distribution analysis of starch granules

Starch granule size distributions were performed using a Malvern Mastersizer 3000 laser diffraction particle size analyzer equipped with a Hydro MV wet dispersion unit (Malvern instruments Ltd, Malvern, Worcestershire, UK). Sample was delivered into the system within the obscuration limit of 0.1-10%. Prior to measurement, the sample was dispersed in distilled water by pulsed ultrasound (on for 4s, off for 2s, for a duration of 10s) and stirred (1300 rpm) for 10 seconds inside the wet dispersion cell. Refractive indices of 1.3 for starch and 1.33 for water, starch density of 1.5g/cm3, and absorption index of 0.10 (per equipment manufacturer's specifications/database) were used as the analytical parameters. Water was degassed after sonication and prior to measurement. Data were collected and analyzed by Malvern software (Version 2.01, Malvern instruments Ltd, Malvern, UK).

2.2.5 Scanning electron microscopy (SEM)

The morphology of starch granules were characterized using scanning electron microscopy. Starch samples were scattered on metal stubs and coated with gold–palladium alloy using a Hummer sputter coating system (Anatech Ltd., Union City, CA). Coated samples were observed under a Hitachi S-3000N variable pressure scanning electron microscope (Hitachi Science Systems, Tokyo, Japan) at an acceleration potential of 25kV. Images were recorded by image capturing software (Version 10-16-2266, Hitachi High-Technologies, Pleasanton, CA, U.S.A.).(Ratnayake and Jackson 2007).

2.2.6 Amylose content analysis

Amylose content was estimated by the dual-wavelength iodine binding method (Zhu and others 2008) with slight modifications . Amylose contents were calculated by the following equation. Starch samples (approximately 100.00mg) were dissolved in ethyl alcohol (1mL) and sodium hydroxide solution (1N, 10mL) in sequence in 100mL volume flask. After samples were completely gelatinized, they were diluted to volume with distilled water. Two mL of the solution were transferred into another 100mL volumetric flask, diluted with about 50mL of distilled water, and titrated to neutral with a 0.2N hydrochloric acid, in the presence of phenolphthalein. Two mL of 0.20% iodine solution was added in to the solution and diluted to volume with distilled water. Absorbance of sample solutions at 620nm and 510nm were determined through a UV-

Visible Spectrophotometer (Biomate 3S, Thermo Scientific, Madison, WI, USA). Amylose standards of 10, 20, 30, 50, and 80 (w/w, d.b) were used to create a standard curve; differential absorbance vs. % amylose. Amylose contents were calculated by the following equation.

% Amylose = 1.214x + 0.0681………………………………..…….. Equation (2.1)

Where x is the difference between the absorbances at 620nm and 510nm, with R^2 = 0.9999.

2.2.7 High-performance size exclusion chromatography (HPSEC)

Samples were dispersed in aqueous (90% v/v) dimethylsulfoxide and filtered following previously published procedures (Ratnayake and Jackson 2007). Samples of 0.050g (d.b) were dispersed in 10ml of 90% (v/v) dimethylsulfoxide (DMSO; BDH, West Chester, PA, U.S.A.) in water solution by keeping at room temperature for 5 days on a multi tube rotator (Model: 4632Q, Thermo scientific, Madison, WI, USA) with a fixed shaker speed at 30rpm. Dispersed samples were filtered through a 1.2μm Magna nylon supported membrane (GE Osmonics, Minnetonka, MN), and 100μL of filtrate was injected into HPSEC system, equipped with Shimadzu LC-20AD pump, Shimadzu CTO-20A column oven (Shimadzu Scientific Instruments Inc., Canby, OR, USA), Shodex DGU-20A Prominence Degasser, Shodex RI-101 detector (Shodex, Showa Denko K.K., Kanagawa, Japan), Size exclusion columns, Shodex OHpak SB-807G, SB-807 HQ, SB-806 M HQ, SB-804 HQ and SB-802.5 HQ (Shodex, Showa Denko K.K., Kanagawa, Japan) connected in series and maintained at 50°C ,were used in the system. Degassed distilled water was used as mobile phase at 1ml/min flow rate. Data was collected and analyzed by Chromatography Data Systems software (Shimadzu Ezstart version 7.43, Shimadzu Scientific Instruments Inc., Canby, OR, USA). Pullulan standards (Standard P-82, Showa Denko K.K., Kanagawa, Japan) P-5, P-10, P-20, P-50, P-100, P-200, P-400, and P-800 representing molecular weights 0.53×10^4, 1.2×10^4, 2.08×10^4, 4.67×10^4, 9.54×10^4, 19.4×10^4, 33.8×10^4, 75.8×10^4, respectively, were used to create the molecular weight standard curve. The molecular weights of samples were calculated using the standard equation given below.

Log MW = -0.2836 x RT + 14.538……………………………..…… Equation (2.2)

Where MW= Molecular Weight (Da), RT= retention time (minutes), with $R^2 = 0.9985$.

2.2.8 X-ray diffraction (XRD)

X-ray powder diffraction profiles were obtained and % relative crystallinity was analyzed by the procedures reported by Ratnayake and Jackson (2008) and Nara and others (1978). The samples were mounted on an aluminum sample plate with thin film of grease applied at the bottom of the cavity to hold the sample and slightly compressed using a spatula to obtain a smooth surface. A Bruker-AXS D8 Discover XRD system (Bruker AXS GmbH, Germany) with a general area detector diffraction system (GADDS), a Gobel mirror, a 0.5mm pinhole collimator, and a Bruker-AXS HI-STAR area detector was used. X-ray tube was set to 40kV and 40mA. Samples were scanned under the following: omega = 4 degrees, detector swing angle = 18°, sample to detector distance = 10.1cm and exposure time = 180s. Bruker-AXS GADDS system software integrated area from $2\Theta = 3$ to 35° and chi = –130 to –50˚. Peak fitting software Origin (version 8.5, OriginLab Corporation, Northhampton, MA, U.S.A.) was used to calculate % relative crystallinities.

2.2.9 Differential scanning calorimetry (DSC)

Starch samples were prepared according to Ratnayake and others (2009) procedure with excess water in hermetically sealed in high pressure DSC pans. Samples were scanned against a blank (empty pan) using a Perkin Elmer Pyris 1 DSC system (Perkin-Elmer Co., Norwalk, CT, USA) from 25 to 135˚C at a 10˚C/min scanning rate. Pyris version 3.50 software (Perkin-Elmer Co.) was used to collect and analyze onset (T_o), peak (T_p), and end (T_c) temperatures, and the transition enthalpy (ΔH). The instrument was calibrated using Indium reference material.

2.2.10 Rapid viscosity analysis (RVA)

Pasting properties of isolated starch were evaluated by RVA. Starch samples of 3.00g (d.b), and 25ml water (adjusted to 14% moisture level) were mixed in an RVA canister followed by prompt vortex mixing (Fisher Scientific fixed speed vortex mixer, Thermo Fisher Scientific Inc., New Jersey, USA) capped with a #8 rubber stopper covering the canister mouth for 10s to avoid formation of clumps. The mixed samples were then analyzed by RVA (Model 4S, Newport Scientific, Warriewood, NSW, Australia), using Standard-1 profile, following AACCI Approved Method 76-21 (AACC International 2002).

2.2.11 Statistical analysis

The study was conducted using completely randomized design (CRD). For each experiment, determinations were replicated at least three times. Analyses of variance were performed and mean separations were performed by Tukey-Kramer HSD test at $p<0.05$ significance level using JMP (Version 10.0.0, SAS Institute Inc. Cary, NC, USA).

2.3 Results and discussion

2.3.1 Proximate composition of whole beans

Proximate compositions of the tested cultivars were analyzed to detect any major differences in compositions. Moisture (~9.4%), ash (~5.2%), crude fiber (~3.8%), and fat (~0.8% w/w, fresh basis) contents were comparable ($p > 0.05$) among the cultivars. The protein content varied in the range 23.0 to 26.2%, with the average being 24.4% (w/w, fresh basis). Total starch content varied in the following order: Orion (37.1%) ≤ Gemini (38.0%) ~ Coyne (39.0%) ≤ Marquis (40.0%) = Beryl-R (40.3%) in dry basis ($p < 0.05$). The approximate average total starch content among all samples was 39%. Regardless of the differences in starch and protein contents, the compositions of the five cultivars were generally comparable to each other. The proximate compositions of the five cultivars of Great Northern bean studied here were comparable to the compositions previously reported for other varieties of *Phaseolus vulgaris* L. dry beans (Rui and others 2011).

2.3.2 Granular morphology and size distribution

Starch granule shape and size influence the functional properties of starch, such as paste viscosity. Scanning electron microscopy (SEM) images of isolated starch granules from each cultivar showed no evidence of starch damage, *i. e.,* devoid of broken granules, no visible cracks or indentations on the surfaces of granules (Figure 2.1). Also, the samples did not contain any foreign materials. All cultivars showed granules of spherical, oval, or elliptical shapes with smooth surfaces. Generally, the smaller granules were spherical in shape compared to their larger counterparts, which were oblong to elliptical in shapes (Figure 2.1). Similar granular morphologies of isolated Great Northern bean starch have been reported previously (Sathe and Salunkhe 1981b; Hoover and Sosulski 1985). Generally, Great Northern bean starch granules have morphologies similar to most other varieties of *P. vulgaris* (Hoover and Ratnayake 2002), but very different from other starches with C-type crystalline patterns, such as tapioca and banana (Ratnayake and Jackson 2007; Carmona-Garcia and others 2009).

The size distributions of starch granules were analyzed by laser diffraction. The average size distributions (i. e., peaks of distribution profiles) spanned between ~4 to 40μm, with a peak ~28μm for each cultivar. The detailed results for selected size classes are given in Table 2.1. Coyne showed a high amount of smaller granules, whereas Gemini and Beryl-R showed the large overall granule sizes. Our results generally agree with most previously published size distribution ranges for Great Northern bean and other *P. vulgaris* starches (Hoover and Ratnayake 2002; Hoover and Sosulski 1985; Sathe and others 1982; Ambigaipalan and others 2011). However, the granular size at the upper 10% of the population was higher than the maximum granular sizes reported in most previously published reports cited above. Variations in analytical methods, especially microscopic techniques *vs.* laser diffraction, and botanical sources of samples could have attributed to the differences observed in granule size distributions.

2.3.3 Polymer composition and molecular weights

Amylose and amylopectin compositions of Great Northern bean starches are given in Table 2.2. A significantly higher level of amylose was detected in Coyne, whereas the other could cultivars were comparable ($p < 0.05$). Starch granule is primarily structured by amylopectin, while amylose being a contributor (Donald 2001; Jane and others 1992). The proportion of amylose has a considerable influence on starch functionalities, especially those related to pasting and product applications (Jane and others 1999; Ambigaipalan and others 2011). Previous studies (Hoover and others 2010; Zhou and others 2004) have reported amylose contents ranging from approximately 30 to 40% for common beans of *P. vulgaris*, whereas this study found amylose proportions in the range 21.0 to 22.6% in the five cultivars of Great Northern bean. The considerable differences in results could be due to the analytical methods used and variations in genetic factors of sources. In amylose analysis by iodine binding, long branch-chains of amylopectin could also bind iodine resulting in an overestimation of amylose content (Jane and others 1999; Zhu and others 2008). Dual wavelength iodine binding method (Zhu and others 2008), which was used in this study, accounts for the iodine affinity by amylopectin, allowing a more accurate (and relatively less, due to the absence of contributions of iodine binding by amylopectin) estimation of amylose.

In general, amylose molecular weights of tested Great Northern bean cultivars were ~10^5Da and amylopectin molecular weights were ~$10^8 – 10^9$Da (Table 2.3). Orion showed the lowest amylose molecular weight, while Marquis and Coyne had high amylopectin molecular weights. The molecular weights observed in this study are different from those previously reported for Great Northern bean starch using Sepharose CL-2B gel filtration; > 2 x 10^6 for amylopectin and 2 x 10^5 Da for amylose (Rayas-Duarte and Rupnow 1993). These differences in molecular weights could be attributed to the source (genetic variations among the cultivars used in each study) as well as the differences between the two analytical methods. Except for the current study and the reports by (Rayas-Duarte and Rupnow 1993) and (Biliaderis and others 1979), information on the molecular weights of *P. vulgaris* starch polymers is scarce in the published literature. Although our results are in general agreement with the molecular weight ranges given in previous reports, the exact molecular weight of Great Northern bean amylopectin has not been reported previously. Chain lengths of amylose and amylopectin are partially responsible for the functionality of starch. The influence of observed differences in molecular weights on functional properties is discussed in the sections below.

2.3.4 Relative crystallinity of granules

Legume starches typically exhibit the characteristic C-type X-ray pattern, which is often referred to as a mixture of A- and B-types (Bogracheva and others 1998; Gernat and others 1990). A-type polymorphism is usually detected in cereal starches, such as maize and wheat, whereas B-type structure is present in tuber starches, such as potato.

These polymorphic structures differ in terms of unit cell organization and the number of water molecules associated with the unit cell (Sarko and Wu 1978).

All five cultivars of Great Northern bean starches displayed C-type polymorphic patterns, typical of legume starches. In the X-ray diffraction profiles, major peaks were observed at $2\theta = 17^\circ$ and $2\theta = 23^\circ$, and a minor peak was observed at $2\theta = 15^\circ$ (X-ray diffractograms are not reported here). The % relative crystallinities among starches isolated from Great Northern bean cultivars followed the order: Marquis ≥ Coyne ~ Orion ≥ Gemini ~ Beryl-R (Table 2.4). Dry bean *(P. vulgaris)* starches generally have relative crystallinities in the range 17 - 25% (Hoover and Ratnayake 2002), and our results for Great Northern bean starches are consistent with previous findings. Cultivars Marquis and Coyne showed high levels of relative crystallinities. Starch structure, being a semi-crystalline material, is primarily dictated by the inter-molecular interactions of the outer branches of amylopectin molecules (Donald and others 1997). Parallel arrangements of outer branches of amylopectin are considered primarily responsible for the crystalline domains of the granule (Jenkins and Donald 1995). Interestingly, the cultivars Marquis and Coyne also displayed high molecular weights of amylopectin (Table 2.3), which could have contributed to the observed high relative crystallinities.

Although negative relationships between relative crystallinity and amylose proportion have been proposed (Jenkins and Donald 1995; Atkin and others 1999) our results did not follow the same pattern. Beryl-R had both the lowest amylose content (Table 2.2) and the lowest relative crystallinity (Table 2.4). Although most cereal (A-type) and tuber (B-type) starches follow the conventional theories on the origins of crystallinity and its dependence on amylose/amylopectin proportions (Cheetham and Tao

1998), C-type starches, such as Great Northern bean studied here, usually do not follow the same patterns (Hoover and Ratnayake 2002; Jenkins and Donald 1995). In addition, a variety of other factors including crystal size, distribution of crystallite regions, orientation of "crystals" within the semi-crystalline domains, and the extent of polymer interactions could collectively determine the relative crystalinity of a given starch (Hoover and others 2010). A better understanding on C-type starch polymorphism and its influence on relative crystallinity may be required to meaningfully explain these observations.

2.3.5 Gelatinization properties

Starch, when subjected to DSC analysis in the presence of excess water, undergoes a phase transition from an ordered to a disordered state with progressive structural breakdown and polymer dispersion in the aqueous phase (Ratnayake and Jackson 2007; Ratnayake and Jackson 2009). The temperatures at which the steps of this phase transition occur are of importance in determining the thermal properties and functionalities of starch. Generally, both granular structural and molecular level features determine the gelatinization properties.

DSC parameters of the five Great Northern bean starches are presented in Table 2.5. Transition temperatures, T_o, T_p, T_c, and range, were statistically equivalent for starches from all cultivars ($p > 0.05$). The T_o values observed for Great Northern bean starches were, however, higher than what has been reported previously for bean starches of the same species (Hoover and Sosulski 1985; Ambigaipalan and others 2011). The transition enthalpies were significantly different among the five cultivars ($p < 0.05$).

Cultivars Orion and Beryl-R had the highest transition enthalpies, while Beryl-R was statistically comparable to Marquis. In general, the transition enthalpies (ΔH) were comparable to those previously reported for bean starches in the range of 8 – 15J/g (Hoover and Sosulski 1985; Ambigaipalan and others 2011). High gelatinization transition enthalpies of Orion, Beryl-R, and Marquis could not be attributed to any specific structural or molecular level properties of Great Northern bean starches studied here. It has been suggested that the presence of high levels of amylose would interfere with structure building polymer associations of amylopectin molecules' outer branches (Jenkins and Donald 1995). Considering the generally accepted starch structure, which is primarily built on amylopectin molecular interactions (Donald and others 1997), the presence of relatively high levels of amylose (Table 2.2) and high amylose molecular weights (Table 2.3) could have caused the cultivars Coyne and Gemini to display low gelatinization enthalpies. Starch gelatinization measured by DSC could represent any and all endothermic events that take place during the phase transition process (Ratnayake and Jackson 2009; Sahai and Jackson 1999). Accordingly, it is possible that a combined effect of several factors, rather than one specific starch property could have contributed to the observed differences of transition enthalpies in Great Northern bean starches.

2.3.6 Pasting properties

To the best of our knowledge, pasting properties of Great Northern bean starches are not readily available in the published literature. Hoover and others (2010) have summarized pasting properties of bean *(P. vulgaris)* starches based on Brabender visco-amylography (BVA). In the present study, Great Northern bean starch pasting properties were analyzed by Rapid Visco Analyzer (RVA), and these results (Table 2.6) may not be readily comparable with those obtained from BVA tests, available in the published literature, due to experimental and data collection differences between the two methods (Suh and Jane 2003). During pasting, starch granules absorb moisture and swell, resulting in a rapid increase in paste viscosity which is measured as “peak viscosity”. The peak viscosity represents starch granules’ ability to absorb water and swell.

Cultivar Coyne showed the lowest peak viscosity, which could be attributed to the higher proportion of amylose, and larger proportion of smaller size granules compared to the other cultivars studied (Tables 2.1 and 2.2). This argument is consistent with previous reports suggesting that larger starch granules could develop higher peak viscosities upon swelling compared to smaller granules during pasting (Okechukwu and Rao 1995), and correlations between lower paste viscosities and high amylose levels in legume starches (Biliaderis and others 1979). “Trough” represents the lowest level of viscosity achieved, while holding at 95°C prior to cooling the paste, during analysis. The progressive decrease in viscosity is due to the physical destruction of swollen starch granules, and the difference between “peak viscosity” and “trough viscosity”, breakdown (the difference between “peak” and “trough”), represents the granules’ susceptibility to applied shear and heat. The significantly low breakdown observed in Coyne is mainly due to low peak viscosity rather than trough viscosity which was equivalent to that of Gemini (Table 2.6).

The conventional theories on starch structure-function properties well explain the observed differences in pasting properties between starch from Coyne and other cultivars.

The presence of high levels of amylose restricts starch granular swelling, during pasting, resulting in a low peak viscosity (Jane and others 1999). The final viscosity and setback were also low in Coyne compared to the other four cultivars studied. This observation is consistent with previous reports which indicated the low setback in pasting profiles of starches with high amylose contents (Jane and others 1999; Gujska and others 1994). Although there were significant differences, the pasting temperatures spanned within a narrow range; approximately 3.1°C (Table 2.6). It appears that starch isolated from cultivar Coyne is relatively more heat-stable (high pasting temperature and low breakdown) compared to the other Great Northern bean cultivars. This could be due to the high proportion of amylose present in Coyne (Table 2.2).

2.4 Conclusions

This study comprehensively investigated physicochemical and thermal properties of Great Northern bean starch, which is an underutilized source of starch for potential food applications. Although most physicochemical properties were comparable to starches from the species *P. vulgaris*, as reported in the literature, polymer molecular weights and pasting properties of Great Northern bean starch were markedly different from other legume starches. The proportion of amylose in starch composition seems to play a major role in determining thermal (*i. e.,* gelatinization enthalpy) and pasting properties of Great Northern bean starch. The proportion of amylose, however, did not relate to the degree of relative crystallinity. The relative crystallinity of Great Northern bean starch is not related to either the amylose content or amylose molecular weights.

The findings reported here would be of interest in selecting Great Northern bean cultivars for specific food and ingredient applications, and also for further investigations on digestibility, hydrolysis patterns, and modifications targeting food ingredient applications.

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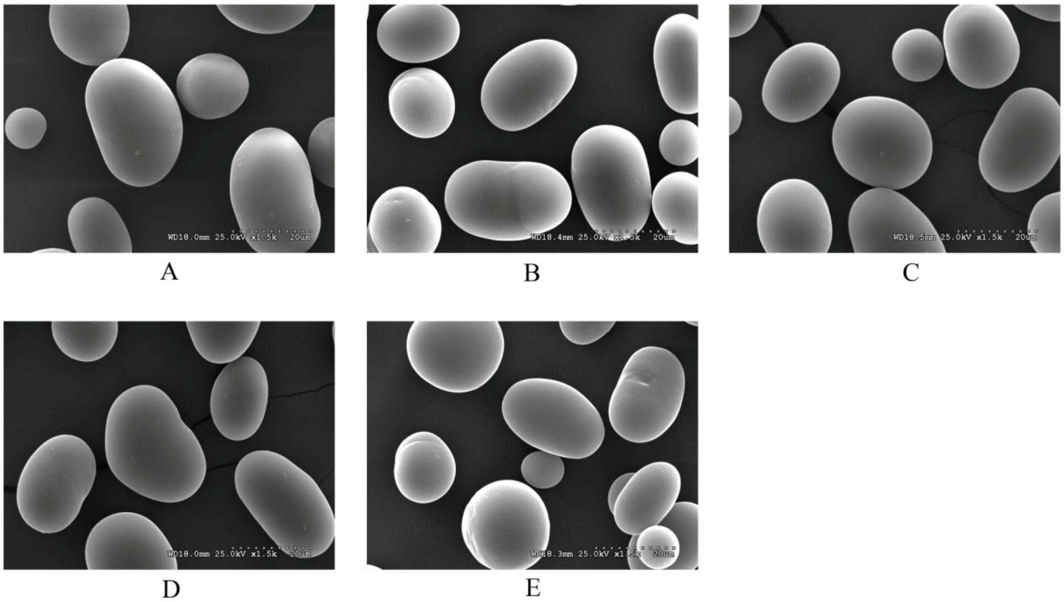

Figure 2.1 SEM images of isolated starches from five cultivars (1500x): (A) Beryl-R; (B) Coyne; (C) Gemini; (D) Marquis; (E) Orion.

Table 2.1. Granule size distributions[1]

Cultivar	Size[2] at 10% (µm)	Size at 50% (µm)	Size at 90% (µm)	Size at 100% (µm)
Beryl-R	17.0 ab	26.1 b	38.6 a	58.9 b
Coyne	15.0 d	24.0 e	35.9 c	51.8 a
Gemini	17.4 a	26.7 a	39.0 a	58.9 b
Marquis	16.8 b	25.6 c	37.3 b	51.8 a
Orion	15.8 c	24.6 d	36.3 c	51.8 a

[1]Means followed by the same letter, within the same column are not significantly different ($p > 0.05$).

[2]Size at the upper end of the specified % of the granule population.

Table 2.2. Starch polymer compositions

Cultivar	Amylose (%)[1]	Amylopectin (%)[2]
Beryl-R	21.0 b	79.0
Coyne	22.6 a	77.4
Gemini	21.5 b	78.5
Marquis	21.1 b	78.9
Orion	21.0 b	79.0

[1] Means followed by the same letter, within the same column are not significantly different ($p > 0.05$).

[2] By subtraction.

Table 2.3. Molecular weights of starch polymers

Cultivar	Amylose molecular weight[1]	Amylopectin molecular weight[1]
Beryl-R	1.71×10^{5} ab	3.59×10^{8} b
Coyne	1.87×10^{5} ab	7.42×10^{8} ab
Gemini	2.25×10^{5} a	3.73×10^{8} b
Marquis	1.14×10^{5} bc	1.28×10^{9} a
Orion	9.01×10^{4} c	5.89×10^{8} b

[1]Means followed by the same letter, within the same column, are not significantly different ($p > 0.05$).

Table 2.4. Relative crystallinity

Cultivar	% Relative crystallinity[1]
Beryl-R	18.2 b
Coyne	23.7 ab
Gemini	18.6 b
Marquis	23.8 a
Orion	21.7 ab

[1]Means followed by the same letter are not significantly different ($p > 0.05$).

Table 2.5. Phase transition properties analyzed by DSC[1]

DSC transition parameter	Cultivar				
	Beryl-R	Coyne	Gemini	Marquis	Orion
Onset temperature, T_o (˚C)	70.71 a	68.40 a	70.07 a	69.69 a	70.21 a
Peak temperature, T_p (˚C)	79.19 a	79.52 a	78.96 a	78.91 a	79.65 a
End temperature, T_c (˚C)	85.84 a	86.39 a	85.62 a	86.39 a	86.30 a
Range, T_c - T_o (˚C)	15.12 a	17.98 a	15.55 a	16.71 a	16.09 a
Enthalpy, ΔH (J/g)	12.94 ab	11.25 c	9.73 d	12.36 b	13.67 a

[1]Means followed by the same letter, within the same row, are not significantly different ($p > 0.05$).

Table 2.6. RVA pasting parameters[1]

RVA Parameter	Cultivar				
	Beryl-R	Coyne	Gemini	Marquis	Orion
Peak (cP)	4356 a	2696 c	3926 ab	3654 b	3779 ab
Trough (cP)	2641 a	2052 c	2155 bc	1953 c	2603 ab
Breakdown (cP)	1715 a	644 c	1772 a	1700 a	1175 b
Final viscosity (cP)	5325 a	3662 c	4947 ab	4631 b	4883 ab
Setback (cP)	2684 a	1610 b	2792 a	2678 a	2280 a
Peak time (min)	4.50 b	4.92 a	4.48 b	4.54 b	4.65 b
Pasting temperature (˚C)	80.9ab	82.3 a	79.3 b	79.4 b	82.1 a

[1]Means followed by the same letter, within the same row, are not significantly different ($p > 0.05$).

CHAPTER 3. IMPROVING NUTRITIONAL VALUE OF INSTANT NOODLES USING GREAT NORTHERN BEANS

Abstract

Traditional instant noodles, made out of wheat flour as the main ingredient, are generally contained simplex nutritional value. The objective of this study was to improve the nutritional profile of instant noodles by substituting wheat flour with Great Northern bean powder. Flour mixtures with selected levels (0, 10, 20, 25, 30, and 60% of hard red winter wheat flour replacement) of Great Northern bean powder were tested by Rapid Visco Analyzer. With the same kinds of flour mixtures, instant noodles were prepared using a pilot-scale noodle processing machine. Acceptable products were tested for color, texture, and cooking properties. Instant noodle prepared with 60% of bean flour failed to make an acceptable product. Bean flour fortification at 30% flour weight resulted in short noodle strands. Products with acceptable quality traits were obtained from 10, 20, and 25% flour replacement with bean powder. Color analysis showed significant differences among tested formulations. Increasing bean flour levels increased the yellow color and darkness of fried noodles. Cooking loss increased significantly with increasing levels of bean flour. Texture profile analysis on cooked noodles showed an increase in springiness with increasing bean flour level, but no apparent trends were observed in the hardness. Water absorption ratio of instant noodle, after cooking, increased with increasing bean flour proportion. The results of this study suggest that replacing a portion of wheat flour with Great Northern bean powder significantly increased protein and fiber contents in instant noodles by 25% and 800% respectively. Fat contents in

noodle product were reduced from 19% to 17%. Wheat flour could be replaced, up to 25% (w/w), with Great Northern bean flour, under normal, pilot-scale processing conditions. Instant noodles with improved nutritional value are comparable to control samples.

3.1 Introduction

Instant noodle was invented in 1958 with the goal of providing a convenient food with tasty and long shelf life (Ando 1976). Under certain circumstances such as travel, disaster, and public emergency situations, instant noodles become a preferred choice over other staples, such as rice, due to convenience in storage, transportation, preparation, and price. The current global consumption is approximately 98.2 billion packs of instant noodles (retail units, such as bags and cups) are consumed every year (WINA 2011). China, Indonesia, Japan, Vietnam, India, and the United States are the major manufacturers and consumers of instant noodles in the world. The United States ranks fifth in consumption (4.03 billion units/year). Along with lifestyle changes, especially in rapidly developing economies, the demand of convenient foods, such as instant noodles, is expected to increase. According to the latest estimates, the global instant noodle market is expected to exceed 154 billion units/year by 2017 (GIA 2013). Inherent advantages of the product, such as low price and minimum cooking time, promote instant noodle products among consumers.

Instant noodle is usually accused of being deficient in nutritional value. This is due to its simple ingredient combination; flour, salt, carbonates, and oil. The viscoelastic properties of noodles are primarily contributed by the wheat flour (Day et al. 2006), but it does not provide appreciable nutritional content. The very low nutritional value of instant noodles is a major issue in expanding the market share of the product. Improving the nutritional value of a widely consumed, relatively cheap item is economically challenging because added nutritional value would increase the cost of ingredients and processing. To improve the nutritional quality of noodles, several studies have been conducted by introducing selected ingredients to the traditional formulation. Soy protein has been tested to improve the lysine and vitamin B contents of instant noodles (Sun and Song 2004). The study found that the emulsifying effect of soy protein reduced the oil content in the final product. Optimum cooking properties of instant noodles were obtained at 5% soy protein addition. Reungmaneepaitoon et al. (2006) investigated selected levels of three types of oat bran in instant noodles. Product analysis for texture and sensory properties showed that acceptable quality instant noodles could be obtained at 5 – 15% level oat bran substitution. These products had significantly high levels of dietary fiber, ranging from 2.12 to 4.50 g, compared to the control (1.25 g). Health benefits, such as lowering blood cholesterol, are associated with dietary fibers (Truswell 1999).

As an ingredient, wheat flour has a protein profile high in leucine and cystine but low in lysine, an essential amino acid. The removal of wheat bran during milling lowers the ash content, as well as the vitamins and dietary fibers. Instant noodle with improved quality could be produced by replacing a portion of the wheat flour in the formulation. Legumes, such as dry beans, are considered the best sources of lysine, and they are rich in dietary fibers. The high protein content and light color of Great Northern bean could be an alternative to fortify the nutrition value of instant noodles.

Although they are well known for superior nutritional quality, dry-edible beans are highly underutilized, especially in commercial food processing operations. The low digestion rate of dry bean starch makes it a lower glycemic food than cereal grains and tubers (Jenkins et al. 1983). In the United States, Great Northern bean ranks third, behind Pinto and Navy beans, in total dry bean production (USDA-NASS 2013). Great Northern bean is preferred for food product applications for its superior nutritional value as well as light color. Great Northern beans have relatively higher amounts of protein (approximately 29%, d.b.) compared to other dry beans (Rui et al. 2011), and ½ cup of cooked Great Northern bean provides more than 20% of the daily recommended amount of fiber (USDA 2009). The lack of dark color and tannins, as well as better thermal stability as an ingredient also are considered advantages of Great Northern beans over other *P. vulgaris* varieties for food applications.

The goal of this study was to improve the nutritional value of instant noodles by substituting a portion of wheat flour with Great Northern bean powder (GNBP). This study was also attempted to maintain acceptable product quality in Great Northern bean incorporated instant noodles, which would be comparable to the traditional product.

3.2 Materials and methods

Hard red winter wheat (HRW) flour (ConAgra Mills, Omaha, NE, USA) and Great Northern bean powder (VegeFull™, Archer Daniels Midland Company, Enderlin, ND, USA) were used for noodle processing. This ingredient is developed by following steps; cooking, drying and milling whole beans. Refined bleached deodorized (R.B.D.)

palm oil (Columbus Foods, Des Plaines, IL, USA) was used for deep frying. All salts and carbonates were of food grade, and obtained from commercial sources.

3.2.1 Flour composition analysis

Proximate compositions of flour ingredients and instant noodles were analyzed by standard AACCI (AACC International 2002) and AOAC (AOAC 2009) methods as follows: Moisture – AACC 44 - 15A, crude protein – AOAC 990.03, crude fiber – AOAC Ba 6a - 05, fat – AOAC 920.39, and ash – AACC 08 - 01. Total starch was analyzed using YSI Select 2700 Biochemistry Analyzer (YSI Life Sciences, Yellow Springs, OH) (YSI 2000). Moisture of instant noodle samples was analyzed by AACC method 44 - 15A (AACC International 1999).

3.2.2 Flour particle size distribution

Flour particle size distributions were performed using a Malvern Mastersizer 3000 laser diffraction particle size analyzer equipped with an Aero S dry dispersion unit (Malvern instruments Ltd, Malvern, Worcestershire, UK). Sample was delivered into the system within the obscuration limit of 0.1-10%. Refractive indices of 1.53 for flour, density of 1.0 g/cm^3, and absorption index of 0.10 (per equipment manufacturer's specifications/database) were used as the analytical parameters. Data were collected and analyzed by Malvern software (Version 2.01, Malvern Instruments Ltd, Malvern, UK).

3.2.3 Flour pasting properties

Pasting properties of flour ingredients were evaluated by rapid visco analyzer (RVA). HRW flour mixed with 0, 10, 20, 25, 30, and 60% GNBP were measured. For analysis, 3.50 g (d.b) of flour, with 25 mL water (adjusted to 14% moisture level) were mixed in an RVA canister capped with a #8 rubber stopper covering the canister mouth for 30 s to avoid formation of clumps followed by prompt vortex mixing (Fisher Scientific fixed speed vortex mixer 945410, Thermo Fisher Scientific Inc., New Jersey, USA). The mixed samples were then analyzed by RVA (Model 4S, Newport Scientific, Warriewood, NSW, Australia), using Standard 1 profile [Temperature was held at 50 ˚C for 1 minute, then increased to 95 ˚C in 3.7 minutes, and held at 95 ˚C for 3.5 minutes, then the sample was cooled down to 50 ˚C and held for 2 minutes. Speed was at 160 rpm.].

3.2.4 Flour water absorption index (WAI) and water solubility index (WSI)

The water absorption index (WAI) and water solubility index (WSI) of flour mixtures and ground uncooked instant noodles were determined according to Anderson et al. (1969) method. Approximately 2.5 g of sample was placed in weighted centrifuge tubes with 30 mL distilled water. After 30 minutes at 30 ˚C shaking water bath, samples were centrifuged at 3000 g for 10 minutes using a bench top centrifuge (Sorvall Legend XTR Centrifuge, Thermo Scientific, Madison, WI, USA). The liquid supernatant was dried in a forced air oven at 103 ˚C for 20 hours to determine the water soluble solids. The remaining gel was weighed to calculate WAI. WSI and WAI were calculated by the following equations.

$$\text{WAI} = \frac{\text{Weight of gel}}{\text{Weight of original sample - Moisture of original sample}} \quad \text{.............Equation (3.1)}$$

$$\%\ \text{WSI} = \frac{\text{Weight of dry solids x 100\%}}{\text{Weight of original sample - Moisture of original sample}} \quad \text{..............Equation (3.2)}$$

3.2.5 Noodle preparation

The basic instant noodle formula is listed in Table 3.1. HRW flour mixtures with 0, 10, 20, 25, 30, and 60% GNBP of flour weight were tested. The noodle making procedure was adapted from the method reported by Hou (2010). Salt and carbonates were dissolved in 100 mL distilled water before adding into flour mixer. A KitchenAid stand mixer (Professional 600, KichenAid, St. Joseph, MI, USA) was used. Dry ingredients were mixed at speed 2 for 2 minutes. Meanwhile, salt solution was blended dropwise into the mixture. Mixing was carried out at speed 4 for another 8 minutes. After clearing the mixing vessel walls, flour was mixed for additional 2 minutes at speed 4. Then, the crumbly mixture was rested in a plastic bag for 30 minutes at room temperature to ensure adequate hydration and dough development. Coarse dough mixture then passed through rollers to develop gluten network and reduced into sheet by a pilot-scale noodle processing machine (HF 06SF, H.F. Kejenteraan SDN. BHD, Johor, Malaysia) at speed 2. The gap distances and number of passes for each rolling are listed in Table 3.2. Prepared raw noodles were steamed at 99 - 100 ˚C for 4 minutes to denature protein and gelatinize starch. Approximately 100 g of steamed noodles were loaded in a frying mold (TMCO/National Manufacturing Co., Lincoln, NE) fabricated according to the dimension given by Hou (2010). The noodle was then immersed in 150

˚C palm oil for 75 seconds. Oil was drained from fried noodle cake by placing on an absorbent paper for 10 minutes. Fresh oil was used every time for frying up to 12 cakes. Cooled noodle cakes were stored in sealed plastic bags and kept at room temperature until further analysis.

3.2.6 Color of instant noodles

CIELAB color space was used to analyze color of prepared noodle samples. The L* (brightness [100], whiteness or lightness), a* (redness [+] and greenness [-]) and b* (yellowness [+] and blueness [-]) values of ground noodle cakes were directly determined by Minolta CR-300 Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan) following the manufacturer's guidelines.

3.2.7 Degree of cook in instant noodles

Instant noodle cakes were ground into fine powder by a coffee grinder (IDS77, Sunbeam Products, Inc. Boca Raton, FL). Samples were prepared according to Ratnayake et al. (2009) procedure with excess water in hermetically sealed in high pressure pans and scanned against a blank (empty pan) using a Perkin Elmer Pyris 1 DSC (Perkin-Elmer Co., Norwalk, CT, USA) from 25 to 135 ˚C at a 10 ˚C/min scanning rate. Pyris version 3.50 software (Perkin-Elmer Co., Norwalk, CT, USA) was used to collect data and analyze onset (T_o), peak (T_p), and end (T_c) temperatures, and the transition enthalpy (ΔH). The instrument was calibrated using Indium (reference material).

3.2.8 Water absorption ratio and cooking loss of instant noodles

Cooking loss of instant noodles was measured by AACC method 66 – 50 (AACC International 2002). Water absorption ratio was calculated based on the method reported by Kamolchote et al. (2010). Fried noodle cake (approximately 85 g) was cooked in a beaker on a hotplate (Super-Nuova Multiplace, Thermo Scientific, Madison, WI, USA) with 600 mL water for 4 minutes. Cooked noodles were collected by draining water through an open Buchner funnel. Water and cooked noodles were dried separately by oven at 103 ˚C for 20 hours. Water absorption ratio % and cooking loss % were calculated by following equations:

Equation (3.3):

$$\text{Water absorption ratio (\%)} = \frac{\text{Noodle weight after cooking - Dry weight of noodles}}{\text{Dry weight of noodles}} \times 100\%$$

Equation (3.4): $$\text{Cooking loss (\%)} = \frac{\text{Weight of residue in cooking water}}{\text{Weight of noodles sample}} \times 100\%$$

3.2.9 Texture analysis of cooked instant noodle

Single instant noodle cake was cooked in a boiling water pot covered with a lid for exactly 4 minutes. Water was decanted 3 minutes after cooking. Texture including hardness, hardness work done, springiness, and adhesiveness were analyzed by CT3 Texture Analyzer equipped with a 1000 g load cell (Brookfield Engineering Laboratories, Middleboro, MA) using equipment settings given in Appendix A. For analysis, five cooked noodle strands were placed perpendicular to long edge of the probe on base table (Appendix B). Wedge shaped probe TA-PFS was used for Texture Profile Analysis (TPA). Flat rectangular probe TA-PTF was used for a single compression test on noodles.

3.2.10 Statistical analysis

The study was conducted using completely randomized designs (CRD). For each experiment, determinations were replicated at least three times. Analysis of variance was performed and mean separations were performed by Tukey-Kramer HSD (honestly significant difference) test, at $p < 0.05$ significance level, using JMP (Version 10.0.0, SAS Institute Inc. Cary, NC, USA).

3.2.11 Results and discussion

Hard wheat flour is the main ingredient used in conventional instant noodle manufacturing (Table 3.1). Substitution levels of 0, 10, 20, 25, 30, 60% were selected based on a series of preliminary studies (preliminary results are not reported here) to cover a wide range of flour substitution. The test formulations were pre-screened by testing under pilot-scale processing conditions, using a noodle processing machine (Table 3.2). All formulations were prepared following the same procedure. Upon completion of preliminary tests, wheat flour substitutions, up to 25% (w/w), were further tested and compared with the control (0% substitution). The highest level of GNBP substation tested was 60%. At 60% substitution, the noodle dough sheet broke into small pieces after coming out of the roller (gap 5.5) (Table 3.2), disabling further processing. At 30% GNBP substitution, flour mixture formed a fragile sheet with less structural integrity.

The uneven hydration was visible on the surface of the noodle sheet. During slitting, the noodle sheet sliced into short fragile strands, which were unacceptable for further processing. Based on the observations, it was inferred that the noodle sheets with higher (i. e., >25%) GNBP substitution did not have sufficient gluten network development to stand multiple rolling passes and slicing processes, and the noodle sheets disintegrated during slitting. Therefore, noodles properties of high substitutions (>30%) were not included in further evaluations during this study. Noodle strands with sufficient strength for further processing and handling were formed with GNBP substitution ratio up to 25%.

Properties of flour ingredients were examined on all selected combinations. Pasting properties of flour mixes are given in Table 3.3. GNBP did not display a typical RVA profile under tested conditions. The viscosity of GNBP sample slightly fluctuated around 40 cP with final viscosity mean at 92.3 cP, essentially as a horizontal line of viscosity change. When mixed together, the viscosities of flour mixtures decreased progressively with increasing GNBP substitution ratio (Table 3.3). It is difficult to compare these observations with previous reports on studies conducted with flour substitution with other types of ingredients, such as oat bran (Reungmaneepaitoon et al., 2006), due to variations in compositions and their effects. High levels of flour substitution was associated with less breakdown, progressively decreasing peak viscosity, and low final viscosity which indicated poor dough structure formation (Table 3.3). This was an issue in noodle processing with high levels (25% wheat flour substitution) of GNBP substitution. The addition of other ingredients seemed to have changed the compositions of noodle formula affecting the pasting properties of flour mixes. It could be possible that less wheat starch in formulations with increased GNBP contributed considerably to the observed low paste viscosities (Table 3.3). In this study, noticeable composition differences between HRW and GNBP were observed in all analyzed compounds (Table 3.4). The differences in particle size distribution may also contribute to the decrease of pasting viscosity. Particle size distribution analysis shows that 50% HRW particles are less than 43 μm, and 90% are less than 121 μm. However, 50% GNBP particles are less than 73 μm, and 90% are less than 244 μm. As the amount of GNBP increase, flour mixtures became more prone to disintegration during sheeting process, which was undesired for noodle manufacturing.

As a convenient food, instant noodles are designed to be rehydrated within 3 - 4 minutes in hot water prior to consumption. Therefore, a fully cooked product is required prior to packaging. The degree of starch gelatinization is a good indicator in determining the degree of cook (Geera. 2009). The starch gelatinization in instant fried noodles is takes place primarily during the steaming process (Hou and Kruk 1998). The gelatinization temperature of wheat flour is usually below 70 ˚C and enthalpy lower than 7.5 J/g (Wu et al. 2006). To evaluate the degree of starch gelatinization, the DSC test was carried out. The results confirmed that after steaming and deep frying, starch in instant noodles was completely gelatinized and proteins were denatured; no enthalpies representing either starch or protein phase transitions, were observed for instant noodles samples prepared with all selected flour combinations. It has been suggested that the denatured proteins and gelatinized starch developed and helped maintaining the structure of noodles (Zhang et al. 2003). The lower gelatinization temperature has also been found to improve noodle texture and reduce the rehydration time (Bhattacharya et al. 1999).

Appearance and texture are two important properties in evaluating noodles quality. Color is a crucial attribute of noodle's appearance. The results showed significant differences in colors among each substitution level were observed ($p<0.05$) (Table 3.5). The results showed that the color of instant noodles changed from creamy-white to strong yellow as the GNBP substitution level increased. Multiple factors could influence the color of instant noodles. Generally, the yellow color of noodles is attributed to the presence of alkaline and flavones in flour (Fortmann and Joiner 1971; Miskelly 1996). The substitution of GNBP increased the yellowness of instant noodles, because of the considerably high quantities of flavones in dry beans (Oomah et al. 2005; Vinson et al. 1998). Other components in flours including ash, protein and starch could also influence the color of noodles. Ash content and crude protein amount of bean flour is considerably higher than that of wheat flour (Table 3.4). The darkness color of instant noodle was significantly increased by the addition of GNBP (Table 3.5). For noodle processing, lower ash content (≤ 0.4%) and "medium" protein content (10% - 13%) is preferred (Kim et al. 2006), because the lightness is negatively affected by protein content and ash content (Hou and Kruk 1998; Park and Baik 2004a). Although Great Northern bean is a white color variety, the L* value (*i.e.*, lightness) in instant noodles observed in this study decreased with increasing GNBP substitution. Other factors including degree of starch damage, and protein properties, which were not evaluated in this study, would also influence noodle colors by affecting the water absorption (Baik et al. 1995; Hatcher 2001; Hatcher et al. 2002).

In noodle processing, water plays a key role in physicochemical reactions, hydrating starch, and developing gluten matrix. Appropriate water addition is crucial in noodle processing in order to produce nonsticky and evenly hydrated noodle strands with smooth surfaces. In commercial noodle processing, the optimum amount of water ranges from 30 to 35% (on flour weight basis) for making an acceptable noodle dough (Wu 1998). The water absorption and water solubility of flour mixtures were analyzed in this study. WAI of flour mixture increased as GNBP level increased due to the high water absorption of GNBP (Table 3.7). In contrast, in prepared noodles, WAI tend to decrease as GNBP substitution level increased (Table 3.8). The progressively decreasing WAI in noodle product might be due to the reduction of starch content, which is mainly responsible to water absorption. Still, at the same GNBP substitution level, the WAI values of noodle samples were higher than that of the corresponding flour mixtures. This may due to high water absorption of GNBP and the porous surfaces of instant noodles created during steaming and frying. The pore on noodle surface might assist in water absorption by capillary action (Pinthus and Saguy 1994) . In terms of WSI, both flour mixtures (Table 3.7) and products (Table 3.8) become more soluble with increasing levels of GNBP substitution. To maintain the integrity of the product during cooking, lower water solubility is desired.

Water absorption ratio and cooking loss are important parameters determined particularly for evaluating the cooking quality of noodles. Rehydration test is of special importance for instant noodles, because the rate of rehydration is associated with cooking quality of the product. A good product would rehydrate in 2 - 4 minutes at a 120 – 150%

ratio (Kamolchote et al. 2010). Mean of rehydration ratios for all instant noodle samples were within the range (Table 3.9). As GNBP proportion increased, more soluble material was dissolved in cooking water. From 0 to 25% of substitution, dry material loss increased by 43.73% (Table 3.9). Cooking water became progressively turbid and cloudy with increasing GNBP levels. The inferior cooking properties are mainly due to the reduction of gluten and low ingredient binding caused by GNBP.

Texture is critical in noodles quality evaluations. In terms of product quality, a rubbery, firm, and chewier texture is desired for instant noodles (Kamolchote et al. 2010). Both processing conditions and ingredient formulation determine noodle texture (Hou and Kruk 1998). For example, starch with low gelatinization temperature, higher pasting viscosities and rapid swelling properties are desired for noodles manufacturing (Hou 2001). In this study, the addition of GNBP influenced the hardness and springiness of noodles. Noodle adhesiveness showed no significant observable difference with increasing level of GNBP (Table 3.10). Springiness value reduced as GNBP proportion increased. Higher protein content contributed by GNBP might have contributed to the increased firmness of instant noodles. Low WAI values, for instant noodles, were associated with increasing hardness. These observations were consistent with the report by Choy et al. (2010) which found that the hardness of cooked instant noodles decreased with increasing water content.

The nutritional value of instant noodles was significantly improved by adding GNBP (Table 3.6). Protein and dietary fiber contents were increased with increasing GNBP levels. Particularly, the fat content in final product appeared to be reduced with addition of GNBP. Oil is picked up by noodles during frying process, after water is removed from the surface of noodles and creating many microscopic pores (Bouchon et al. 2003; Wu et al. 2006). Pinthus and Saguy (1994) explained the oil uptake in noodle by capillary action of those microscopic pores on noodle surfaces. Multiple factors including protein content, protein quality, and amylose content have been attributed to the oil content in the final product (Moss et al. 1987; Park and Baik 2004a; b). Instant noodles prepared with low protein wheat flour have shown higher fat content. Compared to wheat noodles, noodles incorporated with bean flour have lower total starch content and higher protein content (Table 3.6). The high protein content and low amylose content in GNBP might have helped to reduce the oil uptake of noodles during frying. Meanwhile, as proposed by previous studies on spaghetti prepared with common beans, indigestible insoluble fraction values also increased despite the low levels of total starch (Gallegos-Infante et al. 2010; Gallegos Infante et al. 2010).

3.3 Conclusions

Considering both product nutritional value improvement and processing operations, wheat flour substitution up to 25% with GNBP could be recommended. However, additional evaluations on commercial-scale process feasibility would be required. Substitution of GNBP has improved the nutritional profile of instant fried noodles, especially in protein and fiber contents. Instant noodles with GNBP substitution are comparable to the control product, though there are some changes in terms of color, cooking properties and texture. This new instant noodle formulation could be further improved by adding other ingredients to reduce the cooking loss. The characteristics and functionality of Great Northern bean protein in food systems need for further investigation.

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Table 3.1. Basic formulation for instant noodles

Ingredient	Weight (g)	Proportion (%flour basis) (w/w)
Flour	500	100
Water	175	35
Salt	7.5	1.5
Potassium carbonate	0.5	0.1
Sodium carbonate	0.5	0.1

Table 3.2. Gap order and distance for rolling and compounding

Gap number	Gap distance (mm)	Number of passes	Number of compounds[1]
7	<0.06	1	0
5.5	3.8	3	1
6	2.4	2	0
6.5	1.1	2	0

[1]Compounds are the procedure to fold the noodle sheet once and run through the roller to combine and develop gluten.

Table 3.3. RVA pasting profile parameters of flour mixture[1]

RVA Parameter	Substitution ratio of GNBP (%)					
	0	10	20	25	30	60
Peak (cP)	2715.33 a	2266.33 b	1848.00 c	1618.33 cd	1450.00 d	536.00 e
Trough (cP)	1795.67 a	1592.00 ab	1341.00 bc	1155.33 cd	1044.33 d	462.33 e
Breakdown (cP)	919.67 a	674.33 b	507.00 c	463.00 cd	405.67 d	73.67 e
Final Viscosity (cP)	2908.67 a	2594.67 b	2216.67 c	1983.67 cd	1798.67 d	780.33 e
Setback (cP)	1113.00 a	1002.67 b	875.67 c	828.33 cd	754.33 d	318.00 e
Peak Time (min)	6.16 a	6.23 a	6.12 ab	5.96 bc	5.90 c	5.30 d
Pasting Temperature (˚C)	79.38 a	77.45 a	81.55 a	85.87 a	81.52 a	87.73 a

[1]Means followed by the same letters, within the same row, are not significantly different ($p > 0.05$).

Table 3.4. Proximate compositions of flour ingredients

Flour type	Moisture (%)	Starch % (d.b)	Crude protein % (d.b)	Ash % (d.b)	Crude fiber % (d.b)	Fat % (d.b)
HRW	10.99 ± 0.08	75.90 ± 0.36	13.40 ± 0.00	0.52 ± 0.02	0.63 ± 0.21	1.03 ± 0.06
GNBP	6.61 ± 0.06	38.15 ± 0.07	24.90 ± 0.00	4.03 ± 0.03	4.60 ± 0.14	1.60 ± 0.00

Table 3.5. Color space analysis results of instant noodles[1]

% Flour substitution with GNBP	Moisture (%)	Color		
		L* (lightness)	a* (red - green)	b* (blue- yellow)
0	4.139	99.04 ± 0.27 a	-0.57 ± 0.09 d	1.61 ± 0.15 d
10	3.796	97.46 ± 0.51 b	-0.23 ± 0.05 c	7.78 ± 0.24 c
20	3.031	95.39 ± 0.72 c	0.43 ± 0.03 b	9.56 ± 0.60 b
25	6.000	93.28 ± 0.67 d	0.72 ± 0.03 a	12.14 ± 0.61 a

[1]Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

Table 3.6 Proximate compositions of instant noodles prepared with selected proportions of GNBP[1]

% Flour substitution with GNBP	Moisture (%)	Starch % (d.b)	Crude protein % (d.b)	Ash % (d.b)	Crude fiber % (d.b)	Fat % (d.b)
0	4.6	61.6 ± 0.3 a	10.9 ± 0.1 d	1.9 ± 0.0 d	0.1 ± 0.0 c	19.2 ± 0.1 a
10	5.9	56.3 ± 0.6 b	11.7 ± 0.1 c	2.1 ± 0.1 c	0.4 ± 0.1 b	18.9 ± 0.1 ab
20	4.6	55.1 ± 0.5 c	12.7 ± 0.0 b	2.5 ± 0.1 b	0.8 ± 0.1 a	18.6 ± 0.1 b
25	4.1	56.3 ± 0.5 bc	13.4 ± 0.1 a	2.6 ± 0.1 a	1.0 ± 0.1 a	17.1 ± 0.3 c

[1]Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

Table 3.7. WSI and WAI of flour mixture[1]

% GNBP level in flour mixture	WAI	WSI (%)
0	1.82 ± 0.02 d	5.67 ± 0.01 c
10	1.96 ± 0.09 c	6.33 ± 0.00 c
20	2.06 ± 0.01 b	8.00 ± 0.00 b
25	2.12 ± 0.02 b	9.00 ± 0.01 b
100	3.39 ± 0.03 a	17.51 ± 0.00 a

[1]Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

Table 3.8. WSI and WAI of instant noodles[1]

% Flour substitution with GNBP	WAI	WSI (%)
0	3.543 ± 0.04 a	5.275 ± 0.10 d
10	3.493 ± 0.03 a	6.756 ±0.15 c
20	3.329 ± 0.04 b	7.327 ±0.00 b
25	3.246 ± 0.05 b	8.276 ±0.00 a

[1]Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

Table 3.9. Cooking properties of instant noodles[1]

% Flour substitution with GNBP	Water absorption ratio	Cooking loss mg/g
0	1.21 ± 0.1 b	69.40 ± 3.3 b
10	1.28 ± 0.1 ab	72.29 ± 6.3 b
20	1.31 ± 0.1 a	95.20 ± 7.8 a
25	1.36 ± 0.0 a	99.75 ± 4.9 a

[1]Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

Table 3.10. Texture analysis of cooked instant noodles

% Flour substitution with GNBP	Adhesiveness (mJ)	Springiness (mm)	Hardness work (mJ)	Hardness (g)
0	0.03 ± 0.01 a	1.00 ± 0.02 a	3.07 ± 0.08 a	632.50 ± 20.13 b
10	0.02 ± 0.01 a	0.80 ± 0.08 b	1.91 ± 0.14 c	507.60 ± 1.11 c
20	0.02 ± 0.01 a	0.74 ± 0.02 b	2.59 ± 0.09 b	650.50 ± 21.81 b
25	0.03 ± 0.02 a	0.60 ± 0.03 c	2.58 ± 0.07 b	818.93 ± 26.66 a

[1] Means followed by the same letters, within the same column, are not significantly different ($p > 0.05$).

4. Overall summary

The two studies reported in this thesis investigated and addressed major gaps in the current knowledge-base on Great Northern bean, particularly related to its physicochemical properties, functionality, and utilization in food processing.

Starch is a versatile ingredient used in food and beverages industry. Food starches come from a variety of sources, mainly from cereals, roots, and tubers. Legume starches, such as dry-bean starch, are not commonly used in food processing operations as ingredients. The structure and properties of starch impact its functionality and subsequent end-use. In selecting a particular starch for an application, a comprehensive knowledge on its physicochemical properties is essential. The first study found that Great Northern bean starch was unique and distinctly different from cereal, root, and tuber starches in physicochemistry and functionality. Within the variety Great Northern bean, differences were observed in starches isolated form different cultivars. It was found that amylose/amylopectin polymer proportions and molecular weights were responsible in determining thermal and rheological properties of Great Northern bean starches. These findings are of importance in predicting the functionalities and product applications for different Great Northern bean cultivars.

The nutritional value of dry beans is important in assessing their potential for food uses. Flour or powder type ingredients are commonly used in commercial scale food processing operations. Great Northern bean, being a light/white color variety, has much greater potential to be a food ingredient compared to other colored varieties, such as Pinto and black beans. Commercially available bean powder was used in the second study to evaluate the potential of Great Northern bean in instant noodle processing. Although it is completely gelatinized during processing, starch fraction plays a critical role in developing dough, sheeting, slitting, and structure formation of noodles. Promising results were observed at up to 25% wheat flour substitution with Great Northern bean powder in preparation of nutritionally improved instant noodles.

The findings on starch properties and functionalities would assist us developing other food applications for Great Northern beans.

Appendix

Appendix A. Texture analyzer settings used to evaluate texture profiles of cooked noodles

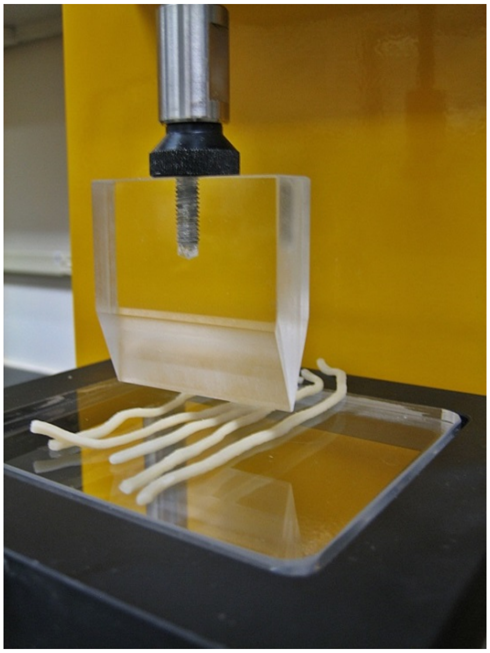
Parameters	Settings
Test Type	Texture Profile Analysis (TPA)
Pre-Test Speed	0.5 mm/s
Test Speed	0.5 mm/s
Post-Test Speed	0.5 mm/s
Target Type	% Deformation
Target Value	50%
Trigger Force	7g

Equipment: CT3 Analyzer with 1000g load cell

Note: When a trigger force of 7gm has been detected at the sample surface, the probe proceeds into the sample at a test speed of 0.5 mm/s to a distance of 50% of the noodle sample's height. Once the target distance is meet the probe returns to the stating position and will repeat the test automatically for the second cycle for TPA test.

Appendix B. Texture analysis of cooked noodles.

A



B

Figure 3A. TPA testing with wedged TA-PFS probe

Figure 3B. Compression testing with rectangular flat TA-PTF probe.