MOLECULAR STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF STARCH ISOLATED FROM *PHASEOLUS VULGARIS*

By

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ABSTRACT

MOLECULAR STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF STARCH ISOLATED FROM *PHASEOLUS VULGARIS*

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The health benefits of legumes have been known for many years. They are considered to be a food staple in the diet of humans. Dry beans have been implicated in the prevention and management of type II diabetes mellitus and in the reduction of risks associated with colon cancer. Dry beans are good sources of complex carbohydrates, protein, dietary fiber, vitamins and minerals. However, use of the starch fraction of dry beans is limited due to the lack of molecular structure-functionality relationships. The specific objectives of this study were: (1) to determine and compare the physicochemical properties of starch isolated from six varieties of *Phaseolus vulgaris*, namely black bean, dark red kidney bean, light red kidney bean, navy bean, pinto bean and small red bean, (2) to determine and compare the digestibility, estimated glycemic index, and molecular weight distributions of isolated raw, canned and stovetop-cooked bean starches, (3) to investigate the effects of canning and a traditional cooking method (boiling) on the digestibility and molecular weight distributions of starch isolated from dried bean.

The physicochemical characteristics of starches isolated from six varieties of *Phaseolus vulgaris* grown in Michigan were determined. Starch yield varied from 22.8% to 34.1% on a whole seed basis (~ 10% moisture content). The raw isolated bean starches exhibited high resistant starch contents ranging from 41.9% (light red kidney bean) to 55.7% (pinto bean), whilst total amylose content varied from 28.0% (pinto bean) to 29.8% (dark red kidney bean). Significant differences were observed for the gelatinization transition temperatures, pasting parameters, and resistant starch contents of the isolated dark red kidney and light red kidney bean

starches when compared to isolated starch from black, navy, pinto and, small red beans. All isolated bean starches displayed the characteristic C-type X-ray diffraction pattern of legumes. The crystallinity and B-type starch polymorph contents ranged from 36.1% to 24.9% and 19.6% to 15.6% respectively. This study demonstrated that the chemical compositions of beans are different for different varieties, and thus the varieties exhibit different physicochemical properties.

The weight average molecular weight distribution (Mw) of starches isolated from native, canned, and stovetop-cooked beans were analyzed using high-performance size exclusion chromatography with multi-angle laser light scattering and refractive index detectors (HPSEC-MALLS-RI). Results revealed that amylose of isolated native dark red kidney bean starch had the smallest Mw (1.0×10^6 g/mol), whereas isolated native pinto bean starch had the largest value of Mw (1.8×10^6 g/mol). The Mw values of amylopectin for isolated native bean starches ranged from 2.4×10^7 g/mol to 3.9×10^7 g/mol. Isolated canned and stovetop-cooked bean starches displayed a mono-modal Mw distribution, with a reduction in high molecular weight fractions, whereas isolated native bean starch Mw distribution was bi-modal. Results of *in vitro* α-amylase starch hydrolysis showed ranges of rapidly digestible starch (RDS) (1.95-2.71%), slowly digestible starch (SDS) (14.36-17.39%), and resistant starch (RS) (78.95-83.7%) among the tested isolated native *Phaseolus vulgaris* starches. However, RDS, SDS, and RS fractions in isolated canned bean starches ranged from 7.58-13.21%, 20.75-24.90%, and 63.17- 68.54%, respectively. Isolated stovetop-cooked bean starches yielded similar results: RDS, 7.37-10.61%; SDS, 22.55-26.84%; and RS, 62.55-68.59%). The hydrolysis indices (HI) and glycemic indices (GI) were marginally greater for isolated canned bean starches than for stovetop-cooked starches.

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Almighty God, my Rock, in whom I live and move and have my being, none of this would have been possible without you. Thank you for always being with me.

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CHAPTER 1

INTRODUCTION

In many countries the incidence of diabetes, obesity and cardiovascular diseases is increasing rapidly. Foods are now consumed for both nutrition and health benefits; their components may potentially exert a positive impact in the prevention and treatment of chronic diseases. The health benefits of legumes have been known for many years. They are considered to be a food staple in the diet of humans (Leterme, 2002). Dry beans have been implicated in the prevention and management of type II diabetes mellitus and in the reduction of risks associated with colon cancer. Several studies have demonstrated that the consumption of dry beans or legumes, low glycemic index foods, may be protective against developing type II diabetes (Broughton, Hernandez, & Blair, 2003; Duranti, 2006; Geil & Anderson, 1994; Guillon & Champ, 2002; Hangen & Bennink, 2002). The glycemic index (GI) of a food is related to the rate at which glucose is absorbed from the small intestine (Jenkins et al., 1981; Jenkins, 1982; Wolever, Jenkins, Thompson, Wong, & Josse, 1987). Low GI foods elicit a more sustained but lower level or degree of increase in post-prandial blood glucose and insulin, and reduce gastric emptying of the stomach (Esfahani et al., 2009; Jenkins, Wolever, Taylor, Barker, & Fielden, 1980; Thorne, Thompson, & Jenkins, 1983). Dry beans give a low glycemic response relative to other high-carbohydrate-containing foods; this may be due to a combination of several factors such as the structure-derived higher resistance to digestion of bean starches, the presence of fiber and/or the partial or incomplete gelatinization of starch during cooking. The physicochemical properties and molecular structures of starch that affect digestion are poorly understood.

Apart from being eaten as a vegetable, grain legumes can be considered as a source for raw material (starch) for the processing industry. For example, isolated starch from peas has been used in extruded bakery products, dressings, instant soups and puddings (Guillon & Champ, 2002). Starch is the most abundant carbohydrate in the legume seed (22-45%, w/w) and can

provide up to 70-80% of the calories consumed by humans worldwide (Mikulíková, Masár, & Kraic, 2008). Starch is a polymeric mixture of essentially linear amylose and branched amylopectin α-D-glucan molecules (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009; Fox & Robyt, 1992; Kim & Huber, 2010). The functionality of starch is dependent on amylose and amylopectin, as well as on the physical organization of these macromolecules into the granular structure (Cheetham & Tao, 1998; Zobel, 1988)

The commercial production of legume starches is still small compared to the overall production of starch (Guillon & Champ, 2002). Bean starch is under-utilized in the food industry due to the high cost of isolation, lack of knowledge of its physical and molecular structures, and limited understanding of how these structures affect its functionality and end-use (Hoover, Hughes, Chung, & Liu, 2010). Starches isolated from different botanical sources (e.g., corn, wheat and potato) differ in chemical structure, morphology and functionality.

Digestibility of native and cooked starches from grain legumes is known to be relatively low when compared to that of most cereals and tubers. The difference in digestibility may be due to legume starches having higher amylose content, higher gelatinization temperature, and higher capacity to retrograde (Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Tovar, Granfeldt, & Bjorck, 1992; Tovar & Melito, 1996). The digestion of starch, which affects the rate of glucose release and absorption in the body, plays an important role in human health and nutrition. Starch can be classified into three main groups based on its rate of digestion, namely rapidly digestible starch (RDS) which provides a quick source of energy, slowly digestible starch (SDS), and resistant starch (RS). Resistant starch is not digested in the small intestine and passes into the colon where it is fermented by natural microflora to produce short chain fatty acids (e.g. butyrate), that retard or reduce colon carcinogenesis (Bennink, Rondini, & Barrett, 2012; Noah et

al., 1998). Determining the physicochemical properties and structural features of starch that are associated with these three starch fractions will provide information to plant breeders to aid in the development of bean varieties with specific functional properties as well as enable food scientists to create bean foods that are high in SDS and RS to prevent or reduce the impact of chronic diseases such as diabetes. In general, results from this study will provide invaluable information to the growing knowledge base on food legumes.

The specific objectives of this study were:

1. To determine and compare the physicochemical properties of starch isolated from six varieties of *Phaseolus vulgaris*, namely, black bean, dark red kidney bean, light red kidney bean, navy bean, pinto bean and small red bean.
2. To determine and compare the digestibility, estimated glycemic index, and molecular weight distributions of isolated raw, canned and stovetop-cooked bean starches.
3. To investigate the effects of canning and a traditional cooking method (boiling) on the digestibility and molecular weight distributions of starch isolated from of dried bean.

The following dissertation is divided into: (1) Literature review, (2) Physicochemical properties of starch isolated from six varieties of *Phaseolus vulgaris* grown in Michigan, (3) Effects of different cooking methods on the digestibility and molecular weight distibutions on isolated bean (*Phaseolus vulgaris*) starch, (4) General conclusions, and (5) Recommendations for future studies. The chapters of this dissertation were written according to the Food Chemistry

journal article format and thus some information such as introduction and laboratory procedures are very similar for certain chapters.

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CHAPTER 2

LITERATURE REVIEW

2.1. Legumes

Dry beans are a part of the plant *Leguminosae* family. The family is generally characterized by edible seeds borne in pods that often open along two seams, by butterfly-like flowers (the flower is irregular and made up of five petals) and by compound stipulate leaves. Both the edible pods and the seeds are called beans or pulses. There are over 18,000 species of legumes though only about 20 are normally consumed by humans (de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006; Leterme, 2002). Most widely used of these include *Phaseolus vulgaris* (common bean), *Vigna unguiculata* (cowpeas), *Pisum sativum* (common pea), *Lens culinaris* (lentil), *Arachis hypogaea* (peanut) and *Glycine max* (soya bean). Legumes are second only to the grasses (cereals) in providing food crops for the world. They are among the most nourishing vegetables eaten by mankind (de Almeida Costa et al., 2006; Sathe & Salunkhe, 1981; Venter & Eyssen, 2001). Grain legumes occupy an important place in human nutrition, especially in the dietary patterns of low income groups in developing countries where animal protein is expensive (Broughton et al., 2003). In general, legumes are good sources of complex carbohydrates, protein, dietary fiber, vitamins and minerals. In addition, the fat content of the majority of legumes falls between one and two percent, except for soybeans, which have a higher fat content. The predominant fatty acid found in legumes is linoleic acid (de Almeida Costa et al., 2006; Tharanathan & Mahadevamma, 2003).

2.2. Production and Utilization

Leguminosae are second to cereal crops in agricultural importance based on area harvested and total production. According to the Food and Agriculture Organization of the

United Nations (FAO), in 2012 more than 24 million metric tons of dry beans were produced worldwide. *Phaseolus vulgaris*, generally known as the common bean, is produced in a range of crop systems and environments in regions as diverse as Latin America, Africa, China, Europe, the United States and Canada (FAO, 2014). North Dakota and Minnesota produce approximately 50% of the total bean crop each year in the United States and the top five beans grown include pinto, navy, black, red kidney (light and dark), and great northern beans (Miller, 2014). The common bean is the most widely cultivated of all beans.

Traditionally, grain legumes are processed and consumed as human food in a variety of ways. Processing improves palatability of beans and also increases the bioavailability of nutrients. Fresh or canned unripe pods or seeds are eaten as vegetables. Pounding, grinding and milling of dry grain is commonly practiced to obtain split seeds (dhals) or cotyledons with or without the seed coat. The most common method of cooking is to boil the entire seed or the dehusked cotyledons in water with salt. The dry beans are usually first soaked overnight (approximately 16 hours) and then cooked in boiling water. The process of soaking softens the seed to reduce cooking time and may also remove anti-nutritional factors such as tannins. Commercial canning has increased the convenience of using dry beans (Anderson, Smith, & Washnock, 1999; Tharanathan & Mahadevamma, 2003). Bean flour is used in food products prepared by baking, steaming or deep frying in oil. Processing methods have been developed using flour or isolated protein concentrates to make instant foods and meat analogs, especially from soybeans (Salunkhe & Kadam, 1983). The use of dry beans for food is dependent upon the seed size, shape, color and flavor characteristics and is often associated with particular social or ethnic groups (Ensminger & Ensminger, 1993). Taste and variety preference vary significantly

from one country to another. Popular uses include soups, mixed-bean salads, rice and peas, beans boiled with meat or other vegetables, or cereals and baked beans (Geil & Anderson, 1994).

2.3. Starch

Starch is the major carbohydrate storage material in many higher plants. Starches isolated from different botanical sources show diverse granule morphology and the granules vary in shape (spherical, oval, polygonal, disk, elongated and kidney shapes), and in size (1 μm-100 μm in diameter). The granule size and shape influence the physicochemical characteristics of starch, as well as its end use (Chibbar, Ambigaipalan, & Hoover, 2010; Hoover, Hughes, Chung, & Liu, 2010). Starch is a polymer in which the monomeric unit is the six-carbon sugar D-glucose. The polymerization of glucose into starch produces two types of polymers, amylose and amylopectin, which represent approximately 98-99% of the dry weight of starch. These polymers have different structures and properties. Amylose is an essentially linear polymer with α–1,4 linkages, whereas amylopectin is a highly branched polymer with short α–1,4 linked chains and 5-6% non-randomly distributed α–1,6 linkages (Figure 2.1.). Amylose has an average molecular weight of approximately 1×10^5 to 1×10^6 g/mol, with a degree of polymerization by number (DP_n) of 324-4920. Conversely, amylopectin is a much larger molecule than amylose with a molecular weight of 1×10^7 to 1×10^9 g/mol and DP_n of 9600-15900 (Sajilata, Singhal, & Kulkarni, 2006; Tester, Karkalas, & Qi, 2004; Wang, Bogracheva, Hedley, Centre, & Asp, 1998). The unit chains present in amylopectins (18-25 units) are shorter than those found in amylose molecules (Tester et al., 2004). Amylose and amylopectin are deposited into starch granules to produce semi-crystalline starch granules that vary in shape, size, and composition

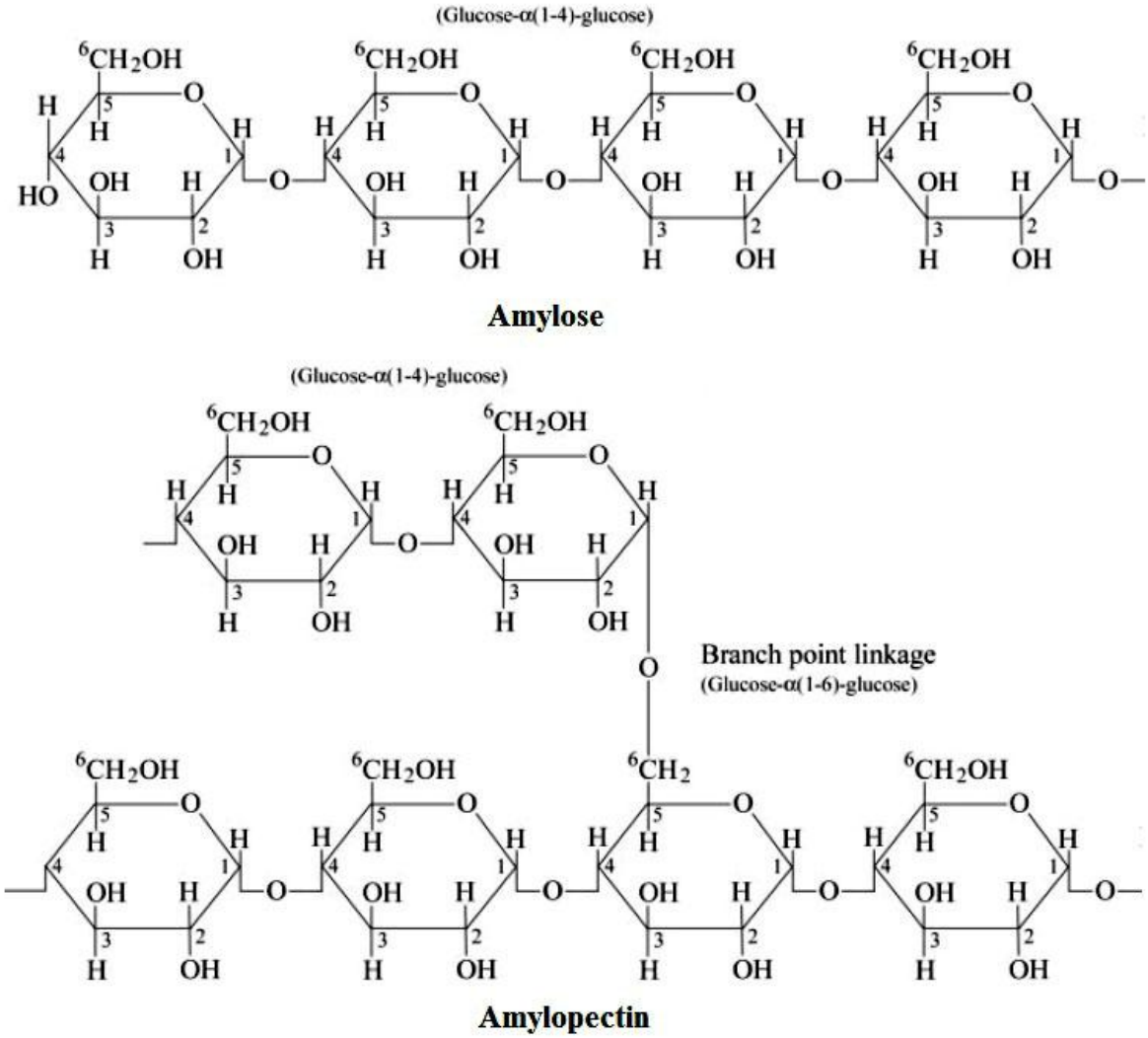

Figure 2.1. Chemical structure of amylose and amylopectin. *Source Tester, Karkalas, and Qi (2004).*

depending on botanical source and growing environment. The amylose and amylopectin contents of normal starches are 20-30% and 70-80%, respectively. Mutant lines of maize starches have been obtained with amylose contents in the range of 0% (waxy maize) to 70% (Hylon VII) (Jane, 1995; Pérez, Baldwin, Gallant, & Jane, 2009; Tester et al., 2004). Starch is semi-crystalline in nature with approximately 70% of the starch granule being amorphous and about 30% crystalline, as shown in diffraction experiments. The main component of the amorphous regions is amylose, while the crystalline region is primarily occupied by amylopectin (Sajilata et al., 2006). Variations in the structure of amylose and amylopectin, such as average molecular size, average degree of polymerization, and chain length distribution can effect differences in the physicochemical properties of starch granules (Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003)

2.4. Legume Starch

The starch contents of beans and other legumes range from 13 to 49% of their dry weight. Extensive research has been conducted on cereal, potato, sweet potato and cassava starches due to their availability and wide usage in food and non-food applications. However, there is a lack of information on structure-property relationships among bean starches. This is in part due to the difficulty encountered in isolating pure starches from legumes because of their high protein content, as well the effect of fine fiber co-settling with the starch and the high cost of isolation (Chibbar et al., 2010; Hoover et al., 2010; Huang et al., 2007).

Legume starch granules vary in shape: round, oval, spherical, elliptical and irregular; and in size: width (5-55 μm) and length (5-70 μm). Most legume starches are comprised of simple

granules (granules are that formed singly). However, a mixture of simple and compound granules (granules that form in aggregates) was reported for wrinkled peas and smooth peas. The amylose contents of legume starches range from 24-48%. Still, it is difficult to compare the amylose contents among and between legumes due to differences in where they were grown, cultivar, physiological state of the seed, and the methodology used for determination (Chibbar et al., 2010; Hoover et al., 2010; Shimelis, Meaza & Rakshit, 2006).

2.5. Crystallinity

Much of the information about starch granule crystalline properties has been acquired from X-ray powder diffraction studies. Starch granules from various botanical origins generally show one of three types of X-ray diffraction patterns: A-type (cereal starches); B-type (tuber, root, high amylose and retrograded cereal, and legume starches) and C-type (legume starches). The C-type pattern is a mixture of both A- and B-types (Cheetham & Tao, 1998). There is a V-type conformation, which results from amylose being complexed with substances such as aliphatic fatty acids, emulsifiers, butanol and iodine to form a double helix (Buléon, Gérard, Riekel, Vuong, & Chanzy, 1998). The main difference between A- and B-types of starches is in their crystalline unit cells. A unit cell is a small repeating entity in a crystal structure. Each type unit cell differs in the three unit cell edge lengths and the three internal angles (Figure 2.2.) (Massa, 2004). The A-type starch adopts a close-packed monoclinic array with eight water molecules present per unit cell, while the B-type is a more open hydrated structure, consisting of double helices packed in a hexagonal array. For the B-type thirty-six water molecules per unit cell fill the large central channel formed by the hexagonically packed double helices;

approximately half of the water molecules are tightly bound to the chains, and the other half are connected only to other water molecules (Figure 2.3.) (Bello-Pérez, Rodriguez-Ambriz, Sanchez-Rivera, & Agama-Acevedo, 2009; Chibbar et al., 2010; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Imberty, Chanzy, & Perez, 1988; Pérez, Baldwin, & Gallant, 2009).

Legume starches upon X-ray diffraction are characterized by strong intensity peaks corresponding to approximately 2θ=15°, 17° and 23°. Also, some legume starches have shown a small peak at 2θ =5.6°, which is characteristic of B-type starches; however, the intensity of peak was very low (Singh, Nakaura, Inouchi, & Nishinari, 2008). In an experiment using microfocus synchrotron wide-angle diffraction mapping on a series of C-type starch granules from smooth peas, it was found that C-type starch granules globally contained 60% of A-type polymorph and 40% of the B- type polymorph; additionally, the two polymorphs were present in each granule. The A-type polymorph was located essentially in the outer part of the granules whereas the B-type polymorph was found mostly in their centers (Buléon et al., 1998). These types of starch polymorphs depend partly on the chain length making up the amylopectin lattice, the density of packing within the granules, and the presence of water (Sajilata et al., 2006). The relative proportions of A- and B- in the C-type polymorphs and how they relate to functional properties (such as gelatinization, pasting and swelling) and digestibility is unknown. Ambigaipalan et al. (2011) studied the structure of faba bean, black bean and pinto bean cultivar starches at different levels of granule organization and their physicochemical properties and found that the B polymorphic content followed the order faba bean >black bean >pinto bean, ranging from 25.0% to 7.9%, while crystallinity ranged from 20.3% to 23.1% for these beans. In general, the crystallinity for legume starches ranges between 17 and 34%, which could be influenced by moisture content, size and number of crystallites arranged in a crystalline array and polymorphic

content (Hoover et al., 2010). Singh et al. (2008) postulated that the proportion of longer amylopectin chains may be responsible for the differences in crystallinity observed among starches from different legumes. High performance liquid chromatography (HPLC) analyses conducted on the distribution of amylopectin chain lengths in starches from 20 species established that there was a close relationship between the weight-average chain lengths of the amylopectins and the crystal type of starch granules. Short amylopectin chain lengths favored A-type crystallinity, while long chain lengths showed B-type crystallinity and intermediate chain lengths were associated with C-type crystallinity (Hizukuri, Kaneko, & Takeda, 1983; Hizukuri, 1985).

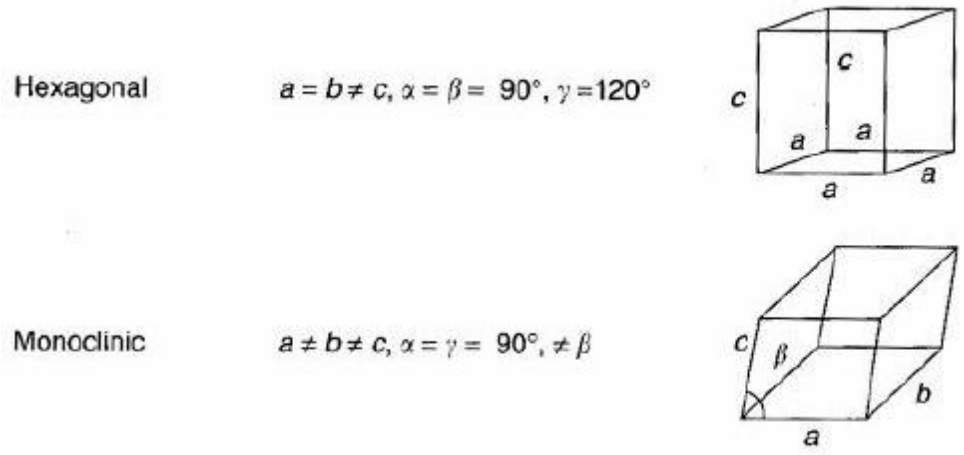

Figure 2.2. Unit cell geometry of hexagonal and monoclinic unit cells. *Source: Massa (2004)*.

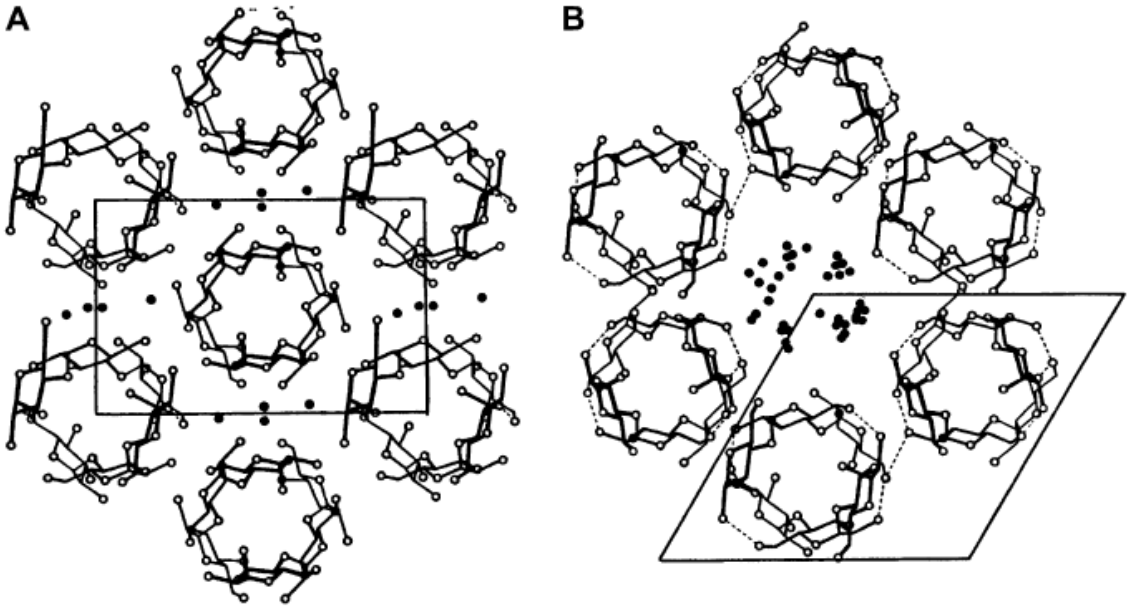

Figure 2.3. Double helices arrangement of A-type and B-type crystallites in starch granules.

Source: Sarko and Wu (1978).

2.6. Gelatinization

Gelatinization has been defined as the phase transition of starch granules from an ordered state to a disordered state, which takes place during heating in excess water (Hermansson & Svegmark, 1996). When starch granules are heated in excess water, the hydrogen bonds between glucose residues weaken, granules take up water resulting in swelling, and granular structure and crystallinity are lost. The starch is dispersed in the heated water and is susceptible to hydrolysis by α-amylase (Bertolini, 2009; Jenkins & Donald, 1998).

The gelatinization process is usually studied by differential scanning calorimetry (DSC). A starch sample is heated at a defined rate and the changes in heat capacity are measured as a function of temperature. Differential scanning calorimetry (DSC) measures the gelatinization transition temperatures (T_o, onset; T_p, peak; T_c, conclusion) and enthalpy of gelatinization (ΔH_{gel}). These transition temperatures are said to be affected by the molecular design of the crystalline region, which is related to the distribution of amylopectin short chains and not the amylose to amylopectin ratio (Jane et al., 1999; Singh, 2011). In general, higher gelatinization temperatures and enthalpies of gelatinization have been observed in starches comprising amylopectin with longer branch chains because longer chains more easily form stable double helical crystallites than shorter chains (Jane et al., 1999).

2.7. Pasting

Pasting is the phenomenon following gelatinization of a starch and involves granular swelling, exudation of amylose and amylopectin, and total disruption of the starch granule. Pasting viscosity profiles are commonly analyzed using a Rapid Visco Analyzer (RVA). A

typical profile is presented in Figure 2.4. Pasting temperature is the point at which the temperature rises above the gelatinization temperature. The peak viscosity indicates the maximum viscosity reached during the heating and holding cycle and is indicative of the water holding capacity of a starch; peak temperature occurs at peak viscosity. The breakdown viscosity is normally regarded as a measure of the disintegration of the starch granules as they are heated due to the rupture of granules and the release of soluble amylose (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002; Gupta, Bawa, & Semwal, 2009; Karim, Norziah, & Seow, 2000). As the starch-water mixture is cooled, re-association between starch molecules, especially of amylose, results in the formation of a gel and the subsequent increase in viscosity (i.e., total setback increases). Total setback involves retrogradation, or re-ordering, of the starch molecules (Kim, Lee, Baik, Joo, & Yoo, 2007; Rupollo et al., 2011).

Pasting properties of starch are affected by amylose and lipid contents and by branch chain-length distribution of amylopectin (Gupta et al., 2009; Jane et al., 1999). Starches with greater amylose, lipid and phospholipid contents have higher pasting temperatures, lower peak viscosities and shear-thinning (breakdown viscosity), and higher setback viscosities (Du, Jiang, Ai, & Jane, 2014; Jane et al., 1999; Zhang, Ao, & Hamaker, 2008; Zhang & Hamaker, 2003). Waxy wheat flour starch, conversely, has been shown to have significantly lower peak and pasting temperatures, higher peak viscosities and lower setback viscosity than non-waxy or normal wheat flour (Abdel-Aal et al., 2002; Gupta et al., 2009). This is because waxy wheat flour starch is essentially made up of 100% amylopectin molecules. Amylopectin with longer branch chains display larger peak viscosity and lower pasting temperatures than their shorter chain counterparts (Rosin, Lajolo, & Menezes, 2002).

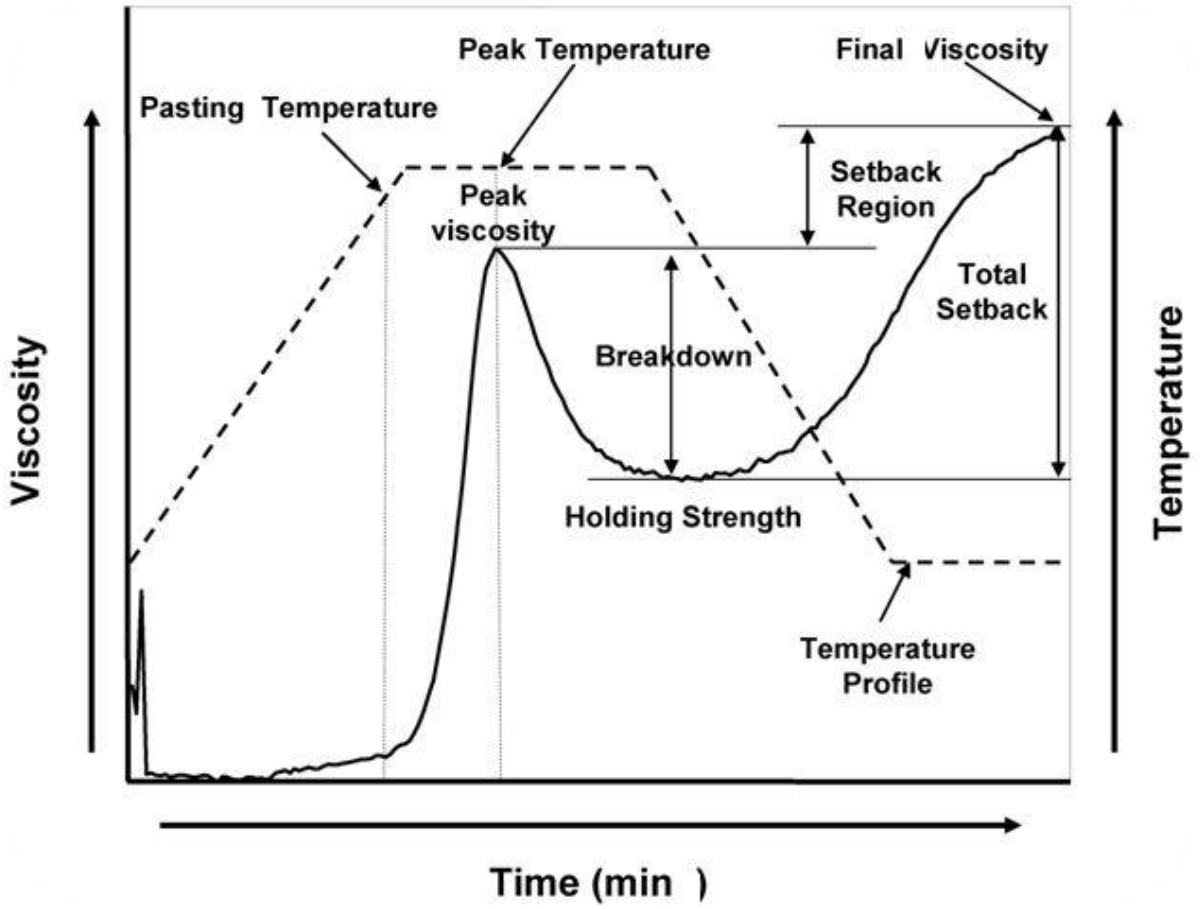

Figure 2.4. Typical RVA curve, showing the main parameters used to describe pasting. *Source: Bertolini (2009).*

2.8. Retrogradation

With continued heating after gelatinization, swollen granules will undergo pasting. When cooled, the starch chains are able to re-associate with each other through hydrogen bonding, forming an ordered structure that is more resistant to digestion. This process is known as retrogradation (Englyst, Kingman, & Cummings, 1992; Englyst & Hudson, 1996; Miles, Morris, Orford, & Ring, 1985; Miles, Morris, & Ring, 1985). Both amylose and amylopectin will re-associate during this process with amylose retrogradation occurring at a faster rate than that of amylopectin. Amylopectin is limited in its retrograding capabilities by its branched structure, and the polymers that do re-associate are less firmly bound than those of retrograded amylose (Copeland, Blazek, Salman, & Tang, 2009; Englyst et al., 1992). Therefore, re-crystallized (retrograded) amylopectin requires a lower temperature (70ºC) to reverse retrogradation upon reheating whereas amylose requires a temperature of 160ºC (Miles, Morris, Orford, et al., 1985; Miles, Morris, & Ring, 1985). Retrogradation can result in effects such as precipitation, gelation, changes in consistency and opacity, and decreased storage stability, all of which are unwanted side effects for industrial applications (Hermansson & Svegmark, 1996). The rate of starch retrogradation is dependent on many factors such as botanical source, ratio of amylose to amylopectin, the structure of both amylose and amylopectin, temperature as well as the presence of other compounds such as lipids and proteins (Thitipraphunkul et al., 2003).

2.9. Resistant Starch

Resistant starch has been defined as the fraction of starch which escapes digestion in the small intestine, and may be fermented in the large intestine (Englyst et al., 1992). The resistance of starch to digestion can be attributed to several factors, which have led to the emergence of

four categories of starch. They are as follows. RS1: starch that is physically inaccessible to digestion by entrapment in a non-digestible matrix (e.g., seeds, grains and tubers with intact cell walls); RS2: native or ungelatinized starch granules protected from digestion due to the conformation of the granule as in raw bananas and potatoes; RS3: retrograded starch produced via heating and subsequent cooling (e.g., corn flakes); and RS4: chemically modified starch, including starches which have been esterified or cross-bonded) (Englyst et al., 1992; Nugent, 2005; Topping et al., 2003). The formation and levels of resistant starch present in foods are greatly influenced by processing techniques, in particular those that use heat and moisture, for example, canning, extrusion, and microwave heating. Dehulling legumes by steam treatment and cooking for instance resulted in higher levels of resistant starch (Tovar et al., 2002). The resistant starch most frequently found in processed foods is mainly retrograded starch.

Research has shown that resistant starch imparts biological benefits, of which some benefits are like those of traditional fiber, and others are unique to resistant starch (Haralampu, 2000). Resistant starch that reaches the large intestine can act as a substrate for microbial fermentation, the end-products being hydrogen, carbon dioxide, methane and short chain fatty acids (SCFA). The short chain fatty acids are thought to be protective against colorectal cancer ((Englyst & Hudson, 1996; Nugent, 2005)

2.10. Starch Hydrolysis and Glycemic Index

In human nutrition, starch plays a major role in supplying the metabolic energy that enables the body to perform energy-requiring functions. It is broken down to glucose by amylase enzymes in the human digestive system, and glucose is absorbed from the small intestine into the bloodstream and used as an energy source. The rate at which glucose is

absorbed from the intestinal tract appears to be an important parameter in controlling the homeostasis of blood glucose. Glycemic index and glycemic load have been used to assess the relative risks of foods that have been implicated in the prevention of diabetes, coronary heart disease and obesity. The glycemic index (GI) is a measure of the extent to which a test food increases blood glucose levels during the 2 hours after consumption, compared to eating an equivalent amount of reference carbohydrate either glucose or white bread (Jenkins et al., 1981; Ludwig et al., 1999; Wolever, Jenkins, Wóng, Josse, & Thompson, 1987). Foods with a high GI cause a more rapid rise in blood glucose and insulin levels compared to foods with a low GI. Dry beans have a relatively low glycemic index, varying from 27-42% relative to glucose and 40-59% that of white bread when compared to other carbohydrate sources (Bennink et al., 2012; Foster-Powell, Holt, & Brand-Miller, 2002). This low GI property can be attributed to the slow rate of bean starch digestion.

Thus, in the context of digestibility, starch is classified into three fractions, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Berry, 1986; Englyst et al., 1992). According to Englyst et al. (1992) RDS is the amount of starch digested in the first 20 min of a standard *in vitro* digestion reaction mixture. RDS causes a rapid increase in blood glucose concentration after ingestion of carbohydrate foods. SDS is the fraction of starch digested slowly but completely in the human small intestine and is defined as the starch that is digested after the RDS fraction, between 20 min and 120 min of a standard *in vitro* digestion reaction. RS is the fraction that escapes digestion in the small intestine, and is analytically defined as the starch that is not digested within the first 120 min of the standard digestion reaction.

The major enzymes involved in starch hydrolysis are amylases and amyloglucosidases. Alpha-amylases are the main endo-splitting enzymes involved in the hydrolysis of α-1,4 bonds of starch (amylose and amylopectin). The nature and the distribution of the hydrolysis products (maltose, glucose, and dextrins of high molecular weights) depend on the source of the amylases and the substrate. Amyloglucosidase is an exo-acting enzyme that hydrolyzes the α-1,6 linkages in starch and produces glucose from the non-reducing end of the starch molecule (Annison & Topping, 1994; Colonna, Leloup, & Buléon, 1992).

Several factors have been proposed to limit starch hydrolysis, including particle size of substrate, amylose content, crystallinity, degree of gelatinization, extent of retrogradation, and the presence of other components interacting with starch (amylose-lipid complexes) (Colonna et al., 1992; Du et al., 2014; Jane et al., 1999). Hydrolysis of native and cooked starches from grain legumes is known to be relatively slower when compared to most cereal starches (A-type crystalline pattern). This characteristic can be attributed to legume starches having larger amylose content, higher crystallinity (when raw), and higher capacity to retrograde (Björck et al., 1994; Noah et al., 1998; Tovar et al., 1992; Tovar & Melito, 1996)

Zhou et al. (2010) conducted a study to determine the changes in the molecular weight distribution of starch fractions following digestion of foods in the human small intestine. Volunteers with ileostomies consumed six selected foods: breakfast cereal (muesli), white bread, oven-baked French fries, canned mixed beans and a custard containing either a low-amylose maize starch (LAMS) or a high-amylose maize starch (HAMS). The digesta total resistant starch contents (expressed as a fraction of ingested starch) were: muesli, 8.9%; bread, 4.8%; fries, 4.2%; bean mix, 35.9%; LAMS custard, 4.0%; HAMS custard, 29.1%. Chromatographic analysis showed that the starches were fractionated into high molecular weight (43,500 kDa),

medium molecular weight (420 kDa) and low molecular weight (8.5 kDa) fractions. It was found that a low molecular weight fraction (8.5 kDa) was predominant in the undigested residue. Canned beans and HAMS custard contained starches with lesser digestibility and consisted mostly of low molecular and medium molecular weight fractions and had very little high molecular weight fractions. These results suggested that not only were the rate and extent of starch digestion, and the SDS and RS contents of a food, affected by the amylose content of starch, but also by the molecular weights of the amylose and amylopectin present in that starch.

2.11. Molecular Weight

Several analytical techniques have been used for the separation and structural characterization of starch including ultracentrifugation, size exclusion chromatography and light scattering (Bello-Pérez et al., 2009; Ratnayake, Hoover, & Warkentin, 2002; Wyatt, 1993; Yoo & Jane, 2002). However, many problems have been linked with the separation and characterization of the two polymer fractions of starch. These include solubilization, degradation aggregation and retrogradation (Chiaramonte, Rhazi, Aussenac, & White, 2012).

2.11.1. Starch Solubilization

In order for starch to be accurately characterized the macromolecule needs to be completely dissolved in the solvent of choice. Solubilization should be under moderate conditions which ensure that the starch structure remains intact. Starch is insoluble in cold water, but becomes soluble in excess water under certain heating conditions; however, depending

on the temperature and length of heating, depolymerization can occur (Bello-Pérez et al., 2009). Starch dissolution is usually accomplished with dimethyl sulfoxide (DMSO) or aqueous DMSO solutions, sometimes with the addition of salts such as LiBr and $LiNO_3$.(Cave, Seabrook, Gidley, & Gilbert, 2009; Othman, Al-Assaf, & Hassan, 2010; Yokoyama, Renner-Nantz, & Shoemaker, 1998). Zhong, et al. (2006) determined the molecular weights of rice starch amylose and amylopectin using DMSO, with either 50 mM LiBr or 10% water or both for batch mode multi-angle laser light scattering. They found that DMSO/50 mM LiBr was a better solvent for dissolving and reducing starch aggregates than DMSO/10% water.

2.11.2. Size Exclusion-Chromatography

Size-exclusion chromatography (SEC) is a well established-method for the analysis of polymers and macromolecules such as starches and proteins. In this type of chromatography, separation is based on molecular size rather than chemical properties. Technically, molecules are separated on the basis of hydrodynamic size and not weight-averaged molecular weight (M_W). The stationary phase in SEC consists of a column packing material (usually microbeads) with pores comparable in size to the molecules to be fractionated. The porous beads of a polymeric gel or silica beads will allow small molecules into their pores but not large ones. When a solute is injected into such a column, the smaller molecules are distributed through a larger volume of solvent than is available to the large molecules. As a result, smaller dissolved molecules flow more slowly through the column because they penetrate deep into the pores, whereas large dissolved molecules flow quickly through the column because they do not enter the pores. Therefore, larger molecules are eluted from the column sooner than smaller

molecules, which enables the separation of molecules by size (Othman et al., 2010; Podzimek, 2014; Thrathnigg, 2000). The elution volume of a molecule is related to its hydrodynamic volume (Cave et al., 2009).

In order to determine the specific molecular weight (Mw) of a molecule, column calibration is required. Standards of known molecular weights are used to convert the elution or retention volume to a molecular weight for a given column set. In SEC analysis of starch, dextrans and pullulans are the standard polymers commonly used (Cave et al., 2009; Huang et al., 2007; Xie et al., 2012). Dextran is branched glucan composed of chains of varying lengths, with molecular weights ranging from 1×10^3 kDa to 410×10^3 kDa. The straight chain consists of α-1,6 glycosidic bonds between glucose molecules, while branches begin from α-1,3 linkages. Pullulans, on the other hand are linear polysaccharides made of repeated units of maltotriose with molecular weights ranging from 3×10^3 kDa to 1600×10^3 kDa (Cave et al., 2009).

2.11.3. Light Scattering

The interaction of light with matter can yield important information about the structure of starch and its polymers. The phenomenon of light scattering occurs when electromagnetic radiation hitting a molecule is partly scattered. Charge separation occurs by the interaction of the electrons within the molecule with the oscillating electric field component of light. An oscillating dipole is created and the molecule emits scattered light. Almost all of the scattered light has the same wavelength as the incident radiation and comes from elastic scattering, known as Rayleigh scattering (Wyatt, 1993). In multi-angle laser light scattering (MALLS) the scattering intensity is measured at several different angles. The two major principles that are used in light scattering to determine the molar mass are:

1. The intensity of light scattered (LS*)* is proportional to the product of the polymer (Mw) and the polymer concentration (C).

$$LS \alpha Mw \times C (dn/dc)^2$$

Where (dn/dc) is the refractive index increment, which expresses the variation of the refractive index of a solution with solute concentration.

2. The angular variation of the scattering is directly related to the radius of the polymer (Wyatt, 1993)

Recently, high performance SEC equipped with both multi-angle light scattering (MALLS) and differential refractometer (RI) detectors has been used routinely to determine absolute molecular weights of starches (Zhong et al., 2006; Yokoyama, Renner-Nantz, & Shoemaker, 1998; Othman, Al-Assaf, & Hassan, 2010; Cave, Seabrook, Gidley, & Gilbert, 2009). HPSEC-MALLS-RI is a rapid method to separate and monitor starch components and debranched fragments. Molecular weight measurements with light scattering is an absolute method and is independent from column calibration (Podzimek, 2014; Wyatt, 1993). The average weight molecular weights of bean, rice, canna, barley, wheat, banana and potato amylopectins determined by this method were found to be in the range of 1.3-56.8 x 10^8 g/mol (Yoo & Jane, 2002).

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CHAPTER 3

PHYSICOCHEMICAL PROPERTIES OF STARCH ISOLATED FROM SIX VARIETIES OF *PHASEOLUS VULGARIS* GROWN IN MICHIGAN

3.1. ABSTRACT

The physicochemical characteristics of starches isolated from six varieties of *Phaseolus vulgaris* grown in Michigan were determined. Starch yield varied from 22.8% to 34.1% on a whole seed basis (~ 10% moisture content). The raw isolated bean starches exhibited high resistant starch contents ranging from 41.9% (light red kidney bean) to 55.7% (pinto bean), whilst total amylose content varied from 28.0% (pinto bean) to 29.8% (dark red kidney bean). Significant differences were observed for the gelatinization transition temperatures, pasting parameters, and resistant starch contents of the isolated dark red kidney and light red kidney bean starches when compared to isolated starch from black, navy, pinto and, small red beans. All isolated bean starches displayed the characteristic C-type X-ray diffraction pattern of legumes. The crystallinity and B-type starch polymorph contents ranged from 36.1% to 24.9% and 19.6% to 15.6% respectively. This study demonstrated that the chemical compositions of beans are different for different varieties, and thus the varieties exhibit different physicochemical properties.

3.2. INTRODUCTION

The common bean (*Phaseolus vulgaris*) is one of the most important group of legumes consumed by humans globally. The genus encompasses more than 50 species and the annual production of dry beans is approximately 24 million metric tons worldwide (Gepts, 2001). Dry beans play an important role in the diets of many people in developing regions, by providing an affordable source of essential nutrients, such as protein (22% of seed weight), vitamins, minerals, and calories, in developing regions (Duranti & Gius, 1997; Geil & Anderson, 1994; Guillon & Champ, 2002; A. Kaur et al., 2013) . Dry beans are considered healthy for all societies because they are high in complex carbohydrates, proteins, dietary fiber, and folate while being low in fat, and sodium (Anderson et al., 1999; Broughton et al., 2003). The inclusion of legumes in the diet have been reported to yield other health benefits including anti-diabetic, hypocholesteremic, antioxidative and anticancer activities (Anderson et al., 1999; Bennink, Rondini &Barrett, 2012; (Englyst, Vinoy, Englyst, & Lang, 2003; Tharanathan & Mahadevamma, 2003).

Starch is arranged in semi-crystalline granules that vary in size, shape and molecular structure among different and within the same plant species. Starch is comprised primarily of two polymers, amylose (linear) and amylopectin (highly branched) (Chibbar, Ambigaipalan, & Hoover, 2010; Hoover, Hughes, Chung, & Liu, 2010; Hughes et al., 2009; Jane, Wong, & McPherson, 1997). The functional properties of starch depend on many factors, including but not limited to the ratio between its two polymers, degree of crystallinity and botanical source. Legume starches provide distinct and unique functional properties to food systems due to their higher gelatinization temperature, greater tendency to retrograde, and greater resistant starch content when compared to that of cereal starches (Mikulíková et al., 2008). Dry bean starch is digested slower, has a lower glycemic index and produces more short chain fatty acids in the

large intestine than cereal or tuber starches (Englyst, Kingman, & Cummings, 1992; Fleming & Vose, 1979; Shekib, 1994). These are important attributes particularly for developed countries, where there is a high prevalence of diabetes and obesity. Due to the importance of legume starches in the human diet, knowledge of their structure-function relationships would be of interest in expanding their efficacy and utilization as new ingredients in the food industry (de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006; Ambigaipalan et al., 2011). Additionally, variation in starch properties among varieties of *Phaseolus vulgaris* could provide valuable information for plant breeders. The objective of this study was to determine the composition and physicochemical properties of starch isolated from six varieties of *Phaseolus vulgaris*.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Six varieties of the species *Phaseolus vulgaris*, black bean (*Zorro*), dark red kidney (*Redhawk*), light red kidney bean (*CELRK*), small red bean (*Merlot*), pinto bean (*P07863*) and, navy bean (*Medalist*) were obtained from USDA-ARS located in East Lansing, MI, USA. The beans were harvested in 2011.

3.3.2. Starch Isolation

Starch was extracted from six varieties of *Phaseolus vulgaris* according to the procedure of Hoover and Sosulski (1985) with some modifications (Figure 3.1.). Beans (450 g) were steeped in distilled water (500 mL, 0.01% sodium metabisulfite) for 24 h at room temperature. The swollen seeds were rinsed with distilled water and homogenized in a Waring blender for 4 min on a low setting. The homogenates were sequentially passed through 250 μm, 180 μm and 75 μm sieves. The filtrate was allowed to settle at room temperature for 18h and the supernatant discarded. The sediment was suspended in excess 0.2% sodium hydroxide, then allowed to stand for 4h, after which the supernatant was removed. This procedure was repeated ten times. The final sediment was suspended in distilled water, passed through a 75 μm sieve, neutralized to pH 7.0 with hydrochloric acid (1 M), filtered and washed with distilled water. The residue was air dried and stored at room temperature in a desiccator for further analyses. Extraction was done in duplicate.

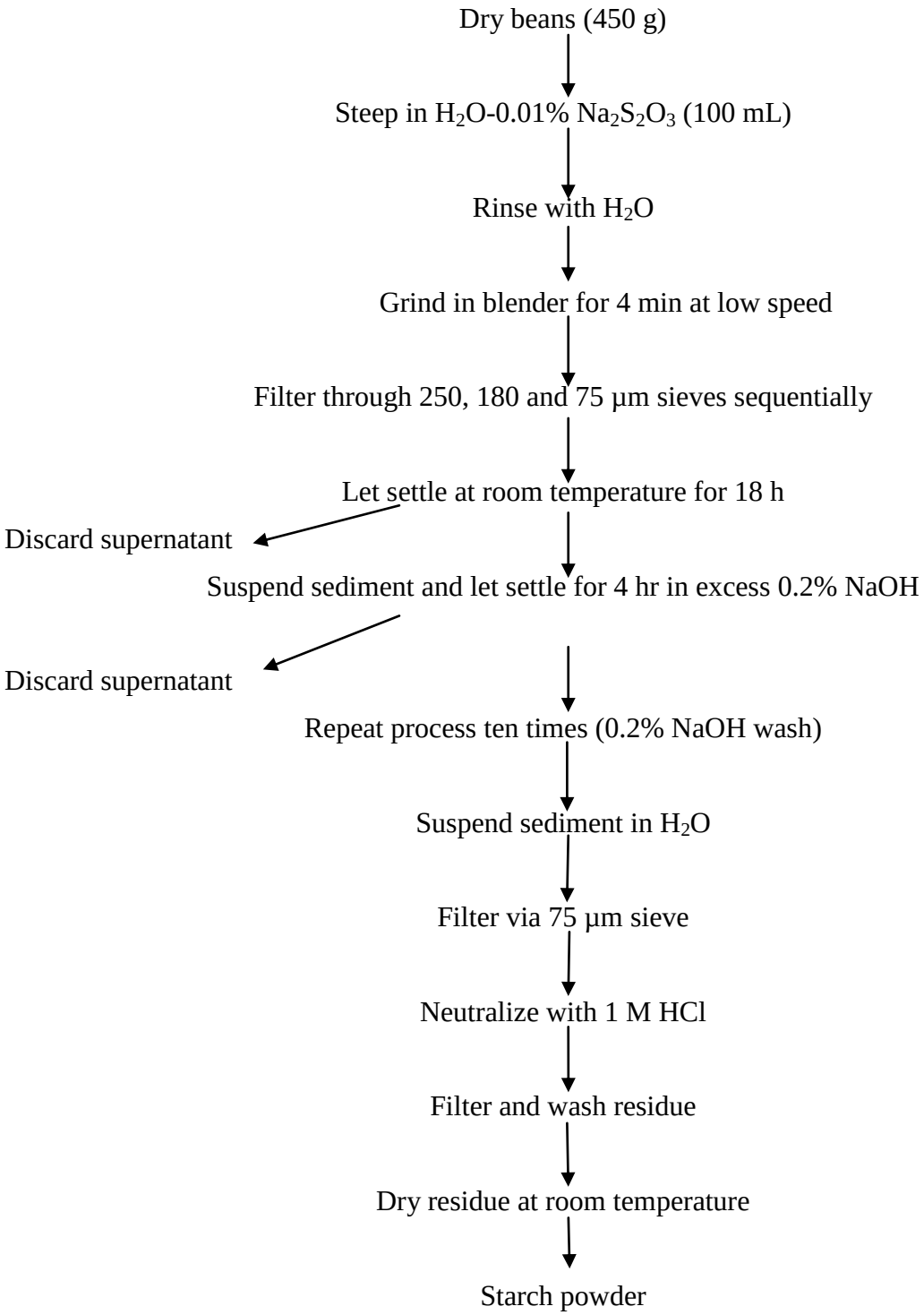


Figure 3.1. Wet isolation of starch from dry beans.

3.3.3. Proximate Analysis of Starch

Quantitative estimations of moisture, fat, and ash of isolated dry bean starches were performed using standard AOAC Methods [925.09, 920.39, 923.03 (2000)] respectively. Analyses were performed in duplicate, and mean values were reported.

3.3.4. Nitrogen Determination

Total nitrogen content of isolated dry bean starch was determined by the Dumas combustion method at A & L Great Lakes Laboratories (Fort Wayne, IN) in accordance with AOAC Method 968.06 (2000). Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.5. Trace Mineral Analysis

For each of the six varieties, isolated bean starch sample (500 mg dry weight) was placed in a test tube and ultra-pure nitric acid (3 mL) added. The mixtures were shaken overnight in a water bath (Dubnoff Metabolic Shaker Incubator, Precision Scientific Co., USA) for 16 h at 25°C. Following extraction, samples were placed in a digestion block (Martin Machine, Ivesdale, IL) and incubated for 4 h at 125°C with refluxing. Following digestion, samples were cooled for 5 min before adding hydrogen peroxide (2 mL) and incubated at 125°C for an additional hour. This step was repeated before raising the digestion block temperature to 200°C and this temperature was maintained until each sample was completely dry. Cooled samples were then resuspended in 2% ultra-pure nitric acid (3 mL) and incubated overnight prior to analysis using ICP-OES (inductively coupled plasma-optical emission spectroscopy; CIROS ICP Model FCE12, Spectro, Kleve, Germany). To ensure batch-to-batch accuracy, all samples were

digested and measured alongside tomato leaf standard purchased from the National Institute of Standards and Technology (SRM 1573A; Gaithersburg, MD). Each of the isolated starch samples per variety was analyzed in duplicate, and mean values were reported.

3.3.6. Physicochemical Properties of Bean Starch

3.3.6.1. Total starch content

Total starch content in isolated bean starch was determined in duplicate according to the procedure in the Total Starch Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). Briefly, isolated bean starch (100 mg) was placed in a glass test tube (16 mL) followed by the addition of 0.2 mL of 80% (v/v) ethanol to aid in dispersing the sample. Thermostable α-amylase (3 mL of 3,000 U/mL of Ceralpha reagent) diluted (1:30) in sodium acetate buffer (100 mM, pH 5.0) was added to the sample and the tube was incubated in a boiling water bath for 6 min with vortexing at 2, 4, and 6 min. The tube was then placed in a water bath at 50ºC and 0.1 mL of amyloglucosidase (3300 U/mL of soluble starch) was added to the tube. The isolated bean starch sample was incubated for 30 min. Following incubation, the contents of the tube were transferred to a volumetric flask (100 mL) and the volume adjusted with distilled water to 100 mL. The contents of the volumetric flask were then transferred to a beaker (250 mL) and 3 mL of the contents were placed in centrifuge tube and a centrifuged at 1500 x g for 10 min at 25ºC. An aliquot (0.1 mL) of the supernatant was pipetted to the bottom of a test tube (15 mL), followed by addition of glucose oxidase-peroxidase-aminoantipyrine (GOPOD) reagent (3 mL) and was then incubated in the water bath at 50^{o}C for 20 min. A spectrophotometer (GENESYS 10S UV-Vis, Thermo Fischer Scientific Inc., USA) was used to measure the absorbance for the

isolated starch sample at 510 nm against the reagent blank (0.1 mL of distilled water and 3 mL of GOPOD reagent). Total percent starch on a dry weight basis was calculated based on formulas outlined in the Total Starch Kit. Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.6.2. Starch Damage

Megazyme Starch Damage Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland) based on the thermostable α-amylase and amyloglucosidase procedure was used according to manufacturer's instructions to determine the starch damage content for all starch samples. During the assay damaged starch granules were first hydrated and then hydrolyzed to maltosaccharides and α-limit dextrins by purified fungal α-amylase. The reaction was terminated with dilute sulfuric acid and aliquots were treated with amyloglucosidase to hydrolyze dextrins to glucose which was measured by glucose oxidase/peroxidase reagent mixture (GOPOD). Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.6.3. Pasting Properties

The pasting properties of the bean starches were determined using a Rapid Visco Analyzer RVA-4 (Newport Scientific Pty Ltd, Warriewood, Australia). Aqueous starch slurries (3 g starch, 25 mL distilled water) were equilibrated at 50 °C for 1 min, heated at a rate of 6 °C/min to 95°C, held at 95°C for 5 min, cooled at a rate of 6°C/min to 50°C, and held at 50°C for 2 min. The spindle speed was 960 rpm for the first 10 s (to disperse the sample) and then 160

rpm for the remaining 23 min. Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.6.4. Gelatinization and Retrogradation

Gelatinization temperatures were measured and recorded on a differential scanning calorimeter (TA Instruments, DSC Q100, DE, USA) equipped with a thermal analysis data station. Isolated starch (5 mg, dry weight) was weighed in a hermetic aluminum DSC pan and distilled water (20 µL) was added with a microsyringe to the DSC pan (TA Instruments, Newcastle, DE, USA). The pan was sealed, reweighed, and allowed to stand at room temperature for 24 h. The scanning temperature range and the heating rate were 20–120°C and 10 °C min^{-1}, respectively. The heated pans were cooled at room temperature for 3 h and kept at 4 °C ± 2°C for 7 days. After the period of storage, samples were scanned under the same conditions as mentioned above. The thermograms were recorded using an empty pan as reference. The transition temperatures reported were the onset (T_o), peak (T_p) and conclusion (T_c) temperatures of the gelatinization endotherm. The enthalpy of gelatinization (ΔH_G) and retrogradation (ΔH_R) were determined by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of Joules per gram (J/g) of dry starch. Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.7. Resistant Starch

The resistant starch contents of duplicate isolated bean starch samples were measured using the Resistant Starch Assay kit by Megazyme (Megazyme International Ireland Ltd. Co., Wicklow, Ireland) according to manufacturer's instructions. Starch samples were incubated in a

shaking water bath with pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37°C; non-resistant starch was solubilized and hydrolyzed to D-glucose by the combined effect of both enzymes. The reaction was terminated by the addition of an equal volume of ethanol and the resistant starch was recovered as a pellet upon centrifugation and washed twice by suspension in aqueous ethanol (50% v/v) followed by centrifugation and decantation of the liquid. The resistant starch in the pellet was dissolved in KOH (2 M) by vigorously stirring in an ice-water bath with a magnetic stirrer and neutralized with acetate buffer. The starch was quantitatively hydrolyzed to glucose with AMG. D-glucose was measured using the GOPOD reagent, thus giving a measure of the resistant starch content of the bean starch samples. The non-resistant starch was determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with the GOPOD reagent.

3.3.8. Amylose and Amylopectin Contents

Megazyme Amylose/Amylopectin Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland) based on the thermostable α-amylase and amyloglucosidase procedure was used according to manufacturer's instructions to determine the amylose and amylopectin content for all starch samples. Starch samples were dispersed by heating in DMSO and starch was precipitated in ethanol. The starch precipitate was dissolved in an acetate/salt solution; amylopectin was specifically precipitated by the addition of the lectin concavalin A and removed by centrifugation. The amylose in the supernatant was enzymatically hydrolyzed to D-glucose which was measured with the GOPOD reagent. The total starch in a separate aliquot of the acetate/salt solution was similarly hydrolyzed to D-glucose and measured using the GOPOD reagent. The concentration of amylose in each starch sample was estimated as the ratio of

GOPOD absorbance at 510 nm of the supernatant of Con A precipitated sample, to that of total starch sample. Each of the isolated starch samples per variety was analyzed in duplicate.

3.3.9. X-ray Diffraction

3.3.9.1. Crystallinity

X-ray diffractograms were obtained using an X-ray diffractometer (XDS 2000, Scintag Inc, CA,USA) with copper K-alpha emission radiation and operating conditions of target voltage 35kV; current 35 mA; scanning range 3-35°; scan speed 0.20°/min; step time 6 s; divergence slit width 2.00 mm; scatter slit width 4.00 mm and receiving slit width 0.200 mm. The moisture contents of all starch samples were adjusted to approximately 11.3% (moisture contents of the isolated starches were in this range, Table 3.1) by storage at room temperature in a dessicator over saturated LiCl for 14 days. Crystallinity of the starches was quantitatively estimated following the method of Nara & Komiya, (1983). The crystallinity (%) was calculated by the equation:

Crystallinity (%) = 100 × Ac/ (Ac + Aa)

where Ac is the crystalline area on the X-ray diffractogram and Aa is the amorphous area.

3.3.9.2. A-type and B-type Polymorphic Composition

Polymorphic composition (proportion of A-type and B-type) of starch samples were calculated using the method outlined by Davydova et al. (1995) and modified by Zhou et al. (2004). The 'B' polymorph content was calculated by determining the ratio of the area under the diffraction peak centered at 15.2º 2θ to the total crystalline area (as described above). Peaks in diffractograms were de-convoluted with eXPFIT (Nix RM, 2010). A calibration curve was

prepared by mixing pure B-type starch (0–100% potato) and pure A-type (100–0% waxy corn) starch. The area of the peak occurring at 15.2º 2θ was directly proportional to the %A polymorph in the calibration mixtures ($r = 0.988$).

3.3.10. Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Analyses of variance (ANOVA) were performed and the mean separations were evaluated by Tukeys HSD test ($p < 0.05$).

3.4. RESULTS AND DISCUSSION

3.4.1. Proximate Analysis

The chemical characteristics of the starches isolated from six varieties of *Phaseolus vulgaris* beans are summarized in Table 3.1. The yields of starches recovered from the dry beans varied from 22.8% (pinto bean) to 34.1% (dark red kidney bean). Isolation of pure starches from legumes is difficult due to the presence of protein and fiber co-settling with the starch during the extraction process (Huang et al., 2007). The disparity in yield may be due to beans before isolation having initially having different starch contents, as well as slight variations in the isolation process. The lipid contents of all the starch samples were less than 1% , which is not surprising since dry beans have a low lipid content. The ash contents ranged from 0.04% to 0.08%. The low ash content suggests that the starches are relatively free from contamination by fine fiber. The nitrogen contents (0.09–0.10%; Table 3.1) were low in all the dry bean starches. Both dark red kidney and light red kidney bean starches yielded the highest level of phosphorus (0.013%), while the other starches contained approximately 0.0030%. These results were all similar to those obtained by other investigators (Ambigaipalan et al., 2011; Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008; Chung, Liu, Pauls, Fan, & Yada, 2008; Hoover & Ratnayake, 2002; Hoover & Sosulski, 1985; Wani, Sogi, Wani, Gill, & Shivhare, 2010; Zhou, Hoover, & Liu, 2004).

Table 3.1. Chemical Composition of Starches from *Phaseolus vulgaris* (g/100g)[a]

Starch Source	Yield (%)[b]	Lipid (%)	Ash (%)	Moisture (%)	Nitrogen (%)	Phosphorus (%)
Black bean	26.7 ± 0.7	0.035 ± 0.0	0.064 ± 0.0	11.45 ±0.5	0.097 ± 0.00	0.0020 ± 0.00
Dark red kidney bean	34.1 ± 5.7	0.015 ± 0.0	0.068 ± 0.0	14.34 ± 0.2	0.101 ± 0.00	0.0130 ± 0.00
Light red kidney bean	31.1 ± 1.1	0.055 ± 0.0	0.078 ± 0.0	12.37± 0.9	0.097 ± 0.00	0.0130 ± 0.00
Navy bean	28.9 ± 0.1	0.055 ± 0.0	0.051 ± 0.0	11.73 ±0.1	0.094 ± 0.00	0.0030 ± 0.00
Pinto bean	22.8 ± 2.3	0.074 ± 0.0	0.035 ± 0.0	12.47 ±0.2	0.089 ± 0.00	0.0020 ± 0.00
Small red bean	25.0 ± 4.4	0.094 ± 0.0	0.075 ± 0.0	11.52 ±0.5	0.092 ± 0.00	0.0020 ± 0.00

[a] Data represent means and standard deviations of duplicate analyses

[b] Yield = (dry mass of isolated starch/ mass of beans used for isolation) x 100%

3.4.2. Total Starch, Starch Damage, Amylose Content and Resistant Starch Contents

All the isolated starch preparations had total starch contents greater than 90% (Table 3.2), with light red kidney bean starch being the highest (96.2%). The variation in total starch results for the isolated starches can be due to fiber (which co-settles with starch during extraction) adhering to some bean starch samples more than others and artificially raising the total starch measured.

The percent starch damage ranged from 0.16% to 0.29%. Starch damage affects the susceptibility of the starch granules to α- amylolysis as well as to hydration and water binding. As starch damage increases, the starch granules become more susceptible to digestive enzymes

(Chung, Lim, & Lim, 2006). In this study the values for starch damage was very low and therefore would not have significant effect on the studied starch characteristics. Based on the data in Tables 3.1.and 3.2., the starches isolated were relatively free from contaminants, such as protein and fat. Legume starches have a high amylose content (24-65%) compared to that of cereal starches (Ambigaipalan et al., 2011; Hoover & Sosulski, 1985; Singh, Singh Sandhu, & Kaur, 2004). The amounts of amylose in the isolated bean starch samples were all around 28% and not significantly different among the varieties studied (Table 3.2.). Others have reported similar values for the common bean (Ambigaipalan et al., 2011; Hoover & Ratnayake, 2002).

Many methods have been developed for estimating the amylose content of various materials. The most common methodologies utilize the iodine binding capacity of amylose. However, amylopectin and other starch contaminants (e.g., lipids) are able to complex with iodine causing an overestimation of the spectrophotometric measurement of amylose (Mestres, Matencio, & Pons, 1996). The method utilized in this study was enzymatic and removed amylopectin before measurement by complexation with concavalin A. Thus, comparison of amylose contents reported in literature among and between legumes is difficult due to differences in the methodologies used to determine amylose and factors affecting amylose content (Chibbar et al., 2010).

Resistant starch (RS) refers to a portion of starch that is resistant to enzymatic hydrolysis in the small intestine and passes into the colon to be fermented by gut microflora (Englyst et al., 1992). In the present study pinto beans contained the greatest amount of resistant starch (55.70%), whilst light red kidney bean had the lowest (41.92%). The resistant starch contents of starches isolated from dark red kidney and light red kidney beans were significantly lower than those of the other bean starches. According to Annison & Topping (1994) amylose content has

been known to be a factor in the formation of resistant starch. In general, starches with high amylose contents are more resistant to enzyme hydrolysis (Li, Jiang, Campbell, Blanco, & Jane, 2008). However, in the current study amylose content was not able to account for the differences observed in resistant starch contents of the isolated starches from the bean varieties. Differences in amylopectin chain lengths may explain the variation in resistant starch contents. Amylopectin with longer chain lengths are less susceptible enzyme hydrolysis due to their ability to form double helices (Li et al., 2008).

The type of resistant starch measured in the raw bean starches was RS2, which refers to native, ungelatinized starch granules. The RS2 content of the native bean starch is reduced once processing conditions cause gelatinization (Bravo, Siddhuraju, & Saura-Calixto, 1998; Ekanayake, Nair, Asp, & Jansz, 2006).

Table 3.2. Physicochemical Properties of Bean Starch of Six *Phaseolus vulgaris* Varieties (g/100g)[a,b]

Starch	Total Starch (%)	Starch Damage (%)	Amylose (%)	Resistant Starch (%)
Black bean	95.96 ± 2.30 a	0.16 ± 0.01 d	28.50 ± 2.19 a	52.89 ± 0.40 b
Dark red kidney bean	90.87 ± 1.92 a	0.25 ± 0.01 b	28.50± 1.44 a	45.20 ± 0.80 c
Light red kidney bean	96.28 ± 1.80 a	0.29 ± 0.01 a	28.63 ± 0.87 a	41.92 ± 0.62 d
Navy bean	91.69 ± 4.51 a	0.16 ± 0.01 d	28.05 ± 1.91 a	51.93 ± 0.77 b
Pinto bean	91.99 ± 5.32 a	0.24 ± 0.01 b	28.01 ± 1.71 a	55.70 ± 0.50 a
Small red bean	92.74 ± 4.22 a	0.21 ± 0.01 c	28.26 ± 1.83 a	51.10 ± 0.80 b

[a] Data represent means and standard deviations (n = 4)

[b] Values within the same column followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference) test

3.4.3. Pasting Characteristics

Pasting properties provide a picture of the functional behavior of a starch in water undergoing heating and cooling periods (Bello-Pérez et al., 2009). Pasting temperature is a measure of the temperature at which a starch suspension starts to thicken (Huang et al., 2007). It also gives an indication of the minimum temperature required for cooking a specific or particular starch. The peak viscosity indicates the water holding capacity of the starch while final viscosity relates to the ability of the starch to form a paste or gel upon cooling (Shimelis, Meaza, Rakshit, 2006).

The RVA profiles of the studied bean starches are presented in Figure 3.1. and the quantitative data are shown in Table 3.3. Bean starches from dark red kidney and light red kidney beans had lower peak viscosities compared to starch samples from navy and pinto beans. Pinto bean had the highest pasting temperature (79.9°C). With respect to the peak viscosity, dark red and light red kidney bean starches had lower peak viscosities than that of navy bean starch. Black bean starch exhibited the highest break down viscosity (1102.5 cP). High breakdown viscosity reflects granular swelling that makes the starch granules more susceptible to shear (Hughes et al., 2009). This indicates that dark red kidney and light red kidney bean starches have more resistance to shear. Setback viscosity values for all bean starches ranged between 2265.5 cP and 4349.5 cP. The faster retrogradation tendency of dark red kidney bean and light red kidney bean starches, indicated by their high setback values, implies that they would be favorable in food products such as gluten-free noodles (Otto, Baik, & Czuchajowska, 1997). The lower pasting temperatures, lower peak and breakdown viscosities, and high setback viscosity observed for light red kidney and dark red kidney bean starches when compared to the other bean starches are indicative of starches with lower amylose content (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002; Chung, Liu, Peter Pauls, et al., 2008; Gupta, Bawa, & Semwal, 2009; Jane et al., 1999). However, in this present study the amount of amylose cannot account for the differences observed since there were no significant differences in amylose content among the starches. Generally, pasting properties of starches have been shown to be influenced by molecular structure (such as molecular weight of amylose and amylopectin, amylopectin chain branch length,and crystallinity) and composition (amylose/ amylopectin ratio and lipid - complexed amylose) (Hughes et al., 2009; Jane et al., 1999). The high weight average molecular

weights of amylopectins (Table 4.3.) for dark red kidney and light red kidney bean starches may contribute to their pasting differences when compared to those of the other bean starches.

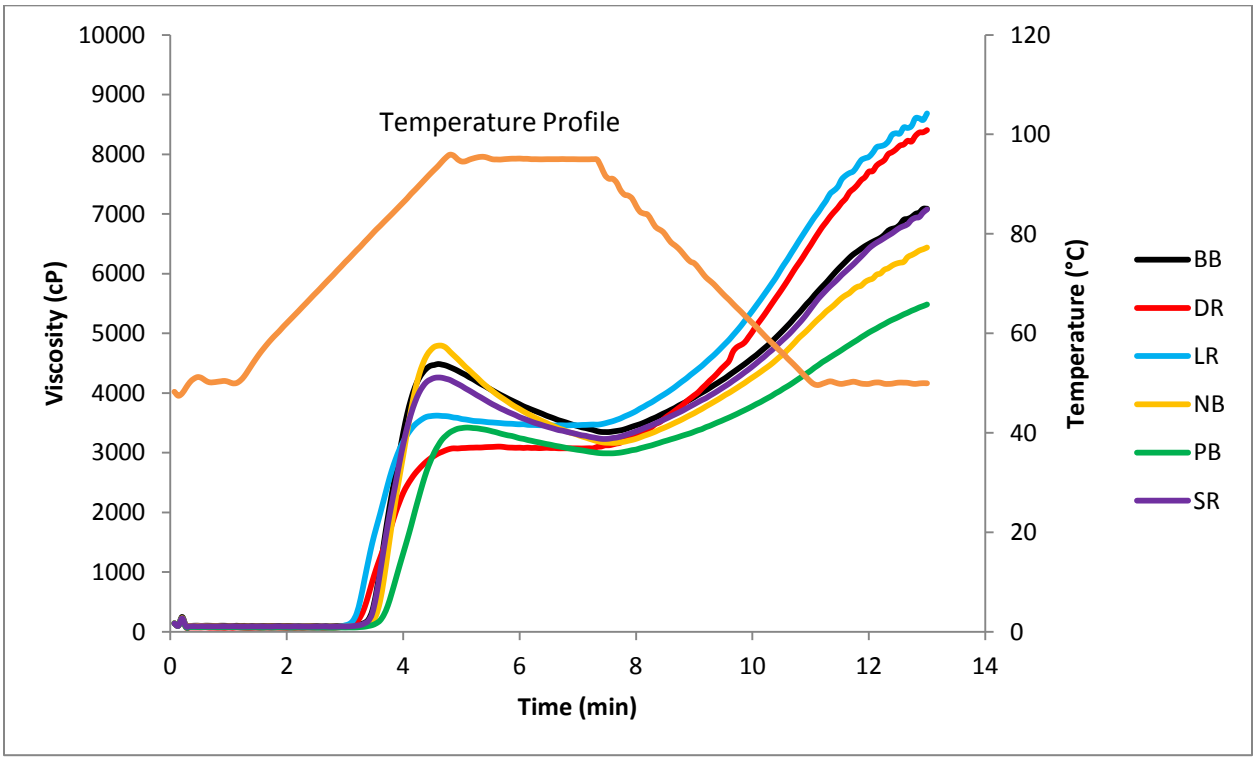


Figure 3.2. Pasting (Rapid Visco Analyzer) profiles of starches isolated from black (BB), dark red kidney (DR), light red kidney (LR), navy (NB), pinto (PB) and small red (SR) beans.

Table 3.3. Pasting Properties of Isolated Bean Starch from Six *Phaseolus vulgaris* Varieties[a,b]

Starch Source	Pasting Temperature (ºC)	Peak Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)	Final Viscosity (cP)
Black bean	76.9 ± 0.3 b	4388.7± 81.0 a	1102.5± 41.7 b	3598.2± 38.5 a,b	6884.5± 84.1 a
Dark red kidney bean	74.9± 0.3 c	2812.3± 213.9 b	118.3± 43.5 d	4349.5± 436.3 a	7043.5± 674.6 a
Light red kidney bean	74.4± 0.6 c	2935.8± 199.8 b	162.2± 16.6 d	4109.5± 197.3 a	6883.0± 413.7 a
Navy bean	78.4± 0.0 b	4793.5± 51.6 a	1631.0± 79.2 a	3273.5± 101.1 a, b	6436.0± 73.5 a
Pinto bean	79.9± 0.3 a	3245.0± 7.8 b	364.0± 38.9 c	2265.5± 33.9 b	5146.5± 13.4 a
Small red bean	77.9± 0.1 b	4234.3± 110.0 a	950.0± 47.4 b	3604.3± 100. a, b	6888.5± 162.6 a

[a] Data represent means and standard deviations (n = 4)

[b] Values within the same column followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference)test

3.4.4. Gelatinization and Retrogradation Properties

Differential scanning calorimetry was used to study the thermal properties of the isolated bean starches. The gelatinization transition temperatures [T_o (onset), T_p (peak) and T_c (conclusion)] and the enthalpy of gelatinization (ΔH) are presented in Table 3.4. There were significant differences ($P<0.05$) in T_o, T_p and T_c among the studied varieties of *Phaseolus vulgaris* starches. The onset gelatinization temperature (T_o) varied from 65.5ºC (light red kidney bean) to 68.8ºC (small red bean). The peak transition temperatures of light red kidney and dark red kidney bean starches were significantly different from those of the other bean starch samples.

A similar trend was also observed for the conclusion transition temperature as well. The smaller transition temperatures may be a result of the low resistant starch contents of light red and dark red kidney bean starches. Conversely, isolated pinto bean starch had the largest resistant starch content and the highest transition temperatures when compared to those of the other starches (Table 3.2.). Black bean starch showed the largest enthalpy of gelatinization (14.5 J/g). Higher gelatinization temperature was an indication of more perfect crystals (Huang et al., 2007). Noda et al. (1998) suggested that the gelatinization transition temperatures are influenced by the molecular organization of the crystalline region, which is related to the distribution of amylopectin short chains, and not by the proportions of crystalline region, which correspond to the amylose/amylopectin ratio. The enthalpy of gelatinization is thought to reflect the total crystallinity of the amylopection polymer (Tester et al., 2004; Tester & Morrison, 1990). Conversely, Cooke & Gidley (1992), proposed that the enthalpy of gelatinization does not show the loss of crystallinity but indicates the loss of the double helical order. Isolated pinto bean starch had the lowest degree of crystallinity (Table 3.5.). The retrogradation properties of bean starches are summarized in Table 3 4. The T_o, T_p and T_c values of retrograded starches were lower than those obtained for the initial gelatinization. Light red kidney and pinto bean starches presented the lowest T_o values and the bean starch isolated from black bean showed the highest T_p. Transition temperatures for all the isolated bean starches were not affected by the amount of amylose present (Table 3.2.).

For retrograded starch, the enthalpy value provides a measure of the energy transformation taking place during the melting of the recrystallized amylopectin chains. Storing gels at lower temperatures results in the formation of starch crystals that melt at lower temperatures. Due to the limited dimensions of the amylopectin chains, the stability of

amylopectin crystallites is lower than that of amylose. Recrystallized amylopectin melts in the range 40-100ºC while amylose is much higher (120-170ºC) (Liu, 2005). Starch samples having amylopectin with longer chains tend to retrograde faster than those with shorter chains (Jane et al., 1999).

Table 3.4. Gelatinization and Retrogradation Parameters for Isolated Bean Starches [a]

Starch Source	T_o [d]	T_p	T_c	ΔH (J/g)
Raw [b]				
Black bean	67.0 ± 0.1 a,b	74.8 ± 0.0 a	87.2 ± 0.1 a	14.5 ± 0.3 a
Dark red kidney bean	66.4 ± 0.3 b,c	72.3 ± 0.4 b	83.6 ± 0.3 c	13.6 ± 0.3 a,b
Light red kidney bean	65.5 ± 0.0 c	71.7 ± 0.1 b	83.0 ± 0.4 c	12.4 ± 0.2 d,c
Navy bean	67.6 ± 0.3 a,b	75.7 ± 0.1 a	86.5 ± 0.6 a, b	11.9 ± 0.0 d
Pinto bean	67.4 ± 0.1 a,b	75.3 ± 0.2 a	87.2 ± 0.3 a	13.1± 0.0 b,c
Small red bean	68.0 ± 0.1 a	75.4± 0.1a	86.2 ± 0.8 b	13.3 ± 0.1 b,c
Retrograded [c]				
Black bean	47.7 ± 0.4 a	61.3 ± 0.4 a	74.5 ± 0.1 a	6.1 ± 0.2 a
Dark red kidney bean	47.3 ± 0.1 a	59.4 ± 0.3 b	72.3 ± 0.4 b,c	5.1 ± 0.1 b
Light red kidney bean	46.2 ± 0.4 b	57.6 ± 0.2 c	71.4 ± 0.3 c	6.0 ± 0.6 a
Navy bean	47.4± 0.1 a	58.6 ± 0.0 b	72.5 ± 0.4 b,c	5.2 ± 0.0 b
Pinto bean	46.9 ± 0.7 a,b	58.6 ± 0.3 b	73.6 ± 0.0 a,b	6.2 ± 0.1 a
Small red bean	47.3 ± 0.1 a	58.6 ± 0.4 b	72.3 ± 0.3 b,c	5.9 ± 0.3 a

[a] Data represent means and standard deviations (n = 2).

[b] Values within the same column for raw samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference) test.

[c] Values within the same column for retrograded samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD test.

[d] T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, ΔH: enthalpy change.

3.4.5. Crystallinity

The proportion of starch present in the crystalline form was the greatest for navy bean starch (36.06%), whereas, pinto bean starch had the lowest value (24.86%) (Table 3.5.). Similar values of crystallinity have been reported for legume starches (black bean starch, 32.1% and pinto bean starch 33% at a moisture content of 19%) (Zhou et al., 2004). Ambigaipalan et al.(2011) studied the structure of faba bean, black bean and pinto bean cultivar starches and crystallinity ranged from 20.3% to 23.1%. Isolated pinto bean starch with its low crystallinity had the largest resistant starch content (55.86%) (Table 3.2.). However, it should be noted that the degree of crystallinity is not always linked with the resistance of starch has to the activity of amylases (Jane et al., 1999). For example, native potato starch has a high degree of crystallinity and is very resistant to amylases whereas, cereal starches are characterized by a high degree of crystallinity but are more susceptible than potato starch to enzymatic activity (Englyst, 2004; Jane et al., 1999).

Crystallinity differences among legume starches is attributed to or influenced by: (1) crystallite size, (2) number of crystallites that are arranged in a crystal array (3) moisture content and (4) proportions of A-type and B-type polymorphs. Crystallites are microscopic crystalline regions found within a solid polymer below the crystalline melting temperature (Hoover et al., 2010a). Some investigators calculate the B-type polymorph based on an X-ray diffraction peak centered at 5.2° 2θ (Davydova et al., 1995). This peak was minimal or non-existent in the X-ray diffractograms of the starches in this study. This could be attributed to the moisture content (11.3%) of the starch samples. Zimeri and Kokin (2002) found that pre-solubilized inulin's crystallinity depended on moisture content and that crystallinity was low at low moisture contents. The A-type polymorph contents of the starches used in this study was determined from

a calibration curve derived from mixtures of pure B-type (0–100% potato) and pure A-type (100–0% waxy corn). An X-ray diffraction peak centered at 15.2º 2θ was directly proportional to the amount of A-type polymorph in the sample (r = 0.988). The amount of B-type polymorph ranged from 15.22 % to 19.59% (Table 3.5). Ambigaipalan et al. (2011) reported that the B-type polymorph contents for faba bean starch (20.6-25.0%), pinto bean starch (21.3-23.1%) and black bean starch (15.4-17.9%). These B-type polymorph values are comparable to the values determined in this study. All dry bean starches show a characteristic C-type X-ray diffraction pattern which is a mixture of A and B-type crystalline structures (Chibbar et al., 2010; Davydova, Leont, Genin, Sasovc, & Bograchevap, 1995; Hoover & Ratnayake, 2002; Hoover & Sosulski, 1985a; Pérez, Baldwin, & Gallant, 2009; Zhou et al., 2004). X-ray diffraction pattern may depend on starch origin as well as environmental growth conditions (Hoover et al., 2010).

Table 3.5. Crystallinity and B-type Polymorph Composition of Starch Isolated from Dry Beans[a]

Starch Source	Crystallinity (%)	B-type Polymorph (%)
Black bean	26.79 ± 3.5 b,c	15.22 ± 0.16 b
Dark red kidney bean	25.35 ± 0.030 b,c	17.36 ± 0.050 a,b
Light red kidney bean	27.94 ± 1.4 b,c	17.04 ± 2.6 a,b
Navy bean	36.06 ± 3.6 a	19.09 ± 0.60 a
Pinto bean	24.86 ± 0.50 c	17.25 ± 1.4 a,b
Small red bean	31.17 ± 1.8 a,b	19.59 ± 0.22 a

[a] Values followed by the same letter within a column are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference) test.

3.5. CONCLUSIONS

The findings of the current study showed that the physicochemical properties of starches isolated from dark red kidney and light red kidney beans differed from those of the other isolated bean starches. Consequently, dark red kidney and light red kidney bean starches may have different end-use applications than the other common bean starches. Dark red kidney and light red kidney bean starches were found to have lower resistant starch contents, lower onset, peak, and conclusion gelatinization temperatures, lower pasting temperatures, lower peak viscosities, lower breakdown viscosities and higher setback viscosities, when compared to starches isolated from black, navy, pinto and small red beans. C-type X-ray diffraction patterns were found for the isolated bean starches of all six studied varieties. Starch from pinto beans displayed the highest resistant starch value (55.7%) and had the lowest degree of crystallinity (24.9%). No

significant differences were observed in amylose contents among the bean starches. Therefore, amylose content did not contribute to the differences observed in the physicochemical properties of the isolated starch samples studied.

APPENDICES

APPENDIX A

X-RAY DIFFRACTION STANDARD CURVE USED IN THE DETERMINATION OF THE POLYMORPHIC COMPOSITION OF ISOLATED NATIVE STARCH

A calibration curve was prepared by mixing pure B-type starch (0–100% potato) and pure A-type (100–0% waxy corn). X-ray diffractograms were obtained using an X-ray diffractometer (XDS 2000, Scintag Inc, CA,USA) with copper K-alpha emission radiation and operating conditions of target voltage 35kV; current 35 mA; scanning range 3-35°; scan speed 0.20°/min; step time 6 s; divergence slit width 2.00 mm; scatter slit width 4.00 mm and receiving slit width 0.200 mm. The moisture contents of all starch samples were adjusted to approximately 11.3% (moisture contents of the isolated starches were in this range, Table 3.1.) by storage at room temperature in a desiccator over saturated LiCl for 14 days. X-ray diffractograms used to generate the calibration curve are displayed in Figure 1-A.

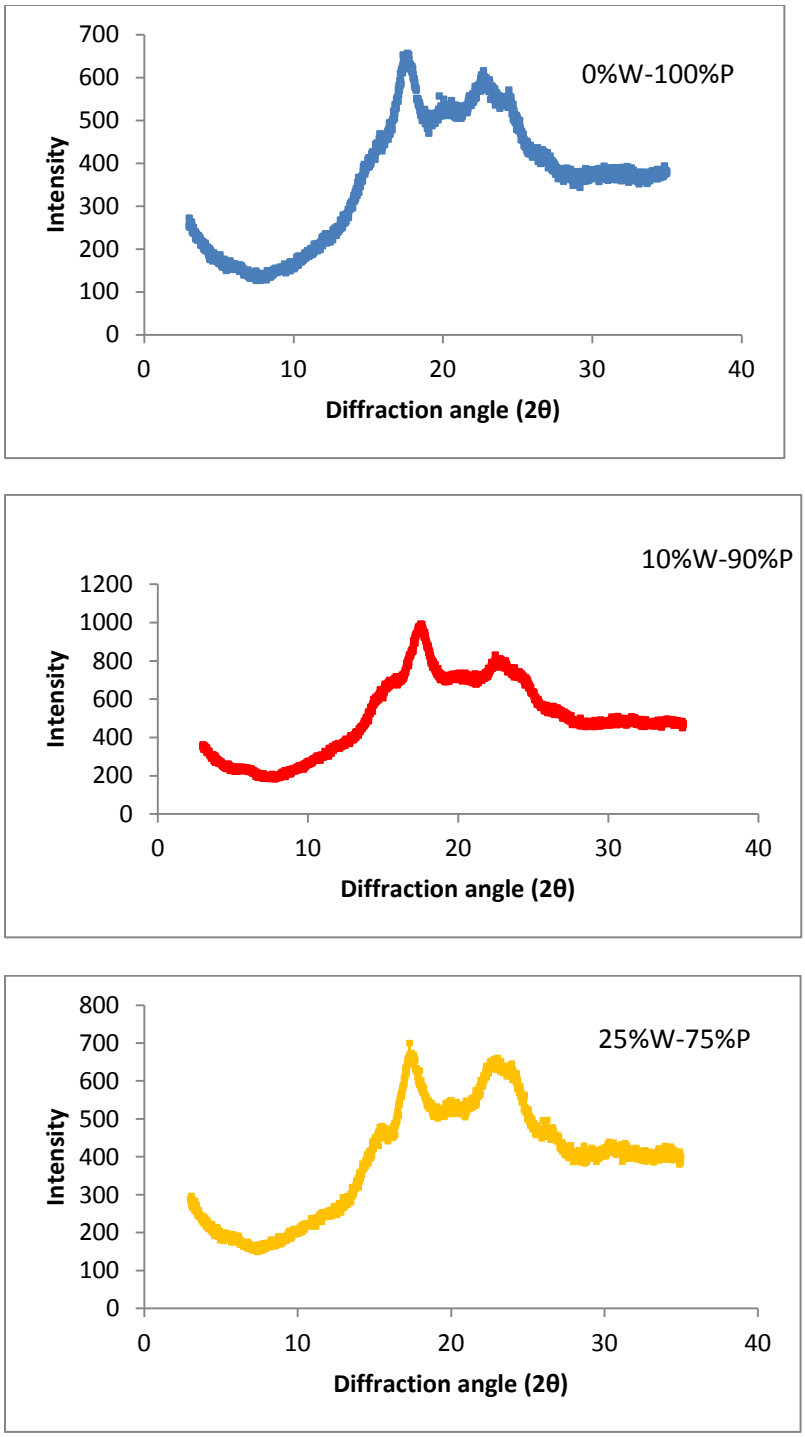
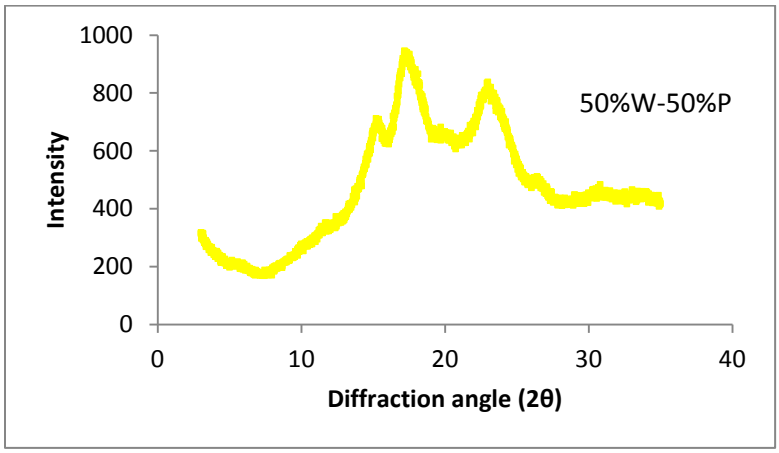
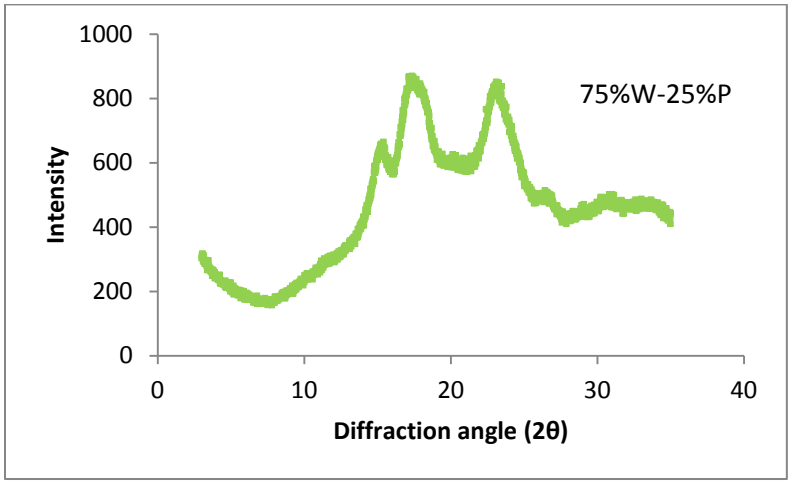


Figure 1-A. X-ray diffractograms of B-type starch (0–100% potato, P) and pure A-type (100–0% waxy corn, W)

Figure 1-A. (cont'd).





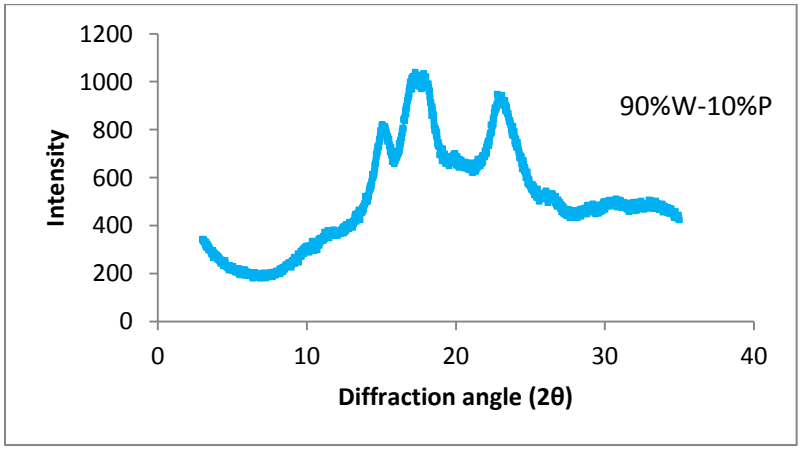
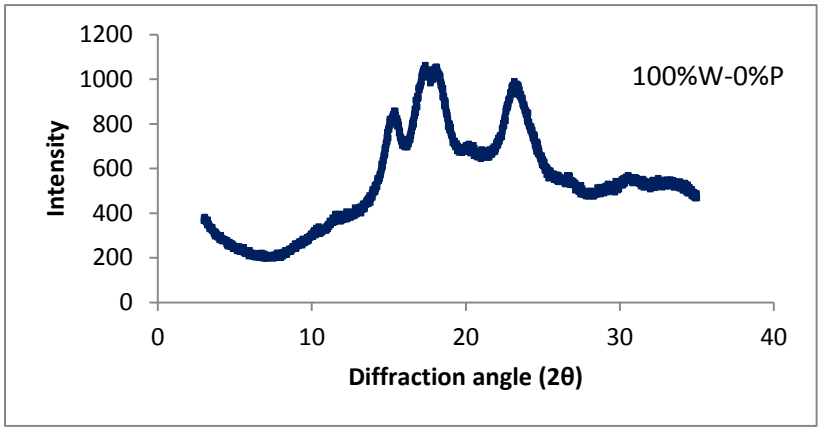


Figure 1-A. (cont'd).



APPENDIX B

PASTING PROPERTIES OF ISOLATED NATIVE BEAN STARCHES

Table 1-B. Pasting Properties of Isolated Native Bean Starches[a,b]

Names	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Setback Viscosity (RVU)	Peak Time (min)	Pasting Temperature (°C)
BB1	369.0833	269.5833	99.5000	585.0000	315.4167	4.5996	76.7500
BB1	378.8333	288.0833	90.7500	596.4167	308.3333	4.5996	76.7000
BB2	362.1667	274.0833	88.0833	560.9167	286.8333	4.5996	76.7500
BB 2	352.8333	263.6667	89.1667	552.5000	288.8333	4.5330	77.5500
DR1	242.0833	231.1667	10.9167	636.3333	405.1667	5.4663	75.1500
DR1	280.4167	277.5000	2.9167	764.3333	486.8333	6.9996	75.1000
DR2	213.5000	199.5833	13.9167	489.0833	289.5000	4.8663	75.1000
DR2	201.4167	189.7500	11.6667	458.0833	268.3333	4.7330	74.3500
LR1	318.6667	306.3333	12.3333	762.0000	455.6667	4.5996	73.5000
LR1	194.1667	181.4167	12.7500	433.9167	252.5000	6.2663	75.2000
LR2	180.7500	167.5833	13.1667	412.6667	245.0833	5.5996	74.3500
LR2	285.0000	269.1667	15.8333	685.7500	416.5833	4.4663	74.4000
NB1	396.4167	265.1667	131.2500	532.0000	266.8333	4.6663	78.3500
NB1	402.5000	261.9167	140.5833	540.6667	278.7500	4.5996	78.4000
NB2	529.0000	357.2500	171.7500	715.6667	358.4167	4.3330	77.5500
NB2	455.5833	310.0833	145.5000	612.5833	302.5000	4.3996	77.5500
PB1	285.5833	252.6667	32.9167	457.2500	204.5833	5.1996	79.2500
PB1	285.0000	245.2500	39.7500	456.5000	211.2500	5.0663	80.0500
PB2	254.9167	231.7500	23.1667	402.0833	170.3333	5.3330	80.0500
PB2	256.1667	230.6667	25.5000	399.6667	169.0000	5.3996	80.1000
SR1	347.9167	265.1667	82.7500	574.6667	309.5000	4.5996	77.5500
SR1	362.2500	273.3333	88.9167	604.9167	331.5833	4.5996	77.4500
SR2	356.4167	281.4167	75.0000	562.3333	280.9167	4.7330	78.4000
SR2	344.8333	274.8333	70.0000	554.2500	279.4167	4.7996	78.3500

[a] RVU: rapid visco units

[b] BB: black bean, DR: dark red kidney bean; LR: light red kidney bean; NB: navy bean; PB: pinto bean; SR: small red bean

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CHAPTER 4

EFFECTS OF DIFFERENT COOKING METHODS ON THE DIGESTIBILITY AND MOLECULAR WEIGHT DISTRIBUTION OF ISOLATED BEAN (*PHASEOLUS VULGARIS*) STARCHES

4.1. ABSTRACT

The weight average molecular weight distribution (Mw) of starches isolated from native, canned, and stovetop-cooked beans were analyzed using high-performance size exclusion chromatography with multi-angle laser light scattering and refractive index detectors (HPSEC-MALLS-RI). Results revealed that amylose of isolated native dark red kidney bean starch had the smallest Mw (1.0×10^6 g/mol), whereas isolated native pinto bean starch had the largest value of Mw (1.8×10^6 g/mol). The Mw values of amylopectin for isolated native bean starch ranged from 2.4×10^7 g/mol to 3.9×10^7 g/mol. Isolated canned and stovetop-cooked bean starches displayed a mono-modal Mw distribution, with a reduction in high molecular weight fractions, whereas isolated native bean starch Mw distribution was bi-modal. Results of *in vitro* α-amylase starch hydrolysis showed ranges of rapidly digestible starch (RDS) (1.95-2.71%), slowly digestible starch (SDS) (14.36-17.39%), and resistant starch (RS) (78.95-83.7%) among the tested isolated native *Phaseolus vulgaris* starches. However, RDS, SDS, and RS fractions in isolated canned bean starches ranged from 7.58-13.21%, 20.75-24.90%, and 63.17- 68.54%, respectively. Isolated stovetop-cooked bean starches yielded similar results: RDS, 7.37-10.61%; SDS, 22.55-26.84%; and RS, 62.55-68.59%). The hydrolysis indices (HI) and estimated glycemic indices (GI) were marginally greater for isolated canned bean starches than for stovetop-cooked starches.

4.2. INTRODUCTION

The common bean (*Phaseolus vulgaris*) is one of the main leguminous plants consumed by humans. Dry beans are rich in proteins, carbohydrates, minerals and dietary fiber. The consumption of dry beans has been linked to reduced risk of diabetes, obesity and heart disease (Anderson et al., 1999). The major carbohydrate found in beans is starch.

Starch is composed of two distinct types of macromolecules: amylose and amylopectin. Amylose is essentially linear with α–1,4 glycosidic linkages, whereas amylopectin is highly branched with short α–1,4 linked glucose chains and 5-6% non-randomly distributed α–1,6 glycosidic linkages. Amylose has an average molecular weight of approximately 1×10^5 to 1×10^6 g/mol, with a degree of polymerization by number (DP_n) in the range of 324-4920. Conversely, amylopectin is a much larger molecule than amylose with a molecular weight of 1×10^7 to 1×10^9 g/mol and DP_n of 9600-15900 (Sajilata, Singhal, & Kulkarni, 2006; Tester, Karkalas, & Qi, 2004; Wang, Bogracheva & Hedley, 1998). The unit chains present in amylopectins (18-25 units) are shorter than those found in amylose molecules (Tester et al., 2004). The amylose and amylopectin contents of normal starches are within the ranges of 20-30% and 70-80%, respectively (Gidley et al., 2010; Han & Lim, 2004; Hasjim, Lavau, Gidley, & Gilbert, 2010a; Yoo & Jane, 2002). The functionality of starch depends to a great extent on the molecular structure, size and weight of these components (Othman et al., 2010). Starch molecular weight is often influenced by botanical source, starch isolation and dissolution procedures, as well as the technique used to determine its molecular weight.

In order for starch to be accurately characterized, the macromolecule needs to be completely dissolved in the solvent of choice. Dissolution is usually accomplished with dimethyl sulfoxide (DMSO) or aqueous DMSO solutions, sometimes with the addition of salts

such as LiBr and $NaNO_3$ (Yokoyama, Renner-Nantz, & Shoemaker, 1998; Othman, Al-Assaf, & Hassan, 2010; Cave, Seabrook, Gidley, & Gilbert, 2009). Several techniques have been used for the separation and structural characterization of starch including ultracentrifugation, size-exclusion chromatography and light scattering (Wyatt, 1993; Bello-Pérez, Rodriguez-Ambriz, Sanchez-Rivera, & Agama-Acevedo, 2009; Ratnayake, Hoover, & Warkentin, 2002; Yoo & Jane, 2002). Size-exclusion chromatography (SEC), which utilizes calibration standards is commonly used to determine the weight average molecular weight (Mw) of starch. Recently, high-performance-size-exclusion chromatography (HPSEC) equipped with multi-angle laser light scattering (MALLS) and refractive index (RI) detectors has been used to determine Mw of polymers. This method provides absolute molecular weight without the need for calibration standards (Podzimek, 2014; Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013; Witt, Gidley, & Gilbert, 2010; Yoo & Jane, 2002).

Starch can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) according to the rate of glucose release through enzymatic action and its absorption in the gastrointestinal tract (Englyst, Kingman, & Cummings, 1992). RDS is the starch fraction that is digested within the first 20 min of incubation with α-amylase and amyloglucosidase. It is also the portion of starch that causes a sudden increase in blood glucose level after ingestion. SDS is the starch fraction that is digested completely, but slowly, in the small intestine and is designated as the portion of starch digested from 20 to 120 min of incubation, RS is the fraction of starch remaining after 120 min of *in vitro* enzymatic digestion and is fermented *in vivo* by bacteria in the large intestine (Englyst, 2004; Englyst et al., 1992; Englyst & Englyst, 2005).

Dietary starch varies greatly in digestibility and studies have shown that legume starches are more resistant to digestion than cereal starches (Englyst, Kingman, & Cummings, 1992). The type and extent of processing treatment can mainly affect the digestibility of starch by influencing its gelatinization and retrogradation (Annison & Topping, 1994; Sajilata et al., 2006). When starch is fully gelatinized and dispersed, the starch becomes easily digestible. However as the gel cools and ages, the polymers once more form a partially crystalline structure which is able to resist digestion (Muir & O'Dea, 1992). The structure formed is known as retrograded resistant starch (Roger & Colonna, 1992). Amylose content, degree of crystallinity, and the presence of other components interacting with starch (amylose-lipid complexes) are also able to influence the digestibility of starch. High amylose starches (containing 70% amylose) and starches with a high degree of crystallinity limit enzymatic starch hydrolysis. (Colonna et al., 1992; Du et al., 2014; Jane et al., 1999).

Beans must be processed (such as by roasting, boiling and canning) prior to consumption in order to improve their palatability. These different heat treatments can cause alterations in the bean structure and also influence its nutritional characteristics (Kaur & Sandhu, 2010; Rehman & Shah, 2005). The heating of starch granules, or dry beans in moderate or excess water, such as during normal home cooking or canning, causes starch to gelatinize. This results in the rupture and disintegration of the semi-crystalline granular structure of starch via the disruptions of inter- and intra-molecular hydrogen bonds of starch molecules (Tester & Morrison, 1990a, 1990b). Gelatinization increases the susceptibility of starch to digestive enzymes, as the swelling of starch granules increases the accessibility of enzymes to penetrate into the granules. Although cooking or another heat treatment gelatinizes granular starch, it is noted that starch granules in cooked bean seeds may be trapped in the protein and cell-wall matrices, inhibiting both the

swelling of starch granules and the solubility of starch molecules during cooking. When the rate of starch digestion is decreased in the human digestive tract, postprandial glucose and insulin responses are reduced and/or delayed (Thorne et al., 1983). Determination of starch digestibility and resistant starch, content of food ingredients and processed foods will provide nutritional information to consumers and others (Perera, Meda, & Tyler, 2010; Roger & Colonna, 1992). The objective of this study is to determine the effect of canning and traditional cooking on the *in vitro* enzymatic digestibility and molecular weight distributions of isolated bean starches.

4.3. MATERIALS AND METHODS

4.3.1. Materials

Six varieties of the species *Phaseolus vulgaris*, black bean (*Zorro*), dark red kidney (*Redhawk*), light red kidney bean (*CELRK*), small red bean (*Merlot*), pinto bean (*P07863*), and navy bean (*Medalist*) were obtained from the USDA-ADRS located in East Lansing, MI, USA. The beans grown in Michigan were harvested in two growing seasons, 2011 and 2012 for each bean variety.

4.3.2. Stovetop-Cooking of Dry Beans

The cooking of the beans was carried out in a traditional manner done in many households. Briefly, dried beans (1.5 kg) was soaked in distilled water (3000 mL) overnight at room temperature. The water was discarded and the soaked beans were rinsed with distilled water. The beans were then placed in a 5-quart stainless steel pot, distilled water was added (3000 mL) and the beans were brought to a boil (100°C)for 15 min on a Kenmore electric stove. The heat was reduced and the beans were allowed to simmer gently (using the simmer option on the stove) for 1h. The cooked beans were allowed to cool to room temperature (approximately 25°C). The cooked beans were stored covered in their cooking water at room temperature (25°C). After two days, starch was extracted from the beans.

4.3.3. Canning of Dried Beans

Dried beans (3 kg) were soaked in distilled water (6000 mL) for 14h. The water was discarded and the beans were rinsed with distilled water. Approximately 450 g amounts of soaked beans were placed in cans (No. 3), hot distilled water (90°C) was added to just cover the

top of the beans, lids were placed on top and the cans were sealed with a Dixie Double Seamer (Athens, Georgia, USA). The sealed cans were then placed in a basket in an FMC retort (FMC Corporation, Food Processing Systems Division, Madera, CA) that heated the cans to 125°C for 15 min and subsequently cooled them to 38°C for over 35 min. The canned beans were stored at room temperature (25°C) for two days before starch extraction. For each variety, starch was extracted from the combined contents of all the cans of that variety.

4.3.4. Starch Extraction

Starch was isolated from native, canned, and stovetop-cooked beans according to the method of Hoover & Sosulski (1985). Air-dried isolated starch samples were stored in a desiccator at room temperature for future analyses.

4.3.5. Moisture Content

Quantitative estimations of moisture of isolated native, canned and stovetop-cooked bean starches were performed using standard[AOAC Method 925.09 (2000)]. Analyses were performed in duplicate, and mean values were reported

4.3.6. Starch Digestibilty

Starch digestibility was analyzed according to the method described by Englyst et al. (1992) with modifications. For each of the six varieties, isolated bean starch (0.5 g, w/w) and 0.1 M sodium acetate buffer (pH 5.2, 20 mL) were added to a 50-mL screw-capped polypropylene centrifuge tube and mixed by vortexing for 5 min. After mixing, guar gum (50 mg) and 6 glass

beads were added to each tube. The tubes were then incubated with pancreatin (P-7545, 8× USP Specifications, Sigma-Aldrich Co., St. Louis, MO), invertase (I-4504, Sigma-Aldrich Co., St. Louis, MO) and amyloglucosidase (from *A. niger*, 3300 U/ml of soluble starch, Megazyme Ltd, Co. Wicklow, Ireland) mixture (5 mL) at 37°C. After 20 min (G20) and 120 min (G120) of incubation of a 0.5 mL aliquot of hydrolysate from the tube were removed and added to absolute ethanol (5 mL) in another tube to stop the enzymatic reaction. The glucose released in each measurement was determined using a glucose oxidase peroxidase diagnostic kit (Megazyme Ltd, Co.Wicklow, Ireland). The remaining contents of each 50 mL tube was heated in a boiling water bath, treated with 7 M KOH (10 mL), and hydrolyzed further with 0.15 mL of amyloglucosidase (50 U/mL) to determine total glucose (TG) after 180 min. Glucose content was also measured using the glucose oxidase peroxidase diagnostic kit. The RDS, SDS, and RS percentages of the total starch were calculated from the values of G20, G120 and TG as follows:

RDS = G20 × 0.9

SDS= (G120-G20) × 0.9

TS = TG × 0.9

RS = TS- (RDS + SDS)

4.3.6.1. Hydrolysis Index (HI) and Glycemic Index (GI)

The hydrolysis curve used to calculate the hydrolysis indices of samples was obtained utilizing the procedure as described in Section 4.3.6, except that hydrolysates were removed and analyzed at 20, 40, 60, 90, 120, and 150 min time points. Percent glucose and starch were calculated for each time point (Englyst et al., 1992) and plotted against time (x-axis) to obtain the hydrolysis curve for each bean variety. The hydrolysis index (HI) was obtained by dividing the

area under the hydrolysis curve (0-180 min) of the isolated bean variety starch sample by the area obtained for the hydrolysis curve of a standard material (white bread, Table 4.1.). The area under the curve was calculated using the trapezoidal method (Microsoft Excel, 2007).

HI ={[Area under curve of isolated bean starch (dry weight)]/ [Area under curve of white bread (dry weight)]} x 100

The estimated glycemic index (GI) was predicted with the formula of Goñi, Garcia-Alonso, & Saura-Calixto (1997):

GI = 39.71 + (0.549 x HI).

Table 4.1. Nutrient Label for Sunbeam Giant Enriched White Bread[a]

Ingredients	Mass per Serving Size (g)[b]
Total carbohydrate	28
Dietary fiber	1
Sugars	3
Protein	4
Total fat	1.5
Trans. fat	0

[a] White bread used in the digestibility study was commercially produced and was purchased from a local supermarket located in East Lansing, MI
[b] Serving size: 57 g

4.3.7. Preparation of Starch Dispersions for the HPSEC-MALLS-RI System

Starch solutions (10 mg/mL) were prepared for HPSEC analyses. Briefly, isolated starch sample (50 mg) was weighed into a screw-capped glass culture tube. 50 mM LiBr in 90%

DMSO-10% de-ionized water solution (5 mL) was added. The tightly capped tube was vortexed for 10 s to disperse the starch. The tubes were placed in a water bath at 80ºC and individual tubes were vortexed every 15min. After two hours, the tubes were removed and shaken on an orbital shaker (Cole-Parmer, IL, USA) at 200 rpm overnight at room temperature. The starch dispersions were centrifuged (Sorvall RT6000B centrifuge, Thermo Scientific Inc., USA) at 4600 x g for 10 min at 20ºC and the supernatants were analyzed with the HPSEC-MALLS-RI system. Analyses for bean starch samples were done in duplicate.

4.3.8. Molecular Weight Determination

The weight-average molecular weights of isolated bean starches were determined using high performance size-exclusion chromatography (HPSEC, Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). HPSEC analyses were conducted using PSS columns: precolumn, Gram10000 and Gram1000 (Polymer Standards Services, Mainz, Germany) with packing materials having pore diameters of 10 μm. A Wyatt miniDawn TREOS multi-angle laser light scattering detector (Wyatt Tech. Corp., Santa Barbara, CA, USA) and a Wyatt Optilab refractive index detector (Wyatt Tech. Corp., Santa Barbara, CA, USA) were used to measure light scattering and refractive index values, respectively. The laser sources of these detectors were operated at 658 nm. The HPSEC analyses of the starches isolated from bean samples were performed at 70ºC for 120 min, with sample concentrations of 10 mg/mL, injection volume of 100 μL and eluent flow rates of 0.2 mL/ min. 50 mM LiBr in 90% DMSO-10% de-ionized water was used as the solvent. The data processing was performed using the ASTRA 5.3.4.18 software package (Wyatt Tech. Corp., Santa Barbara, CA, USA). Pullulan standards: (342 g/mol, 57191;

6 x 10^3 g/mol, 18179; 110 x 10^3 g/mol, 47053; 800 x 10^3 g/mol, 18789; 1600 x 10^3 g/mol, 61943) and dextran standards: 1 x 10^3 g/mol, 00268; 5 x 10^3 g/mol, 00269; 50 x 10^3 g/mol, 00891; 410 x 10^3 g/mol, 00895) (Polymer Standards Services, Mainz, Germany) were used to verify the light scattering method. Four runs were done for each variety and sample treatment.

4.3.9. Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Analyses of variance (ANOVA) were performed and the mean separations were evaluated by Tukeys HSD test ($p < 0.05$).

4.4. RESULTS AND DISCUSSION

4.4.1. *In vitro* Digestibility

The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents of the isolated native, canned and stovetop-cooked bean starch samples are presented in Table 4.2. All isolated native bean starch samples were lower in RDS (1.95-2.71%) and higher in RS (78.95-83.71%) when compared to canned bean starch samples and stovetop-cooked bean starch samples. The RDS contents of isolated stovetop cooked and canned bean starches ranged between 7.58-13.21% and 7.37-10.61% respectively. RDS is rapidly and completely digested in the small intestine and is associated with more rapid elevation of postprandial plasma glucose. The RDS and RS values for native isolated bean starches were comparable to the respective RDS and RS contents of raw potato starch determined by Englyst et al., (1992). Native starches are very resistant to hydrolysis by pancreatic amylase (Chung, Liu, & Hoover, 2009). Ancona, Campos, Guerrero, & Ortiz (2011) postulated that the crystalline structure of native starch protects their glycosidic bonds and limits enzymatic hydrolysis. The RS levels of isolated native starches determined by the Megazyme method (Table 3.2.) were considerably lower than those obtained using Englyst et al. (1992) protocol. The disparity may be due to a significant reduction in the duration of enyme digestion (2 h) used in Englyst et al. (1992) protocol as opposed to the 16 h of enzyme digestion employed in the Megazyme protocol. Additionally, instead of directly measuring glucose originating from RS as in the Megazyme protocol, Englyst et al. (1992) method indirectly estimates total RS. This indirect measurement of RS includes any RS2 and RS3 in the sample (Perera, Meda, & Tyler, 2010).

In starches that have undergone heat-moisture treatments, gelatinization has taken place, which occurs with a loss in crystallinity. During gelatinization the starch granules are swollen

and ruptured and are more susceptible to hydrolysis by α-amylase (Bertolini, 2009; Jenkins & Donald, 1998). It can be postulated that a greater degree of crystallinity in starch reduces enzymatic starch hydrolysis (Hoover & Zhou, 2003). This phenomenon was observed for isolated native navy bean starch which had the highest level of crystallinity (36.1% , Table 3.5.). However, in this present study, the reverse also occurred where isolated native pinto bean starch had the lowest degree of crystallinity (24.9%, Table 3.5.) also had the lowest level of RDS and the highest level of RS (Table 4.2.). It is possible that enzymatic hydrolysis is not only dependent on overall crystallinity but on an interplay of many factors such as amylose content and amylopectin branch chain lengths. Amylose contents of the isolated native bean starch samples did not affect the enzymatic digestibility in this study since no significant differences were observed in the amylose contents of the six bean varieties. Slowly digestible starch is a desirable form of dietary starch. Isolated native light red kidney bean starch had the largest amount of SDS (17.39%) and the smallest amount of RS (78.95%). Only marginal differences in the RDS, SDS and RS contents were observed among isolated canned and stovetop-cooked starches. In general, isolated starches from canned and cooked beans contained greater amounts of RDS, SDS and RS when compared to their native starch counterpart samples.

The extent of hydrolysis (after 180 min) among the native starches ranged from 24.9% for black beans to 27.3% for light red kidney beans (Figure 4.1.). All isolated native bean starches showed similar hydrolysis rates during the initial 40 min with small differences in the latter stages (Figure 4.1.). Hoover and Sosulski (1985) showed that after 6h digestion with porcine pancreatic α-amylase, four dry bean starches had hydrolysis rate values ranging from 26% for isolated native navy bean starch to 35% for isolated native black bean starch. In this current study isolated native black bean starch was enzymatically hydrolyzed to a lesser extent

(7.7%) than in the study conducted by Hoover and Sosulski (1985). Variations in the extent of starch hydrolysis may have been due to different enzyme and substrate concentrations in this study compared to the study conducted by Hoover and Sosulski (1985). The higher degree of hydrolysis for light red kidney bean starch appears to be associated with its higher RDS content. A similar phenomenon was also observed with isolated native small red bean and dark red kidney bean starches. The enzyme starch hydrolysis profiles for isolated stovetop-cooked and canned bean starches are illustrated in Figures 4.2. and 4.3. Isolated starches from stovetop-cooked and canned beans displayed a greater extent of starch hydrolysis (39.8-8.4%) than the starches that were isolated from raw whole beans (24.9-27.3%) (Figure 4.1.). Although, the extent of starch hydrolysis was found to be higher in the processed starch samples than the native starches; however, when compared with the degree of starch hydrolysis for corn starch (74%) (Hoover & Sosulski, 1985a), the processed dry bean starches were more resistant to α-amylase hydrolysis. This may be due to bean starch not being fully gelatinized when processed and therefore the physical nature of the starch granule limits starch hydrolysis and/or leads to the formation of retrograded resistant starch (RS3) (Björck et al., 1994; Tovar et al., 1992; Tovar & Melito, 1996). The resistant starch content of the processed isolated bean starches ranged from 62.5% to 68.6%.

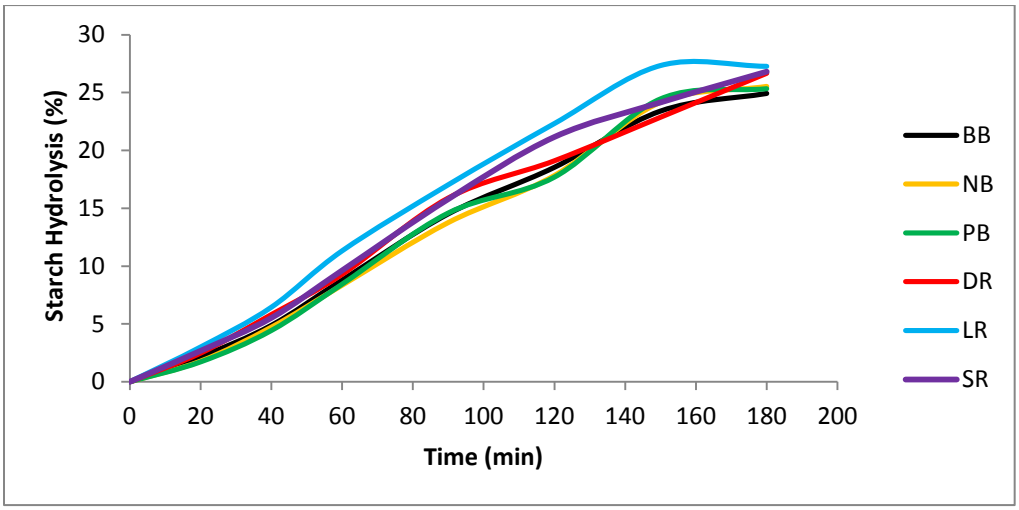


Figure 4.1. Starch hydrolysis curves for isolated native bean starches of six *Phaseolus vulgaris* varieties.[a]

[a]BB: isolated native black bean starch; NB: isolated native navy bean starch; PB: isolated native pinto bean starch; DR: isolated native dark red kidney bean starch; LR: isolated native light red kidney bean starch; SR: isolated native small red bean starch

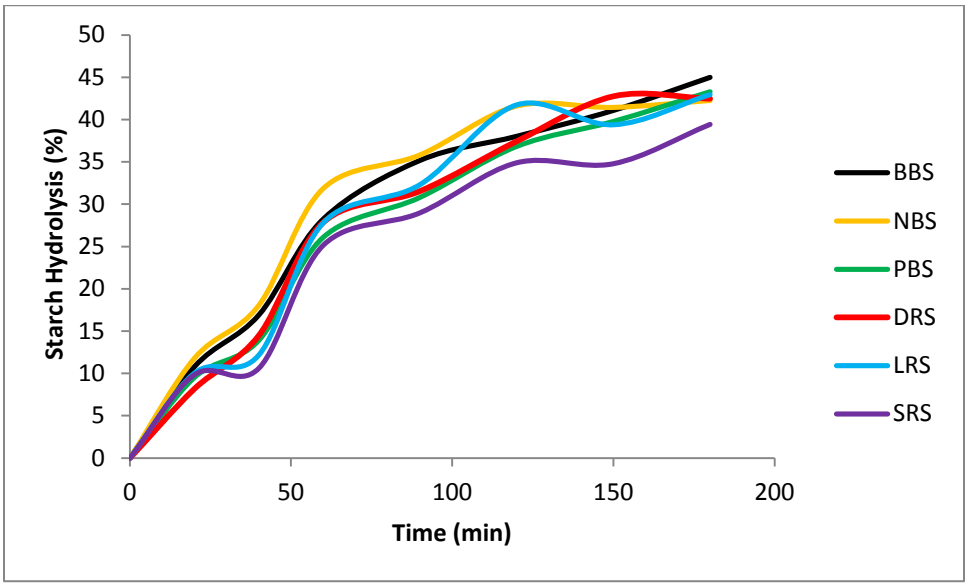


Figure 4.2. Starch hydrolysis curves for isolated stovetop-cooked bean starches of six *Phaseolus vulgaris* varieties.[a]

[a] BBS: isolated stovetop-cooked black bean starch; NBS: isolated stovetop-cooked navy bean starch; PBS: isolated stovetop-cooked pinto bean starch; DRS: isolated stovetop-cooked dark red kidney bean starch; LRS: isolated stovetop-cooked light red kidney bean starch; SRS: isolated stovetop-cooked small red bean starch.

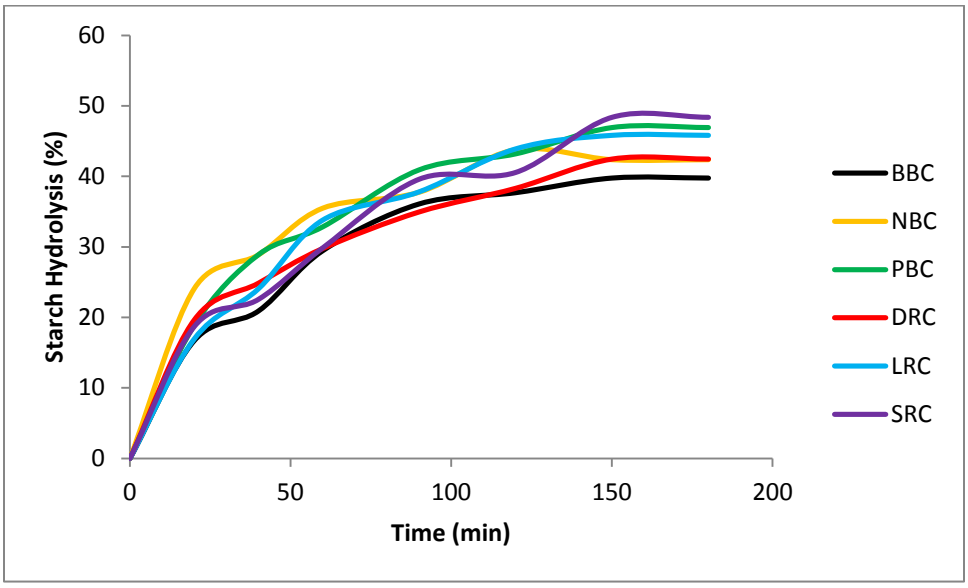


Figure 4.3. Starch hydrolysis curves for isolated canned bean starches of six *Phaseolus vulgaris* varieties. [a]

[a] BBC: isolated canned black bean starch; NBC: isolated canned navy bean starch; PBC: isolated canned pinto bean starch; DRC: isolated canned dark red kidney bean starch; LRC: isolated stovetop cooked light red kidney bean starch , SRC: isolated canned small red bean starch.

Table 4. 2. Rapidly Digestible Starch, Slowly Digestible Starch and Resistant Starch Contents of Isolated Starch from Raw, Stovetop-Cooked, and Canned Beans [a,b]

Starch Source	RDS (%)	SDS (%)	RS (%)
Ra w [c]			
Black bean	1.95 ± 0.02 b,d c	14.69 ± 0.05 a,b	83.34 ± 0.03 a,b
Dark red kidney bean	2.23± 0.01 a,b,c	14.96 ± 0.41 a,b	82.50 ± 0.42a,b
Light red kidney bean	2.71 ±0.12 a	17.39 ±1.45 a	78.95±1.34 c
Navy bean	1.61 ± 0.16 d,c	14.44 ±0.51 b	83.70 ±0.35a
Pinto bean	1.55 ± 0.25 d	14.36 ± 0.47 b	83.56 ±0.71 a
Small red bean	2.41 ±0.24 a,b	16.64 ±0.25a,b	80.95 ±0.01b,c
Canned [d]			
Black bean	7.58 ±0.66 c	24.90 ±0.82 a	67.52 ±1.50 a
Dark red kidney bean	8.82 ±0.78 b,c	22.64 ±0.56 a	68.54 ±0.22 a
Light red kidney bean	9.96 ±1.08 a,b,c	24.05 ±1.58 a	65.99 ±0.49 a
Navy bean	13.21 ±1.93 a	20.75 ±0.73 a	66.03 ±2.66 a
Pinto bean	11.96 ±0.67 a,b	24.87 ±3.99 a	63.17 ±3.32 a
Small red bean	12.89 ±0.24 a,b	22.75 ±1.49 a	64.37 ±1.73 a
Stovetop-Cooked[e]			
Black bean	9.72 ±1.90 a	24.51 ±1.67a,b	65.77 ±0.23 a,b
Dark red kidney bean	7.37 ±0.49 a	26.32 ±0.31 a,b	66.32 ±0.80 a,b
Light red kidney bean	8.87 ±1.03 a	22.55 ±1.58 a	68.59 ±0.54 b,
Navy bean	10.61 ±1.53 a	26.84 ±2.33 a,b	62.55 ±0.80 b
Pinto bean	8.58 ±1.65 a	24.58 ±0.49 a,b	66.85 ±2.14 b
Small red bean	8.87 ±1.03 a	22.55 ±1.58 b	68.59 ±0.54 a

[a] RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch, all calculated as percentage of total starch based on Englyst et al., (1992).

[b] Data represent means and standard deviations of duplicate measurements.

[c] Values within the same column for raw samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference) test

[d] Values within the same column for canned samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD test

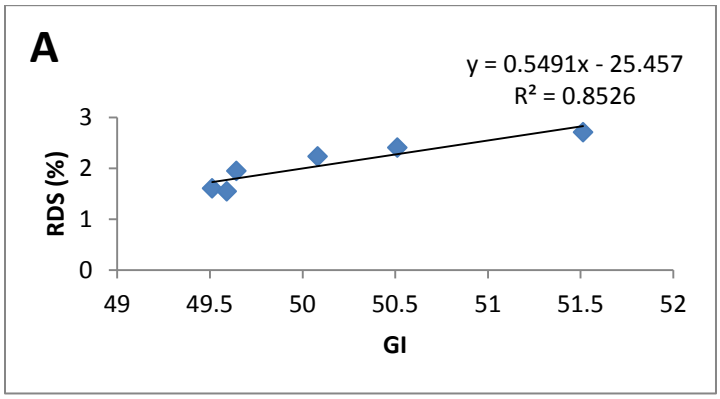
[c] Values within the same column for stovetop-cooked samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD test

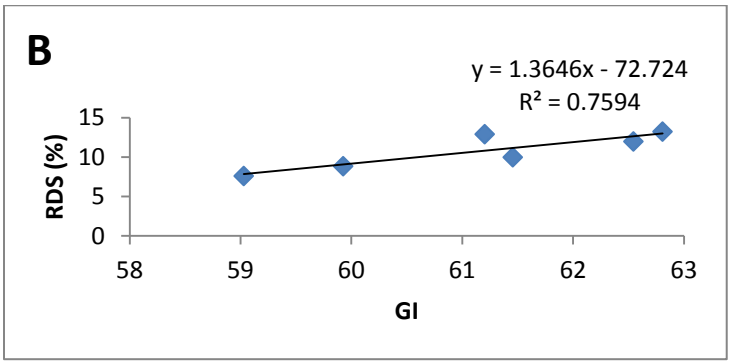
The calculated hydrolysis index (HI) and glycemic index (GI) of isolated native, canned and stovetop-cooked bean starches are reported in Table 4.3. The HI expresses the digestibility of starch in foods in relation to the digestibility of starch in white bread (Thorne et al., 1983). Values obtained for HI for isolated raw bean starches are in the range of 17.9 to 21.5 with raw light red kidney bean starch having the lowest hydrolysis index. For isolated stovetop- cooked and canned bean starches the hydrolysis index values were in the range of 35.2 to 42.1.

The glycemic index (GI) concept is a tool for ranking foods with respect to their blood glucose raising potential (Thorne et al., 1983). The estimated glycemic index (GI) is derived from *in vitro* digestion kinetics (Goñi et al., 1997). The calculated GI values for isolated native bean starches were higher in the present study than those reported by Ambigaipalan et al. (2011) for isolated native starch from different cultivars of black bean (49.6 vs ~39%) and pinto bean (49.7 vs ~36%). The substantial difference could be ascribed to the methods used for analysis. The Englyst (1992) method was used in this study because it mimicked *in vivo* digestion, whereas Ambigaipalan et al. (2011) used an American Association of Cereal Chemists approved method (AACC, 32-40) and also used the following equation to predict glycemic index: GI = 8.198 + 0.862HI. The derivation of this equation is based on the regression analysis of HI and GI under the conditions of that particular experiment (Granfeldt, Björck, Drews, & Tovar, 1992).

The main determinant of the glycemic index of foods has been found to be RDS (Englyst & Hudson, 1996; Englyst, Vinoy, Englyst, & Lang, 2003). Regression analyses of results from the three processing treatments (native, canned, and stovtop-cooked beans) of the six bean varieties in the present study (Figure 4.4.) confirmed that RDS (r^2 = 0.83, 0.76, 0.49) is strongly correlated to GI. Glycemic indices have been positively correlated to RDS and SDS contents of

starches (Madhusudhan & Tharanathan, 1995, 1996). Dried beans are low glycemic index foods, which makes them beneficial in the management of diabetes (Thorne et al., 1983).





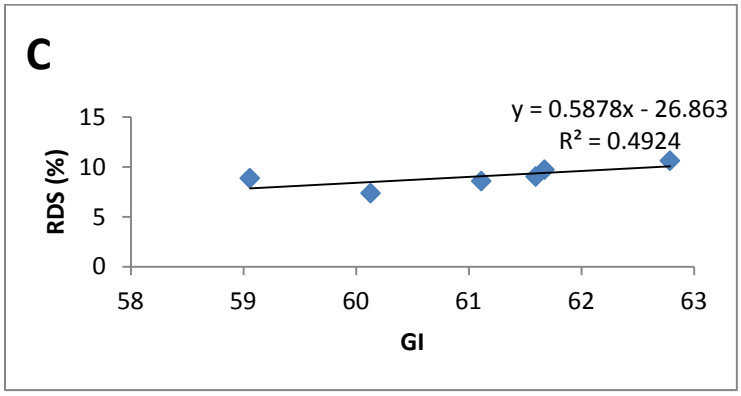


Figure 4.4. Relationship of RDS with GI of raw (A), canned (B) and stovetop-cooked (C) bean starch isolated from six *Phaseolus vulgaris* varieties.

Table 4.3. Hydrolysis Index and Glycemic Index of Starch Isolated from Six Bean Varieties[a]

Starch Source	HI [b]	GI [c]
Raw[d]		
Black bean	18.29 ± 0.25 c,d	49.64 ± 0.14 c,d
Dark red kidney bean	18.91± 0.12 b,c	50.08± 0.07 b,c
Light red kidney bean	21.52 ±0.48 a	51.51 ±0.26 a
Navy bean	17.87 ± 0.52 d	49.49 ± 0.03 d
Pinto bean	18.02 ± 0.20 c,d	49.67 ± 0.11 c,d
Small red bean	19.69 ±0.17 b	50.51 ±0.09 b
Canned[e]		
Black bean	35.2 ±1.98 a	59.03 ±1.09 a
Dark red kidney bean	36.84 ±0.67 a,b	59.93 ±0.37 a
Light red kidney bean	39.63 ±0.39 a,b	61.45 ±0.21 a
Navy bean	42.09 ±2.53 a	62.81 ±1.39 a
Pinto bean	41.61 ±0.69 a	62.54 ±0.38 a
Small red bean	39.17 ±1.21 a, b	61.20 ±0.66 a
Stovetop-Cooked[f]		
Black bean	40.02 ±1.69 a,b	61.67 ±0.92 a,b
Dark red kidney bean	39.00 ±0.14 a,b,c	61.11 ±0.07 a,b,c
Light red kidney bean	39.88 ±0.027 a,b	61.59 ±0.015 a,b
Navy bean	42.05 ±0.18 a	62.79 ±0.096 a
Pinto bean	37.21 ±1.48 b,c	60.13 ±0.81 c
Small red bean	35.26 ±0.34c	59.06 ±0.18 c

[a] Data represent means and standard deviations of duplicate

[b] HI: hydrolysis index =[area under hydrolysis curve (white bread)/ area under hydrolysis curve (starch)] x 100

[c] GI: glycemic index = 39.71 + 0.549 x HI

[d] Values within the same column for raw samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD (honest significant difference) test

[e] Values within the same column for canned samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD test

[f] Values within the same column for stovetop-cooked samples followed by the same letter are not significantly different ($P < 0.05$) from each other by Tukey's HSD test

4.4.2. Molecular Weight Determination

Starch is composed of two macro-molecules, amylose and amylopection (Hoover & Sosulski, 1985; Hughes et al., 2009). When starch is fractionated by size-exclusion chromatography and viewed on a chromatogram, two peaks are observed. The first peak represents the branched and higher molecular weight amylopectin, while the second peak corresponds to the mainly linear and lower molecular weight fraction of amylose. The weight average molecular weights (Mw) of amylose and amylopectin of isolated native bean starches are shown in Table 4.4. and the Mw distribution profile is displayed in (Figure 4.5.). Amylose fraction of isolated native dark red kidney bean starch had the smallest Mw (1.0×10^6 g/mol), whereas native pinto bean starch had the largest Mw (1.8×10^6 g/mol). The weight average molecular weights of amylopectin for isolated native bean starch in the present study ranged from 2.4×10^7 g/mol to 3.9×10^7 g/mol. The above Mw values for amylopectin were lower than those reported by Du et al. (2014) for native pinto bean starch (5.3×10^8 g/mol), native red kidney bean starch (8.3×10^8 g/mol) , native black bean starch (3.1×10^8 g/mol) and native navy bean starch (3.27×10^8 g/mol). Du et al. (2014) used aqueous starch dispersions for HPSEC-MALLS-RI to determine the Mw of the starches. Heating starch in water can cause degradation as well as the formation of aggregates which result in starch polymers having higher measured Mw (Cave et al., 2009). The lower Mw values for amylopectin measured in this study may be attributed to the method used to dissolve starch samples as well as shear scission and degradation during SEC separation (Cave et al., 2009). Regression analyses of results from the six bean varieties in the present study showed that Mw for amylose was strongly correlated to GI ($r^2 = 0.55$), RDS ($r^2 = 0.50$), SDS($r^2 = 0.49$), RS ($r^2 = 0.42$). Mw for amylopectin was not correlated with GI, SDS, RS and RDS values for isolated native starches (data not shown).

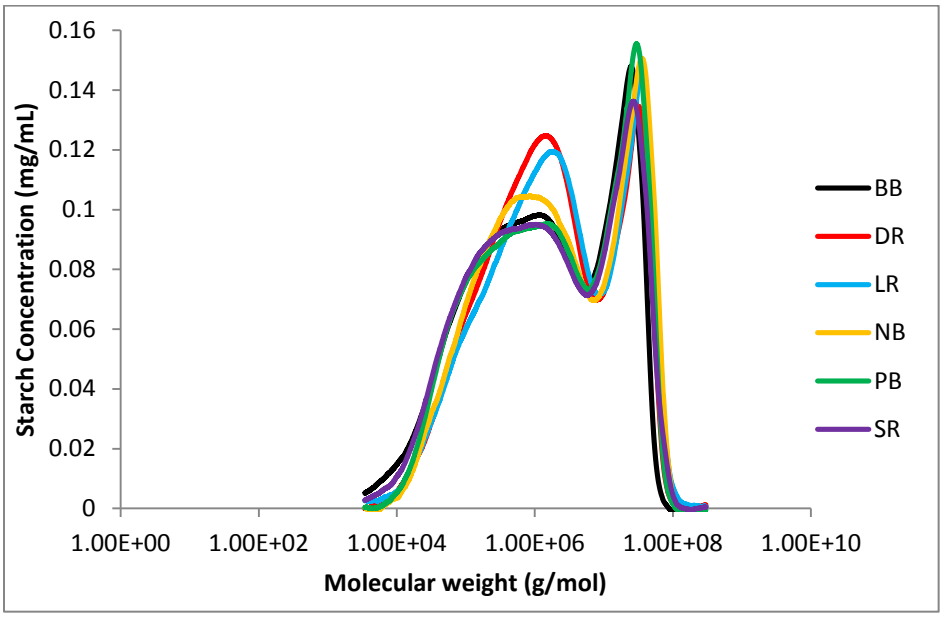


Figure 4.5. Molecular weight distributions of isolated native starch from six *Phaseolus vulgaris* varieties.[a]

[a] For abbreviations, see Figure 4.1.

Table 4. 4. Weight Average Molecular Weight (Mw) of Amylose and Amylopectin in Isolated Native Bean Starch [a]

Starch Source	Weight Average Molecular Weight (x 10^6 g/mol)	
	Amylose	Amylopectin
Black bean	1.2	24.0
Dark red kidney bean	1.4	31.0
Light red kidney bean	1.8	34.0
Navy bean	1.0	35.0
Pinto bean	1.6	29.0
Small red bean	1.5	26.0

[a] Results of 4 injections. A calibration curve was used to calculate the weight average molecular weights.

The weight average molecular weight distribution profiles for isolated stovetop-cooked and canned bean starches are displayed in Figures 4.6. and 4.7., respectively. Almost all samples of the isolated canned bean starches exhibited a monomodal distribution, that is, where only one peak was evident; indicating that starch degradation had occurred. Seemingly, the high pressures and temperatures during the canning process (Bravo et al., 1998) were able to modify the starch structure of both amylose and amylopectin, which led to reduction in the high molecular weight starch fractions when compared to those isolated native bean starches (Figure 4.5.). It is possible that the molecular degradation affected amylopectin more than amylose (compare Figures 4.5., 4.6. and 4.7.). The weight average molecular weight distribution of isolated stovetop cooked

bean starches showed some remnants of a high molecular weight fraction (Figure 4.7.). The exact mechanisms of the molecular weight reduction of starch are not completely clear. In general, some amount of polymer degradation takes place with a subsequent reduction in high molecular weight fractions. This reduction in molecular weight appears to occur through the breaking of covalent bonds by the heating process (Einde, Goot, & Boom, 2003).

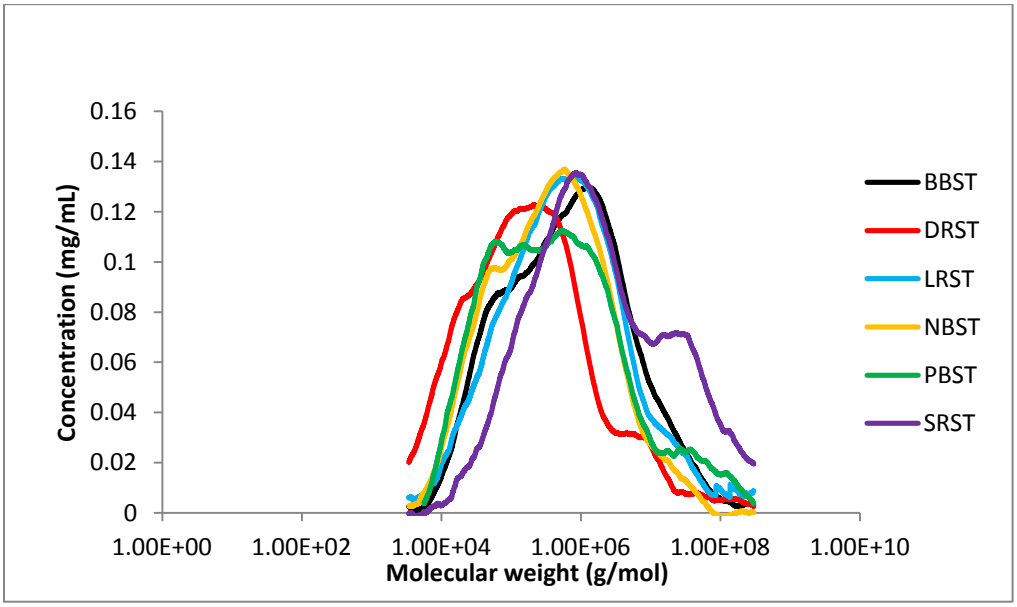


Figure 4.6. Molecular weight distributions of starch isolated from stovetop cooked beans of six *Phaseolus vulgaris* varieties.[a]

[a] For abbreviations, see Figure 4.1.

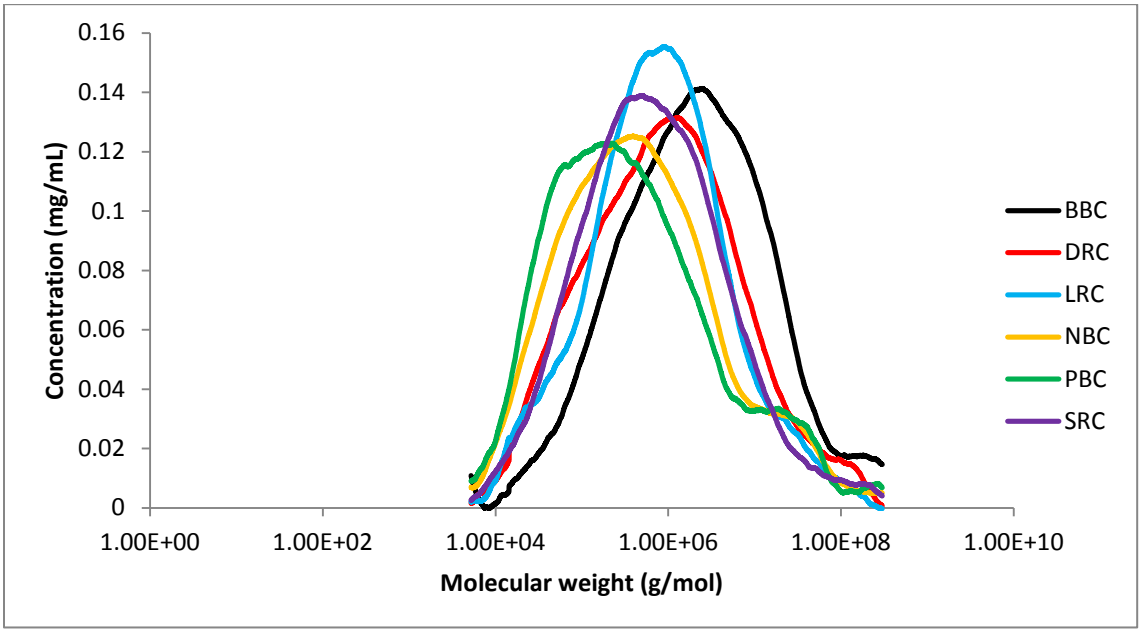


Figure 4.7. Molecular weight distributions of starch isolated from canned beans of six *Phaseolus vulgaris* varieties.[a]

[a] For abbreviations, see Figure 4.3.

4.5. CONCLUSIONS

Information regarding starch digestibility, such as digestion rate and enzyme resistance, are of importance for diet-related disorders including obesity and diabetes (Hasjim, Lavau, Gidley, & Gilbert, 2010). All isolated native bean starches were low in rapidly digestible starch (1.95-2.71%) and high in resistant starch (78.95.0-83.71%). *In vitro* digestibility showed that light red kidney bean starch, dark red kidney bean starch and small red bean starch were more susceptible to hydrolysis by pancreatic α-amylase than the other three studied varieties. The main determinant of the glycemic indices of the isolated native bean starches, isolated canned bean starches and isolated stovetop-cooked bean starches was found to be the proportion of rapidly digestible starch. The weight average molecular weights of amylose for the starches were comparable to isolated starches reported in the literature. However, amylopectin Mw results were lower and this could be due to shear scission during SEC analysis or the method used for dissolution. The weight average weight molecular distribution for amylose was found to be strongly correlated to glycemic index. The correlation between amylopectin Mw and GI was weak. In general, isolated canned and stovetop-cooked starches had higher amounts of RDS, lower amounts of SDS and RS, and larger glycemic indices than those of native isolated bean starch. This indicates that the hydrothermal treatments of stovetop-cooking and canning significantly alter the digestibility and molecular weight of the isolated starch studied.

APPENDICES

APPENDIX C

ENZYMATIC HYDROLYSIS OF ISOLATED NATIVE, CANNED AND STOVETOP-COOKED BEAN STARCHES

Table 1-C. Absorbance of Glucose Released During Enzymatic Hydrolysis of Isolated Native Bean Starches

	ABS											
Time (min)	BB1	BB1	BB2	BB2	NB1	NB1	NB2	NB2	PB1	PB1	PB2	PB2
20	0.123	0.123	0.122	0.122	0.109	0.111	0.118	0.117	0.115	0.116	0.102	0.107
40	0.200	0.210	0.192	0.196	0.191	0.196	0.206	0.209	0.188	0.183	0.188	0.190
60	0.314	0.321	0.304	0.303	0.307	0.304	0.303	0.317	0.301	0.307	0.296	0.301
90	0.467	0.479	0.467	0.479	0.453	0.457	0.474	0.487	0.475	0.479	0.469	0.482
120	0.591	0.582	0.588	0.588	0.599	0.592	0.579	0.581	0.571	0.59	0.544	0.553
150	0.739	0.741	0.715	0.706	0.782	0.78	0.766	0.772	0.766	0.743	0.74	0.779
180	0.742	0.789	0.759	0.784	0.793	0.846	0.771	0.852	0.772	0.788	0.759	0.809
	DR1	DR1	DR2	DR2	LR1	LR1	LR2	LR2	SR1	SR1	SR2	SR2
20	0.148	0.15	0.142	0.155	0.158	0.15	0.157	0.163	0.134	0.137	0.145	0.148
40	0.260	0.269	0.265	0.272	0.283	0.294	0.241	0.249	0.219	0.219	0.232	0.235
60	0.388	0.388	0.389	0.392	0.421	0.458	0.405	0.405	0.333	0.340	0.356	0.366
90	0.605	0.620	0.624	0.642	0.586	0.6	0.59	0.642	0.515	0.539	0.537	0.542
120	0.740	0.756	0.719	0.731	0.809	0.803	0.731	0.75	0.678	0.710	0.686	0.701
150	0.842	0.877	0.868	0.892	0.948	0.919	0.901	0.965	0.804	0.794	0.763	0.771
180	0.972	1.023	0.979	1.042	0.905	0.944	0.897	0.978	0.782	0.914	0.861	0.893

[a] ABS: absorbance

[b] BB: black bean, DR: dark red kidney bean; LR: light red kidney bean; NB: navy bean; PB: pinto bean; SR: small red bean

Table 2-C. Absorbance of Glucose Released During Enzymatic Hydrolysis of Isolated Canned Bean Starches

	ABS											
Time (min)	BB1	BB1	BB2	BB2	NB1	NB1	NB2	NB2	PB1	PB1	PB2	PB2
20	0.2169	0.267	0.257	0.275	0.385	0.378	0.447	0.464	0.373	0.378	0.355	0.347
40	0.429	0.431	0.449	0.465	0.643	0.637	0.661	0.647	0.523	0.518	0.461	0.459
60	0.498	0.489	0.582	0.591	0.738	0.76	0.777	0.781	0.746	0.738	0.689	0.699
90	0.684	0.687	0.787	0.785	0.864	0.876	0.956	1.004	0.754	0.739	0.869	0.869
120	0.868	0.852	0.907	0.923	0.932	0.926	1.007	1.055	0.931	0.935	1.054	1.046
150	0.91	0.917	0.929	0.94	1.047	1.063	1.189	1.208	1.01	1.019	1.061	1.082
180	0.951	0.978	0.978	0.979	1.134	1.145	1.036	1.054	1.234	1.239	0.996	1.043
	DR1	DR1	DR2	DR2	LR1	LR1	LR2	LR2	SR1	SR1	SR2	SR2
20	0.28	0.277	0.307	0.309	0.274	0.278	0.313	0.311	0.397	0.393	0.385	0.387
40	0.531	0.533	0.52	0.52	0.399	0.404	0.434	0.433	0.516	0.506	0.477	0.466
60	0.646	0.656	0.648	0.649	0.577	0.576	0.555	0.561	0.558	0.565	0.584	0.614
90	0.755	0.83	0.731	0.749	0.75	0.771	0.788	0.78	0.748	0.757	0.738	0.75
120	0.881	0.887	0.893	0.895	0.854	0.875	0.847	0.85	1.009	0.999	0.924	0.957
150	1.002	0.995	0.941	0.935	0.986	0.991	0.983	0.978	0.999	1.014	0.973	0.991
180	1.061	1.071	1.047	1.087	1.001	1.002	1.043	1.054	1.245	1.267	1.094	1.091

[a] ABS: absorbance

[b] BB: black bean, DR: dark red kidney bean; LR: light red kidney bean; NB: navy bean; PB: pinto bean; SR: small red bean

Table 3-C. Absorbance of Glucose Released During Enzymatic Hydrolysis of Isolated Stovetop-cooked Bean Starches

	ABS											
Time (min)	BB1	BB1	BB2	BB2	NB1	NB1	NB2	NB2	PB1	PB1	PB2	PB2
20	0.318	0.332	0.274	0.249	0.331	0.337	0.283	0.285	0.236	0.244	0.257	0.334
40	0.42	0.427	0.412	0.434	0.446	0.428	0.434	0.449	0.368	0.35	0.347	0.382
60	0.689	0.701	0.628	0.638	0.733	0.743	0.712	0.717	0.619	0.618	0.624	0.627
90	0.818	0.821	0.789	0.816	0.81	0.787	0.805	0.81	0.685	0.723	0.75	0.733
120	0.881	0.87	0.857	0.878	0.924	0.907	0.933	0.95	0.82	0.811	0.878	0.897
150	0.975	0.984	0.891	0.896	0.935	0.957	0.962	0.937	0.909	0.896	0.919	0.922
180	1.026	1.056	0.995	1.0008	0.972	0.982	0.968	0.939	0.989	0.931	1.014	1.011
	DR1	DR1	DR2	DR2	LR1	LR1	LR2	LR2	SR1	SR1	SR2	SR2
20	0.207	0.238	0.233	0.243	0.239	0.269	0.262	0.282	0.256	0.232	0.277	0.276
40	0.321	0.348	0.405	0.408	0.286	0.318	0.311	0.307	0.295	0.309	0.245	0.253
60	0.582	0.661	0.635	0.632	0.606	0.612	0.623	0.62	0.563	0.559	0.57	0.569
90	0.726	0.719	0.689	0.685	0.703	0.711	0.703	0.702	0.65	0.667	0.62	0.629
120	0.809	0.815	0.831	0.842	0.908	0.882	0.909	0.867	0.723	0.812	0.768	0.734
150	0.973	0.994	0.963	0.944	0.907	0.919	0.911	0.915	0.789	0.825	0.812	0.832
180	0.991	0.963	0.956	0.941	1.008	0.988	0.972	0.991	0.906	0.92	0.941	0.892

[a]ABS: absorbance

[b] BB: black bean, DR: dark red kidney bean; LR: light red kidney bean; NB: navy bean; PB: pinto bean; SR: small red bean

APPENDIX D

WEIGHT AVERAGE MOLECULAR WEIGHT DETERMINATIONS

One of the two major principles that are used in light scattering to determine the molar mass is:

1. The intensity of light scattered (LS*)* is proportional to the product of the polymer (Mw) and the polymer concentration (C).

$$LS \alpha Mw \times C (dn/dc)^2$$

Where (dn/dc) is the refractive index increment, which expresses the variation of the refractive index of a solution with solute concentration (Wyatt, 1993).

Differential Index of Refraction (dn/dc) Determination

Isolated starch samples were vacuum oven dried at 70°C for 18 h. Starch samples (25mg) were placed in a screw capped glass tube (14mL) and DMSO-50 mM LiBr-H_2O (90% DMSO-10% H_2O) (5mL) was added. The tubes were placed in a water bath held at 80°C for 2 h. The tubes were votexed every 5 min to mix for the first 30min, then vortexed every 10min. The tubes were then taken and were allowed to shake at 200rpm overnight (~16hrs) at room temperature (25°C). The tubes were centrifuged (4000 rpm) at 20°C for 10 min (Eppendorf model 5810R). The supernatants were decanted into scintillation vials and serial dilutions of 0 (blank), 1, 2, 3, 4 and 5mg/mL were made using DMSO-50 mM LiBr-H_2O (90% DMSO-10% H_2O)as the solvent. Mixtures were vortexed for 10 s and injected (400 µL) using Agilent G1329A autosampler to an Agilent 1200 system (G1311A Quaternary Pump with a G1322A

degasser) directly connected from pump to the dRI detector (Wyatt Corp., Optilab rEX, wavelength at 658nm). The samples were kept at room temperature (~25C). The HPLC flow rate was at 0.1mL/min (25°C), while the dRI detector temperature was set at 40°C. Data collection and dn/dc calculation was done by using ASTRA software.

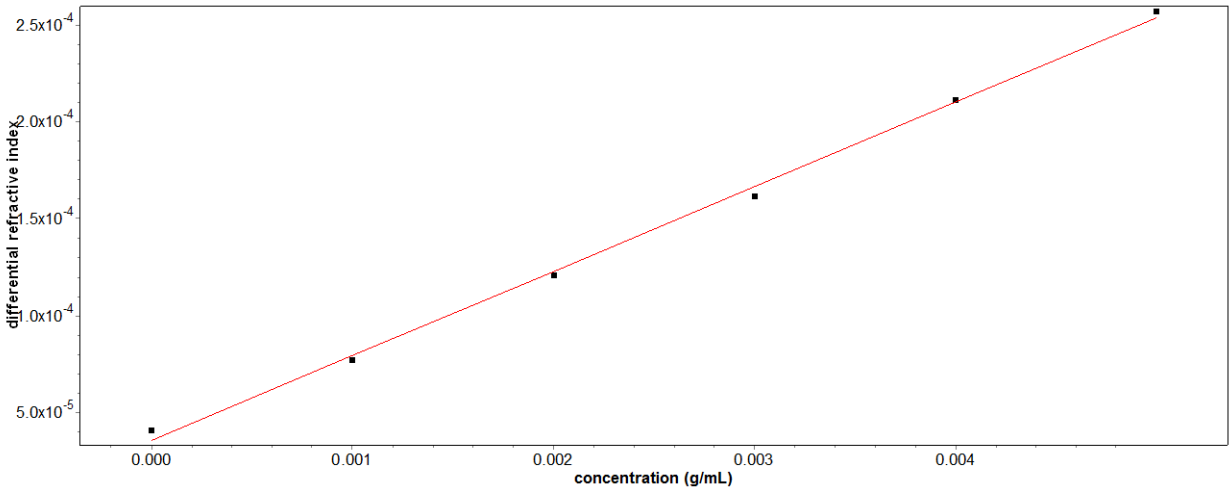


Figure 1-D. Isolated native black bean starch dn/dc.

BB starch dn/dc: 0.0438±0.0010 mL/g **r^2:** 0.997

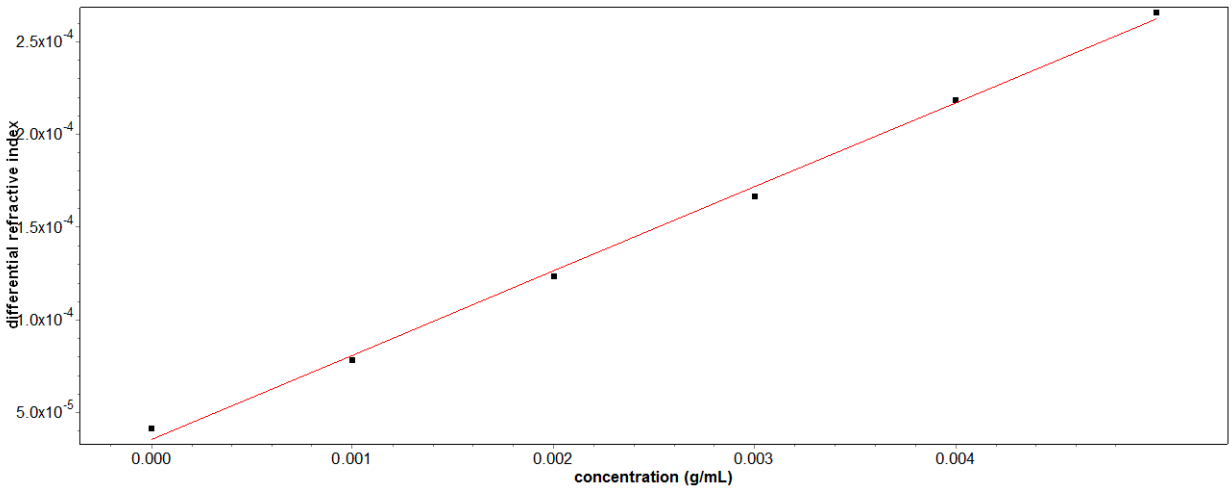


Figure 2-D. Isolated native navy bean dn/dc.

NB starch dn/dc: 0.0454±0.0011 mL/g **r^2:** 0.997

Table 1-D. Light Scattering Weight Average Molecular Weights of Isolated Native, Canned and Stovetop-cooked Bean Starches

Starch Source[a]	Molecular Weight (g/mol)		
	Native	Canned	Stovetop-cooked
BB1	2.34E+08	9.89E+06	6.26E+06
BB1	2.55E+08	6.98E+06	9.23E+06
BB2	1.89E+08	1.50E+07	1.09E+07
BB2	6.46E+07	1.15E+07	1.06E+07
DR1	4.83E+07	8.38E+06	8.29E+06
DR1	4.73E+07	7.69E+06	9.45E+06
DR2	2.54E+07	1.70E+07	1.66E+07
DR2	9.96E+07	1.63E+07	5.45E+06
LR1	5.67E+07	1.26E+07	1.07E+07
LR1	5.29E+07	9.89E+06	6.92E+06
LR2	1.11E+08	1.06E+07	7.50E+06
LR2	7.25E+07	1.07E+07	6.88E+06
NB1	3.13E+07	7.85E+06	1.56E+07
NB1	3.07E+07	8.36E+06	5.80E+06
NB2	3.54E+07	1.56E+07	1.65E+07
NB2	1.92E+07	1.06E+07	9.33E+06
PB1	8.68E+07	5.52E+06	1.16E+07
PB1	5.20E+08	9.51E+06	4.67E+07
PB2	1.00E+08	6.60E+06	1.09E+07
PB2	1.97E+07	1.60E+07	1.11E+07
SR1	2.25E+07	8.52E+06	8.83E+06
SR1	2.49E+07	7.27E+06	1.30E+07
SR2	2.20E+08	1.13E+07	8.38E+06
SR2	2.48E+07	1.11E+07	1.12E+07

[a] BB: black bean; DR: dark red kidney bean; LR: light red kidney bean; NB: navy bean; PB: pinto bean; SR: small red bean

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CHAPTER 5

GENERAL CONCLUSIONS

In the present study, starches were isolated from native, canned and stovetop-cooked dried beans and their physicochemical properties, digestibility and molecular structures, and how these features are interrelated were determined. The general findings include:

All isolated native starch samples had total starch contents greater than 90%, with light red kidney beans having the highest total starch (96.2%). This indicated that the major component of the starch isolates was in fact starch. The disparity in the total starch contents for the native isolated bean starches could be due to fiber co-settling with the starch during extraction and adhering to some bean starch samples more than others both of which could decrease the total starch content. The amounts of amylose in the isolated native bean starches varied from 28.01 to 28.63% and were not significantly different from each other, thus it is unlikely the amylose content had a significant influence on any differences noticed in the studied starch characteristics. Resistance starch determined by the Megazyme assay revealed that isolated native pinto bean starch contained the greatest amount of resistant starch (55.70%), whilst light red kidney bean had the lowest (41.92%). The resistant starch contents of starches isolated from dark red kidney bean and light red kidney were significantly lower than those from the other isolated native dry bean starches. Therefore, these two isolated native bean starches would have a different nutritional impact when compared to the other four isolated native bean starches from *Phaseolus vulgaris*.

Significant differences ($P<0.05$) were observed in gelatinization onset (transition) temperature ($T_{o)}$, peak temperature (T_p) and conclusion temperature (T_c) among the studied isolated native bean starches. The onset gelatinization temperature (T_o) varied from 65.5ºC (isolated native light red kidney bean starch) to 68.8ºC (isolated native small red bean starch). The peak transition temperatures of isolated native light red kidney and dark red kidney bean

starches were significantly different from those of the other bean starch samples. A similar trend was also observed for the conclusion transition temperatures as well. The ranges of T_o (46.2-47.7°C), T_p (58.6-61.3°C) and T_c (71.4-74.5°C) values of retrograded isolated native starches were lower than those obtained for the initial gelatinization. Both amylopectin and amylose were involved in the retrogradation process, since isolated native bean starches were allowed to retrograde for 7 days at 4°C.

Results revealed that isolated native bean starches from dark red kidney and light red kidney beans had lower peak and break down viscosities than native starch isolated from navy and pinto beans. This indicated that dark red kidney and light red kidney bean starches would swell less and have more resistance to shear. Therefore they would be suitable in foods that require excessive stirring or need to be pumped through a pipe. The setback viscosity values for the isolated native bean starches ranged between 2265.5 cP and 4349.5 cP. The smaller resistance starch contents of isolated native light red kidney and dark red kidney bean starches when compared to the other isolated native bean starches, may account for their lower pasting temperatures. All isolated native bean starches exhibit a C-type X-ray diffraction pattern. Crystallinity was the greatest for navy bean starch (36.06%), whereas, pinto bean starch had the lowest value (24.86%). In the present study no relationship was observed between crystallinity and the other starch characteristics studied.

The findings of the current study showed that the physicochemical properties of native starches isolated from dark red kidney and light red kidney beans differed from those of the other isolated bean starches. Consequently, native dark red kidney and light red kidney bean starches may have different end-use applications than the other common bean starches.

The digestibility of the isolated native, canned and stovetop-cooked bean starches were investigated using the *in vitro* Englyst method. The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents, and the glycemic indices of the isolated native, canned and stovetop-cooked bean starches were determined. All isolated native bean starch samples were lower in RDS (1.55-2.71%) and higher in RS (80.95-83.70%) when compared to isolated canned bean starch samples and isolated stovetop-cooked bean starch samples. The RDS contents of isolated stovetop-cooked and canned bean starches ranged between 7.3-10.61% and 7.58-13.21%, respectively. Native starches are in a relatively no-swollen form, without having undergone heating, and thus is less susceptible to enzymatic hydrolysis whereas isolated canned and stovetop-cooked starches have undergone gelatinization and have lost some degree of crystallinity. Isolated native light red kidney bean starch had the largest amount of SDS (17.39%) and the smallest amount of RS (78.95%). Only marginal differences in the RDS, SDS and RS contents were observed among isolated canned and stovetop-cooked starches. In general, isolated starches from canned and stovetop-cooked beans contained greater amounts of RDS and SDS and lower amounts of RS when compared to their isolated native starch counterpart samples.

The extent of hydrolysis after 180 min among the native starch samples ranged from 24.9% for black beans to 27.3% for light red kidney beans. All isolated native bean starches showed similar hydrolysis rates during the initial 40 min with small differences in the latter stages.. Isolated starches from stovetop-cooked and canned beans displayed a greater extent of starch hydrolysis (39.8-48.4%) than the starches that were isolated from raw whole beans (24.9-27.3%). The glycemic index (GI) values for isolated native bean starches ranged from 49.41 to 51.5l. Isolated native light kidney bean starch had the highest GI value. The glycemic indices

for isolated canned and stovetop-cooked starches from all six bean varieties ranged from 59.03 to 62.81. There were only marginal differences in the GI values among the isolated canned and stovetop-cooked bean starches. Strong relationships were found between glycemic index and RDS content for all the bean starches studied. Results, indicate that dry beans are low glycemic index foods, which make them beneficial in the management of diabetes.

The weight average molecular weights (Mw) of amylose and amylopectin of isolated native bean starches were determined. Amylose of isolated native dark red kidney bean starch had the smallest Mw (1.0×10^6 g/mol), whereas amylose of native pinto bean starch had the largest Mw (1.8×10^6 g/mol). The Mw of amylopectin for isolated native bean starch samples in the present study ranged from 2.4×10^7 g/mol to 3.9×10^7 g/mol. The above amylopectin Mw values were lower than those reported in literature (5×10^8-8×10^8). The lower Mw values for amylopectin may be attributed to the method used to dissolve starch samples as well as to shear scission and degradation during size-exclusion chromatography (SEC) separation. Findings from this present study showed the Mw for amylose was postively correlated to GI ($r^2 = 0.55$), RDS ($r^2 = 0.50$), SDS($r^2 = 0.49$), and RS ($r^2 = 0.42$) for isolated native bean starches. Mw for amylopectin was weakly correlated with GI, SDS, RS and RDS values for isolated native bean starches which suggests that amylopectin Mw is unable to predict the variability observed in GI, SDS, RS and RDS values obtained.

Isolated canned bean and stovetop-cooked bean starches generally exhibited a monomodal distribution in their Mw distribution, which indicated that the hydrothermal treatments degraded starch, which is bimodal in nature upon evaluation with size-exclusion chromatography.

Beneficial properties of dry bean starch confirmed in this research indicate that dry bean starch can be utilized as a new starch source in the food industry, with unique functional properties. Findings from this study will not only add to the growing knowledge base of dry bean starches, but provide information regarding bean starch digestibility, such as digestion rate RDS, SDS and RS contents, which are of importance for diet-related disorders including obesity and diabetes.

CHAPTER 6

FUTURE RECOMMENDATIONS

1. `Findings from this study showed that isolated bean starches have low glycemic indices (GI). *In vitro* methods to measure GI may prove helpful for the initial screening of *Phaseolus vulgaris* varieties; however, in accordance with the definition, the GI must be confirmed *in vivo* by clinical trials. Therefore, studying the oral glucose tolerance and insulin response (more sensitive) of cooked and canned bean starches in healthy individuals is recommended.
2. In order to fully link the molecular structure of bean starch to functional properties (eg., digestibility) and to make meaningful comparisons with the structures of cereal starches, it is recommended for future studies that the fine structures of amylose and amylopectin be characterized. This should include, but not be limited to, average chain length, average chain length distribution, degree of polymerization and level of branching.
3. The present study examined the molecular weight distribution and physicochemical properties of isolated starch from single lines of six varieties of *Phaseolus vulgaris*. Investigating these same parameters using several lines for each bean variety as well different genus (e.g., *Vigna unguiculata*) may yield results that truly represent the species in general.
4. The weight average weight molecular distribution is affected by the method used to dissolve starch. Investigating the effects of various methods for starch dissolution is recommended to determine an optimum method which reduces starch degradation.