Structural Characterization and Glycemic Attributes of Intermediate Wheatgrass (*Thinopyrum intermedium*) Flour and Extracted Starch

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Abstract

Intermediate wheatgrass (IWG) is an environmentally sustainable perennial crop with potential food applications. This study investigated the starch hydrolysis kinetics of IWG grown in Roseau (IWG-RS) and Rosemount (IWG-RM), Minnesota, USA and the molecular structure of their residual (resistant) starch after 2 hr hydrolysis. Hard red wheat (HRW) and Jasmine rice (JR) were compared to the IWG samples. Molecular size distribution and unit chain profiles of the RS fraction of raw starches after enzymatic hydrolysis were determined with gel permeation chromatography and high-performance anion-exchange chromatography respectively. Moreover, thermal properties, size distribution, granule size and morphology, as well as the unit and internal chain profile of extracted starches were evaluated. IWG flour had significantly lower total starch, lower RDS and higher lipid and protein contents compared to JR and HRW. JR flour had the highest eGI (49.2), with IWG-RM recording the lowest (40.6). Significant differences were observed in the glucan chain lengths of the RS fraction. JR had the shortest average chain length (DP=4.75) compared to HRW (DP=7.46), IWG-RS (DP=5.72) and IWG-RM (DP=4.85). IWG flour had slower starch hydrolysis kinetics compared to JR and HRW flour. The RS fraction of the samples consisted mostly of short chains. The glucan chain length of IWG RS fraction was also significantly affected by location. The amylose contents of IWG-RS and IWG-RM were 30.7% and 30.4%, respectively. IWG starches had the lowest gelatinization temperatures. Enthalpy of gelatinization (ΔH) of HRW was similar to that of IWG-RM. The λ_{max} of the starches suggests that the amylose chains and internal chains of the IWG starches were longer than those of HRW and JR. IWG-RM has

the least β-limit dextrin and longer external chain. Unit and internal chain profiles of amylopectins between IWGs were similar. This study revealed that IWG could potentially be exploited for the preparation of foods with lower glycemic responses. IWG starches properties were similar to those of wheat. Differences in some starch properties were also observed between the IWG grown at different locations. Understanding the microstructure of starch from Intermediate wheatgrass can potentially optimize its chemical functionality.

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Chapter 1. Literature Review

1.1. Introduction

Cereal grains, legumes and oilseed are the three main categories of annual crops that make up human diets. Cultivated crop systems could provide less positive impact than sustainable crop systems because of its low carbon sequestration, water pollution and less biodiversity (Cassman & Wood, 2005). Cultivated croplands also requires significant amounts of pesticides and chemical fertilizers that mainly lead to natural habitats alteration and harmful byproducts (Cassman & Wood, 2005). A perennial crop, such as Intermediate wheatgrass (*Thinopyrum intermedium*), has a massive root system (Figure 1.1) to retain water and utilize fertilizer more effectively (Cox et al., 2006).

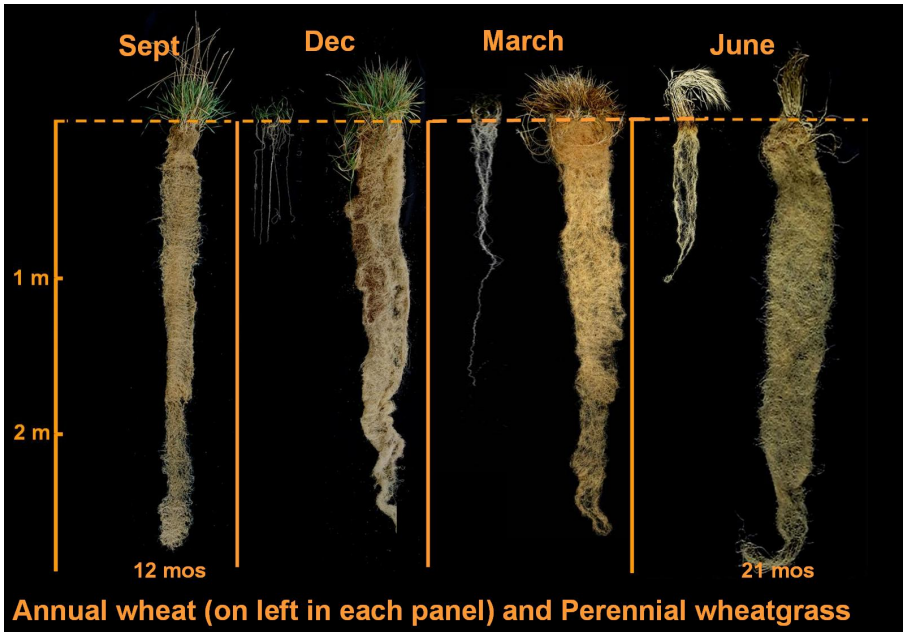


Figure 1. 1 Comparison of root system length between annual wheat (left) and perennial wheatgrass (right) root system.

Intermediate wheatgrass (IWG) is a cool season, perennial grass that is native to the Mediterranean and Asian regions (Wagoner, 1995). The Rodale Research Center (Kutztown, PA) selected Intermediate wheatgrass in 1983 as a potential perennial grain

crop because of its breeding potential, environmental benefits and nutritional values. Its breeding potential includes consistent seed maturity, lodging resistance, edible seeds and mechanical harvesting possibility (Wagoner, 1988). One of the most significant benefits of perennial crops is that they do not require to replant and reseed which would primarily enhance economic efficiency (Wagoner & Schaeffer, 2008; Watt, 1989). Perennial crops have long growing seasons because they can regrow on their existing rooting system. Compared to annual crops, which need to be replanted every year, their root system will require longer time to grow and grasp the soil. The loosen soil particles could be washed away easily as well as the fertilizers and might generate pollution problem during the growing season (Dehaan & Ismail, 2017). IWG can significantly improve the ecosystem by increasing nitrogen fixation and reducing soil erosion using its massive root system (Culman, Snapp, Ollenburger, Basso & DeHaan, 2013). The perennial crops can potentially uptake 30 to 50 times of nitrogen in fertilizers than annual crops (Randall & Mulla, 2001). The development of perennial crops can be a solution to the soil erosion caused by the annual crops production (Wagoner & Schaeffer, 2008). Research showed that perennial crops are 50 times more effective in covering topsoils (Gantzer, Anderson, Thompson & Brown, 1990). Higher amounts of carbon storage and chemical fertilizer utilization in perennial crops generate less burden to the environment that could lead to climate change (Robertson, Paul & Harwood, 2000).

The Rodale Institute (Kutztown, PA) performed two selection cycles in 1988, followed by the Land Institute, starting breeding studies using the previously selected grains in the year 2003 (Cox, Van Tassel, Cox & Dehaan, 2010). Seed yield and size are the two critical traits when it comes to edible crops (Zhang et al., 2017). Most perennial

crops produce low yield and smaller seed size than annual crops which poses a challenge for large scale food production. Wild perennial crop domestication and improvements are the primary methods for perennial crop breeders to increase seed yield and size (Dehaan et al., 2014). Kernza® is the trademark name created by the Land Institute for sales and production of Intermediate wheatgrass that came from particular growing locations in the United States (Dehaan & Ismail, 2017). A study conducted by Culman and colleagues (2013) reported that, the yield of Kernza® grain increased to 33% of annual wheat in 2011 under their management, from a previous grain yield of 4.5% in 2010. Seed yield was increased by 77% and seed mass by 2.3% after two selection cycles at the Land Institute in Kansas, USA (Dehaan et al., 2014). Dehaan et al., (2004) predicted that IWG could potentially have a similar yield as wheat in 20 years. On the contrary, seed size might take much longer (about 110 years) to achieve seed size similar to that of wheat at the current rate of seed size increments (Dehaan et al., 2014).

Perennial crops can generally improve agricultural efficiency and reduce economic costs (DeHaan et al., 2014). The *Thinopyrum* species have been hybridized with modern wheat varieties so that the perennial property can be retained. The genetic codes of IWG are substantially similar to that of wheat (Guo et al., 2016). Current studies have been focusing on using IWG to partially or fully substitute wheat in baked goods (Dehaan & Ismail, 2017).

1. 2. Objectives

1. Study starch hydrolysis kinetics of Intermediate wheatgrass flour and its effects on the unit chain profile of its resistant starch fraction
2. Investigate the unit and internal chain profile of Intermediate wheatgrass amylopectin

1.3. Significance of the Project

This research will provide information on the starch hydrolysis kinetics of Intermediate wheatgrass starches, as well as information on the structural characteristics of Intermediate wheatgrass amylopectin. The data will fulfill our understanding of the starch component in developing perennial crops, such as Intermediate wheatgrass. In order to partially or fully utilize Intermediate wheatgrass in different food applications, it is essential to understand the structural characteristics of its starch and hypoglycemic property.

1.4. Commercial use of IWG

Food industries are excited about utilizing perennial crops such as IWG in commercial products because customers are paying attention to sustainable food sources progressively. Functional and chemical properties of IWG utilized in different food systems have been studied to provide the breeding program for further development. Companies like General Mills and PepsiCo are interested in knowing how this crop could be incorporated or replaced annual wheat. The first industrial-scale commercial product of Kernza® is the Long Root Ale developed by Patagonia Provisions (Dehaan & Ismail, 2017). Also, IWG has been used by local breweries and bakeries for small scale food

production as well (Dehaan & Ismail, 2017). Bang Brewery (St. Paul, MN, USA) has been incorporating 15.8% Kernza® flakes in their mash to brew a local craft beer called Gold. They had experienced difficulties while fully converting the starch in IWG to fermentable sugars. A new project has been funded by Forever Green Initiative to investigate and document the change in flavor and sensory profile of IWG-incorporated craft beer by four mashing techniques.

1.4.1. IWG Food Applications

IWG flour has been reported to have desirable flavor characteristics and high protein content that could be used for baked food applications, such as muffins, cookies or bread (Wagoner, 1995; Marti, Bock, Pagani, Ismail & Seetharaman, 2016). IWG has the potential to become an alternative product to replace common edible grains, such as wheat, rye and barley (DeHaan & Ismail., 2017). Although IWG has poor gluten forming properties, adding IWG whole flour to hard wheat flour increased dough consistency because of its high fiber content (Marti, Qiu, Schoenfuss & Seetharaman, 2015). The addition of fiber also resulted in higher water absorption, higher torque and lower dough development time when investigating the dough mixing properties (Marti et al., 2015). Rahardjo et al (2018) reported low stability, resistance to extension, extensibility and low rising capacities in IWG dough. The addition of dough conditioners such as vital wheat gluten, wheat protein isolate and transglutaminase could increase the dough extensibility and resistance to extension in some extent in IWG-enriched flour (Banjade, Gajadeera, Tyl, Ismail & Schoenfuss, 2019).

1.4.2. IWG Chemical Composition

The chemical composition of Intermediate wheatgrass has been studied. However, each particular harvest has a slightly different composition because of improvement in breeding (Rahardjo et al., 2018). With the breeding cycle being improved by multiple selections over the years, the seed size and yield have been increased. Larger seed size also means a higher amount of endosperm can be milled (Becker, Wagoner, Hanners & Saunders, 1991; Rahardjo et al., 2018). Interestingly, the protein, lipid, dietary fiber and carotenoids contents in IWG are significantly higher than conventional wheat cultivar regardless of the differences among harvests (Becker et al., 1991; Rahardjo et al., 2018; Tyl & Ismail, 2018). The chemical composition of IWG in each location and harvest varied. The growing locations for current IWG research include Salina, KS; St Paul, MN; Roseau and Rosemount, MN, etc (Marti et al., 2015; Marti et al., 2016; Rahardjo et al., 2018; Tyl & Ismail, 2018; Banjade et al., 2019; Mathiowetz, 2018). For instance, IWG from Roseau location had more protein than from Rosemount, while Rosemount IWG had more fat, ash and insoluble fiber than Roseau IWG (Banjade et al., 2019). Some IWG lines had low lipase activities, and all lines were low in lipoxygenase activities (Tyl & Ismail, 2018). Most IWG population found to have high antioxidant activities than wheat (Tyl & Ismail, 2018).

1.4.2.1. IWG Proteins

IWG has significant amounts of proteins, yet it lacks the high molecular weight glutenin to strengthen the gluten structure during bread-making (Marti et al., 2015). Marti et al (2015) reported 20% of protein in their study. However, Tyl & Ismail (2018), Banjade

et al (2018) and Rahardjo et al (2018) reported 19.76 – 24.84 %, 16.74 – 18.50 % and 18.01 – 21.15 % protein, respectively. IWG has low gluten forming properties because it lacks high molecular weight glutenin subunits (Becker et al., 1991; Dehaan et al., 2014). Multiple studies in the University of Minnesota have been attempting to investigate the protein structure of IWG to improve its functional properties (Marti et al., 2016; Rahardjo et al., 2018; Banjade et al., 2018). Lysine was reported to be the limiting essential amino acid in both IWG and wheat proteins. Other than lysine, IWG has substantially more essential amino acids than wheat (Becker et al., 1991). IWG has 1.4 times cysteine (higher thiols) and methionine compared to wheat (Becker et al., 1991). However, IWG proteins consist of mostly low molecular weight glutenin subunits and α, ɣ gliadins which make it difficult to form a viscoelastic gluten network (Marti et al., 2016; Bunzel, Tyl & Ismail, 2014; Ismail et al., 2015; Rahardjo et al., 2018). Marti et al (2015) concluded that proteins in IWG dough that were stabilized by non-covalent interactions appeared to have higher solubility and larger amounts of accessible and total thiols. Marti et al (2015) blended IWG flour into hard wheat flour (HWF) in four ratios (IWG: HWF): 0:100, 50:50, 75:25, and 100:0. The high amount of IWG-enriched flour would result in faster aggregation time and lower peak torque in GlutoPeak tester, which indicated weak gluten strength (Marti et al., 2015). IWG-enriched wheat flour dough model did not establish a proper gluten network (Rahardjo et al., 2018; Banjade et al., 2018). The high amount of fiber and deficiency of high molecular weight glutenins could be contributing to the undesirable gluten network, resulting in poor bread-making qualities (Becker, 1991; Ismail et al., 2015; Rahardjo et al., 2018). A hard wheat flour to IWG ratio of 50:50 was suggested to have a good balance between the functional characteristic and nutritional qualities (Marti et al., 2015). However, cookies

remained similar in quality as those made from all-purpose wheat flour (Engleson & Atwell, 2008). New research has been conducted aimed by enhancing IWG flour with chemical modifiers, while others attempted to utilize IWG kernels in innovative ways (Banjade et al., 2018).

1.4.2.2. IWG Lipid

Research has shown that Intermediate wheatgrass has more lipid than whole wheat flour (Marti et al., 2016; Rahardjo et al., 2018; Banjade et al., 2018). Higher fat content had also been reported in Banjade et al (2018) of 5 – 5.5 % in 2015 harvest than those from Rahardjo et al (2018) of 2.6 – 4.79 % and Tyl & Ismail (2018) of 2.23 – 3.89 %. The fatty acids in flour can generate complexes with amylose in starch which would act as resistant starch that could lower the starch hydrolysis kinetics (Tufvesson & Eliasson, 2000). Mathiowetz (2018) conducted a study on the fatty acid profile of IWG and reported IWG had high amount of polyunsaturated linoleic and oleic fatty acids which would make IWG more susceptible to hydrolytic and oxidative rancidity. However, the high antioxidant activities in IWG can slower the rate of oxidative rancidity over storage (Mathiowetz, 2018).

1.4.2.3. IWG Dietary Fiber

Dietary fibers are complex polysaccharides presented in plant tissues. It is not digestible by the human small intestine but can be fermented in the human large intestine by degree (AACCI 2001). Resistant starch can also be classified as dietary fiber because it

cannot be digested in the human small intestine but can be fermented in the large intestine (Slavin, 2013). The two major categories of dietary fiber are soluble and insoluble dietary fiber. Soluble dietary fiber includes water-soluble arabinoxylans, β-glucan, fructans, and pectin. Insoluble dietary fiber includes water-insoluble arabinoxylans, cellulose, lignin, and resistant starch (Gebruers et al., 2008). Higher total dietary fiber content has been reported in IWG flour (16.4%) than in whole wheat flour (11%) (Marti et al., 2015). A certain amount of dietary fiber intake in the human diet can be beneficial in controlling postprandial blood glucose levels (Karl et al., 2015). Modern lifestyle has encouraged the public to seek healthier options for their diets. Whole IWG flour has a remarkable amount of dietary fiber and antioxidants which would be beneficial to human health (Becker et al., 1991; Marti et al., 2016). Besides, IWG-enriched flour was also evaluated as overall favorable when being made into different baked goods (Becker et al., 1991).

1.5. IWG Starch

Due to its small seed size, the starch content of IWG is substantially lower than that in wheat. However, the seed size of IWG has been increased over the years which leads to an increase in the endosperm to bran ratio in IWG. Total starch content has increased with an increase in endosperm size (Becker et al., 1991; Rahardjo et al., 2018). Marti et al (2015) found lower total starch content in IWG flour (46.7%) than refined (73.9%) and whole wheat flour (72%) and higher amount of proteins. Rahardjo et al (2018) and Tyl & Ismail (2018) also reported similar starch content of 46.74 – 52.45% and 42.53 – 52.96 % in their study. IWG starch granules presented poor affinity to iodine (Marti et al., 2015). The starch granule is a layered structure consists of crystalline growth rings and

amorphous growth rings, which is between 100 and 400 nm thick (Gallant, Bouchet & Baldwin, 1997). The crystalline growth ring which is considered as the "hard shell" is the linear region of double helices. The amorphous growth ring is the "soft shell" which is the highly branched region (Gallant et al., 1997). Amylose and amylopectin are the two major components in starch. Amylose is a linear polymer made up of glucose units which are linked by α-1,4 glucosidic linkages (Tester, Karkalas, & Qi. 2004.). Amylopectin is a branched polymer made up of a linear chain and multiple branches. The linear chain consists of glucose units that are linked by α-1,4 glucosidic linkages. The branches, which are also made up of glucose units, are connected to the linear chain by α-1,6 glucosidic linkages (Tester, Karkalas, & Qi, 2004). Rahardjo et al (2018) reported amylose content in IWG whole flour ranged from 22-25 %, whereas the amylose content ranged from 19.9 – 27.3 % in 2012 harvest (Tyl & Ismail, 2018). Amylose could be correlated to bread firmness during baking, whereas amylopectin might be related to bread firmness as it retrogrades in storage (Alcázar-Alay & Meireles, 2015).

1.5.1. Granule Morphology of IWG Starch

Hard wheat flour showed separated large round A-type granules and smaller size B-type granules, whereas the starch granules seemed to be infused together in IWG (Marti et al., 2015). IWG starch granules showed maltese cross under polarized light which indicates a perfectly aligned crystalline structure in the starch granule (Marti et al., 2015). Hard wheat starch typically has a spherical structure (Singh, Singh, Kaur, Sodhi & Gill, 2003). However, little information has been investigated in IWG starch granules.

1.5.2. Thermal Properties of IWG

The Differential Scanning Calorimetry (DSC) would indicate the thermal properties of starch granules (Wankhede et al., 1990; Beleia et al., 1980; Yanez, Walker & Nelson, 1991). The gelatinization temperatures (T_o, T_p, T_c) are measured when starch is being gelatinized with excess water in the heating process. The enthalpy (ΔH) is calculated by the area under the endothermic peak of melting the crystalline structure of amylopectin in starch. The amount of enthalpy indicates the amount and length of the double helices in starch amylopectin (Cooke & Gidey, 1992). High gelatinization temperature represents how perfect the crystalline structure is (Tester, 1997). Jane et al. (1999) reported that the gelatinization temperatures (T_o, T_p, T_c) of wheat starch were 57.1°C, 61.6°C and 66.2°C, respectively. The gelatinization temperature range was 9.1°C. The enthalpy (ΔH) was 10.7 J/g. Similar ranges were also reported in Singh, Singh, Isono & Noda (2010) when they conducted a study with thirteen wheat varieties. However, little information exists in the thermal properties of IWG starch.

1.5.3. IWG Starch Pasting Properties

The amylose to amylopectin ratio and their molecular weight distribution might play a role in functionality differences, such as pasting profile of starch (Mua & Jackson, 1997). Starch pasting profile studies the gelatinization properties of starch/water mixture as a function of time and temperature (Park et al., 2009). The crystalline region would unfold, and the amorphous region would swell in the starch granule during starch gelatinization (Jacobs & Delcour, 1998; Jacobs et al., 1998; Tester & Debon, 2000).

Pasting temperature was significantly higher as more IWG flour (around 96.5 °C) was blended in whole wheat flour which might be related to starch characteristics because the addition of bran to refined wheat flour did not change the pasting temperature (Marti et al., 2015). The high protein and lipid contents in IWG might contribute to the high pasting temperature because of the complexes formed between protein and starch or lipid and starch (Lim, Lee, Shin & Lim, 1999; Kaur & Singh, 2000). Amylose contents of different IWG lines were lower (21-25%) compared to whole wheat flour (Rahardjo et al., 2018; Mathiowetz, 2018). Amylose content is positively correlated to the final viscosity and setback, whereas amylopectin content contributes to the peak viscosity (Jane et al., 1999; Kowittaya & Lumdubwong, 2014). A decrease in peak viscosity was observed when more IWG whole flour was incorporated into refined whole wheat flour when examining the pasting properties which were related to the presence of protein and fiber in higher IWG-enriched flour (Marti et al., 2015; Rahardjo et al., 2018). Proteins in a food matrix can negatively influence the peak viscosity and positively affect the pasting temperature because it can form a film around the starch granules and prevent the starch granule from disruption (Lim et al., 1999). Also, the higher amount of starch in whole wheat flour might contribute to the higher peak viscosity (Rahardjo et al., 2018). The breakdown is the leaching process of amylose and amylopectin after the starch granule reaches its peak viscosity (Rahardjo et al., 2018). Higher breakdown value was shown on wheat flour than IWG flour indicates higher stability in the IWG starch granule structure (Rahardjo et al., 2018). The setback shows the retrogradation tendency of starch during the cooling of the gelatinized starch paste (Tester & Morrison, 1990). Higher final viscosity means the linear amylose chains would re-associate more rapidly. The more amylose in a food system would

generate firmer gels during retrogradation (Ji, Zhu, Zhou & Qian, 2010; Rahardjo et al., 2018). Also, when more IWG flour was added, a low retrogradation tendency was observed which would relate to bread staling during storage (Marti et al., 2015; Collar, 2003). Rahardjo et al (2018) found that different IWG harvests had their unique functional characteristics for different food applications. Pasting temperature was observed to be higher in hard wheat flour than IWG but lower than bulk IWG (Rahardjo et al., 2018). The formation of starch-lipid complexes might result in higher pasting temperature (Kaur & Singh, 2000).

1.5.4. IWG Starch Microstructure

The unit chain distribution of amylopectin can be studied by debranching the α-1,6 glucosidic linkages of starches or amylopectin using isoamylase and pullulanase. The analysis can be conducted on a high-performance anion-exchange chromatography (HPAEC). The results can be classified into two main categories: short chains (DP < 36) and long chains (DP > 36) (Bertoft, 2004). Unit chain distribution can also reveal the chain length of starches, which can affect their enzymatic digestion. Longer glucan chains in amylopectin showed to have higher amounts of resistant starches (You et al., 2014).

Internal and external chains are the two types of chains presented in amylopectin. The branches are believed to contain in the internal chains which are the amorphous part of the molecule, and the external chains represent the crystalline part of the molecule (Pérez & Bertoft, 2010). β-amylase is used to produce internal chains by cleaving the external chains around it. β-amylase can produce maltose units by cleaving the polymer from their non-reducing ends, but it fails to hydrolyze the branched point of amylopectin molecule.

The part where β-amylase is not able to hydrolyze, called β-limit dextrin, is the internal chain of amylopectin (Manners, 1989). Phosphorylase *a* from the rabbit is used to produce φ-limit dextrin (Bertoft, 2004). Degree of polymerization (DP) is used when discussing the chain length of the amylopectin molecule. A study by Hizukuri (1986) classified the amylopectin microstructure into several groups: A-chain is the shortest chain; B1-chain is from DP 20-24; B2-chain is from DP 42-48; B3-chain is from DP 69-75. Bertoft, Piyachomkwan, Chatakanonda & Sriroth, (2008) hypothesized B_{fp} would be DP ≤ 7, BS_{major} would be from DP 8-25, and BL would be B2 (DP 26-50) and B3 (DP > 50). A study by Singh, Singh, Isono, Noda & Singh (2009), which used twenty-four Indian wheat lines, presented all wheat starches had polymodal chain length distribution that peak at DP 11. They also reported that there were 41.1-49.1% short chains (DP 6-12), 47.3-52.3% medium chains (DP 13-24) and 3.6-7.0% long chains (DP>24) (Singh et al., 2009). In contrast, Jane et al (1999) recorded the wheat starch had peak at DP 12, and chain length distribution of 24.18% (DP 6-12), 41.7% (DP 13-24) and 29.2% (DP>24). A ratio of A to B chains indicates the extent of multiple branching, which can be used to analyze the structure of amylopectin (Marshall & Whelan, 1974). However, little information has been investigated on the unit and internal chain profile of IWG starch granules.

1.6. Glycemic Attributes of Intermediate wheatgrass

In-vitro starch hydrolysis kinetics stimulates the breakdown of starch by digestive enzymes into glucose units in the human body after starch intake for two hours (Jenkins et al., 1987; Englyst, Kingman & Cummings, 1992). This model assists in understanding how different starch fractions and expected glycemic indices could influence the functionality

and quality attributes of a food matrix (Englyst et al., 1992). Englyst et al (1992) described three starch fractions in in-vitro starch digestibility which were rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Rapidly digestible starch corresponds to starch being hydrolyzed after the initial 20 minutes after consumption. Slowly digestible starch is the amount of starch hydrolyzed between 20 minutes and 120 minutes of digestion. Resistant starch is the extra starch that did not get hydrolyzed after two hours period (Englyst et al., 1992). In-vitro starch digestibility can be affected by several factors. Retrogradation and drying can reduce the rate of starch hydrolysis, which resulted in a decrease in RDS, and increase in SDS and RS (Hsu et al., 2015). Englyst & Hudson (1996) found that RDS was positively correlated with the GI of a food. Starch hydrolysis rate from low to high by the α-amylase (a digestive enzyme) reported as follows: rice > wheat > tapioca > waxy rice > maize (Singh & Ali, 2006). The higher fiber content in cereal grain would lead to low glycemic response (Brennan, 2005). The high fiber amount would contribute to the increase in digesta viscosity and result in a lower starch hydrolysis rate. Fiber might also form a physical barrier around the starch granules to inhibit starch hydrolysis (Juntunen et al., 2002; Tharakan, Norton, Fryer & Bakalis, 2010). Protein fractions might form a film around the starch granules to prevent enzymes from accessing the granule to lower the starch hydrolysis rate (Rooney & Pflugfelder, 1986; Lim et al., 1999). Amylose in starch can form helical complexes with free fatty acids and monoglycerides that could significantly lower the starch hydrolysis rate (Ai, Hasjim & Jane, 2012; Fanta, Shogren & Salch, 1999; Kawai, Takato, Sasaki & Kajiwara, 2012; Tufvesson & Eliasson, 2000). Phenolic acids presented in the food matrix could influence starch

hydrolysis by acting as a non-competitive inhibitor of hydrolytic enzymes (Shobana, Sreerama & Malleshi, 2009).

Glycemic index, which is defined as the postprandial incremental glucose increase after a meal, has used to classify food in the extent of how the glucose is being released to the bloodstream after consumption (Goñi, Garcia-Alonso & Saura-Calixto, 1997; Jenkins et al., 1987). The glycemic response is expressed as the percentage of the corresponding area after consumption of a reference food, such as white bread (Jenkins et al., 1987). The classification of glycemic foods is: low (GI<55), medium (GI 56-69) and high (GI>70) (Jenkins et al., 1987; Englyst et al., 1992). People with type II diabetes can consume low glycemic food to control the postprandial blood glucose levels in their body (Karl et al., 2015). The glycemic index of bread from refined wheat flour is 67.8 (Chhavi & Sarita, 2012). Biscuits from refined wheat flour is 68 (Anju & Sarita, 2010). However, little information has been investigated on starch hydrolysis kinetics of IWG starch, flour and food matrix.

1.7. Conclusion

Intermediate wheatgrass, which has been explored as an innovative crop for its food application, has high fiber, proteins and lipid contents. Due to its chemical composition, Intermediate wheatgrass can be exploited in the management of type II diabetes. Other seed components in Intermediate wheatgrass might be related to low starch hydrolysis rate. However, the hypoglycemic property in Intermediate wheatgrass has not been investigated. There is also little information on the physical and molecular characteristics of its starch. Understanding the microstructure of Intermediate wheatgrass starch could potentially give

a better background knowledge in explaining the hypoglycemic property of Intermediate wheatgrass. Furthermore, the hypoglycemic property of Intermediate wheatgrass can necessitate more in-depth research in utilizing Intermediate wheatgrass in food applications.

Chapter 2. Starch Hydrolysis Kinetics of Intermediate Wheatgrass (*Thinopyrum intermedium*) Flour and Its Effects on The Unit Chain Profile of Its Resistant Starch Fraction

2.1. Introduction

Intermediate wheatgrass (IWG) (*Thinopyrum intermedium*) is a perennial grass native to Europe and Asia (Hybner & Jacobs, 2012). Currently used as hay and pasture in the northern Great Plains, some parts of Washington, Colorado, Kansas, New Mexico and Arizona in the United States, IWG has the potential to be used for food applications (Culman, Snapp, Ollenburger, Basso & DeHaan, 2013). In addition to its food applications potential, IWG has some environmental advantages such as soil erosion reduction and nitrogen fixation (Culman et al., 2013).

Efforts to explore IWG for food application has been hindered by its low grain yield and small seed size compared to wheat (Hybner & Jacobs, 2012). Significant progress has however been made in recent years on increasing yield and seed size, domestication and development of IWG for food applications. IWG breeders, led by the Land Institute in Kansas, USA, have been able to increase grain yield by approximately 77% and seed size by 23% after two cycles of selection (DeHaan et al., 2014).

The Forever Green Initiative, University of Minnesota and USDA Agricultural Research Service (ARS) program are developing new crops and high-efficiency cropping systems. The Department of Food Science and Nutrition, at the University of Minnesota, Twin-Cities has been exploring the use of IWG for various food applications. As part of this effort, the chemical composition and functional characteristics of whole grain IWG flour were recently investigated and reported (Marti, Bock, Pagani, Ismail & Seetharaman, 2016). These studies reported significant differences in the chemical composition and

functional characteristics between IWG and hard red wheat (HRW). IWG kernel has an average weight of 8.32 mg in Minnesota after the third cycle of breeding while the mean weight of commercial bread wheat is over 30 mg (Zhang et al., 2017). IWG was found to have significantly higher bran content of 47.8% to 56.0% compared to bran content of 16.8% for HRW. The higher bran content was attributed to its small kernel size (Becker, Wagoner, Hanners & Saunders, 1991).

The starch contents of IWG harvested in 2014 ranged from 46.74 - 52.45 g/100 g in dry basis (Rahardjo et al., 2018). The starch hydrolysis kinetics of IWG has however not been investigated. This is important in determining how the different starch fractions and expected glycemic index (eGI) of IWG will be affected in different food systems. Measurement of *in-vitro* starch digestibility and glycemic index (GI), allows for the classification of foods into low (GI<55), medium (GI 56-69) and high (GI>70) glycemic index based on the extent to which their hydrolysis release glucose into the bloodstream when consumed (Jenkins et al., 1987; Englyst, Kingman & Cummings, 1992). Frequent consumption of foods with low glycemic indices has been reported as an important strategy in the control of postprandial blood glucose levels in people with type II diabetes (Karl et al., 2015).

In-vitro starch digestibility and glycemic index can be affected by various factors such as: degree of starch gelatinization (Marangoni & Poli, 2008) and retrogradation (Hsu, Chen, Lu & Chiang, 2015), viscosity of the food matrix (Kaur & Singh, 2009), anti-nutritional variables (Yoon, Thompson & Jenkins, 1983), presence of dietary fiber (Jenkins et al., 1987), lipids (Kawai, Takato, Sasaki & Kajiwara, 2012; Annor, Marcone, Corredig, Bertoft & Seetharaman, 2015) and proteins (Rooney & Pflugfelder, 1986), etc. With the

relatively high fiber, protein and lipid contents of IWG, it is hypothesized that the *in-vitro* starch digestibility and eGI of fully gelatinized IWG whole flour and extracted starches will be significantly lower than those of other cereals. Recent research also revealed a novel finding that retrogradation can induce more slowly digestible starch with the external A and B chains forming intermolecular associations (Martinez et al., 2018).

Cereal starches with greater amounts of shorter chains and branches are comparatively slowly hydrolyzed by amylolytic enzymes than starches with fewer branches. It has also been reported that short chains in amylopectin induce weak points in the structure of starch resulting in higher susceptibility to starch degrading enzymes (Jane, Wong & Mcpherson, 1997). Starches with long glucan chains in their amylopectin exhibited relatively higher slowly digestible starches (Zhang, Ao & Hamaker, 2008). Unit chain profile of starches or amylopectin can be determined by hydrolyzing specifically α-(1,6) linkages with pullulanase and isoamylase and the resulting chains analyzed by high-performance anion-exchange chromatography. Very little information exists on the unit chain profile of the resistant starch fraction of starches after digestion with α-amylase and amyloglucosidase. This study focused on investigating starch hydrolysis kinetics of IWG starch from two locations and the molecular structure of residual (resistant) starch after 2 hr hydrolysis. Information generated from this study is important in understanding the fine structural characteristics of IWG resistant starches.

2.2. Experimental

2.2.1. Materials

IWG whole grain kernels were obtained from two growing locations: Roseau (IWG-RS) and Rosemount (IWG-RM), MN, USA. HRW whole grain kernel was obtained from Grain Millers, Eden Prairie, MN USA. Dynasty® Jasmine rice (JR) was purchased in a local store (St Paul, MN). It is worth noting that the JR was polished. HRW and polished JR were selected as controls because they are the most commonly consumed cereal grain. All kernels were kept at room temperature for this study.

2.2.2. Sample preparation

IWG, HRW and JR were milled into flour using the UDY cyclone mill (Fort Collins, CO, USA) with a 100 μm sieve. Note that IWG from both locations and HRW were milled from whole grain kernels. JR was milled from polished grain because it was purchased from a local store as stated above. Milled samples were then stored at 4ºC throughout the study.

2.2.3. Starch extraction

Starch was extracted from flour samples according to the method reported by Waduge, Xu & Seetharaman (2010) with modifications. Grain samples were frozen with liquid nitrogen and immediately milled for 1 min with a coffee grinder (Bodum® Bistro, NY, USA 10001) into a flour. An alkaline extraction buffer solution (12.5 mM, pH 10, containing 0.5% SDS and 0.5% $Na_2S_2O_5$ (w/v) was added to the flour and the mixture became a 5% (w/v) slurry. The mixture was stirred for 10 min, and the samples were recovered by centrifugation at 4000 rpm for 10 min (at 4ºC). The extraction step was then repeated. The resulting residue was washed three times with distilled water, and recovered

again by centrifuging at 4000 rpm for 10 min (at 4ºC). The residue was then suspended in distilled water and the starch slurry was passed through four layers of cheesecloth and then through a 70 μm nylon mesh. The slurry was centrifuged at 4000 rpm for 10 min (at 4ºC), and the top brown layer was scraped off with a spatula. These steps were continued until all the brown layer was removed from the starch fraction. The extracted starch was then washed with acetone and centrifuged at 4000 rpm for 10 min (at 4ºC). The extracted starches were then air dried.

2.2.4. Chemical analysis

Ash content of the samples was determined by dry ashing method using muffle furnace (AOAC 923.03). Moisture content was determined by force draft oven drying (AOAC 935.29). Crude fat content was determined by Soxhlet extraction method using petroleum ether for 6 hr. Protein content was determined by the Dumas combustion method (AACC 46-30.01). Total carbohydrate was determined by subtraction. Results were reported on a dry weight basis.

2.2.5. Total starch

Total starch contents of whole flour samples were determined by Megazyme total starch assay kit (Megazyme International Ireland, Bray, Wicklow, Ireland). All total starch results were reported on a dry weight basis. Total starch assays were performed on the extracted starch from each sample to ensure the purity of extracted starch for further analysis.

2.2.6. Resistant starch

Resistant starch was carried out by Megazyme resistant starch assay kit (Megazyme International Ireland, Bray, Wicklow, Ireland). All resistant starch results were reported on a dry weight basis.

2.2.7. In-vitro starch digestibility and expected glycemic index

In-vitro starch digestibility of whole flour and starch samples were carried out based on a method developed by Englyst et al., (1992). About 0.7 g raw whole flour and starch samples were weighed based on total starch content, 10 mL double distilled water added and cooked. Ten (10) mL of sodium acetate buffer (0.1M pH 5.2) and 5 mL enzyme solution were added to samples. Enzyme solution was prepared by a mixture of pancreatin from porcine pancreas (Sigma Aldrich, P-1625, activity 3×USP/g), Invertase from baker's yeast (*Saccharomyces cerevisiae*) (I450A-1G, Sigma-Aldrich) and amyloglucosidase (200 U/mL p-nitrophenyl β-maltoside, Megazyme International Ireland, Bray, Wicklow, Ireland) according to the method described by Englyst et al. (1992). Samples were incubated at 37°C for 2 hr. At intervals of 20 min, 0.1 mL aliquots of hydrolyzed samples were pipetted and added to 0.9 mL 80% ethanol to stop the hydrolysis. The amount of glucose released from the samples was determined with glucose oxidase peroxidase (GOPOD). Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) are the three main categories in hydrolyzed starch (Englyst et al., 1992). RDS = glucose detected at 20 min × 0.9; SDS = (glucose detected at 120 min – glucose detected at 20 min) × 0.9 and RS = total starch - (RDS + SDS). The hydrolysis kinetics of samples was described

using a nonlinear first-order equation established by Goñi, Garcia-Alonso & Saura-Calixto (1997). C is the starch hydrolyzed at a chosen time t; $C\infty$ is the equilibrium concentration at the final time (120 min); k is the kinetic constant. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (AUC) of the samples by AUC of white bread, which serves as a reference sample, as reported by Goñi et al. (1997). The AUC was calculated by the equation: AUC = C_∞ (t_f–t_o) – (C_∞/k) [1–$e^{-k(t^f-t^o)}$], where t_f is the final time and t_o is the initial time. The eGI was calculated by the equation: eGI = 8.198 + 0.862 *HI as described by Granfeldt, Bjorck, Drews & Tovar (1992).

2.2.8. Enzymatic hydrolysis of raw starch samples

About 0.7 g raw starch samples were weighed based on total starch content. Ten (10) mL of sodium acetate buffer (0.1 M pH 5.2) and 5 mL enzyme solution were added to samples. Enzyme solution was prepared by a mixture of pancreatin from porcine pancreas (Sigma Aldrich, P-1625, activity 3×USP/g), invertase from baker's yeast (Saccharomyces cerevisiae) (I4504-1G, Sigma-Aldrich) and amyloglucosidase (200 U/ml *p*-nitrophenyl β-maltoside, Megazyme International Ireland, Bray, Ireland) according to the method described by Englyst et al. (1992). Samples were incubated at 37°C for 2 hr. Eighty (80) mL of 95% ethanol was added to the samples to stop the hydrolysis. Contents were then transferred to 150 mL centrifuge tubes and centrifuged at 1500 × g for 10 min and precipitate dried at 50˚C in the forced air oven.

2.2.9. Size distribution of resistant starches

Hydrolyzed starch samples were prepared from hydrolyzing native starches. Two (2) mg hydrolyzed starch samples were dissolved in 90% dimethyl sulfoxide (DMSO; 100 μL), heated in a hot water bath (80°C) for 5 min and then stirred overnight at room temperature (25°C). Water (750 μL, 80°C) and 100 μL of 0.01 M sodium acetate buffer (pH 5.5) were then added. One (1) mL of sample was applied to a column (1 × 90 cm) of Sepharose CL-6B gel (GE Healthcare, Uppsala, Sweden), and eluted with 0.5 M NaOH at 1 mL/min. Fractions (1 mL) were analyzed for carbohydrates with phenol–sulfuric acid reagent (Dubois et al., 1956). Absorbances were taken at 490 nm with the WPA S800 Diode Array Spectrophotometer, (Biochrom Ltd., Cambridge, United Kingdom). Pullulan standards were used to calibrate the Sepharose CL-6B gel column with the following molecular weights (P-5: 0.59 x 10^4 g/mol; P-20: 2.28 x 10^4 g/mol; P-100: 11.2 x 10^4 g/mol; P-200: 21.2 x 10^4 g/mol; and P-800: 78.8 x 10^4 g/mol) (Shodex Denko America, Inc, New York, USA).

2.2.10. Starch granule morphology

The surface characteristics of native and hydrolyzed raw starches were viewed with a Hitachi S-570 scanning electron microscope (Hitachi Scientific Instruments, Rexdale, Ontario, Canada) after starches were sputtered with 15 nm of gold dust on a stub. The working distance used was 15 mm with a voltage of 10 kV.

2.2.11. Unit chain profiles of hydrolyzed starches

Freeze dried hydrolyzed starch (2.0 mg) were dissolved in 90% DMSO (50 μL) with gentle stirring overnight. The solution was diluted by adding warm water (400 μL,

80°C), after which 0.01 M sodium acetate buffer (50 μL, pH 5.5) was added. Isoamylase (1 μL) and pullulanase M1 (1 μL) (Megazyme International Ireland, Bray, Wicklow, Ireland) were added to the mixture, which then was stirred overnight at room temperature. After debranching, the enzymes were inactivated by boiling for 5 min, the volume adjusted to obtain a final concentration of 1 mg/mL, and the sample filtered through a 0.45 μm nylon filter. The filtered sample (25 μL) was injected into the Dionex ICS 3000 HPAEC system (Dionex Corporation, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector, CarboPac PA-100 ion-exchange column (4 × 250 mm), and a similar guard column (4 × 50 mm). The samples were then eluted with a flow rate of 1 mL/min. The two eluents used were 150 mM sodium hydroxide (A) and 150 mM sodium hydroxide containing 500 mM sodium acetate (B). An eluent gradient was made by mixing eluent B into eluent A as follows: 0-9 min, 15-36% B; 9-18 min, 36-45% B; 18-110 min, 45-100% B; 100-112 min, 100-15% B; and 112-130 min, 15% B. The system was stabilized by elution at 15% B for 60 min between runs. The areas under the chromatograms were corrected to carbohydrate concentration following the method of Koch, Anderson & Åman (1998).

2.2.12. Statistical analysis

All data were collected at least in duplicate. ANOVA one-way test was used to determine significant differences between sample means when $p < 0.05$. All statistical analysis was conducted using Statgraphics Centurion XV, version 15.1.02 (StatPoint, Warrenton, VA, USA).

2.3 Results and Discussion

2.3.1. Chemical composition

The chemical composition of the samples used in this study is shown in Table 1.1. Except for protein content, the ash and fat contents of the IWG-RS and IWG-RM were not significantly different. The ash content of the samples ranged from 0.2% to 2.3%. IWG-RS and IWG-RM had similar ash contents of 2.3% and 2.0%, respectively. The significantly higher amount of ash in both IWG samples suggested they are good sources of inorganic minerals compared to HRW and JR (Rahardjo et al., 2018). The fat contents of samples ranged from 0.8% to 2.4%, with IWG samples from both locations having similar fat contents (2.3% and 2.4%). The protein content of IWG-RS (17.9%) was significantly different from that of IWG-RM (15.5%). The amount of fat and proteins present in IWG flours were significantly higher than that of HRW and JR, which also aligned with the results reported by Rahardjo et al. (2018). Comparably, less carbohydrate was observed in both IWG and HRW flours compared to JR.

Table 1.1. Chemical composition of JR, HRW and IWG-RS and IWG-RM flour samples[z]

	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
JR	8.1±0.004[b]	0.2±0.0006[a]	0.8±0.0007[a]	7.2±0.4[a]	83.7[c]
HRW	6.2±0.0009[a]	1.5±0.0007[b]	1.9±0.0006[b]	13. 5±0.1[b]	76.9[b]
IWG-RS	5.9±0.0008[a]	2.3±0.0002[c]	2.3±0.0007[c]	17.9±0.07[d]	71.6[a]
IWG-RM	6.0±0.002[a]	2.01±0.001[c]	2.4±0.0005[c]	15.5±0.8[c]	74.1[a]

[z]Values are expressed as mean percentages (n=2). Values with different letters in columns are significantly different ($p < 0.05$) from each other. Carbohydrate contents were calculated by difference. JR: Jasmine rice, HRW: Hard red wheat, IWG-RS and IWG-RM: Intermediate wheatgrass grown in Roseau and Rosemount, Minnesota, USA respectively.

Table 1.2 shows the total and resistant starch contents of whole flour samples as well as their extracted starches. JR had the most amount of total starch (78.4%). This was followed by HRW (61.1%) and then IWG-RM (52.5%) and IWG-RS (51.7%). Resistant starch contents ranged from 2.2 to 6.6%. Extracted starches from the samples were relatively pure with total starch contents of above 90%. It is important to note that all samples were subjected to the same starch extraction protocol. HRW flour had the highest amount of resistant starch of 6.6%; followed by IWG-RM (4.3%). Resistant starch content of IWG-RS and JR were similar, while that of IWG-RM was twice as high as that of IWG-RS. This observation suggests that the glycemic index of IWG-RS and that of JR could be similar and higher than that of HRW and IWG-RM due to the suggested strong correlation between resistant starch and glycemic index (Kumar et al., 2017).

Table 1. 2. Total and resistant starch of JR, HRW and IWG-RS and IWG-RM flour samples[z]

	Total Starch (%)		Resistant Starch (%)
	Flour	Extracted starch	Flour
JR	78.4±0.1[c]	89.6±1.2[a]	2.3±0.2[a]
HRW	61.1±1.0[b]	98.1±0.6[b]	6.6±0.1[c]
IWG-RS	51.7±0.4[a]	88.9±0.6[a]	2.2±0.1[a]
IWG-RM	52.5±0.6[a]	94.1±1.3[a]	4.3±0.1[b]

[z]Values are expressed as mean percentages (n=2). Values with different letters in columns are significantly different ($p < 0.05$) from each other. JR: Jasmine rice, HRW: Hard red wheat, IWG-RS and IWG-RM: Intermediate wheatgrass grown in Roseau and Rosemount, Minnesota, USA respectively.

2.3.2 In-vitro starch digestibility and eGI.

RDS, SDS, RS, HI, *k* and eGI of flour and starch samples measured by the modified Englyst et al. (1992) method are shown in Table 1.3. The hydrolysis kinetics of the samples over 2 hr is also shown in Figure 1.2 [A]. At 120 min, about 50% of JR flour starch had been hydrolyzed compared to about 40% from both IWG flour samples (Figure. 1.2 [A]). The amounts of IWG hydrolyzed in his study were similar to that hydrolyzed for Kodo millet as reported by Annor, Marcone, Bertoft & Seetharaman, (2013). The relatively low amounts of starch hydrolyzed in IWG flour samples might be due to the high dietary fiber, protein and lipid contents (Annor et al., 2013). A review by Brennan, (2005) also indicated that high dietary fiber content (mainly soluble fiber) leads to a lower amount of hydrolyzed starch resulting in a lower glycemic index. The low starch hydrolysis rate might be caused by an increase in digesta viscosity in the human intestinal tract because of the high soluble fiber content (Juntunen et al., 2002; Tharakan, Norton, Fryer & Bakalis, 2010). Soluble fiber might also form physical barriers around starch granules which would limit the access of amylolytic enzymes and lead to lower hydrolysis rate (Ellis, Dawoud & Morris, 1991). The effect of proteins on starch digestibility as reported in a study by Rooney & Pflugfelder, (1986) asserted that proteins form a film around the starch granules and thus prevents starch hydrolyzing enzymes from accessing the starch granules. On the other hand, lipid could form amylose-lipid complexes that could limit the rate of enzymatic degradation in starch

(Jane & Robyt, 1984). The RDS of HRW and JR flour samples were similar and significantly higher than that of IWG-RS and IWG-RM. RDS is defined as rapidly digestible starch and is absorbed in the duodenum and proximal regions of the small intestine resulting to a spike of blood glucose and usually a subsequent episode of hypoglycemia (Zhang, Ao & Hamaker, 2008). Interestingly, RDS of IWG-RS (22.8%) was significantly ($p< 0.05$) higher than the RDS of IWG-RM (20.7%). The SDS of IWG-RS (18.6%) and IWG-RM (20.2%) were lower than those of JR (23.0%) and HRW (21.1%). The consumption of food incorporated with slowly digestible carbohydrates and resistant starch could be beneficial to weight management for an individual because it could delay gastric emptying and digestion rate (Frost & Dornhorst, 2000; Scheppach, Luchrs & Menzel, 2001). RS for JR flour was significantly higher than all other flour samples. Starch hydrolysis rates of the flour samples as indicated by *k* was the lowest for IWG-RM (0.0353) and the highest for IWG-RS (0.0404). HRW and JR had similar rates of hydrolysis; 0.0391 and 0.0392 respectively. Based on the linear relationship between hydrolysis index and glycemic index, the faster hydrolysis rate will correspond to higher glycemic index (Granfeldt et al., 1992; Goñi et al., 1997). The eGI indices of the flour samples were in the following order: JR> HRW >IWG-RS>IWG-RM. All flour samples had eGI that were lower than 55 and can be classified as low glycemic (Brennan, 2005). The eGI observed for JR in this study was significantly lower than that reported for different varieties of African and some Asian rice varieties (Gayin et al., 2017). In their study, eGI of above 70 was reported. These differences in eGI observed in this study and those reported by Gayin et al. (2017) could be due to varietal differences, as the same method was used in both studies. The significantly lower eGI of the IWG flour samples used in this study could also

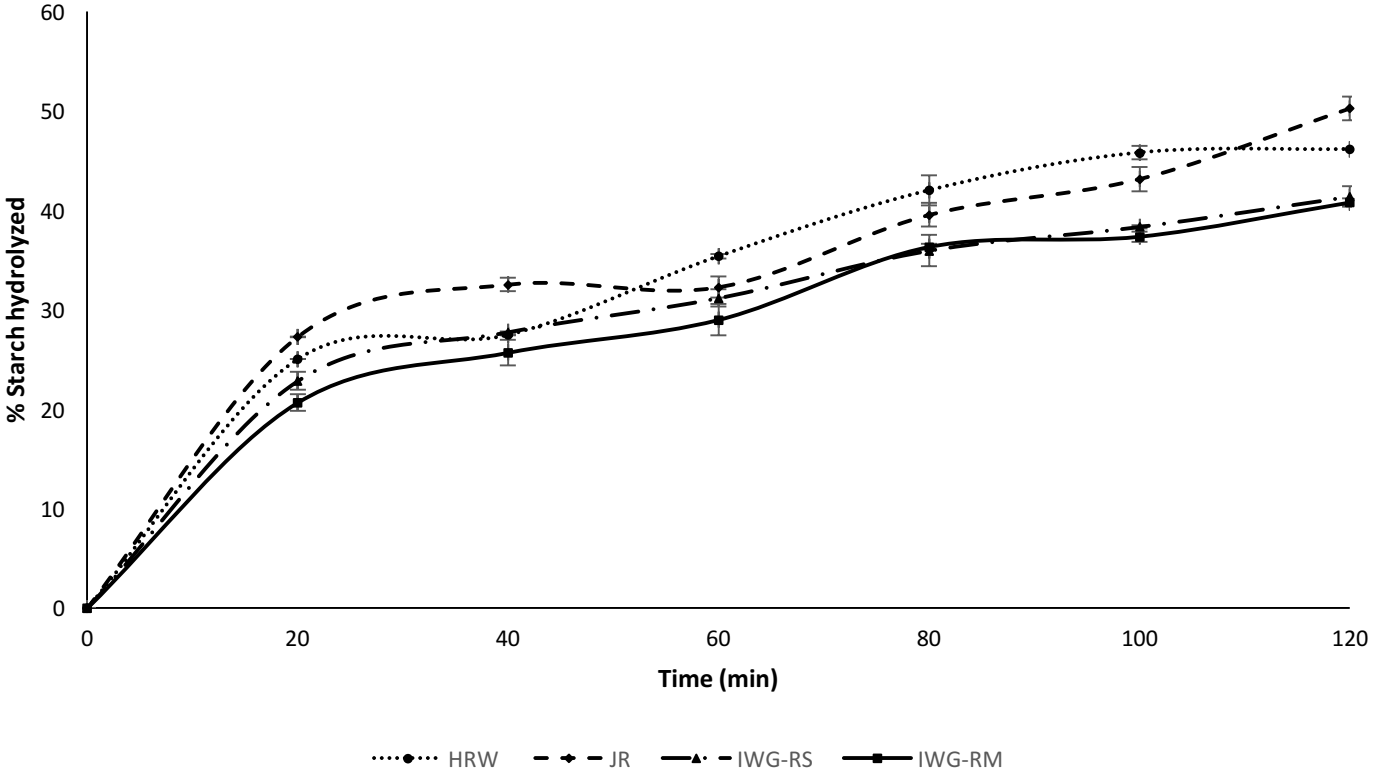
be due to the presence of significantly more lipids and protein (Annor et al., 2013). JR, which had the highest eGI had the least amounts of proteins and lipids. Seneviratne & Biliaderis (1991) reported an inverse relationship between the rate and extent of hydrolysis and amylose-lipid complexes.

To investigate the starch hydrolysis rates of the samples without the confounding effects of other grain components such as lipids, proteins and fiber, the *in-vitro* starch hydrolysis of cooked extracted starches of the samples was carried out and results are shown in Table 1.3 and Figure 1.2 [B]. Results showed an increase in the starch hydrolysis rates of the cooked extracted starches vs the flour samples. This observation confirms the effects of other seed components on starch hydrolysis (Annor et al., 2013). Cooked extracted starch of IWG-RS had the highest amount of starch hydrolyzed after 2 hr (about 60%). RDS of cooked extracted starches of IWG from both locations (IWG-RS: 35.23 and IWG-RM: 32.99) were similar to that of cooked extracted starch from JR (34.03). SDS of cooked extracted starches were similar, except for that of IWG-RS. With respect to eGI, higher values were observed for cooked starch samples compared to that of the cooked flour samples (Table 1. 3). While eGI of cooked flour from IWG-RS was only higher than that of IWG-RM flour, its cooked extracted starch had the highest eGI of all the samples (59.2). The cooked extracted starch of HRW had the lowest eGI. The difference in the hydrolysis kinetics of the cooked extracted starches could be due to differences in their starch structure and their ratio of amylose and amylopectin. Since these samples were cooked, the possible effects of granular and the supramolecular effects of starch on starch hydrolysis rates were lost (Zhang & Hamaker, 2009).

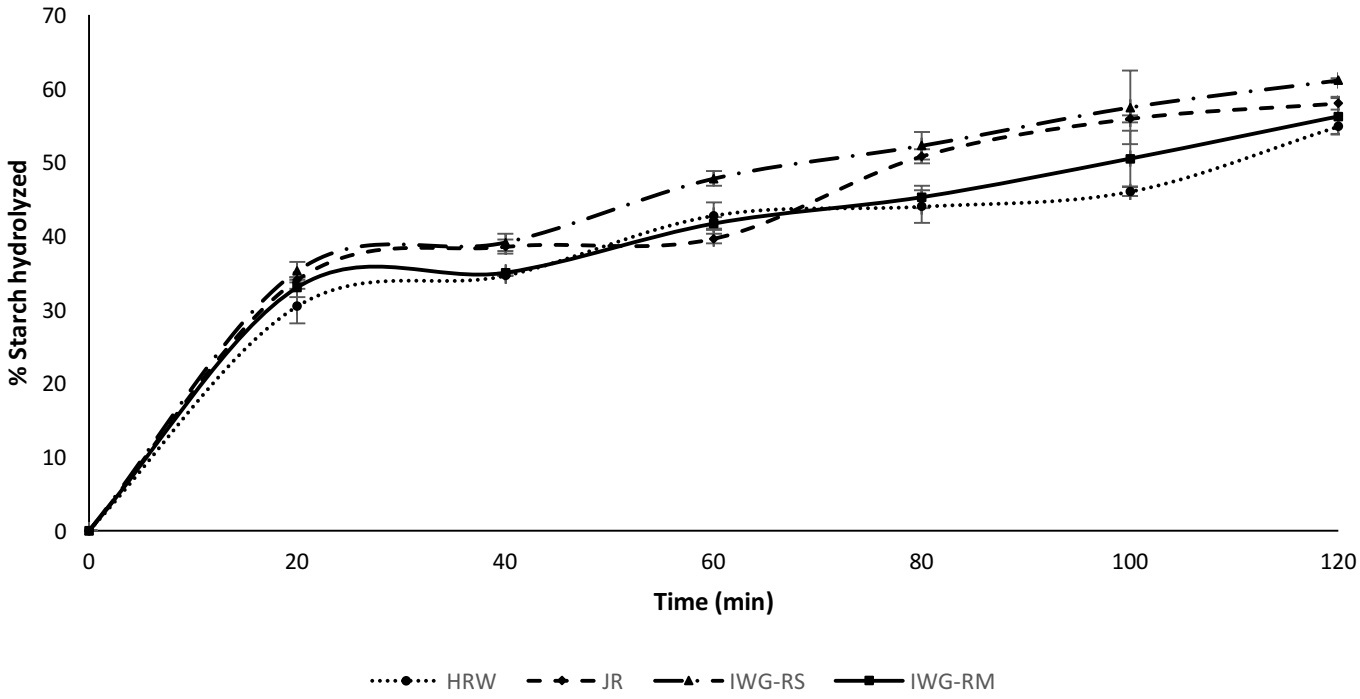
Table 1. 3 RDS, SDS, RS, HI, *k* and eGI of JR, HRW and IWG-RS and IWG-RM flour and starches

Sample	RDS	SDS	RS	HI	*k*	eGI
HRW flour	25.0±0^{b,c}	21.1±0.07^{a,b,c}	14.93±0.1^{b}	43.7±0.04^{b}	0.0391±0^{a,b}	45.9±0.03^{a,b}
JR flour	27.3±0.03^{c,d}	23.0±1.2^{b,c,d}	28.17±1.2^{c,d}	47.6±0.7^{b}	0.0392±0.001^{a,b}	49.2±0.6^{a,b}
IWG-RS flour	22.8±0.9^{a,b}	18.6±1.9^{a}	10.30±1.0^{a}	39.4±0.01^{a}	0.0404±0.004^{a,b}	42.2±0^{a,b}
IWG-RM flour	20.7±0.8^{a}	20.2±0.4^{a,b}	11.72±0.4^{a,b}	37.6±0.8^{a}	0.0353±0.002^{a}	40.6±0.7^{a}
HRW starch	30.5±2.4^{d,e}	24.4±1.4^{c,d}	43.30±1.0^{f}	52.3±2.2^{c}	0.0406±0.004^{a,b}	53.3±1.9^{a,b}
JR starch	34.0±0.4^{e,f}	23.9±0.5^{c,d}	31.67±0.9^{d}	55.7±0.8^{d,e}	0.0445±0.0003^{b}	56.9±0.7^{b}
IWG-RS starch	35.2±1.2^{f}	25.8±0.9^{d}	27.83±0.3^{c}	59.2±0.9^{e}	0.0430±0.002^{b}	59.2±0.8^{b}
IWG-RM starch	33.0±1.3^{e,f}	23.2±1.1^{b,c,d}	37.90±2.5^{e}	54.8±2.4^{c,d}	0.0442±0.0003^{b}	55. 4±2.0^{b}

Values are expressed as mean percentages (n=2). Values with different letters in columns are significantly different ($p < 0.05$) from each other. JR: Jasmine rice, HRW: Hard red wheat, IWG-RS and IWG-RM: Intermediate wheatgrass grown in Roseau and Rosemount, Minnesota, USA respectively. RDS: Rapidly digestible starch; SDS: Slowly digestible starch; RS: Residual starch; HI: Hydrolysis index; *k*: Kinetic constant; eGI: Expected glycemic index.



A. Starch hydrolysis kinetics of JR, HRW, IWG-RS and IWG-RM flour samples



B. Starch hydrolysis kinetics of JR, HRW, IWG-RS and IWG-RM starches

Figure 1. 2. Starch hydrolysis kinetics of JR, HRW, IWG-RS and IWG-RM flour and starch samples

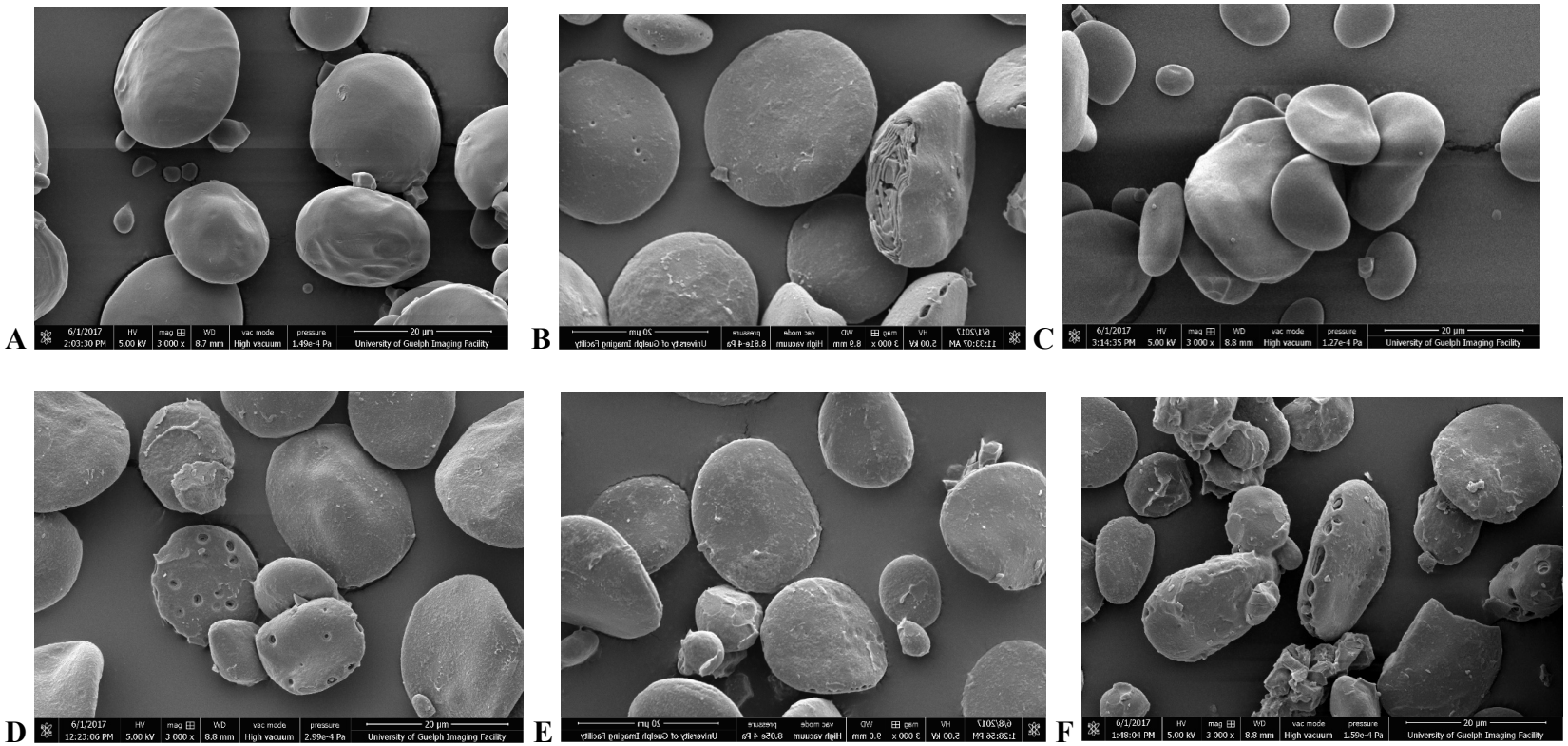
2.3.3. Surface morphology of native and enzymatically hydrolyzed starch granules

The surface morphology of native and hydrolyzed starch granules of the samples except for JR are shown in Figure 1.3. Starch granules of IWG and HRW had similar surface morphological characteristics. This is expected as IWG is related to wheat. The native starches of IWG and HRW were observed to be large disc-shaped granules with some smaller spherical and very few polygonal granules. The flat side of the disc-shaped granules of IWG and HRW appeared to have large indentations. These indentations seemed to be more prominent in HRW compared to IWG. Pinholes were also observed at the sides of the disc-shaped granules as seen in Figure 1.3 [E]. The morphological characteristics of IWG and HRW are very different from that of JR starch, which has been reported to be mainly of small polygonal granules (Wani et al., 2012).

A look at the hydrolyzed starch granules of HRW and IWG shows the development of pinholes on the flat side of the granules (Figure 1.3 [B, D and F]). These pinholes appeared to be bigger on the disc-shaped starch granules of IWG compared to that of HRW. The pinholes on the sides of the disc-shaped granules were also larger on the hydrolyzed starch granules compared to their native counterparts. The appearance of the pinholes on the surface of the hydrolyzed granules did suggest an in-out enzymatic digestion by the starch hydrolyzing enzymes. In general, the hydrolyzed starch granules of HRW seemed to be more intact compared to those of IWG (Figure 1.3 [B]). This observation seems to correlate with the expected glycemic indices of HRW and IWG starches, where the latter had higher eGI.

2.3.4. Size distribution of the resistant starch fraction

To investigate the molecular size distribution of the resistant starches produced after the two hours of enzymatic hydrolysis, hydrolyzed starches were run on Sepharose CL-6B after dissolution in 90% DMSO. The column was calibrated with pullulan standards with the following molecular weights (P-5: 0.59 x 10^4 g/mol; P-20: 2.28 x 10^4 g/mol; P-100: 11.2 x 10^4 g/mol; P-200: 21.2 x 10^4 g/mol; and P-800: 78.8 x 10^4 g/mol). Appendix B Figure 3.1 shows the chromatograms of the pullulan standards and the resistant starches. The results showed that the resistant starch fractions of the samples differed in their size distribution. All the samples eluted in the void of the column before the pullulan standard with the highest molecular weight. This observation indicates the resistant starch fractions of the samples are relatively large molecules. The two IWG samples had similar size distributions. They eluted within a small range, compared to the resistant starch fraction of HRW which had the largest size distribution. The resistant starch fraction from JR also had a broad size distribution and also was the first to elute. Results from the gel permeation chromatography did suggest significant differences in the structure of the resistant starch fraction of the samples.



A: native HRW; B: enzyme hydrolyzed HRW; C: normal IWG-RM; D: enzyme hydrolyzed IWG-RM; E: normal IWG-RS; F: enzyme hydrolyzed IWG-RS

Figure 1. 3 Scanning electron micrographs of native and enzyme hydrolyzed HRW, IWG-RS and IWG-RM starches

2.3.5. Unit chain profiles of hydrolyzed starch samples

The determination of unit chain profile of the resistant starch fractions of the samples was necessitated by observations made from the determination of the molecular size distribution of the fractions on gel permeation chromatography. The use of debranching enzymes and high-performance anion-exchange chromatography allowed for more detailed analysis of the fractions. The unit chain profiles of the resistant fraction of the samples are shown in Appendix C Figure 4.1. The mole percent and chain lengths of short (DP<36) and long (DP>36) (Bertoft, 2004) chains are shown in Table 1.4. The results indicated that the samples had more short chains than long chains. The average chain lengths of the resistant starch fractions ranged from 4.75 to 7.47. These values were significantly less than that average values of 17 to 18 reported for amylopectins from millet, rice, maize and potato (Annor, Marcone, Bertoft & Seetharaman, 2014; Bertoft, 2004; Bertoft, 2013). The significantly shorter average unit chains of the resistant starch fractions were consistent with the fact that they were hydrolyzed by the starch degrading enzymes. Interestingly, the long chains had similar lengths as amylopectin samples reported in the aforementioned references. This observation suggests that the starch hydrolyzing enzymes hydrolyzed mostly the shorter chains such as the A-chains and the short B-chains in the starches, leaving the longer B-chains virtually intact. A-chains in the amylopectin molecule are chains that do not carry any other chains (Peat, Whelan & Thomas, 1952). They are mostly short chains, and some of them are involved in the formation of the crystalline structure of amylopectin (Hizukuri, 1986; Bertoft, 2013). The B-chains, on the other hand, carry other chains and can be classified into short and long B-chains (Hizukuri, 1986). The

short chains observed in the resistant starch fractions were likely to be the A-chains that are involved in the crystalline structure of amylopectin. These A-chains are referred to as $A_{crystal}$ (Bertoft, 2013). The chains in the crystalline structure of cereal starches have been reported to have more short chains and branches hence resistant to enzymatic hydrolysis (Zhang & Hamaker 2009). The mole ratio of short to long chains of the resistant starch fractions were significantly different. HRW had the least ratio of 35.6 compared to 66.77, 53.27 and 66.63 for JR, IWG-RS and IWG-RM respectively. This observation shows that the resistant starch fraction of HRW has the most mole percent of long chains. The chromatograms of the unit chain profiles of the samples as indicated by Appendix C Figure 4.1 shows differences in the unit chain profiles of the resistant starch fraction of the samples. Every sample had a significantly high amount of glucose after each was debranched, with HRW having the lowest amount. The chromatograms also indicated that the short chains of the chromatograms were divided into two categories at about DP 19. This division was less pronounced in JR. Apart from the presence of small chains of DP from 1 to 5, the unit chain profiles of the resistant starch fraction of the samples were similar to the typical amylopectin unit chain profiles. Appendix A Table 3.1 shows the correlation matrix between the resistant content of IWG starch determined by the Englyst et al. (1992) protocols and the unit chain profiles of the resistant starch fractions. It is important to note that the Megazyme resistant starch method was performed on the flour samples whilst the of the Englyst et al. (1992) procedure was for the raw starches. Resistant starch obtained from Englyst et al. (1992) procedure showed a significant positive correlation (0.9379) to resistant starch performed using the Megazyme method. It was observed that the resistant starch fraction of the starches was positively correlated (0.4996) with the molar amounts

long chains but negatively correlated (-0.3033) with the length of the long chains (Appendix A Table 3.1). The negative correlation (-0.3809) of the molar amounts of short chains to the amount of resistant starch fraction contradicts the observation made by Jane et al. (1997) that greater amounts of shorter chains and branches in cereal starches are slowly hydrolyzed by starch degrading enzymes.

Table 1. 4 Short and long chains mole percentages, chains length of JR, HRW and IWG-RS and IWG-RM hydrolyzed starch samples[z]

	Mole (%)		Mole Ratio	Chain length		
Sample	Short chains (DP <36)	Long chains (DP >36)	Short chains: Long chain	Short chains (DP <36)	Long chains (DP >36)	All chains
JR	20.72±0.08[c]	0.32±0.03[a]	65.77±6.98[b]	4.07±0.07[a]	48.77±0.58[a]	4.75±0.01[a]
HRW	13.05±0.04[a]	0.36±0.01[a]	35.97±0.37[a]	6.29±0.00[c]	49.58±0.24[ab]	7.46±0.02[c]
IWG-RS	17.18±0.64[b]	0.30±0.01[a]	53.37±2.65[b]	4.90±0.17[b]	50.37±0.07[b]	5.72±0.21[b]
IWG-RM	20.34±0.32[c]	0.31±0.01[a]	66.63±1.35[b]	4.18±0.06[a]	48.92±0.13[a]	4.85±0.07[a]

[z]Values are expressed as mean percentages (n=2). Values with different letters in columns are significantly different ($p < 0.05$) from each other. JR: Jasmine rice, HRW: Hard red wheat, IWG-RS and IWG-RM: Intermediate wheatgrass grown in Roseau and Rosemount, Minnesota, USA respectively; DP: Degree of Polymerization.

2.4. Conclusions

This study, for the first time, documented the starch hydrolysis kinetics and the unit chain profile of the resistant starch fraction of IWG. The eGI of fully gelatinized IWG flour was found to be significantly lower than both HRW and JR. This observation makes IWG flour a better alternative to HRW and JR in the management of type II diabetes. The same observation was however not observed for fully gelatinized extracted starches. The study also highlights the importance of using IWG as whole grains rather than refined grained due to the possible effects of other seed components such as lipid and protein in maintaining their hypoglycemic property. The resistant starch fraction of the samples consisted more of shorter chains.

Chapter 3. Structural characterization of intermediate wheatgrass (*Thinopyrum intermedium*) starch

3.1. Introduction

Intermediate wheatgrass (IWG) is a native plant from Europe and Asia, but it is also grown in the United States (Hybner & Jacobs, 2012). It produces a large biomass and is used as cool-season forage in the western part of the United States (Harmoney, 2015). Annual grains provide around 70% of food needed for humans, but annual grain production is less sustainable and has less beneficial impact for the environment (Cox et al., 2006). Perennial grains require less herbicide and do not require annual plowing (Culman, Snapp, Ollenburger, Basso & DeHaan, 2013). IWG is a perennial grain with multiple environmental benefits including reducing soil erosion, lowering nitrate leaching, and increasing carbon sequestration (Culman et al., 2013). The long root system of IWG can help in capturing fertilizers easily (Culman et al., 2013). The yield of IWG is about 1500 kg/hectares, while wheat is about 4500 kg/hectares (Culman et al., 2013).

The Forever Green Initiative of the University of Minnesota is interested in producing crops with potential food values that benefit the ecosystem. Over the past few years, the Forever Green Initiative has been promoting IWG for food applications, and this has resulted in the chemical and functional properties of IWG being studied (Marti, Bock, Pagani, Ismail & Seetharaman, 2016; Rahardjo et al., 2018; Banjade, Gajadeera, Tyl, Ismail & Schoenfuss, 2019). Baked products, such as bread, crackers and cookies, are one of the most popular food items made from cereal grains (Rahardjo et al., 2018; Banjade et al., 2019). To better understand the functional differences between using wheat and IWG in such products, the physical and molecular characterization of IWG need to be studied and compared with wheat and rice because they are the most commonly consumed cereal grains.

Starch is one of the major components in cereal grain. The micro-structure in starch could have an impact in the functionality of a food matrix. Starch granule has a layered structure that made up of crystalline and amorphous lamellae. Crystalline lamellae, which is the non-reducing end of the molecule, is the linear region that consisted of double helices (Pérez & Bertoft, 2010). Whereas, the amorphous lamellae, which is the reducing end of the molecule, is consisted of highly branched chains (Pérez & Bertoft, 2010). The major components in starch are amylose and amylopectin. Amylose is a linear polymer made up of glucose units linked by α-1,4 glucosidic linkages (Tester, Karkalas, & Qi., 2004.). Amylopectin is a highly branched polymer consisted of a linear chain and multiple branches. The linear chain is made up of glucose units linked by α-1,4 glucosidic linkages, and the branches are made up of glucose units linked to the linear chain by α-1,6 glucosidic linkages (Tester et al., 2004.).

The micro-structure of starch can be studied by analyzing the unit and internal chain distribution of amylopectin. The unit chain distribution of amylopectin can be conducted on a high-performance anion-exchange chromatography system (HPAEC) by debranching α-1,6 glucosidic linkages of the amylopectin with isoamylase and pullanase (Bertoft, 2004). The results can be classified into short chain (DP < 36) and long chain (DP > 36) (Bertoft, 2004). Amylopectin is consisted of two types of chains: internal and external chains. These chains are also classified into A and B chains. The entire A chains are external. However, B chains are consisted of one external and one internal segment (Manners, 1989). The internal chains, where most of the branches are considered to be, are presented in the amorphous part of the amylopectin molecule, and the external chains are found in the

crystalline part of the molecule (Pérez & Bertoft, 2010). The internal chains are produced by the removal of external chains by β-amylase and phosphorylase *a* from rabbit muscle (both are exo-acting enzymes) (Manners, 1989; Bertoft, 1989). β-amylase cleaves the glucan units at the non-reducing ends and produces maltose. β-amylase is not able to hydrolyze the branched point of amylopectin resulting in the production of β-limit dextrins which is the internal part of the amylopectin structure consists of branches (Manners, 1989). φ-limit dextrin is produced by the use of phosphorylase *a*, which would remove the glycosyl residues. Because exo-acting enzyme cannot hydrolyze the branched point of amylopectin, the production of φ and β-limit dextrins can be used to study as the internal chain profile of amylopectin.

In this study, the physical and molecular characterization of IWG starches from two growing locations were investigated and compared with Hard Red Wheat and polished Jasmine Rice. IWG is more sustainable compared to other cereal crops, and it has also been used for cool-season forage. It can possibly be used in multigrain and organic products because of its biodynamic property. Like other cereal grains, starch is the main component in IWG. It is critical to understand the structure components in newly developed environmentally-friendly crops to optimize its uses. Little has known on the structural component of IWG starch and its gelatinization properties. To the best of the authors' knowledge, this is the first attempt at investigating the IWG starch characteristics in detail.

3.2. Experimental

3.2.1. Materials

IWG whole grain kernels provided by Forever Green Initiative, were from two growing locations: Roseau (IWG-RS) and Rosemount (IWG-RM), MN, USA. HRW whole grain kernel was provided by Grain Millers, Eden Prairie, MN USA. Dynasty® polished Jasmine rice (JR) was purchased in a local store (St Paul, MN). HRW and polished JR were selected as controls because they are the most commonly consumed cereal grain. All kernels were kept at room temperature for this study.

3.2.2. Starch Extraction

IWG grains were frozen with liquid nitrogen and then milled for 1 min into flour with a Hamilton Beach Fresh-Grind Coffee Grinder. The starch was extracted from the flour samples based on a method reported by Waduge, Xu & Seetharaman (2010) with modifications. An alkaline extraction buffer solution (12.5 mM, pH 10, containing 0.5% SDS and 0.5% $Na_2S_2O_5$ (w/v)) was added to the milled IWG flour as a 5% (w/v) slurry. The mixture was stirred for 10 min. and flour recovered by centrifugation at 4000 rpm for 10 min (at 4°C). The extraction step was then repeated. The resulting residue was washed three times with distilled water and recovered by centrifugation 4000 rpm for 10 min (at 4°C). The residue was then suspended in distilled water and the starch slurry was passed through four layers of cheesecloth and then through a 70 μm nylon mesh. The slurry was centrifuged at 4000 rpm for 10 min (at 4°C), and the brown layer that formed on top of the starch layer was scraped off with a spatula. The scraping step was repeated until all the brown top layer was removed from the starch fraction. The extracted starch washed with

acetone and, recovered by centrifuging at 4000 rpm for 10 min (at 4°C). The starch was then air dried at room temperature.

3.2.3. Thermal properties

The gelatinization properties of the samples were measured with a differential scanning calorimeter Q1000 equipped with thermal analysis data and recording software facility (TA Instruments, Universal Analysis 2000, DE, USA). Sample dispersion in water (1:3) was allowed to equilibrate for at least 3 hr at room temperature in aluminum pans before thermal analysis was conducted. The thermograms were recorded with an empty aluminum pan as a reference, and then scanned from 20–120°C at a heating rate of 10°C/min. The onset (T_o), peak (T_p), and conclusion (T_c) transition temperatures were reported. The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and the base line under the peak and was expressed as J/g of dry starch.

3.2.4. Size Distribution of Debranched IWG Starches

Starch samples (6 mg) were dissolved in 90% dimethyl sulfoxide (DMSO; 150 µL) and heated in a warm water bath (80°C) for 5 min and then stirred slowly overnight at room temperature (25°C). The next day, warm water (900 µL, at 80°C) was added to the sample, and then cooled to room temperature. A 100 µL of 0.01 M sodium acetate buffer (pH 5.5) was added followed by the addition of 1 µL of 1000 U, 260 U/mg isoamylase enzyme (Megazyme International Ireland, Bray, Wicklow, Ireland) and 1 µL of 700 U, 34 U/mg pullulanase M1 (Megazyme International Ireland, Bray, Wicklow, Ireland). The resulting

mixture was then stirred slowly overnight at room temperature (25°C). The samples were boiled in a warm water bath for 5 min to deactivate the enzyme. The sample (1 mL) was then injected on to a column (1 × 90 cm) of Sepharose CL-6B gel (GE Healthcare, Uppsala, Sweden), and eluted with 0.5 M NaOH at 1 mL/min. Fractions (1 mL) were collected and analyzed for total carbohydrates with the phenol–sulfuric acid reagent (DuBois, Gilles, Hamilton, Rebers & Smith, 1956). Chromatograms were divided into amylose and amylopectin fractions according to Sargeant (1982).

3.2.5. Size Distribution of IWG Starch Components and Their β-Limit Dextrins.

Starch samples (6 mg) were dissolved in 90% DMSO (150 μL) and heated in hot water (80°C) for 5 min and then stirred slowly at room temperature (25°C) overnight. The samples were then diluted with warm water (1.85 mL, at 80°C) to a concentration of about 3 mg/mL. The size distribution of the starch components was chromatographed on a Sepharose CL-2B column (1.6 × 32 cm) (Pharmacia, Uppsala, Sweden). A dissolved starch solution of 700 μL was eluted through the column with 0.01 M NaOH at a rate of 0.5 mL/min. Odd-numbered fractions (1 mL) were collected, and tested for the carbohydrate content, using the phenol-sulfuric acid reagent (Dubois et al., 1956). The even-numbered tubes were tested for the wavelength maxima (λmax) of the glucan–iodine complex after addition of 1 mL of 0.01 M HCl to neutralize the fractions and then, 0.1 mL of 0.01 M I_2/0.1 M KI. The absorbance of phenol-sulfuric acid reagent and glucan-iodine complex were determined with a WPA Spectrawave S800 diode array spectrophotometer (Biochrom Ltd., Cambridge, U.K.) at 490 nm.

β-limit dextrins (β-LD) of the IWG starches were prepared according to the method reported by Bertoft (2004). The starch samples (6 mg) were dissolved in 90% dimethyl sulfoxide (DMSO; 150 μL) and heated in a warm water bath (80°C) for 5 min and then stirred slowly overnight at room temperature (25°C). The next day, warm water (900 μL, at 80°C) was added to the sample, and cooled to room temperature. A 100 μL of 0.01 M sodium acetate buffer (pH 6) was added and 2 μL of of β-amylase (4 U/mg) (Megazyme International Ireland, Bray, Wicklow, Ireland) was added directly. The tubes were then stirred slowly overnight at room temperature (25°C). The samples were boiled in a water for 5 min to inactivate the enzyme. About 700 μL of the mixture was injected onto the Sepharose CL-2B column and analyzed.

3.2.6. Amylopectin Fractionation of IWG starches

Amylopectin fractionation was conducted according to the method reported by Klucinec & Thompson (1998) with modifications. Granular starch (2 g) was dispersed in 90% dimethyl sulfoxide (DMSO) (40 mL) in a boiling water bath for 10 min and stirred overnight with a magnetic stirrer. The starch slurry was then boiled for 10 min, then four volumes (160 mL) of ethanol was added. The starch–ethanol mixture was cooled to room temperature (25°C) and centrifuged at 8,000 × *g* for 10 min at 4°C. The supernatant was discarded, and the precipitated starch was washed once again with ethanol to obtain non-granular starch. The non-granular starch was dissolved in 90% DMSO (56 mL) at 90°C. A mixture of 1-butanol (23.5 mL), isoamyl alcohol (23.5 mL), and water (324 mL) [Mixture A] being stirred vigorously was added dropwise to the dissolved starch after it had been cooled to 85°C. After the addition of mixture A, the entire mixture was cooled to about

28°C for 20 h. The entire mixture was then centrifuged at 22,000 × *g* at 4°C for 30 min. The supernatant, which was the amylopectin fraction, was decanted carefully and reduced in volume to about 50 mL by rotary evaporation at 50°C. Three volumes (about 150 mL) of methanol was then added to the amylopectin solution and left overnight at room temperature to be precipitated. The precipitated amylopectin was obtained after centrifugation at 22,000 × *g* at 4°C for 30 min. The amylopectin was dissolved in hot water (20 mL), precipitated with three volumes of ethanol for 1 hr at room temperature, and collected by centrifugation. The precipitated amylopectin was dissolved in hot water (20 mL) and then freeze dried.

3.2.7. Analysis of Amylopectin Purity by Gel-Permeation Chromatography

The purity of the amylopectin samples was analyzed after debranching of the sample by gel-permeation chromatography on Sepharose CL-6B according to the procedure previously reported (Laohaphatanaleart, Piyachomkwan, Sriroth & Bertoft, 2010). The total carbohydrate content of the various test tube fractions was determined with the phenol–sulfuric acid method (DuBois et al., 1956).

3.2.8. Production of φ, β-Limit Dextrins

The φ, β-limit dextrins were produced according to the method of Bertoft (2004) with modifications as reported by Kalinga et al (2013). It involved removal of the external chains of the amylopectin by the successive use of phosphorylase *a* and β-amylase. Starch granules (100 mg) were dissolved in 90% DMSO (3 mL) by stirring in a boiling water bath for 10 min and left overnight at room temperature. The sample slurry was diluted in warm

water (32.7 mL, 70°C) on the second day, followed by the addition of 1.1 M sodium phosphate buffer (3.6 mL, pH 6.8), 2.8 mM ethylenediaminetetraacetic acid (EDTA; 1.7 mL) and freshly prepared 0.9 U/mL phosphorylase *a* (9 mL) (Sigma-Aldrich, MO, USA). The sample was stirred overnight at room temperature. On the third day, the sample was boiled for 5 min to inactivate enzyme mixture, then filtered in a tangential flow filtration system (Model 910-0025, Thermo Scientific, Waltham, MA, USA; Minimate™ TFF Capsule, Pall Corporation, New York, USA). A mixture of 1.1 M sodium phosphate buffer (3.6 mL, pH 6.8), 2.8 mM ethylenediaminetetraacetic acid (EDTA; 1.7 mL) and freshly prepared 0.9 U/mL phosphorylase *a* (9 mL) (Sigma-Aldrich, MO, USA) was added. The sample was stirred overnight at room temperature. On the fourth day, the sample was boiled for 5 min to inactivate enzyme, then filtered in a tangential flow filtration system (Model 910-0025, Thermo Scientific, Waltham, MA, USA; Minimate™ TFF Capsule, Pall Corporation, New York, USA). Approximately 10 mL of 0.01 M sodium acetate buffer (pH 6.0) was added to the filtered sample along with 5 μL of β-amylase (Megazyme International Ireland, Bray, Wicklow, Ireland). The sample was stirred overnight at room temperature. On the fifth day, the sample was boiled for 5 min to inactivate enzyme, then filtered in a tangential flow filtration system (Model 910-0025, Thermo Scientific, Waltham, MA, USA; Minimate™ TFF Capsule, Pall Corporation, New York, USA). Approximately 10 mL of 0.01 M sodium acetate buffer (pH 6.0) was added to the filtered sample along with 5 μL of β-amylase (Megazyme International Ireland, Bray, Wicklow, Ireland). The sample was stirred overnight at room temperature. On the sixth day, the sample mixture was boiled for 5 min to inactivate enzyme, then filtered in a tangential flow

filtration system (Model 910-0025, Thermo Scientific, Waltham, MA, USA; Minimate™ TFF Capsule, Pall Corporation, New York, USA). The sample was then freeze-dried.

3.2.9. Unit and Internal Chain Distribution of IWG Amylopectin

Freeze dried hydrolyzed starch (2.0 mg) were dissolved in 90% DMSO (50 μL) with gentle stirring overnight. The solution was diluted by adding warm water (400 μL, 80°C), after which 0.01 M sodium acetate buffer (50 μL, pH 5.5) was added. Isoamylase (1 μL, 1000 U, 260 U/mg) and pullulanase M1 (1 μL, 700 U, 34 U/mg) (Megazyme International Ireland, Bray, Wicklow, Ireland) were added to the mixture, which then was stirred overnight at room temperature. After debranching, the enzymes were inactivated by boiling for 5 min, the volume adjusted to obtain a final concentration of 1 mg/mL, and the sample filtered through a 0.45 μm nylon filter. The filtered sample (25 μL) was injected into the Dionex ICS 3000 HPAEC system (Dionex Corporation, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector, CarboPac PA-100 ion-exchange column (4 × 250 mm), and a similar guard column (4 × 50 mm). The samples were then eluted with a flow rate of 1mL/min. The two eluents used were 150 mM sodium hydroxide (A) and 150 mM sodium hydroxide containing 500 mM sodium acetate (B). An eluent gradient was made by mixing eluent B into eluent A as follows: 0-9 min, 15-36% B; 9-18 min, 36-45% B; 18-110 min, 45-100% B; 100-112 min, 100-15% B; and 112-130 min, 15% B. The system was stabilized by elution at 15% B for 60 min between runs. The areas under the chromatograms were corrected to carbohydrate concentration following the method of Koch, Andersson & Åman (1998).

3.2.10. Particle Size Analysis

The dry starch samples (<1 gram) were analyzed for their particle size distribution by light scattering using a Horiba LA-960 laser particle size analyzer (Horiba, Japan). The refractive index used for starch samples was 1.53.

3.2.11. Starch Granule Morphology

The surface characteristics of native and hydrolyzed starches were viewed with a Hitachi S-570 scanning electron microscope (Hitachi Scientific Instruments, Rexdale, Ontario, Canada) after the starches were sputtered with 15 nm of gold dust on a stub. The working distance used was 15 mm with a voltage of 10 kV.

3.2.12. Statistical Analysis

The data presented in this are collected in duplicate. ANOVA one-way test was used to determine significant differences between sample means when $p < 0.05$. All statistical analysis was conducted using Statgraphics Centurion XV, version 15.1.02 (StatPoint, Warrenton, VA, USA).

3.3. Results and Discussion

3.3.1. Thermal properties

The gelatinization transition characteristics of the granular starch samples are presented in Figure 2.1 and Appendix D Table 4.1. The onset temperatures (T_o) were significantly different ($p \leq 0.05$) and in the following order: IWG-RS (58.9°C) < IWG-RM (60.2°C) < HRW (62.4°C) < JR (68.3°C). A similar trend was observed for T_p with

IWG-RS having the lowest of 63.5°C and JR recording the highest of 73.0°C. The conclusion temperatures (T_c) ranged from 71.5 to 80.8 °C. T_c of HRW and JR starches were significantly higher ($p \leq 0.05$) than both IWG starches. The higher gelatinization temperature observed with both control samples (JR and HRW) suggests that IWG starches may have less perfectly aligned crystallinity than JR and HRW starches. The differences observed in the gelatinization temperature range (T_c-T_o) among all starch samples was not significant ($p \leq 0.05$). Gelatinization temperature range has been reported to demonstrate the quality and uniformity of amylopectin crystals (Ratnayake, Hoover, Shahidi, Perera & Jane, 2001). The enthalpy of gelatinization (ΔH), which indicates the energy required to unwind the double helices in amylopectin of starch granules, ranged from 5.3–8.1 J/g. While a similarity was observed with the enthalpies of the starches from JR and IWG-RS on the one hand and HRW and IWG-RM on the other hand, enthalpies of HRW and IWG-RM starches were significantly ($p \leq 0.05$) higher than JR and IWG-RS starches. This may suggest that the amylopectin crystals of the latter are likely to be more stable compared to the former (Vamadevan, Bertoft & Seetharaman, 2013; Gayin et al., 2017). The enthalpy of HRW and IWG-RM starches were significantly higher than those of JR and IWG-RS starches which gives an indication of more and longer double helices in HRW and IWG-RM samples (Qi, Tester, Snape, & Ansell, 2003). T_o values from 53.3 to 75.9°C have been reported for non-waxy rice starches which are consistent with results obtained from this study (Wickramasinghe & Noda, 2008; Wang, Xie, Shi & Xue, 2010). The transition temperatures for the HRW starch sample in this study were higher than those reported by Yoo & Jane (2002) for

native wheat starches except for the ΔH which was relatively lower. The difference might be related to the varietal and growing location of HRW.

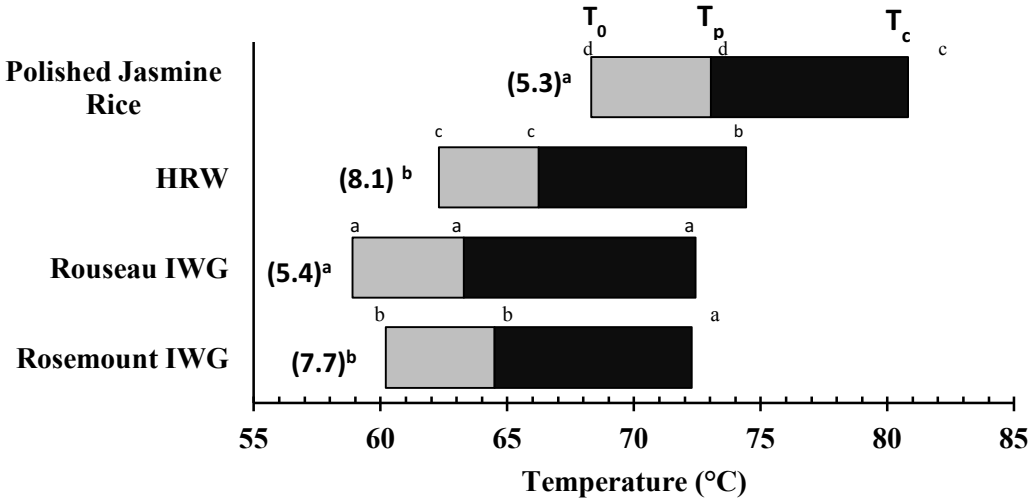


Figure 2.1: Thermal properties of starches from polished Jasmine Rice, HRW and Intermediate wheatgrass. Values in parenthesis are enthalpies of gelatinization (ΔH) (J/g). T_0 = onset temperature; T_p = peak temperature; T_c = conclusion temperature; and ΔH = enthalpy of gelatinization. Means with the same letters are not significantly different ($p \leqslant 0.05$).

3.3.2. Size distribution of Debranched IWG Starches.

The IWG starch samples were compared to JR and HRW starches. The amylose (long-chain and short-chain) and the amylopectin contents were determined by analyzing the chain profile of the debranched starches with gel-permeation chromatography using the Sepharose CL-6B gel shown in Table 2.1 and Appendix E Figure 5.1. Amylose eluted earlier than amylopectin because debranched amylopectin has smaller molecular size compared to amylose. Amylose showed on the first peak on Appendix E Figure 5.1, while

amylopectin showed on the second peak. The amylose content from this study for IWG-RM, IWG-RS, JR, HRW were 30.39, 30.73, 12.07 and 28.58%, respectively. The amylose contents for IWG-RS, IWG-RM and HRW starches were similar but JR starch had a lower amylose content. It has been reported that the amylose content for IWG ranged from 22-25% of the starch content and their HRW controls were 25.5% of the starch content (Rahardjo et al., 2018). The differences observed from previous work and this study might be related to the different varietals and methods used for amylose determination for HRW and IWG. Higher amount of LC_{am} were recorded than SC_{am} for all samples (Appendix E Figure 5.1). The amylopectin peak for JR eluted slower than the other samples which indicates the molecular size of JR amylopectin was smaller compared to other samples after debranching. IWGs and HRW had significantly higher amount of LC_{am} than JR. IWG-RM had the highest LC_{am} of 21.46. However, LC_{am} to SC_{am} ratio for all samples were similar. The ratios for JR, HRW, IWG-RS, IWG-RM were 1.17, 2.03, 1.90, and 2.40, respectively.

Table 2. 1 Amylose Content and Composition of Rice, HRW, IWG Starches[z]

Index	**JR**	**HRW**	**IWG-RS**	**IWG-RM**
Amylose (%)	12.07[a]	28.58[b]	30.73[b]	30.39[b]
LC_{am}:SC_{am}	1.17[a]	2.03[a]	1.90[a]	2.40[a]
LC_{am} (%)	6.51[a]	20.01[b]	20.15[b]	21.46[c]
SC_{am} (%)	5.56[a]	9.42[a]	10.59[a]	8.93[a]

[z]Means with the same letters are not significantly different within columns ($p \leqslant 0.05$). Long-chain amylose (LCam), short-chain amylose (SCam).

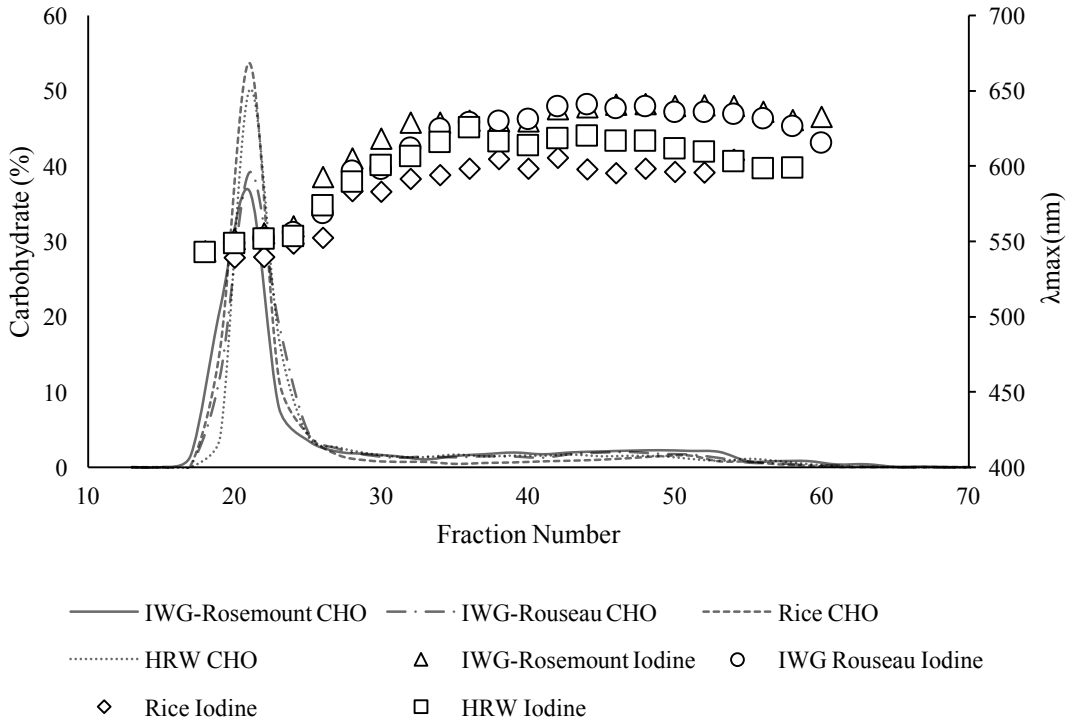


Figure 2. 2 Sepharose CL-2B gel-permeation chromatographs of IWG, Rice, HRW starches. The lines represent the carbohydrate contents and the symbols represent the λ_{max} (nm), Carbohydrate (CHO).

3.3.3. Size Distribution of native IWG Starch Components and Their β-Limit Dextrins

Gel-permeation chromatography using the Sepharose CL-2B gel shows the molecular distribution of starch before debranching on Figure 2.2 and 2.3. Amylopectin eluted faster than amylose component without debranching because of its larger molecular size. Therefore, the peak represents the amount of amylopectin in each sample. Figure 2.2 shows that IWG-RS and IWG-RM had less amylopectin than the controls (HRW and JR). The markers on Figure 2.2 indicate the iodine affinity of each sample. Higher λ_{max} indicates

that longer chain amylose presented in the sample to bind with iodine (Banks, Greenwood & Khan, 1971). The λ_{max} of IWG-RM, IWG-RS, HRW and JR ranged from 540 – 640 nm, 540 – 640 nm, 540 – 620 nm and 540 – 600 nm, respectively. The lowest λ_{max} in JR corresponds to the lowest amylose content in Table 2.1. Gel-permeation chromatography using the Sepharose CL-2B gel shows the molecular distribution of β-LD on Figure 2.3. The β-LDs showed on the first peak because of their larger molecular size, and the maltose showed on the second peak in this bimodal distribution. The maltose peak was presented on Figure 2.3 because of the removal of the linear amylose and external chains of branched starch polymers (Takeda, Shitaozono & Hizukuri, 1990). IWG-RM had a lower β-LD peak than the rest of the samples, which indicates IWG-RM had the lowest amount of β-LD among all four samples. On the other hand, IWG-RM and JR had higher maltose peak than the other two samples. The higher maltose peak reveals that the external part of amylopectin in IWG-RM and JR might be longer so that more maltose could be cleaved off by β amylase. The λ_{max} among all samples were similar in respect to the β-LD peak, which means the internal chain length between samples were similar.

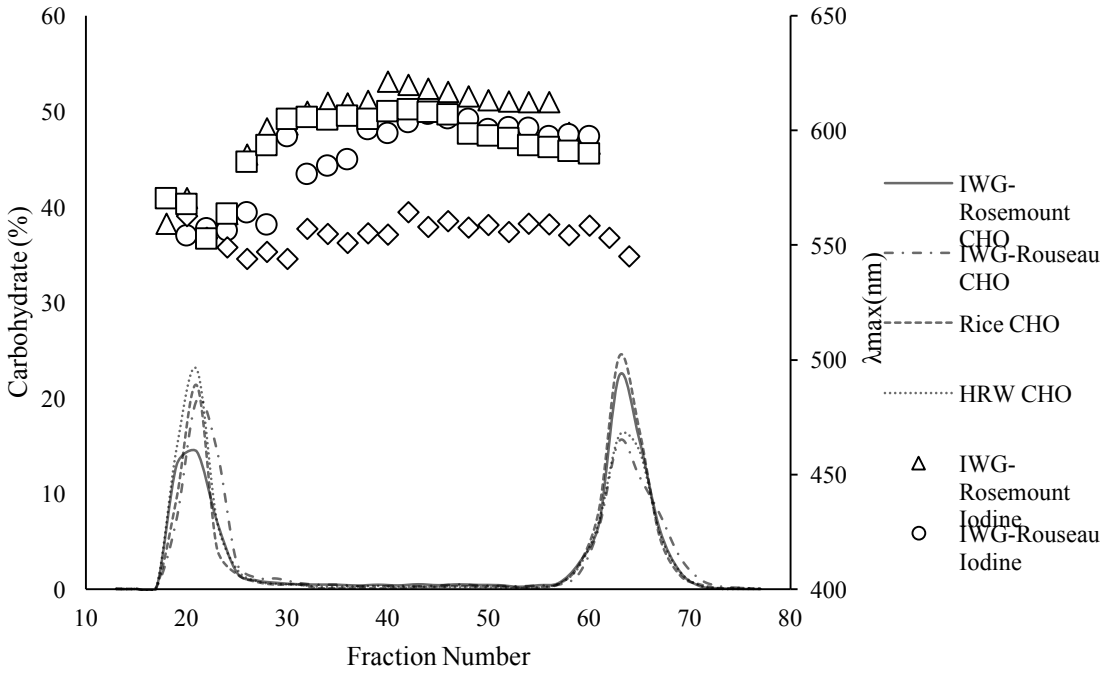


Figure 2. 3 Sepharose CL-2B gel-permeation chromatographs of IWG, Rice, HRW β-limit dextrin (right). The lines represent the carbohydrate contents and the symbols represent the λ_{max} (nm), Carbohydrate (CHO).

3.3.4. Particle size distribution of Intermediate Wheatgrass Starches.

The particle size distribution of all samples is shown in Appendix F Figure 6.1. Starch granule size of all samples ranged from 5 – 60 μm. IWG-RM and HRW had the largest granule size of 20 μm. The particle size of HRW is similar to Evers & Lindley (1977) in which one third of the wheat starch had particle size ranged from 15 – 20 μm.

In addition, IWG-RS was relatively smaller with a size of 17 μm, and JR had the smallest granule size of 15 μm. This also can be seen from the SEM image Figure 1.3. In Figure 1.3, the JR starches are considerably smaller compared to the IWG and HRW with the same magnification. The HRW starch granule has spherical structure, while the rice starch granule has polygonal structure. Interestingly, both spherical and polygonal

structures were observed in IWG starch granules. Likewise, Singh, Singh, Kaur, Sodhi & Gill (2003) also reported spherical structure in wheat starch and polygonal structure in rice starch.

3.3.5. Unit and Internal Chain Distribution of IWG Amylopectin

IWG amylopectins from both locations were extracted from raw starches. The purity of IWG amylopectins were measured using gel permeation chromatography on Sepharose CL-6B. The purity of IWG amylopectins from both locations were about 94% (raw data not shown). The small amount of amylose existed in extracted amylopectin could be actual amylose or amylopectin with long linear backbone (Takeda, Hizukuri & Juliano, 1987; Inouchi et al., 2005; Laohaphatanaleart et al., 2010).

The unit chain profiles of IWG amylopectins are shown on Appendix G Figure 7.1 [A] and [B]. IWG-RS and IWG-RM had similar unit chain profiles, which indicates that they have similar short and long chain alignments. The groove position, which is around DP 36, is the division between short and long chains (Bertoft, Piyachomkwan, Chatakanonda & Sriroth**,** 2008). IWG from both locations had similar transition around DP 36. The peak DP of long chain of IWG-RM at DP 44 was lower than that of IWG-RS at DP 45. Unit chain length distribution of IWG amylopectins were similar to a typical hard red spring wheat sample reported by Kalinga et al (2013).

The internal chain profiles of IWG amylopectins that were analyzed on HPAEC system are shown on Appendix H Figure 8.1 [A] and [B]. The division of each chain category on internal chain profiles obtained from IWG-RS and IWG-RM were generally

similar. The only exception would be the peak DP of B chains from IWG-RM was slightly higher at DP 36 than that of IWG-RS at DP 35. Internal chain length distribution of IWG amylopectins were also similar to a typical hard red spring wheat sample reported by Kalinga et al (2013).

Table 2.2 shows the average chain length (CL) of various chain categories and segments of IWG amylopectins. The average CLs of IWG-RS and IWG-RM were 17.73 and 17.64, respectively. This observation is also corresponded to rye, normal barley, medium and waxy rice that were reported by Bertoft et al (2008) because cereal starch with A-type X ray allomorph pattern has shorter CLs compared to root or tuber amylopectin (Hizukuri, 1985). The short and long chain CLs were similar between IWG amylopectins from both locations. As discussed in Section 3.3.3 and Figure 2.3, IWG-RM showed a longer external part of amylopectin, yet the ECLs between IWG amylopectins from the internal chain profile were not significantly different. Still, there could be a correlation between the external chain length of amylopectin and the corresponding enthalpies when melting starches (Figure 2.1) (Vamadevan et al., 2013). Also, the internal structure of starch could affect the gelatinization temperature of starch granules (Vamadevan et al., 2013). φ, β-limit value from IWG-RS (56.30) and IWG-RM (57.00) were similar to each other. φ, β-limit value gives an indication of the length of external chains (Bertoft et al., 2008; Zhu, Corke & Bertoft, 2011). The external chains other than A_{fp} could participate in the formation of crystalline lamellae (Gidley & Bulpin 1987). The external chain length of IWG-RS (11.48) and IWG-RM (11.55) had similarity compared to Bertoft et al (2008). The total internal chain length of IWG amylopectins from both locations are similar to oat,

waxy barley and waxy rice reported by Bertoft et al (2008). The similar average chain length in φ, β-limit dextrins from IWG-RS (7.75) and IWG-RM (7.58) indicates that the branched structure in their amorphous lamellae were similar. CL from short B chains was longer in IWG-RS (9.89) than IWG-RM (9.71). In general, hard red spring wheat from different maturity stages also recorded similar amylopectin chain length on Kalinga et al (2013).

Table 2. 2 Average Chain Lengths (CLs) of Different Chain Categories and φ, β -Limit Values of IWG Amylopectin Obtained by High-Performance Anion-Exchange Chromatography[z]

Sample	**IWG-RS**	**IWG-RM**
CL	17.73[a]	17.64[a]
SCL	15.09[a]	15.09[a]
LCL	53.10[a]	52.29[a]
ECL	11.48[a]	11.55[a]
ICL	5.25[a]	5.08[a]
TICL	13.31[a]	12.77[a]
φ, β -limit value	56.30[a]	57.00[a]
CL_{LD}	7.75[a]	7.58[a]
BS-CL_{LD}	9.89[b]	9.71[a]
BL-CL_{LD}	39.36[a]	38.26[a]

[z] SCL = CL of short chains; LCL = CL of long chains; ECL (external CL) = CL × (φ, β-limit value/100) + 1.5; ICL (internal CL) = CL – ECL – 1; TICL (total internal CL) = B-CL_{LD} – 1; φ, β -limit value was calculated from the difference in CL of amylopectin and its φ, β-limit dextrin; CL_{LD} = average CL of φ, β-limit dextrin; BS-CL_{LD} = CL of short B-chains; and BL-CL_{LD} = CL of long B-chains. Values with different letters are significantly ($P < 0.05$) different within each row.

Table 2.3 shows the relative molar amounts of various chain categories of IWG amylopectins. The molar amounts of A chains from IWG-RS (53.32) and IWG-RM (52.55) were similar. Similar molar amounts of A chains were also observed in Rye and Rice reported by Bertoft et al (2008). A_{fp}, which is unique to amylopectin from different sources, is too short to participate in the crystalline lamellae (Koizumi, Fukuda & Hizukuri, 1991;

Bertoft et al., 2008; Bertoft, 2012; Gidley & Bulpin, 1987). A-chains that are involved in the crystalline lamellae are called $A_{crystal}$ (Bertoft, 2008). A_{fp} of IWG-RS and IWG-RM were 7.19 and 7.18, respectively. This observation is similar to waxy rice but lower than rye, oat and barley (Bertoft et al., 2008). A_{fp} of IWG amylopectins also had similar relative molar amount as small granule hard red spring wheat at 35 days after anthesis (Kalinga et al., 2013). Within the B-chain categories, IWG-RS and IWG-RM generally had similar characteristics with the exception of BS_{major}. IWG-RS had similar amount of BS_{major} compared with that of IWG-RM. BS_{major} is the interconnection of two or three building blocks (Bertoft, Koch & Åman, 2012). B_{fp}, which participates in tightly branched building blocks in clusters (Bertoft & Koch, 2000), were similar between IWG amylopectins from both locations. In general, the relative molar amounts of different chain categories had similar characteristics as hard red spring wheat, rye, barley and rice (Kalinga et al., 2013; Bertoft et al., 2008).

Table 2. 3 Relative Molar Amounts (%) of Chain Categories in Amylopectins of IWG Starches[r]

Sample	**IWG-RS**	**IWG-RM**
A-chains	53.32[a]	52.55[a]
A_{fp}	7.19[a]	7.18[a]
$A_{crystal}$	46.14[a]	45.37[a]
B-chains	46.68[a]	47.45[a]
BS	39.68[a]	40.71[a]
BL	7.00[a]	6.74[a]
B_{fp}	15.15[a]	16.25[a]
BS_{major}	24.90[a]	24.83[a]
B2	5.78[a]	5.76[a]
B3	1.22[a]	0.98[a]

[r] Values with different letters are significantly different ($P < 0.05$) within each row. DP = degree of polymerization. Detected as maltose after debranching of φ, β-limit dextrins. "Fingerprint" A-chains at DP 6–8 in the original amylopectin sample; $A_{crystal}$-chains calculated as all A-chains – A_{fp}; B-chains correspond to DP > 3 in φ, β-limit dextrins and were divided into short (BS) and long (BL) chains at between DP 22–25 and above, respectively, depending on the sample; "Fingerprint" B-chains at DP 3–7 in φ, β-limit dextrins; The major group of short B-chains at DP 8 to 22–24, depending on the sample; Long chains between DP 22 or 25 and 55, depending on the sample; Long chains at DP > 56.

Table 2.4 shows the selected molar ratio of the different chain categories from IWG amylopectins and their φ, β-limit dextrins. Relative molar composition can be used in the classification of the four types of amylopectins proposed in Bertoft et al (2008). A to B chain ratio were close to 1 between IWG amylopectins which is similar to the Haworth et al 1937. BS to BL ratio were similar between IWG-RS (5.67) and IWG-RM (6.04). It gives an indication of the proportion of B-chains involved in the internal structure of the clusters and the chains interconnecting them (Hizukuri, 1986). BLs are the major part of the backbone, while BSs are side chains to the backbone and interconnect building blocks (Bertoft, 2013). The ratio of $A_{crystal}$ to BS should be around 1 if one crystalline A chain forms double helix with one BS chain from the external segment (Imberty & Pérez, 1989; O'Sullivan & Pérez, 1999). The fact that $A_{crystal}$ to B-chain ratio is below 1 indicates that long B chains did not form double helix with crystalline A chains (Imberty & Pérez, 1989; O'Sullivan & Pérez, 1999). B_{fp} to BS_{major} ratio was higher in IWG-RM (0.65) than IWG-RS (0.61).

Table 2. 4 Selected Molar Ratios of Different Chain Categories of IWG Amylopectins and Their φ, β-Limit Dextrins[z]

Sample	**IWG-RS**	**IWG-RM**
A:B	1.14[a]	1.11[a]
S_{ap}:L_{ap}	13.29[a]	13.86[a]
BS:BL	5.67[a]	6.04[a]
$A_{crystal}$:BS	1.16[a]	1.12[a]
$A_{crystal}$:B	0.99[a]	0.96[a]
B_{fp}:BS_{major}	0.61[a]	0.65[b]

[z] Values with different letters are significantly different ($P < 0.05$) within each row. A = A-chains; B = B-chains; S_{ap} = short amylopectin chains; L_{ap} = long amylopectin chains; BS_{major} = the major group of short B-chains at DP 8 to 22–24, depending on the sample; and B_{fp} = "fingerprint" B-chains at DP 3–7 in φ, β-limit dextrins. $A_{crystal}$-chains were calculated as all A-chains – A_{fp}. B-chains correspond to DP > 3 in φ, β-limit dextrins and were divided into short (BS) and long (BL) chains at between DP 22–25 and above, respectively, depending on the sample.

According to Bertoft et al (2008), IWG amylopectins from both locations can be classified into the "group 1", "group 2" category based on their internal unit chain profiles obtained by HPAEC. Oat, rye, normal barley and waxy barley were in "group 1". On the other hand, Medium rice, waxy rice and waxy maize were classified into "group 2". This comparison shows that IWG amylopectins had similar features as other granular cereal starches. "Group 2" generally had higher amount of B2, B3, B_{fp}, and higher B_{fp} to BS_{major} ratio compared to "group 1" (Bertoft et al., 2008).

3.4. Conclusions

This study is the first to document the unit and internal chain profile of IWG starch. The gelatinization transition temperatures of IWGs starch were relatively lower than HRW and JR but the range of the gelatinization was similar with the HRW and JR starches. IWGs and HRW starch have a similar amount of amylose and amylopectin, but IWG-RM has a significantly different long chain amylopectin. There are slight differences observed in the unit and internal chain profiles of IWG-RS and IWG-RM amylopectin.

Chapter 4. Conclusion and Future Work

In conclusion, there was a higher amount of protein measured in IWG-RS than IWG-RM. The ash and fat contents were similar between IWG samples. Less total starch content had also observed from IWG flour samples than HRW and JR, which was due to the low carbohydrate contents in IWG compared to the controls. HRW had the highest amount of resistant starch followed by IWG-RM. IWG flour had low hydrolysis rate than JR which could be due to the high amount of other seed components (fat, protein and fiber) presented in the flour. IWG flour had a lower GI than the controls. However, GI of all samples were lower than 55, which made them all in the category of "low glycemic food".

On the contrary, IWG-RS starch had the highest GI, while HRW starch had the lowest GI. There was no significant difference in IWG starch GI between the two growing locations. The difference observed between IWG and the controls might be related to the different microstructure in starch among different samples, which made it interesting to investigate further on the starch microstructure of IWG starch. The normal IWG and HRW starch had disc-shape granules, while normal JR starch had polygonal shape granules. Hydrolyzed starch granules of all starch samples had more and larger pin-holes than normal starches because the digestive enzymes accessed and consumed the granules. In unit chain profile of the resistant starch fractions, the average chain length of the resistant starch fractions was shorter in all starch samples because the starch samples had been previously hydrolyzed. HRW has more mole percent of long-chain than the rest of the samples. The high amount of resistant starch in HRW might be one of the reasons that its starch had a lower GI than other starch samples.

IWG starch had lower gelatinization temperature than controls which means IWG had less perfectly aligned crystallinity. HRW and IWG-RM had higher enthalpies than JR and IWG-RS which indicates HRW and IWG-RM had more and longer double helices. JR had the lowest amylose content among the rest of the samples. IWG-RM had the lowest amount of internal chains and possibly a longer external part of its amylopectin. IWG-RS and IWG-RM had similar unit chain profile. The majority parameters of the internal chain distribution of IWG amylopectin were also similar to each other except the chain length of short B-chains.

This study showed the chemical composition could be one of the reasons that IWG have low glycemic response. Also, the expected glycemic index of Intermediate wheatgrass can be related to the fine structure of its resistant starch fraction. There were differences in the internal structure of IWG amylopectin. Future work can investigate the relationship between in the internal structure of IWG amylopectin and other functional properties, such as starch pasting profile and swelling and solubility characteristics.

Chapter 5. Reference

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Chapter 6. Appendices

Appendix A. Correlations between resistant starch and unit chain profile parameters.

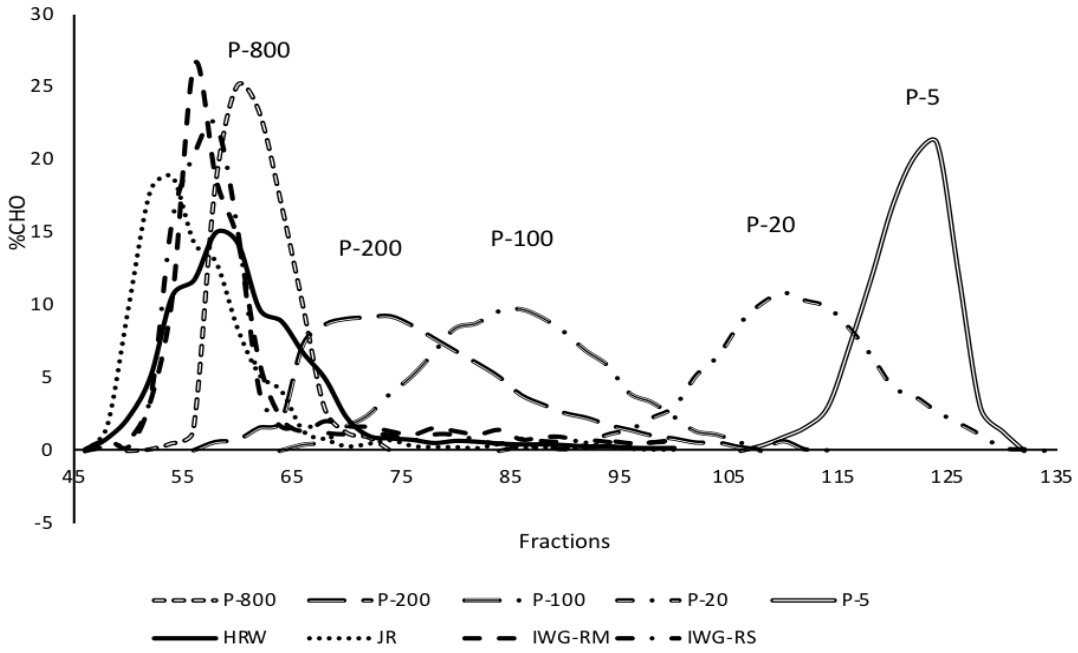
Table 3. 1 Correlations between resistant starch and unit chain profile parameters

	Resistant starch[1]
Resistant starch	0.9379**
Resistant Starch[1]	
Mole (%) short chain	-0.3809
Mole (%) long chain	0.4996
Short chain length	0.5728
Long chain length	-0.3033
Average chain length	0.5723

* and ** indicate that correlation is significant at the 0.05 and 0.01 levels (two-tailed). N = 8.

[1]Determined by the Englyst et al. (1992) procedure.

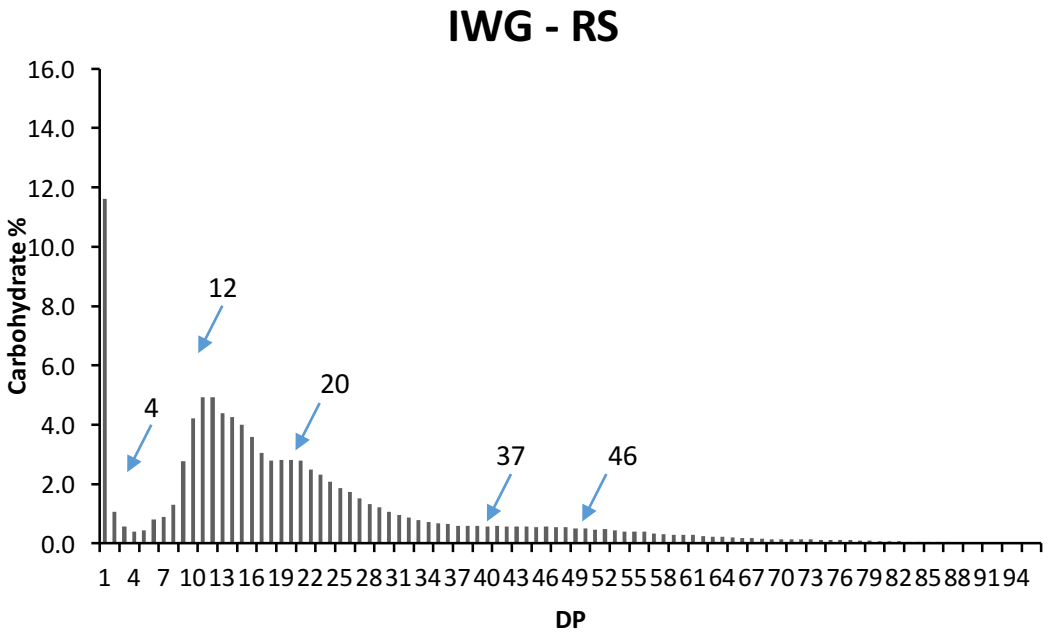
Appendix B. Sepharose CL-6B gel permeation chromatogram of enzymatically hydrolyzed JR, HRW, IWG-RS and IWG-RM starches.



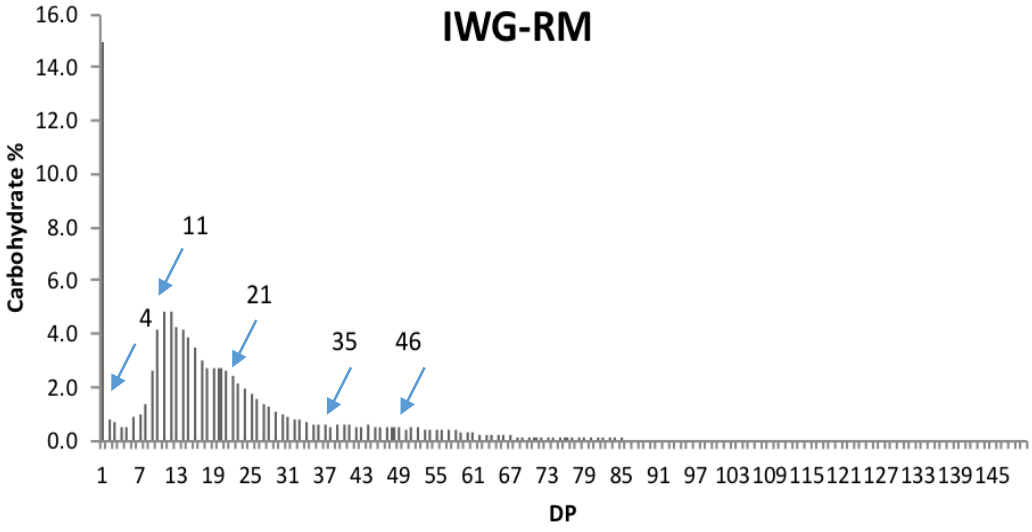
P-5: $0.59\times10^4\ g/mol$; P-20: $2.28\times10^4\ g/mol$; P-100: $11.2\times10^4\ g/mol$; P-200: $21.2\times10^4 g/mol$; P-800: $78.8\times10^4\ g/mol$.

Figure 3. 1 Sepharose CL-6B gel permeation chromatogram of enzymatically hydrolyzed JR, HRW, IWG-RS and IWG-RM starches

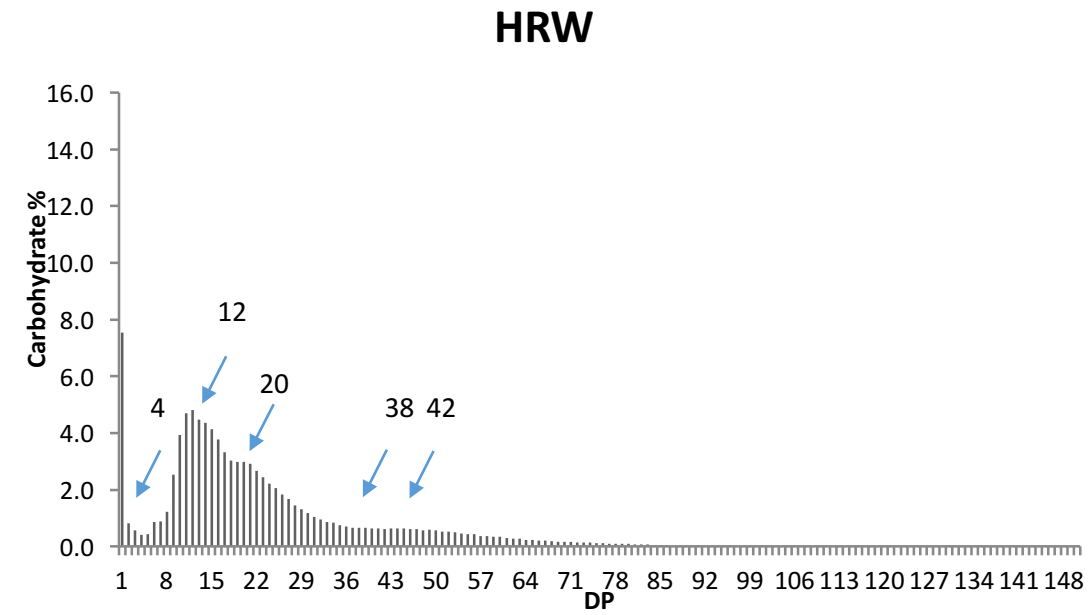
Appendix C. Unit chain profile of IWG-RS, IWG-RM, HRW and JR hydrolyzed starch.



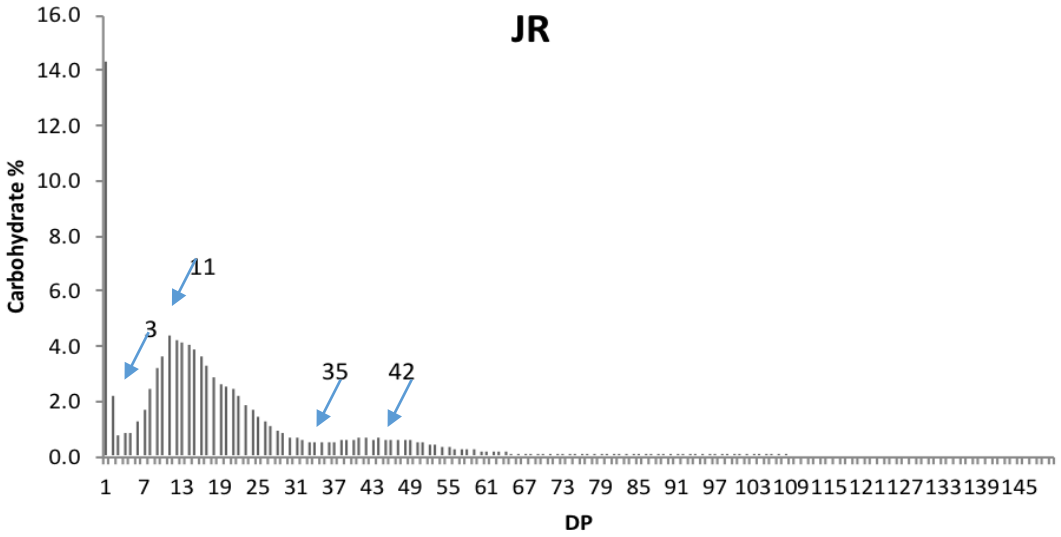
A



B



C



D

A: IWG-RS; B: IWG-RM; C: HRW; D: JR

Figure 4. 1 Unit chain profile of IWG-RS, IWG-RM, HRW and JR hydrolyzed starch

Appendix D. Thermal properties of starches from polished Jasmine Rice, HRW and Intermediate wheatgrass.

Table 4. 1 Thermal properties of starches from polished Jasmine Rice, HRW and Intermediate wheatgrass[z]

	T_o (°C)	T_p(°C)	T_c (°C)	ΔH	Gelatinization range (T_c-T_0)
JR	68.3[d]	73.0[d]	80.8[c]	5.3[a]	12.5
HRW	62.4[c]	66.5[c]	74.3[b]	8.1[b]	11.9
IWG-RS	58.9[a]	63.5[a]	71.5[a]	5.4[a]	12.6
IWG-RM	60.2[b]	64.5[b]	72.3[a]	7.7[b]	12.1

[z]Means with the same letters are not significantly different within columns ($p \leqslant 0.05$).

Appendix E. Sepharose CL-6B gel-permeation chromatography of IWG, Rice, HRW debranched starches.

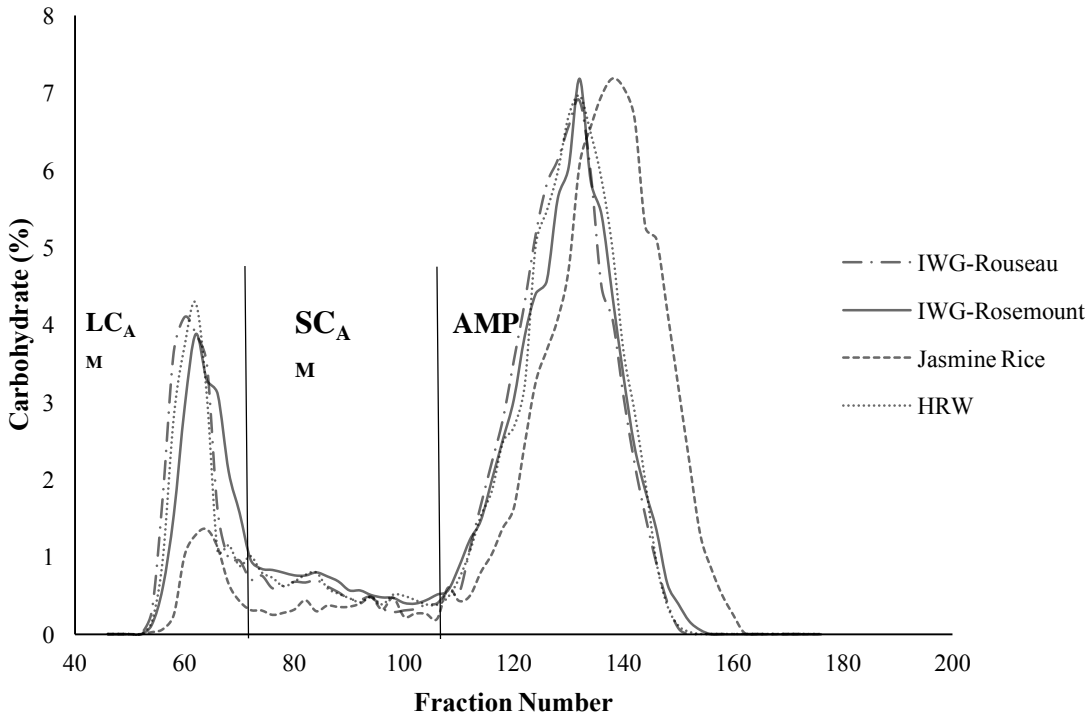


Figure 5. 1 Sepharose CL-6B gel-permeation chromatography of IWG, Rice, HRW debranched starches. Long-chain amylose (LCam), short-chain amylose (SCam), amylopectin (AMP).

Appendix F. Particle size distribution of IWG, Rice, HRW starches.

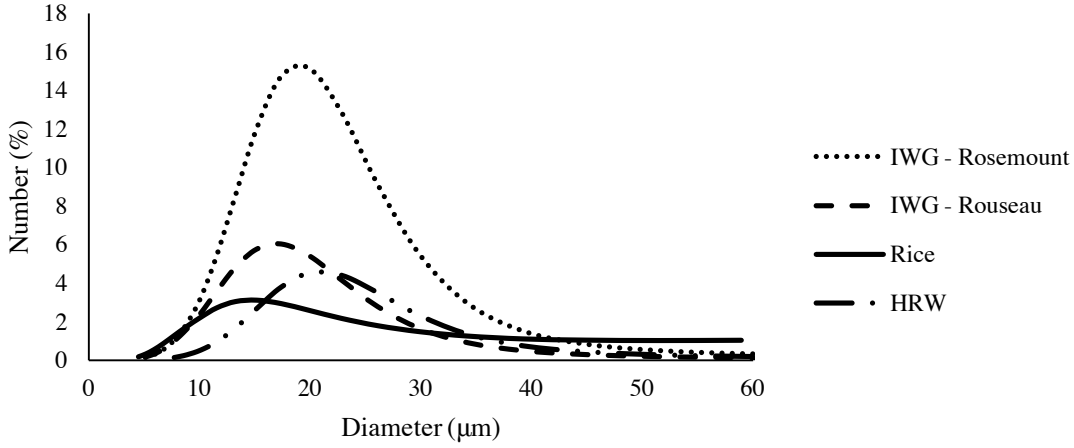
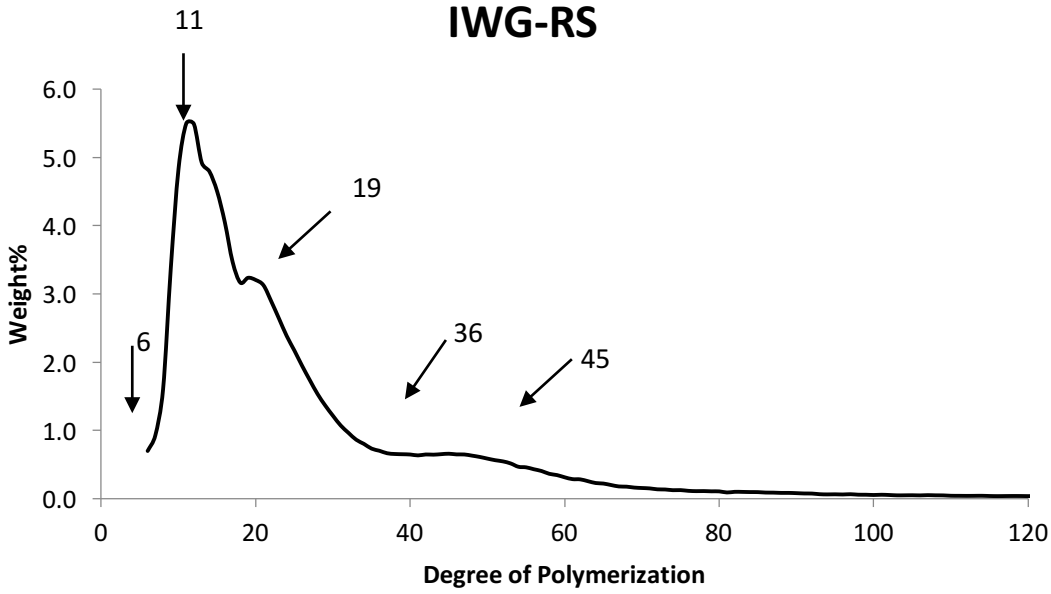
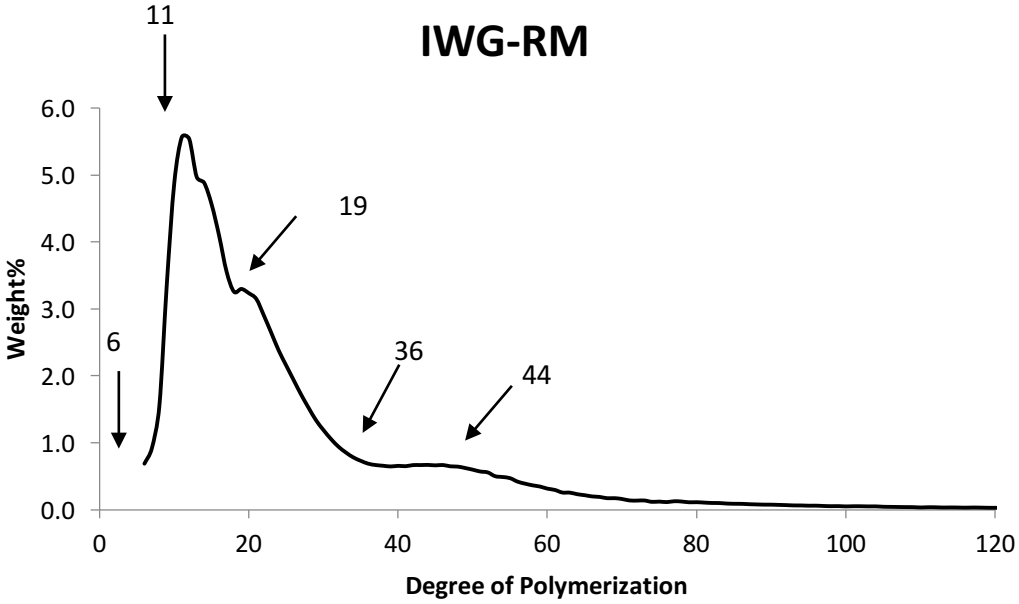


Figure 6. 1 Particle size distribution of IWG, Rice, HRW starches.

Appendix G. The unit chain profile of debranched IWG amylopectins obtained by high-performance anion-exchange chromatography.



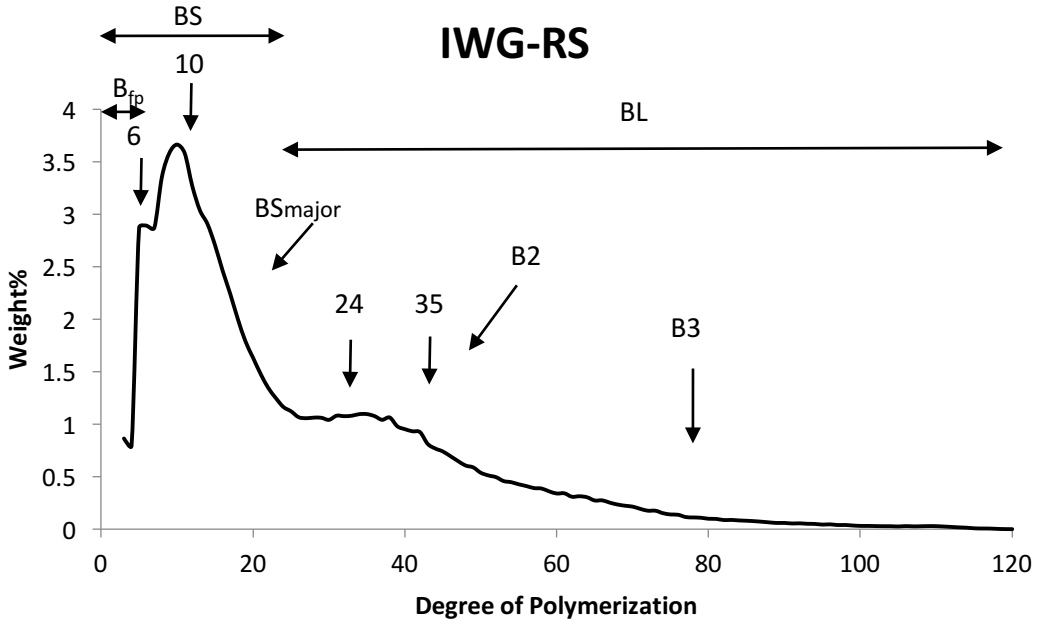
A



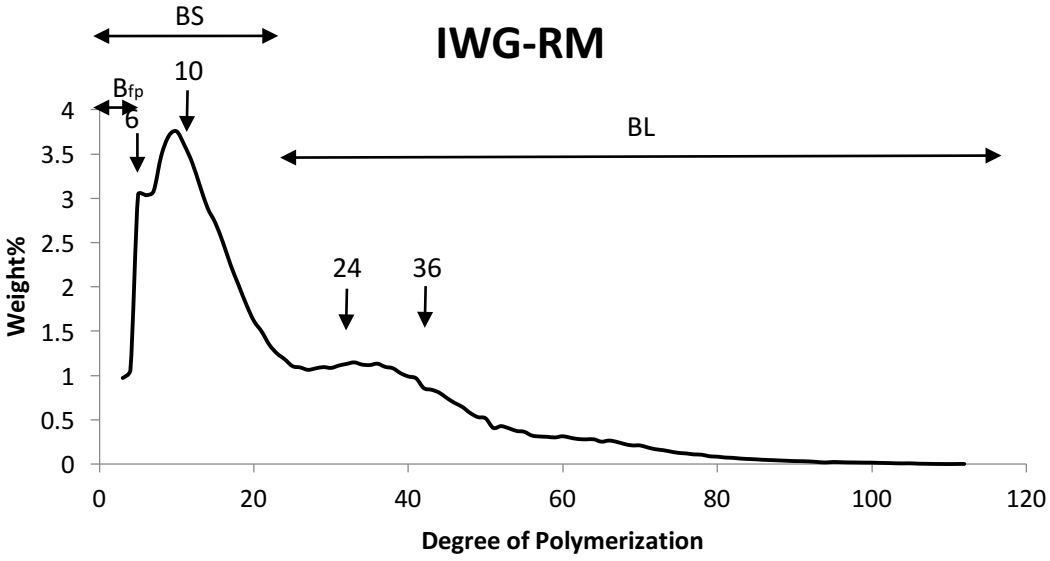
B

Figure 7. 1 The unit chain profile of debranched IWG amylopectins obtained by high-performance anion-exchange chromatography, with arrows and numbers indicating degree of polymerization. A: IWG-RS; B: IWG-RM.

Appendix H. The unit chain profile of debranched φ, β-limit dextrins of IWG amylopectins obtained by high-performance anion-exchange chromatography.



A



B

Figure 8. 1 The unit chain profile of debranched φ, β-limit dextrins of IWG amylopectins obtained by high-performance anion-exchange chromatography, with arrows and numbers indicating degree of polymerization. Short B-chains (BS) are subdivided into "fingerprint" B-chains (B_{fp}) and a major group (BS_{major}), whereas long B-chains (BL) are subdivided into B2- and B3-chains. A: IWG-RS; B: IWG-RM.