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

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Abstract

Background and objectives

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.

Findings

IWG flour had significantly lower total starch, lower RDS and higher lipid 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).

Conclusions

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.

Significance and Novelty

IWG could potentially be exploited for the preparation of foods with lower glycemic responses.

Keywords

expected glycemic index, hydrolysis kinetics, intermediate wheatgrass, starch, unit chain profile

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. Experimental

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 commonly consumed cereal grains. All kernels were kept at room temperature for this study.

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. IWG from both locations and HRW were milled from whole grain kernels. JR was milled from polished grain. Milled samples were then stored at 4^{o}C throughout the study.

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 to form 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 4^{o}C). 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 4^{o}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^{o}C), 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 4^{o}C). The extracted starches were then air dried.

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.

2.5. 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.

2.6. 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.

2.7. 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_∞ 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: $\mathrm{AUC} = C_\infty (t_f - t_o) - (C_\infty/k)\,[1 - e^{-k(t_f - t_o)}]$, 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).

2.8. 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.

2.9. 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).

2.10. 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.

2.11. 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).

2.12. 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).

3. Results and Discussion

3.1. Chemical composition

The chemical composition of the samples used in this study is shown in Table 1. Except for protein content, the ash and fiber 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 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)

3.3. 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 3. The hydrolysis kinetics of the samples over 2 hr is also shown in Figure 1 [A]. At 120 min, about 50% of JR flour starch had been hydrolyzed compared to about 40% from both IWG flour samples (Figure. 1 [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. This observation is due to an increase in intestinal digesta viscosity due to 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 3 and Figure 1 [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 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).

3.4. 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 2. 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 2 [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 2 [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 2 [B]). This observation seems to correlate with the expected glycemic indices of HRW and IWG starches, where the latter had higher eGI.

3.5. 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 $10^4 g/mol$). Supplementary figure 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.

3.6 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 Supplementary figure 2. The mole percent and chain lengths of short (DP<36) and long (DP>36) (Bertoft, 2004) chains are shown in Table 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 Supplementary figure 2 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. Supplementary table 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 (Supplementary table 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.

4.0 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.

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List of Tables

Table 1.

	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]

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 2.

	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	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]

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.

Table 3.

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}

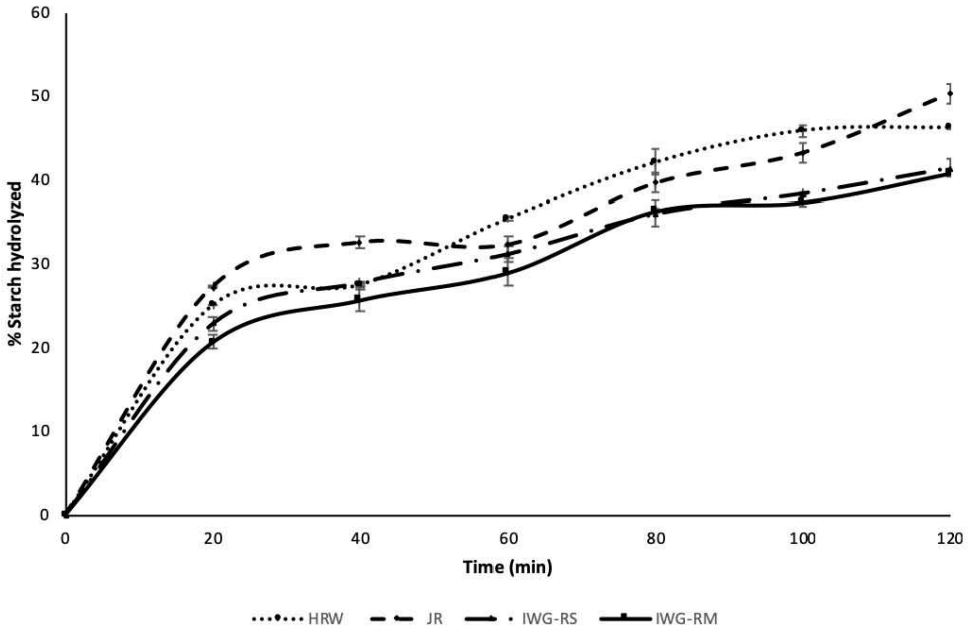
Values are expressed as mean percentages (n=2). Values with different letters in columns are significantly different ($p < 0.05$) from each other. JR: Jasmine rice, HRW: Hard red wheat, IWG-RS and IWG-RM: Intermediate wheatgrass grown in Roseau and Rosemount, Minnesota, USA respectively. RDS: Rapidly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

Table 4.

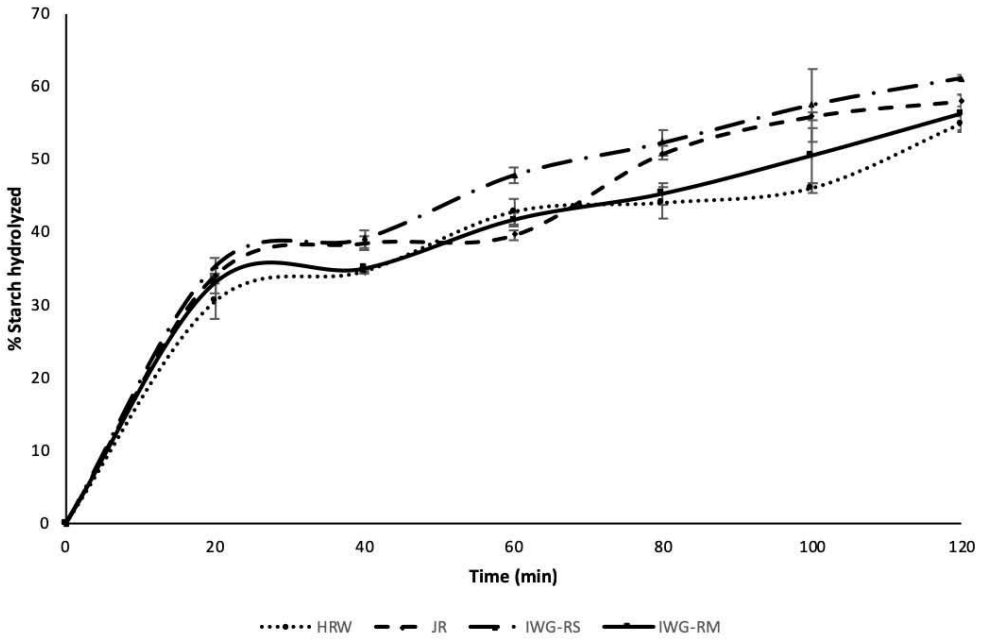
Sample	Mole (%) Short chains (DP <35)	Long chains (DP >35)	Mole Ratio Short chains: Long chain	Chain length Short chains (DP <35)	Long chains (DP >35)	All chains
JR	20.72±0.08^{c}	0.32±0.03^{a}	65.77±6.98^{b}	4.07±0.07^{a}	48.77±0.58^{a}	4.75±0.01^{a}
HRW	13.05±0.04^{a}	0.36±0.01^{a}	35.97±0.37^{a}	6.29±0.00^{c}	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}

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.

List of Figures



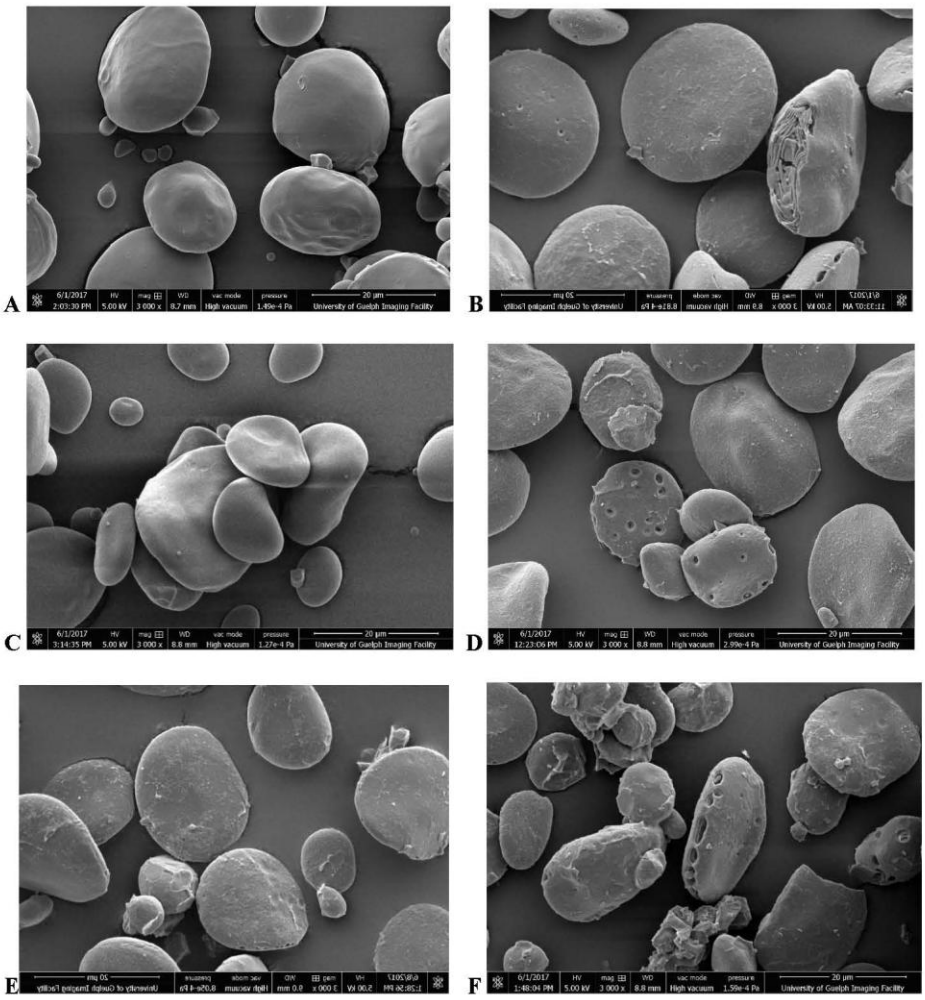
A.



B.

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.



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 2.