# Characteristics of perennial wheatgrass (*Thinopyrum intermedium*) and refined wheat flour blends: the impact on rheological properties

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## ABSTRACT

Intermediate wheatgrass (IWG) (*Thinopyrum intermedium*) is a perennial grass with desirable agronomic traits and positive effects on the environment. It has high fiber and protein contents, which increase the interest in using IWG for human consumption. In this study, IWG flour was blended with refined wheat at four IWG:wheat ratios (0:100, 50:50, 75:25, 100:0). Samples were analyzed for proximate composition, microstructure features, pasting properties (using Micro Visco-Amylograph), protein solubility, and total and accessible thiols. Gluten aggregation properties (using GlutoPeak) and mixing profile (using Farinograph) were also evaluated. IWG flour enrichment increased the pasting temperature and decreased peak viscosity of blended flours. IWG proteins exhibited higher solubility than wheat, with a high amount of accessible and total thiols. GlutoPeak highlighted the ability of IWG proteins to aggregate and generate torque. Higher IWG flour enrichment resulted in faster gluten aggregation with lower peak torque, suggesting weakening of wheat gluten strength. Finally, the addition of IWG to refined wheat flour resulted in a decrease in dough development time and an increase in consistency, likely due to the higher levels of fiber in IWG. The 50% IWG flour enrichment represents a good compromise between nutritional improvement and maintenance of the pasting properties, protein characteristics and gluten aggregation kinetics.

Keywords: perennial wheatgrass; gluten aggregation; pasting properties; protein structural features; *Thinopyrum intermedium*

The development of perennial crops has received great attention from agronomists, breeders and environmentalists because they can be used as alternative crops for marginal lands due to the low environmental impact (Wagoner and Schauer 1990). In 1983, the Rodale Research Center began studying a number of perennial crops and *Thinopyrum intermedium* - commonly called intermediate wheatgrass (IWG) - was selected as one of the best perennial crop candidates according to compositional and nutritional analyses. IWG is well known to possess many favorable agronomic characteristics, including resistance of various diseases present in common wheat, drought and frost resistance, and high biomass (Wagoner and Schauer 1990; Vogel and Jensen 2001). As a perennial species, IWG processes a longer growing season and greater root mass than annual crops, which can greatly reduce erosion risks and nitrate leaching (Glover et al 2010; Culman et al 2013). Therefore, it has the potential to positively impact on environment, and it could successfully replace annual crops - as cereals are - for food production, especially on marginal agricultural lands.

From a nutritional point of view, IWG seed was found to have a higher concentration of protein; although IWG seed protein is nutritionally poor in lysine as is wheat, it has higher amount of all other essential amino acids than wheat (Becker et al 1991).

In a recent study, the chemical properties of IWG have been investigated (Bunzel et al 2014; Schoenfuss et al 2014)), highlighting the superiority of IWG compared to whole wheat in terms of protein and fiber content. The gluten protein profile of IWG, however, differs significantly from that of conventional wheat. IWG gluten proteins is comprised mostly of α and γ gliadins, and some low molecular weight (LMW) glutenins (Bunzel et al 2014). All the IWG varieties tested were deficient in high molecular weight (HMW) glutenins (Bunzel et al. 2014), suggesting a poor gluten forming ability.

Based on nutritional characterization, IWG-based food products, to a greater extent, might have nutritional benefits for consumers. Nevertheless, as for improvement of IWG potential for food production, efforts are tied to the bottleneck of the functional properties of IWG seed grain that are critical for the functionality of foods. It has been reported that no gluten aggregation was found in IWG (Becker et al 1991), indicating that IWG flour is not very suitable for preparing gluten-based foods, such as bread and pasta. More recently, unextractable polymeric proteins, which closely affect wheat rheological properties, were found in hybrid crosses of common wheat and IWG (Hayes et al 2012). More information from a molecular standpoint - starch and protein - on this crop is required in view of a possible application of IWG flour cereal-based products. In this regard, as well-established, the property that makes wheat unique is the ability of its proteins to form a viscoelastic dough. The interactions leading to the formation of the visco-elastic network of gluten involve rearrangement of hydrophobic contacts among proteins (or within individual proteins) and rearrangement of intra- and intermolecular disulfides and thiols in a disulfide exchange process (Morel et al 2002). In regards to starch, changes in viscosity of cereal flours or starches during heating and cooling provides information on molecular changes promoted by processing conditions (Marti et al 2013) or ingredient interactions (Marti et al 2011).

The aim of this study was to evaluate the rheological properties of IWG and refined wheat flour blends, with the goal of increasing the amount of IWG that can be included in a baked-product. Through this study, it is expected to determine the functionality and potential use of IWG blends in baked product systems, which can benefit both the environment and consumers' health.

## MATERIALS AND METHODS

**Materials.** Commercial refined flour from hard wheat (HWF) was kindly provided by Horizon Milling LLC (Mankato, MN, USA). Intermediate wheatgrass (IWG) was kindly provided by the Land Institute (Salina, Kansas, USA). IWG kernels were ground in a whole grain flour Cyclone

Sample Mill (UDY Corp., Fort Collins, CO) equipped with a 0.25 mm screen. Blends at four IWG:HWF ratios (0:100, 50:50, 75:25 and 100:0) were prepared. These percentages of enrichment were chosen in order to provide a flour with a fiber content higher than 10%.

**Proximate composition.** Starch, proteins and fiber contents were determined in triplicate according to the approved methods AACC 76-13, 46-30.01, and 32-07.01, respectively (AACC 2000). Moisture content was measured by drying the sample at 180°C for 4 min by an infrared balance (MB 45, OHAUS, Parsippany, NJ).

**Microstructural features.** Microscopy images of refined wheat flour and IWG (whole grain) were obtained by means of an Olympus BX40 microscope (Olympus Co., Tokyo, Japan) using Lugol ($I_2KI$) as staining.

**Pasting Properties.** The pasting properties of blended flours were determined using a Micro-Visco Amylograph (C. W. Brabender Instruments, South Hackensack, NJ). Fifteen grams of flour (14% moisture) were dispersed in 100 mL of distilled water and stirred at 250 rpm during the test. The following temperature profile was applied: mixing at 30°C for 3 min, heating from 30 °C to 95 °C at a heating rate of 7.5 °C/min, holding at 95 °C for 5 min, cooling from 95 °C to 30 °C at a cooling rate of 7.5 °C/min, and holding at 30°C for 2 min. Measurements were performed in triplicate. The following indices were considered: *i)* pasting temperature (temperature at which an initial increase in viscosity occurs); *ii)* peak viscosity (maximum viscosity achieved during the heating cycle); *iii)* peak temperature (temperature at the maximum viscosity); *iv)* breakdown (index of viscosity decrease during the holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95 °C); *v)* final viscosity; *vi)* setback (index of the viscosity increase during cooling corresponding to the difference between the viscosity at 30 °C and the viscosity reached after the first holding period).

**Protein Solubility.** An aliquot of flour containing ~1 mg protein were suspended in 1 mL of a 0.05 M sodium phosphate buffer (pH 6.8) in presence/absence of 2.0% sodium dodecyl sulfate (SDS) and containing 0.01 mol $L^{-1}$ dithiothreitol (DTT) where indicated and transferred to a shaker for 60 min at room temperature. After centrifugation (12,200×g for 5 min), the amount of protein in the supernatant was determined colorimetrically using the RC-DC Protein Assay (Bio-Rad, Hercules, CA, USA). All samples were measured in triplicate and results were expressed as mg soluble protein/g protein.

**Accessible and Total Thiols.** Accessible thiols were determined following the method described by Iametti et al (2006). A 100 mg aliquot of flour was suspended in 5 mL of buffer containing 0.05 M sodium phosphate, 0.1 M NaCl and 0.5 mM DTNB (5,5'-dithiobis(2-nitrobenzoic acid)). The suspensions were incubated for 60 min at 25 °C and centrifuged for 3 min at 11,000 x *g*. The absorbance of the supernatant was determined at 412 nm. Total thiols were measured by the same method but in the presence of 1% SDS.

**Gluten Aggregation Properties.** Gluten aggregation properties of flours were measured using the GlutoPeak (C.W. Brabender Inc., South Hackensack, NJ, USA), as reported by Kaur Chandi and Seetharaman (2012). An aliquot of 8.5 g of flour was dispersed in 9.5 g of 0.5M $CaCl_2$, scaling both water and flour weight on a 14% flour moisture basis. Sample temperature was maintained at 34 °C by circulating water through the jacketed sample cup. The paddle was set to rotate at 1900 rpm and the test was carried out for 10 minutes. All the measurements were performed in triplicate. The main indices automatically evaluated by the software are: *i)* the maximum torque (expressed in Brabender Equivalents - BE), corresponding to the peak occurring as gluten aggregates; *ii)* the peak maximum time (expressed in seconds), corresponding to the time before torque falling off when gluten breaks down.

**Mixing Properties.** The behavior of the dough during mixing was measured using a Farinograph-AT (C.W. Brabender Inc., South Hackensack, NJ, USA) equipped with a 50 g mixing bowl. The AACC 54-21 standard method (AACC 2000) was used for the identification of optimal water absorption for wheat flour. Dough samples containing intermediate wheatgrass were prepared at the same water absorption as wheat dough. Measurements for each sample were performed in triplicates.

**Statistical Analysis.** All experiments were performed in triplicates. Analysis of variance (ANOVA) was performed using Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA, USA). The level at which significant differences are reported is $p \leq 0.05$.

## RESULTS AND DISCUSSION

**Proximate composition.** Chemical composition of refined wheat flour (HWF) and intermediate wheatgrass (IWG) is shown in Table I. As reference, data of whole wheat flour (USDA 2015) were shown. IWG flour exhibited a significantly ($p \leq 0.05$) lower total starch content compared to bothrefined and whole wheat flour (47.7% , 73.9%, and 72% respectively) and a higher amount of protein (20%, 15% and 13.2% for IWG, refined and whole wheat flours, respectively). The protein data corresponds with results previously collected where IWG has been reported to have protein contents ranging from (17 – 21%; Becker et al 1991, 1992; Bunzel et al 2014; Schoenfuss et al 2014). Despite having more protein, it has been reported that perennial grasses are characterized by a low gluten content (Becker et al 1991). Insoluble and soluble dietary fiber of IWG flour are approximately 8-fold and 4-fold more than those of refined wheat flour (Table I). The differences in dietary fiber are likely related to the nature of flour - whole grain for IWG and refined flour for HWF. Indeed, the kernels of perennial grains are smaller than those of wheat, thus they have a greater surface area per gram of seed and consequently more bran (Becker et al 1991). Published data for whole wheat flour show total dietary fiber contents of approximately 11% (USDA, 2015). Bunzel et al (2014) reported IWG to have 16.4% total dietary fiber. Curiously, Becker et al (1991) reported crude fiber of only 1.69% but this is most likely method related. In this study the chemical composition of IWG was compared to that of HWF since the following sections the rheological properties of IWG and its blends with common wheat were assessed.

**Microstructural features.** Pictures of HWF and IWG starch granules in refined wheat flour and intermediate wheatgrass are shown in Fig. 1. Starch granules in HWF exhibited the bimodal distribution typical of wheat flours, with the presence of large-sized round starch granules (A-type) accompanied by many smaller round granules (B-type) (Fig. 1a). In IWG flour, starch granules appear assembled together, and in some cases embedded within the cell wall, so that they do not appear well distributed in the field of view (Fig. 1b). Moreover, IWG starch granules in the presence of iodine solution did not appear in blue/violet color as wheat starch granules did, suggesting poor affinity to iodine; this is an aspect that needs further investigation. Schoenfuss et al (2014) reported that the ratio of amylose/amylopectin in a bulk IWG sample was 23/77 (0.298). This is comparable to what has been reported for various cultivars of hard red spring wheats (Labuschagne et al 2007). Pictures of samples taken under polarized light (Fig. 1c, 1d) highlighted in IWG the presence of a maltese cross, reflecting an ordered and crystalline structure of starch granules.

**Pasting Properties.** Pasting characteristics of blended flours are shown in Fig. 2 while viscosity data are summarized in Table II. HWF exhibited a typical peak of viscosity at about 85.1°C. During the holding period at 95 °C, the product slurries were subjected to high temperatures and mechanical shear stress causing starch granule disruption and amylose leaching, which led to a slight decrease in viscosity (evaluated by the breakdown index). IWG is characterized by a small peak at 96.5°C and by very low breakdown value (Table II). These differences between the samples were present even when the test was carried out with a constant starch:water ratio (data not shown), to avoid the effect of starch concentration on pasting properties. The variation in starch granules size and shape are also known to significantly influence pasting properties (Singh et al 2003). The predominant presence of starch granules in IWG that are assembled together could provide an explanation for the onset temperature of gelatinization phenomenon in IWG being 4°C higher that in HWF (Table II). Schoenfuss et al (2014) reported IWG peaked earlier in comparison to whole wheat pastry flour (5.5 vs. 5.8 min), which is the opposite of what we saw with refined wheat flour. They also reported a peak temperature of (95°C), which is a lower temperature than we report (97°C). The likely explanation for these discrepancies are that the values were obtained by different methods (Rapid Visco Analyser versus Micro-Visco Amylograph), and the flours that were used. As expected, as we increased IWG concentrations, effects on pasting properties occurred. Pasting temperature significantly ($p \leq 0.05$) increased in the presence of high levels of IWG ($\geq 50\%$). This result was exclusively related to starch characteristics, since adding bran to refined flour did not significantly affect the pasting temperature (data not shown). Whereas, the decrease ($p \leq 0.05$) in peak viscosity in IWF blends was related to differences in chemical composition – protein and fiber content – and  to the presence of components - mainly protein and fiber - that are competitive with starch for water. According to Collar et al (2006), the replacement of wheat with soluble and insoluble dietary fibers would reduce initial starch granule swelling accounting for the lower peak viscosities of the pastes. During cooling, the viscosity increased as a result of the formation of a gel structure indicating the tendency of the granules to associate or retrograde. This is evaluated by the setback index. Low setback values indicate low rates of starch retrogradation and syneresis (Ji et al 2010). As the substitution levels of IWG flour increased the setback and final viscosities decreased, suggesting low retrogradation tendency. This aspect could be relevant for bread properties during storage, since setback values can be considered as valuable predictors at dough level for bread staling kinetics during storage (Collar 2003).

**Protein Solubility.** Solubility of IWG blends proteins in phosphate buffer in the absence and in the presence of denaturing and reducing agents is reported in Fig. 3. Protein solubility in solvent systems with various dissociating ability has been used to discriminate among cereals (Iametti et al 2006) and more recently to describe the effects of technological treatments and ingredients on cereal-based products (Bonomi et al 2012). In this study, this approach was used in order to provide information about the type of interactions in IWG. This is of great interest because it has been demonstrated that there is a correlation between the aggregation properties of proteins and their behavior during processing (Ciaffi et al 1996).

IWG exhibited a higher protein extractability in phosphate buffer compared to HWF (Fig. 3), suggesting that albumins and globulins are present in a greater amount in IWG. Indeed, extractability in phosphate buffer provides information about proteins held together by ionic interactions. No significant differences ($p>0.05$) were detected between 50% and 75% IWG and between 75% and 100% IWG flours. As expected, the addition of detergent resulted in increased soluble protein levels in all the samples. Indeed, SDS facilitates the breakdown of hydrophobic interactions and makes soluble those proteins that form homo and heteropolymeric aggregates based exclusively on these kinds of interactions (Bonomi et al 2013). No significant differences in protein solubility were detected between refined flour and 50% IWG sample; on the contrary, the amount of soluble proteins increased as IWG substitution level were greater than 50%. In particular, significant ($p\leq0.05$) differences were observed between HWF and 75%-100% IWG. Furthermore, the amount of IWG flour protein extracted in the presence of a reducing agent was significantly ($p <0.05$) higher than that of HWF and 50% IWG blend. The addition of disulphide-reducing agents such as DTT to the buffer containing SDS provides the solubilization even of those proteins that form or are trapped within aggregates stabilized by disulfide bonds (Bonomi et al 2013). Treatment with denaturant and disulfide reducing agent solubilized about the 80% of the proteins present IWG, but only 60% in the case of common flour. In general, regardless the type of extraction buffer, the degree of extractability of flours was the following: 0%IWG (HWF) < 50% IWG < 75% IWG < 100% IWG. Previously it has been demonstrated that protein insolubility positively affects dough tenacity and consequently dough strength but not its extensibility (Ciaffi et al 1996). A high percentage of IWG enrichment (50%) did not negatively affect protein solubility, which is a promising result from the perspective of being able to incorporate IWG to produce baked-goods with high fiber and protein contents.

**Total and Accessible Thiols.** The content of accessible and total thiols in flours is shown in Fig. 4. The amount of accessible thiols per gram of protein increased as the percentage of IWG increased. In the presence of SDS, the thiol content showed a marked increase. Indeed, thiols buried within the structure of a protein (or a protein aggregate) may become available to suitable reagents only upon protein denaturation by physical or chemical agents (Iametti et al 2013).

The total content of thiols significantly increased as the IWG enrichment increased. This result is not related to IWG solubility, since the procedure measures protein thiols independently of protein solubility (Iametti et al 2006). The higher content of thiols in IWG blends compared to HWF is in agreement with the higher number in cysteine residues found in IWG compared to common wheat (Becker et al 1991). Cysteine thiols and cysteine disulfides are the key for generating covalently-linked protein networks in many diverse foods (Shewry and Tatham 1997). It has been demonstrated that flours differing in their technological performances traits differ in terms of accessible and total thiols. In particular, soft flours show a lower amount of total thiols compared to hard and durum wheat flour (Bonomi et al 2013). The high level of thiols in IWG is encouraging in view of its use in cereal-based products, such as bread, since the network-forming capacity of proteins involved in thiol–disulfide exchange reactions in individual food systems is related to a multiplicity of factors, that include their relative abundance (Iametti et al 2013). However, the amount (and location) of reactive thiols and disulfides, and their availability to exchange events in IWG should be further investigated.

**Gluten aggregation properties.** The GlutoPeak is a new instrument for testing gluten quality. It provides a measurement of the aggregation behaviour of gluten, as it is present in wheat flour, coarse grain or vital gluten (Kaur Chandi and Seetharaman 2012). The gluten aggregation profile of HWF, IWG and their blends are shown in Fig. 5. During the test, the sample is mixed with water (ratio of flour : water about 1:1) and subjected to intense mechanical action by the rotating element. These conditions allow the development of gluten that result in a strong increase in the consistency of the slurry up to a maximum peak. From that moment, the continuous mechanical stress causes the breakdown of the gluten network, a phenomenon recorded as a decrease in consistency. While the amount of glutenin dictates gluten strength, gliadin to glutenin ratio is related to the maximum time to peak (Melnyk et al 2012).

Interestingly, IWG was able to aggregate and generate a peak. On the contrary, very early studies did not find any gluten forming ability in intermediate grass (Becker et al 1991). Research in our laboratory has demonstrated that gluten free flours did not show any peak or aggregation phenomenon when tested using the GlutoPeak (data not shown). However, gluten in IWG exhibited a lower peak torque (19.2 ± 0.63 BE and 42.0 ± 0.99 BE, for IWG and HWF, respectively) and a lower peak time (44.5 ± 2.2 s and 74.5 ± 3.4 s, for IWG and HWF, respectively), suggesting weaker protein aggregation properties compared to HWF. Blending refined flour with IWG seemed to affect torque more significantly rather than peak maximum time in agreement with previous work on flour blending (Lu & Seetharaman, 2014). This result could be related to the differences in protein profile. According to Melynk et al (2012), glutenin fraction was more important in dictating gluten strength (peak torque) with only small effect on peak maximum time.

Increasing the substitution levels of hard wheat flour with IWG led to a decrease in gluten peak torque and a shorter gluten aggregation time. Peak maximum time is indicative of the time required for gluten to aggregate and exhibit maximum torque on the spindle. The addition of IWG significantly decreased this parameter ($p<0.05$). Adding IWG to wheat flour (50%) resulted in a gluten aggregation profile similar to winter wheat varieties characterized by acceptable bread-making performances (data not shown). The effect of IWG enrichment was greater when IWG made up 75% of the formula (31.0 ± 4.0s). A similar trend was also observed when materials such as fiber or germ were added and resulted in the weakening of the gluten network (Goldstein et al 2010; Marti et al 2014). Previous studies demonstrated that replacing good quality gluten fractions with those from a lower quality wheat variety decreased gluten quality. These cultivar specific differences in gliadin and glutenin were important in dictating gluten strength (torque), and a lesser effect was observed on peak maximum time (Melnyk et al 2012; Lu and Seetharaman 2014). Interestingly, 50% IWG exhibited a torque value similar to that of HWF (42.9 ± 2.96 BE and 42.0 ± 0.99 BE for 50%IWG and HWF, respectively), suggesting that 50% IWG flour blend might have the potential to be used for preparing baked-products without worsening flour performance in terms of gluten aggregation.

**Mixing Properties.** The effects of incorporation of IWG on mixing characteristics were determined by the farinographic test and shown in Fig. 6. Farinograph profiles are a critical indicator of flour quality in various wheat-based product applications. In this study, all the dough samples were prepared at constant water absorption (70%) that was optimal for HWF to reach 500 BU. The wheat flour used for preparing the blends was a very strong flour with a very high dough development time (6 min) and a very high stability (14 min). The use of so strong a flour was driven by our goal to prepare a dough containing a high level of IWG.

Adding IWG to the HWF significantly ($p < 0.05$) increased dough consistency likely due to the higher levels of fiber in IWG (Table I). The peak torque values increased from 489 ± 8.5 BU (refined wheat flour) to 780 ± 5.6, 850.5 ± 4.9, and 862.5 ± 10.6 BU, respectively for 50%, 75%, and 100% IWG. Fiber-rich preparations are known for their ability to absorb considerable amounts of water, leading to an increase in the mixing torque. That ability is mainly determined by the presence of a large number of hydroxyl groups which enter into interactions with water via hydrogen bonds (Rosell et al 2010). However, continuously increasing the substitution level of IWG in blends did not result in significant increase in dough consistency. It seems that increment in proportion of IWG flour does not affect the strength of the gluten network when 70% water absorption was set for all the IWG blends.

Addition of increasing quantities of IWG significantly decreased (3-fold) the dough development time (5.8 min for HWF and 1.85 min for IWG), which is likely explained by the dilution of gluten fractions due to addition of the whole grain IWG and caused interference in the development of the gluten network. However, no significant ($p>0.05$) differences were observed in the dough development time as increasing the replacement of hard wheat flour with IWG flour (1.66, 1.75, and 1.85 for 50%, 75%, and 100% IWG respectively). Interactions among HWF and IWG proteins should be further investigated. According to Matsuo and Irvine (1970), some of the differences in mixing properties could be attributed to protein content, while the differences in dough development time and stability could be attributed to different types of gluten (Irvine et al 1961). Addition of 50% and 75% IWG greatly affected dough stability during mixing, highlighting the weakening effect of IWG addition on the rheological characteristics. Stability is known to be related to the quality of the protein matrix, which is easily damaged by the addition of other ingredients, due to gluten dilution (Marti et al 2014). Interestingly, 100% IWG was more resistant to consistency loss due to mixing compared to 50% or 75% IWG blends, as shown by the curve profile. This is in agreement with the ability of fiber to assume a rigid conformation, improving the strength of the dough (Peressini and Sensidoni 2009). However, the role of IWG proteins is unclear. Indeed, previous studies on wheat flours indicated gluten protein from different wheats possess different properties, replacing good quality gluten fractions with those from a lower quality wheat decreases gluten quality (Melnyk et al 2012; Lu and Seetharaman 2014).

## CONCLUSIONS

Functionality of intermediate wheatgrass substituted in wheat flour was studied using rheological instruments. The overall results highlighted the ability of IWG protein to aggregate forming a gluten-like network that was less strong than common wheat flour. This is related to the high protein solubility of IWG. Despite the high level of thiol groups, these seem to not to be as available for aggregating as in wheat. The 50% IWG-enrichment allows nutritional improvement of cereal-based products without dramatically changing starch pasting properties, gluten aggregation kinetics, and protein characteristics. Future studies should focus on the suitability of IWG-blends to prepare baked-products with nutritional functionality.

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**Figure Legends**

**Fig. 1.** Microscope images of refined wheat flour (a, c) and IWG (b, d) taken under unpolarized (a, b) and polarized (b, c) light.

**Fig. 2.** Pasting properties of HWF and IWG blends using a Micro-Visco Amylograph (C. W. Brabender Instruments, South Hackensack, NJ). Fifteen grams of sample (14% moisture) dispersed in 100 mL of distilled water and stirred at 250 rpm.

**Fig. 3.** Amount of protein solubilized in 1 mL of buffer after a 60 min. incubation followed by centrifugation. Buffer = 0.05 M sodium phosphate buffer (pH 6.8); SDS = 2.0% sodium dodecyl sulfate (SDS) added; DTT = 0.01 mol $L^{-1}$ dithiothreitol (DTT).

**Fig. 4.** Accessible and total protein thiols. Accessible thiols determined following the method described by Iametti et al (2006), and total thiols measured by the same method with the addition of 1% SDS.

**Fig. 5.** Gluten aggregation properties of hard wheat flour and intermediate wheatgrass blends using the GlutoPeak (C.W. Brabender Inc., South Hackensack, NJ, USA). 8.5g of sample (14% moisture) dispersed in 9.5 mL of 0.5M $CaCl_2$ and mixed at 1900 rpm and 34°C.

**Fig. 6.** Mixing profile of hard wheat flour and intermediate wheatgrass blends using a Farinograph-AT (C.W. Brabender Inc., South Hackensack, NJ, USA).

## Table I

**Composition traits of refined wheat flour, whole wheat flour, and intermediate wheatgrass. Values expressed as g/100g sample d.b.**

| | Refined wheat flour | Intermediate wheatgrass | Whole wheat flour[4] |
|---|---|---|---|
| Starch[1] | 73.9 ± 0.4 | 46.7 ± 0.8 | 72.0 |
| Protein[2] | 15.0 ± 0.08 | 20.0 ± 0.3 | 13.2 |
| Total Dietary Fiber[3] | 2.57 ± 0.13 | 16.87 ± 0.36 | 10.7 |
| Insoluble Dietary Fiber | 1.68 ± 0.08 | 13.58 ± 0.08 | - |
| Soluble Dietary Fiber | 0.89 ± 0.05 | 3.29 ± 0.28 | - |

means n=3; ± = standard deviation

[1] Measured by AACC method 76-13

[2] Measured by AACC method 46-30.01

[3] Measured by AACC method 32-07.01

[4] Data from http://ndb.nal.usda.gov

## Table II

### Pasting properties of hard wheat flour (HWF) and intermediate wheatgrass (IWG) blends[1]

| | HWF | 50% IWG | 75% IWG | IWG |
|---|---|---|---|---|
| Pasting Temperature (°C)[2] | 61.0 ± 0.2a[3] | 63.5 ± 0.4b | 64.1 ± 0.4b | 65.2 ± 0.3c |
| Peak viscosity (BU)[4] | 500 ± 11a | 363 ± 9b | 308 ± 7c | 257 ± 4d |
| Peak Temperature (°C)[5] | 85 ± 0a | 89 ± 0b | 92 ± 0c | 97 ± 1d |
| Breakdown (BU)[6] | 310 ± 7a | 147 ± 2b | 76 ± 5c | 35 ± 3d |
| Final viscosity (BU) | 739 ± 26a | 692 ± 43ab | 649 ± 12b | 504 ± 4c |
| Setback (BU)[7] | 548 ± 23a | 476 ± 36b | 416 ± 12c | 282 ± 3d |

[1] Measured on a Micro-Visco Amylograph (C. W. Brabender Instruments, South Hackensack, NJ). 15g of sample flour (14% moisture) dispersed in 100 mL of distilled water and stirred at 250 rpm. Profile 30°C for 3 min, heating from 30 °C to 95 °C at ramp rate of 7.5 °C/min, hold at 95 °C for 5 min, cooling to 30 °C at a rate of 7.5 °C/min, and holding for 2 min.

[2] Temperature at which an initial increase in viscosity occurs.

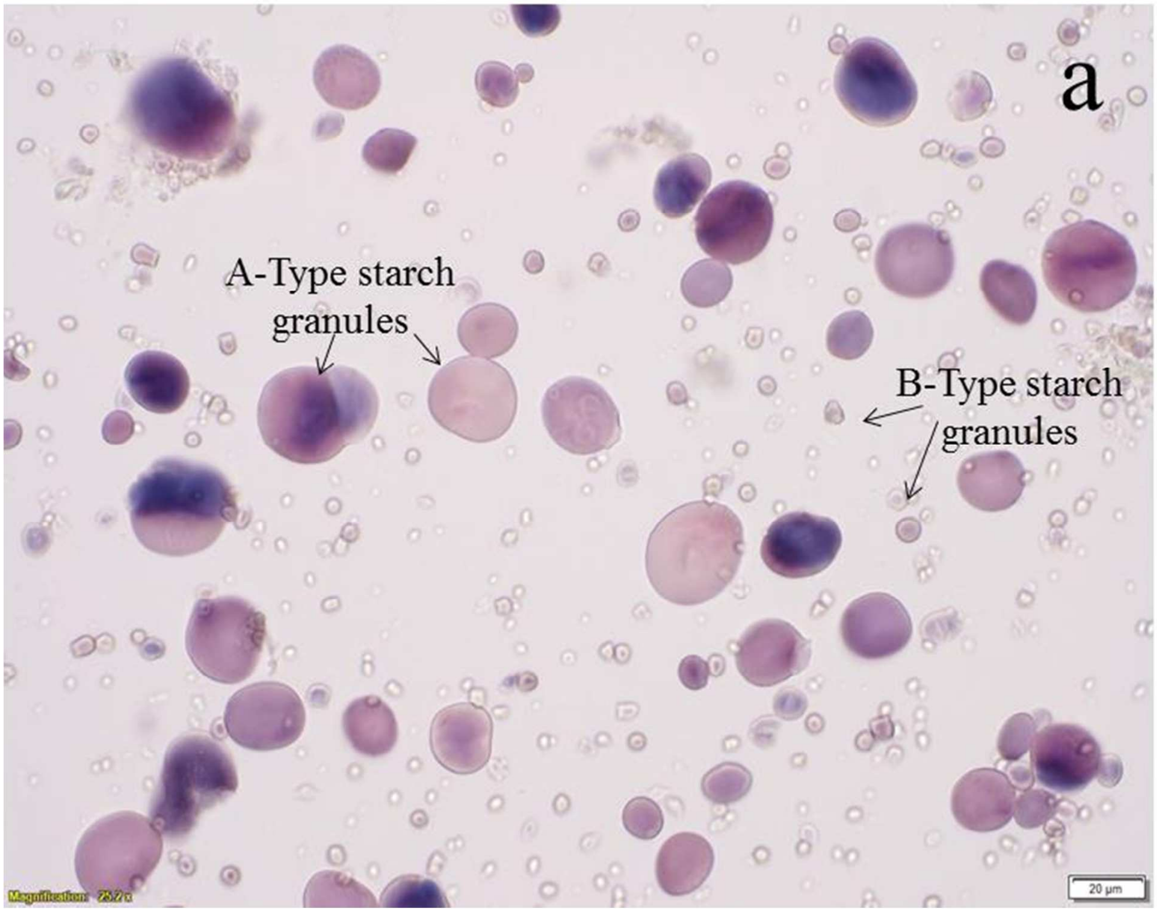
[3] Means (n=3) followed by standard deviation with a different letter for each index are significantly different ($p<0.05$).

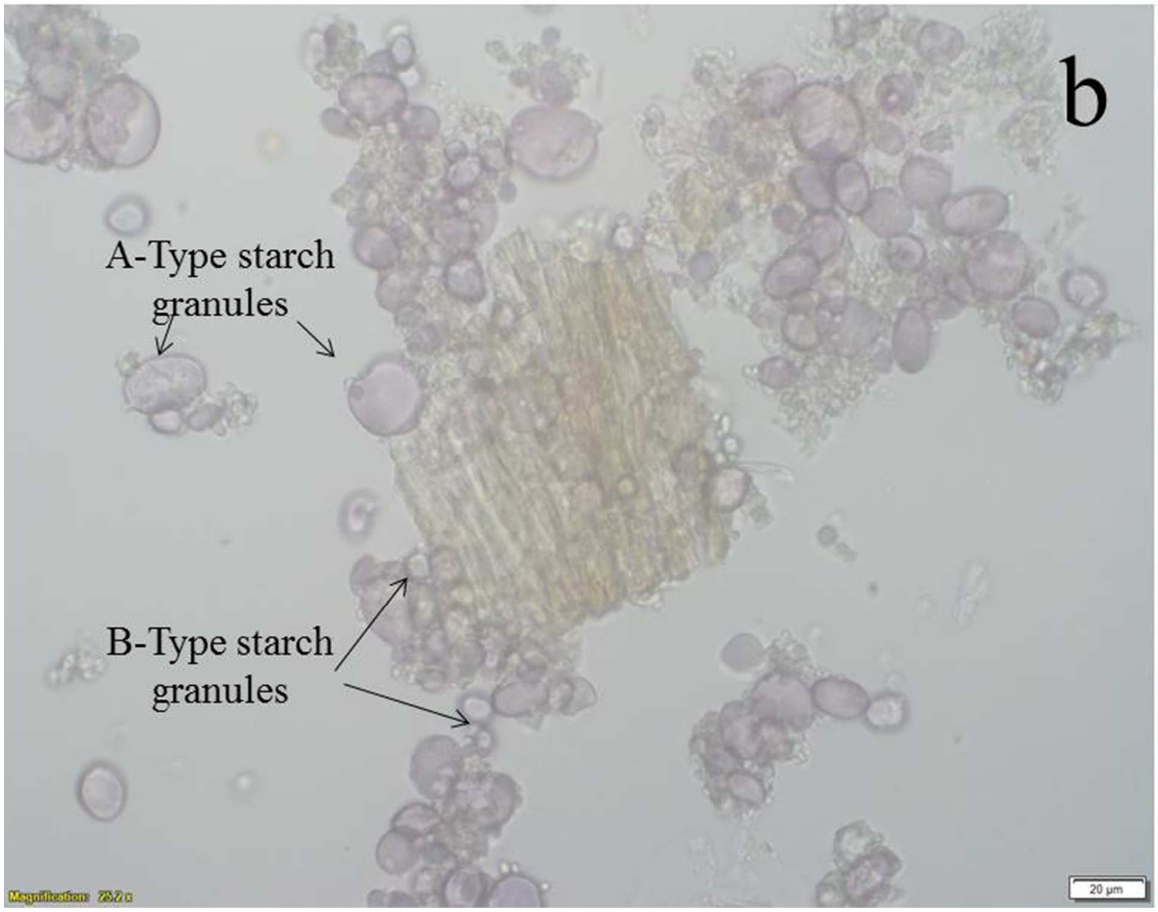
[4] Maximum viscosity achieved during the heating cycle.

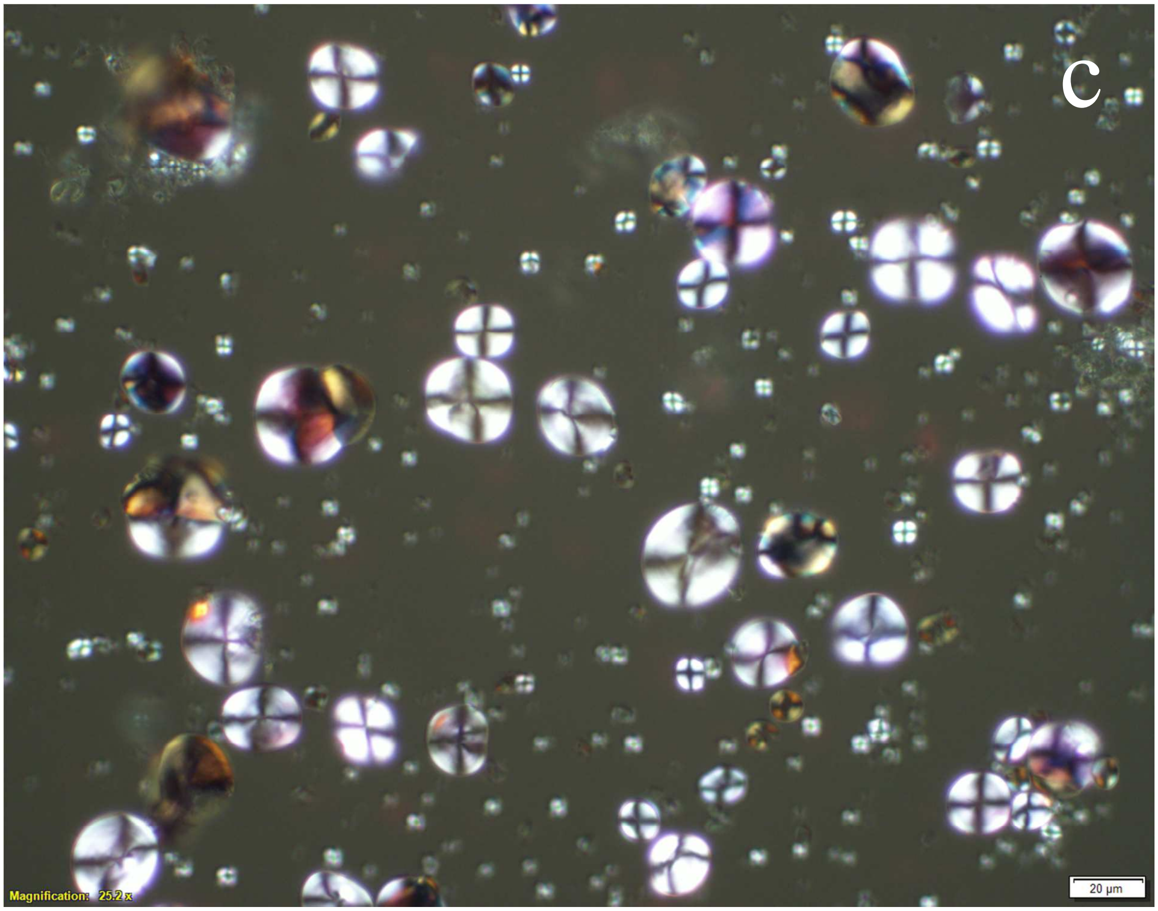
[5] Temperature at the maximum viscosity.

[6] Difference between the peak viscosity and the viscosity after the holding period at 95 °C.

[7] Difference between the viscosity at 30 °C and the viscosity reached after the first holding period.


a
A-Type starch granules
B-Type starch granules
Magnification: 25.2 x
20 µm


b
A-Type starch granules
B-Type starch granules
Magnification: 25.2 x
20 µm


c
Magnification: 25.2 x
20 µm

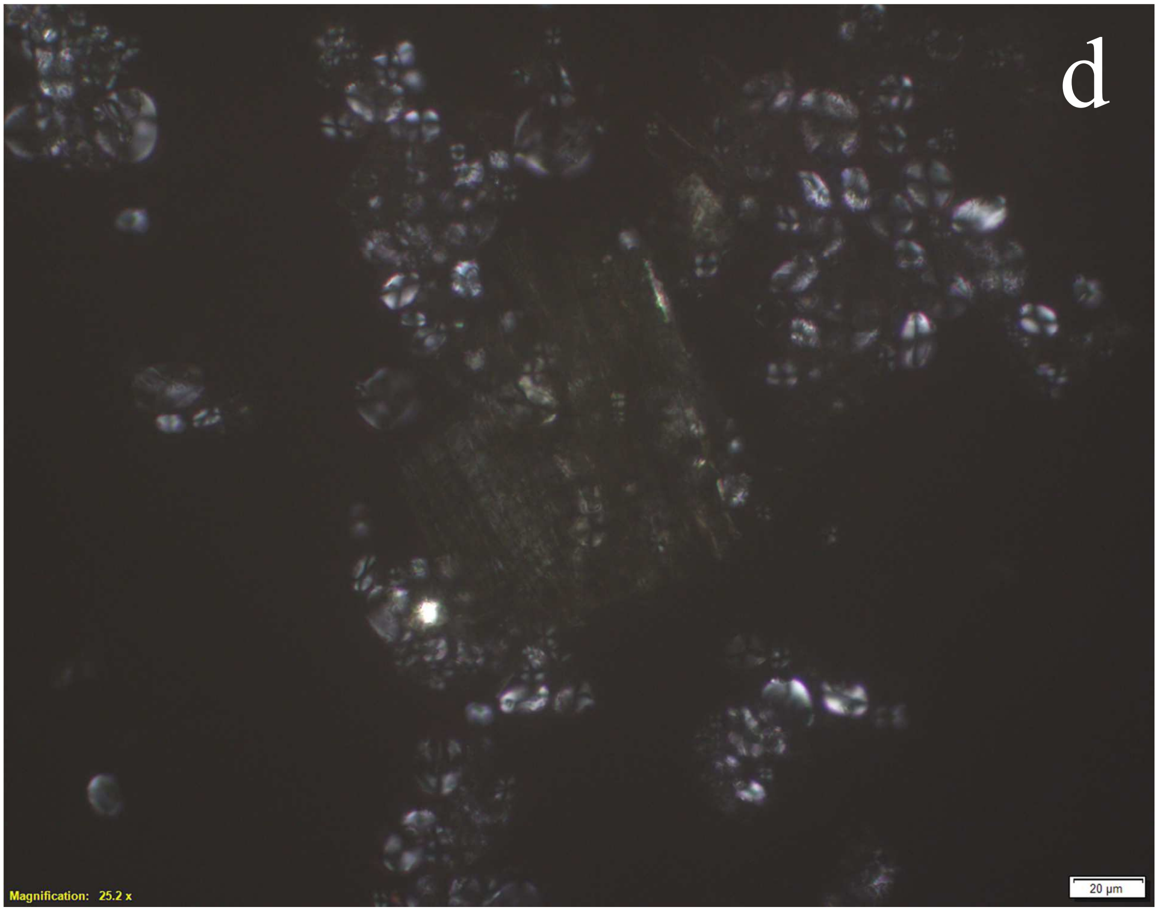
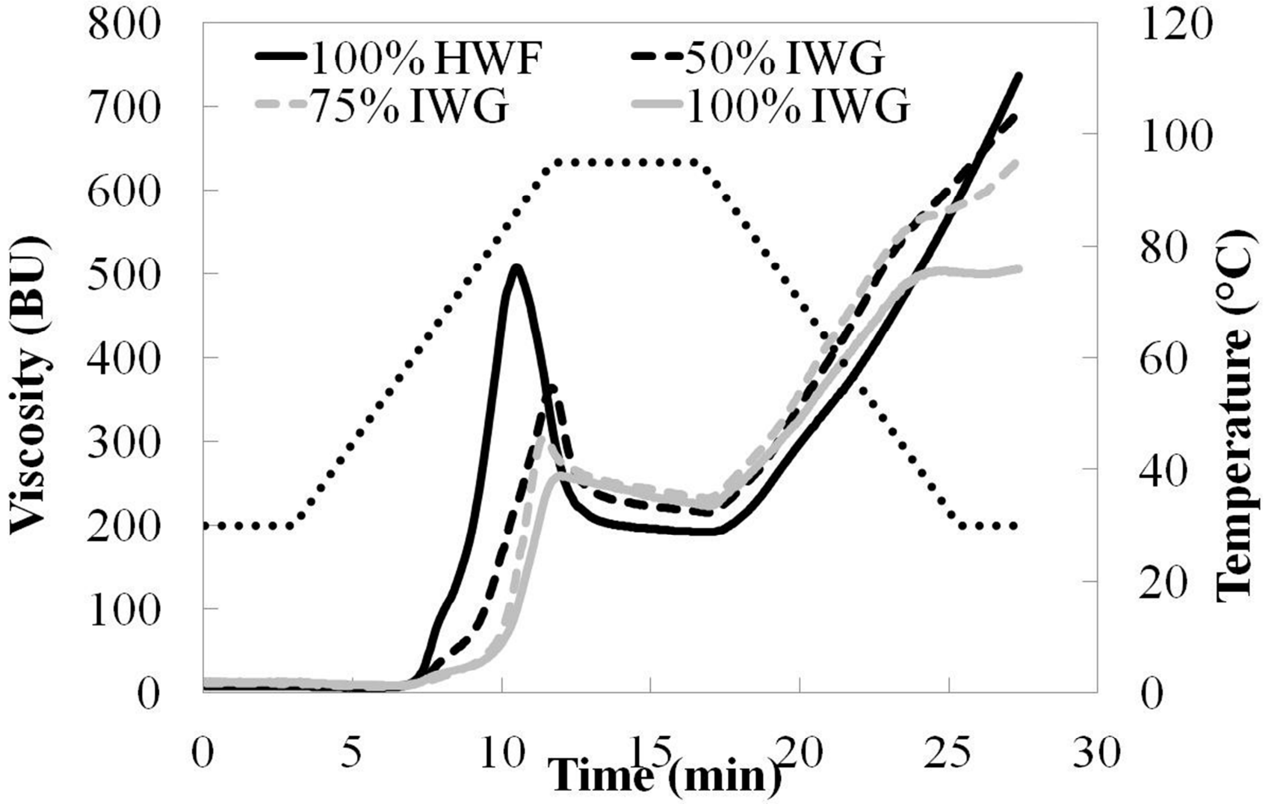


Fig. 1.



100% HWF
50% IWG
75% IWG
100% IWG
Viscosity (BU)
Temperature (°C)
Time (min)
0
100
200
300
400
500
600
700
800
0
20
40
60
80
100
120
0
5
10
15
20
25
30

**Fig. 2.**

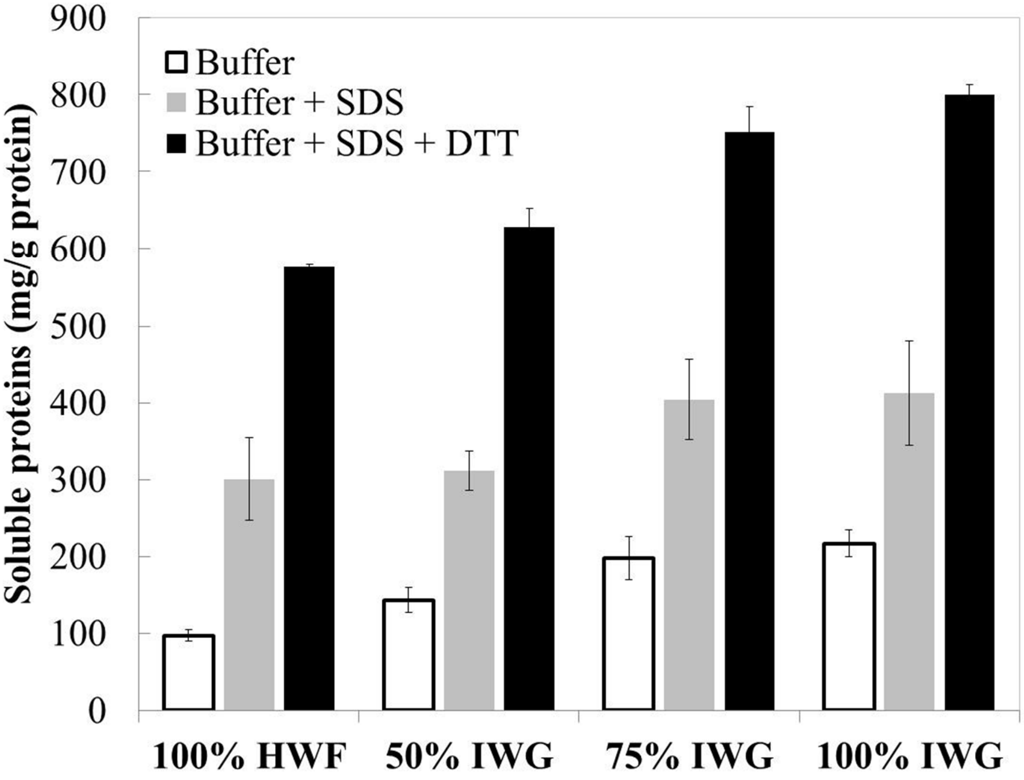
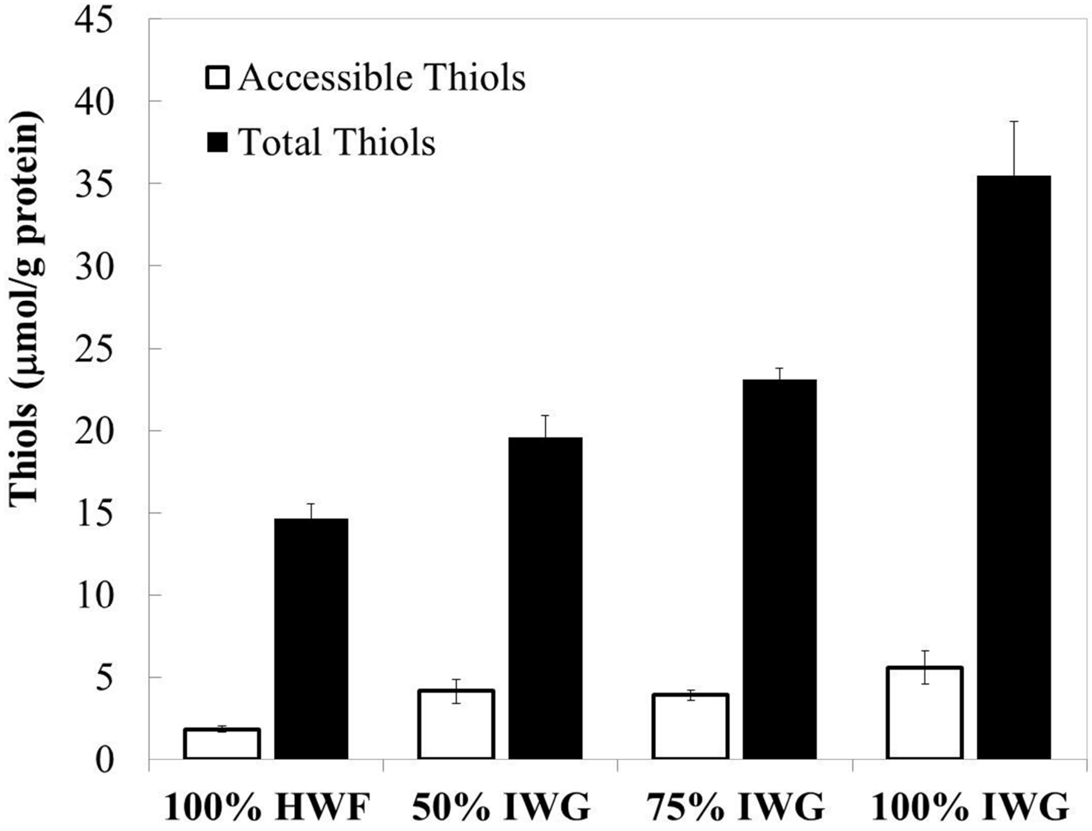
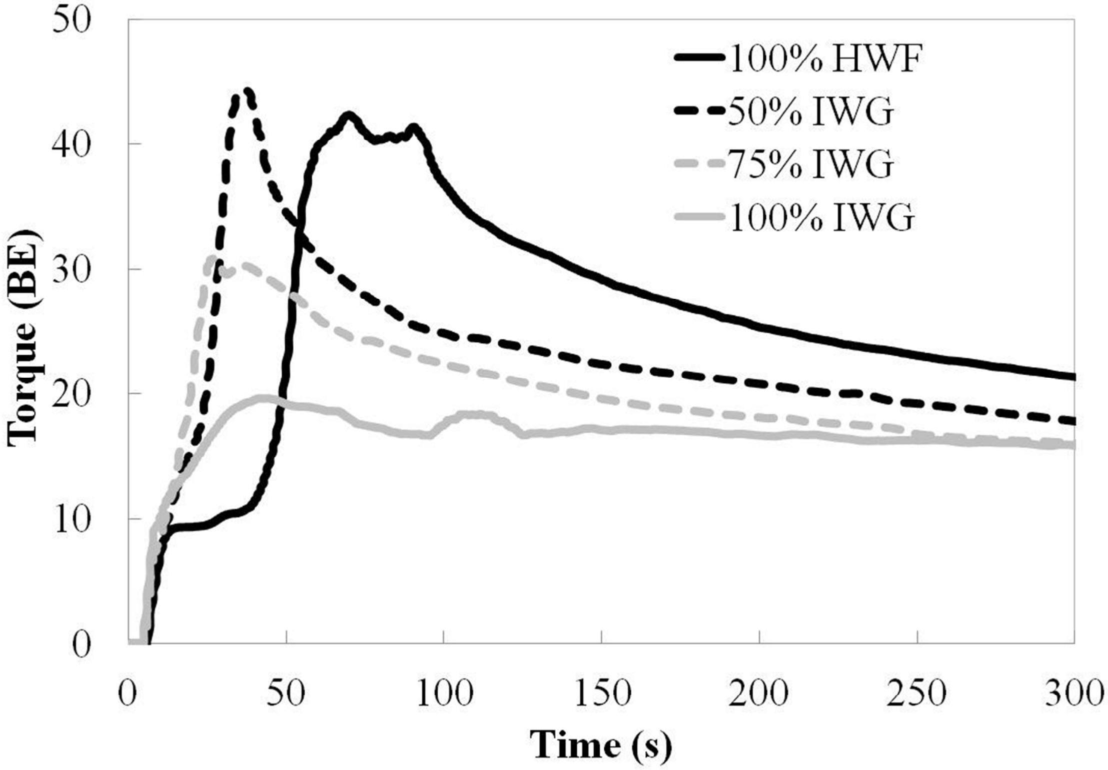

900
800
700
600
500
400
300
200
100
0
Soluble proteins (mg/g protein)
☐ Buffer
▨ Buffer + SDS
■ Buffer + SDS + DTT
100% HWF
50% IWG
75% IWG
100% IWG

**Fig. 3.**



45
40
35
30
25
20
15
10
5
0
Thiols (µmol/g protein)
☐ Accessible Thiols
■ Total Thiols
100% HWF
50% IWG
75% IWG
100% IWG

**Fig. 4.**



469

470 **Fig 5.**

471

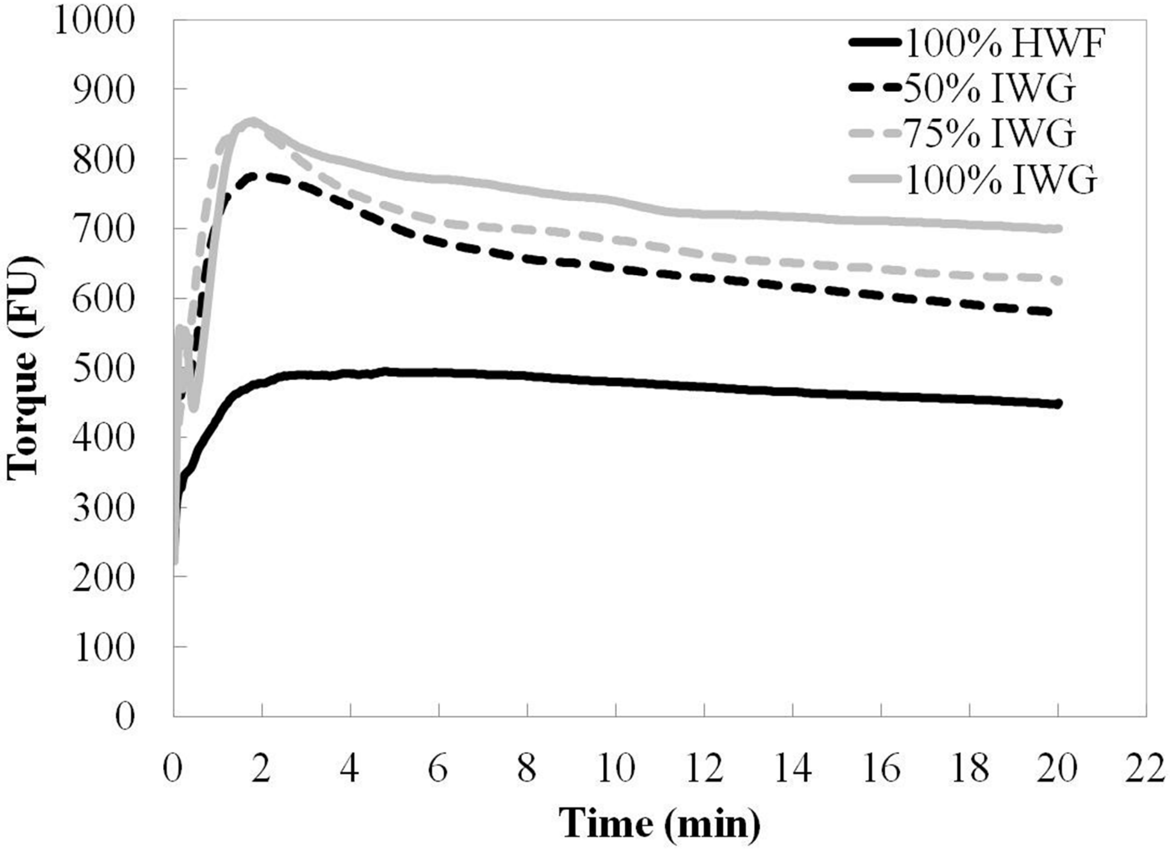
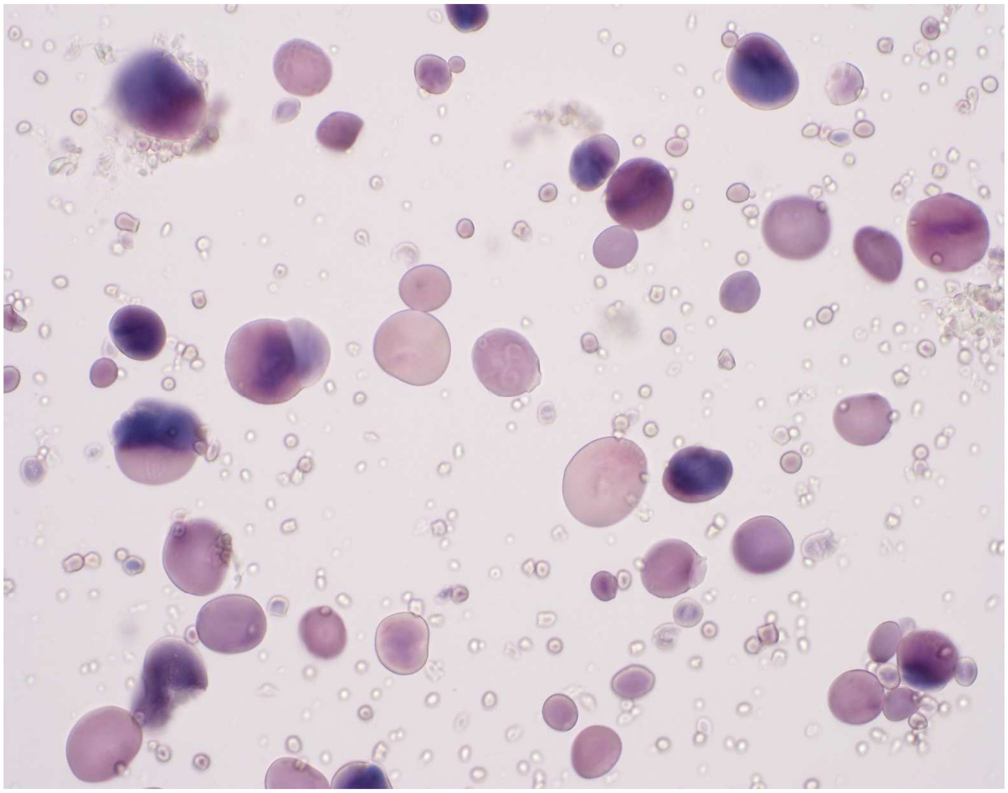
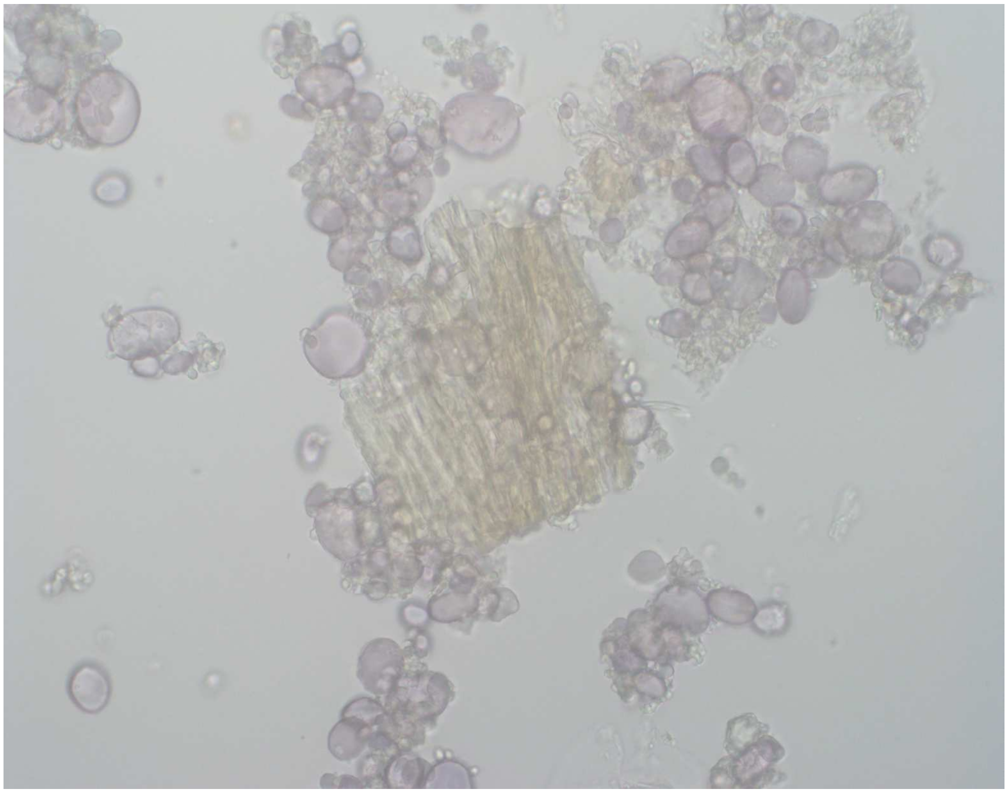

100% HWF
50% IWG
75% IWG
100% IWG
Torque (FU)
0
100
200
300
400
500
600
700
800
900
1000
Time (min)
0
2
4
6
8
10
12
14
16
18
20
22

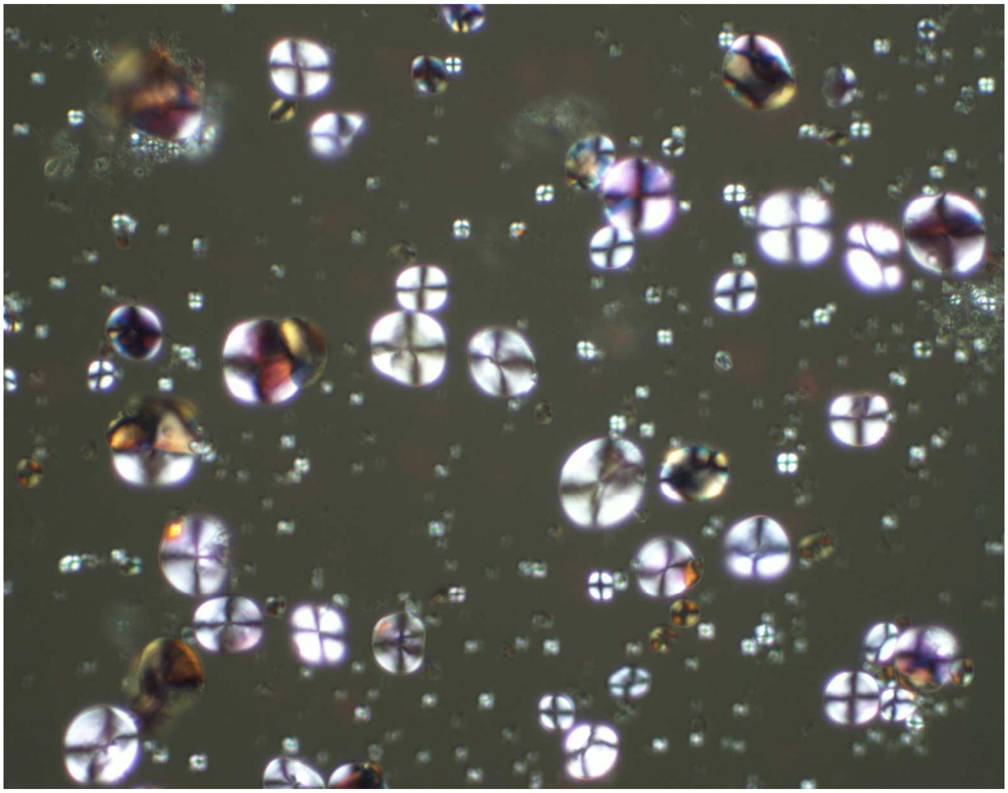
**Fig. 6.**



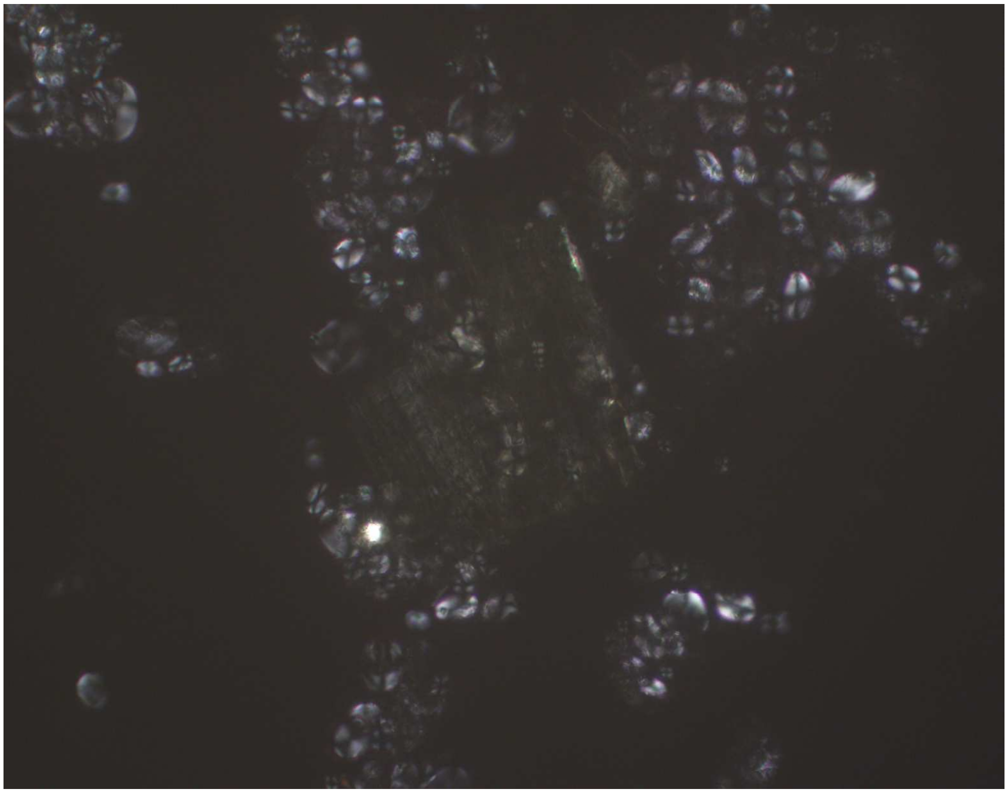
863x677mm (72 x 72 DPI)



863x677mm (72 x 72 DPI)



863x677mm (72 x 72 DPI)



863x677mm (72 x 72 DPI)