Functional Characteristics and Quality of Intermediate Wheatgrass for Food Applications

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Abstract

Thinopyrum intermedium, commonly known as intermediate wheatgrass (IWG), is genetically related to wheat, belonging to the Triticeae tribe. However, IWG is a perennial crop that produces a large biomass that can be used for hay or forage, shows great potential to be developed as a grain crop, and provides sustainable environmental benefits. The environmental benefits of perennial crops in comparison to annual grain crops include reduced soil and water erosion, reduced soil nitrate leaching, increased carbon sequestration, and reduced inputs of energy and pesticide. IWG is being bred by The Land Institute and the University of Minnesota for increased seed yield so that it can be used as a perennial grain crop. Breeding progress has resulted in increased seed size and seed yields two times larger than those of original populations. However, farmers will be reluctant to plant the crop if there is not a strong market for these crops, and this market pull cannot be established unless the crop is characterized and developed for food use. While breeders are intensifying efforts to enhance the yield and size of the grain as well as its quality parameters based on characterization data, it is crucial to determine ways to optimize utilization of the grain in order to expand its market potential as a stand-alone flour or as a replacement for wheat in commercial applications. Studies have shown that consumers are seeking transparency and sustainability in their food supply and companies of all sizes are being receptive. The use of a sustainable crop, such as IWG, in food products will allow consumers to feel good about their purchase, and their role in supporting a sustainable agricultural system. Accordingly, food industries, such as General Mills and PepsiCo, are interested in commercializing products formulated with ingredients derived from these crops. Our U of M food science team is working with companies such as General Mills, Inc. to determine how intermediate wheatgrass can be incorporated into their food products as a green and earth-friendly crop. Our team is focusing efforts on characterizing chemical, functional and flavor properties as affected by refinement, storage, milling and various processing conditions. Understanding nutritional and flavor attributes as well as the functionality of perennial grains for food applications is vital if the dual use of these crops is to be realized. Our work showed that IWG is high in protein, fiber and antioxidants. While IWG has a superior nutritional profile as compared to wheat, it poses challenges for baked products that require dough rising properties. We are currently investigating the effect of conditioners on enhancing dough functionality, determining the effect of fiber on dough development, researching the effect of blending with wheat, determining the effect of thermal treatment on the grain/flour shelf life, and exploring several food applications that would enhance its potential utilization as a food crop. Data gathered will provide necessary information to the breeding program for the continued screening and breeding efforts to ultimately develop IWG with good yield, size and quality traits for food applications.

A. Background of the project:

Our collaborators (breeders and geneticists) from the Agronomy Department at the University of Minnesota and from the Land Institute are working on developing a new perennial grain crop by domesticating Thinopyrum intermedium. Thinopyrum intermedium, commonly known as intermediate wheatgrass (IWG), is genetically related to wheat, belonging to the Triticeae tribe.

However, IWG is a perennial crop that produces a large biomass that can be used for hay or forage, shows great potential to be developed as a grain crop, and provides sustainable environmental benefits. The environmental benefits of perennial crops in comparison to annual grain crops include reduced soil and water erosion, reduced soil nitrate leaching, increased carbon sequestration, and reduced inputs of energy and pesticide. IWG is well adapted to Minnesota, producing biomass and grain yields approximately 50% higher than when grown in Kansas. The dense root system and rapid regrowth after harvest seen with perennial grasses ensures that the system is environmentally benign relative to annual grain cropping. In general, IWG possesses high winter hardiness and is nearly immune to most diseases that affect wheat.

From a consumer perspective, the engagement in purchasing habits that can improve the environment is gaining prominence. The use of perennial crops in sustainable cereal, snack and bakery products will allow consumers to feel good about their purchase, and their role in supporting a more sustainable agricultural system. Our collaborators from General Mills stated that a project focused on characterizing food quality attributes and developing improved food varieties "will advance the state of our knowledge and the prospects for commercializing Intermediate wheatgrass for food use".

We have previously tested several IWG lines for proximate composition, total starch, total dietary fiber, soluble dietary fiber, insoluble dietary fiber, amino acid profile, and carbohydrate composition. Protein profiling, starch functionality testing, and baking tests were performed followed by flavor profiling. The data was promising regarding the grain's acceptability. However, the data also revealed some characteristics that should be improved to expand its market potential as a stand-alone flour or as a replacement for wheat in commercial applications. Therefore, additional characterization of selected IWG lines (of varying grain characteristics) was warranted to provide detailed feedback to the breeders to modify, key traits to enhance the quality of IWG.

We anticipate that a strong partnership among the Land Institute, University of Minnesota breeders and Food Scientists, as well as end-use industry partners like General Mills will be the engine that drives development of a successful IWG crop. Working together will aid tremendously in developing desirable traits in perennial crops. ***Therefore, the overall objective*** of this project was to characterize advanced breeding lines for grain/flour chemical characteristics, functional attributes, storage stability, and flavor quality as well as identify new technologies to incorporate selected lines and their by-products in a variety of food products that can serve as a new food supply.

B. Progress made toward the original goals of the project:

We have made excellent progress in all activities outlined in the project. Below is a summary of the different activities.

Chemical Characterization and Functionality

Sixteen IWG experimental lines along with one bulk IWG sample and two wheat controls (Arapahoe and commercial hard red wheat) were analyzed for proximate composition following standard methodologies. Dietary fiber, total starch content, and percent damaged starch were determined using Megazyme kits. Amylose/amylopectin ratio and their molecular weight distribution were determined using size exclusion chromatography. Gluten forming proteins

profile and molecular distribution were determined using gel electrophoresis and size exclusion chromatography. Dough rheology was assessed using a farinograph and a texture analyzer equipped with a Kieffer rig, while starch pasting properties were monitored using a rapid visco analyzer. Bread baking tests were performed following the AACCI 10-10.03 method. Compared to wheat controls, IWG samples had higher protein, dietary fiber, and ash contents, yet were lower in starch content and deficient in high molecular weight glutenins (HMWG), important protein components responsible for dough strength and elasticity. Specifically, wheat controls had more high molecular weight polymeric proteins (HMWPP), while IWG samples had more albumins and globulins. The ratios of amylose to amylopectin among the IWG samples and the wheat controls were similar. On the other hand, the soluble to insoluble dietary fiber ratio was higher in wheat controls than in IWG samples. Dough rheology data showed that IWG dough was weaker than that of the controls. Farinograph and Kieffer data demonstrated that doughs made from wheat controls were more stable, more resistant to extension, and more extensible than doughs made from IWG samples. In terms of starch pasting properties, wheat controls had higher peak, hold, and final viscosities than the IWG samples, indicating the superiority of wheat controls over IWG samples as viscosity builders. The starch pasting properties data illustrated the samples' behavior upon heating and cooling treatments, which are important characteristics to consider when evaluating IWG for commercial applications. Even though IWG had similar specific volumes to one of the wheat controls (hard red wheat), both wheat controls had a higher rising capability due to the wheat's gluten network forming ability. Deficiency in HMWG and high fiber content of IWG samples, contributed to the poor gluten network and consequently inferior baking quality. Overall results suggest that IWG has a superior nutritional profile as compared to wheat, but poses challenges for baked products that require dough rising properties. Further studies on IWG, such as investigating the effect of conditioners on enhancing protein functionality, determining the effect of fiber on dough development, effect of blending with wheat, and exploring other food applications would enhance its potential utilization as a food crop.

Residues of concern, antioxidants, and storage stability

IWG and hard red wheat (HRW) control were analyzed for residues of concern, namely pesticides and mycotoxins, and for antinutritive components, namely as phytate and trypsin inhibitor. No significant differences were found between IWG and HRW sample. The same samples were subjected to six treatments consisting of two steam treatments (present/absent) and three incubation temperatures (4°C, 23°C, and 45°C). Antioxidants namely carotenoids and hydroxyxcinnamic acids were quantified. Antioxidant activity pre- and post-steam treatment was analyzed using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and leucomethylene blue (LMB) assays. Lipoxygenase activity was measured using the ferrous oxidation-xylenol orange (FOX) assay, and lipase activity was determined through a spectrophotometric method. IWG had significantly higher antioxidant content and activity than HRW. On the other hand, IWG higher lipase activity yet lower lipoxgenase activity than HRW. Throughout storage, samples were pulled for analysis of indicators of hydrolytic and oxidative rancidity, including free fatty acids, hydroperoxides, and malondialdehydes. The antioxidant activity was not significantly affected by storage conditions. Minimal increase in rancidity was observed, indicating a good shelf life despite higher lipase and fat contents.

IWG bread flavor characterization

Nineteen odorants were selected and identified in the bread crusts based on two criteria, compounds that reported a flavor dilution value of $\geq$ 32 and present in at least one of the bread crust extracts. All compounds were positively identified based on comparisons of linear retention indices (LRIs) from two different column chemistries, mass spectra, and odor descriptors to authentic compounds. Based on the chemical structures of the odorants, the Maillard reaction, lipid oxidation, and yeast fermentation were suggested as the main pathways of flavor generation. Quantification of the identified odorants indicated that 16 compounds were significantly different between the two bread crusts, with the majority significantly higher in WW bread. Specifically, 2-methylpropanal, 3-methylbutanal, 3-methyl-1-butanol, methional, 2-acetylfuran, 2-acetyl-1-pyrroline, 1-octen-3-one, 2-acetylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-diemthylpyrazine, 2,3-diethyl-5-methylpyrazine, ethyl octanoate, and (*E*,*E*)-2,4-decadienal were present in higher concentration in WW than IWG bread crust by 2.5, 2.09, 1.28, 1.41, 4.2, 3.76, 1.39, 4.89, 5.10, 4.47, 6.72, 3.22, and 7.69-fold respectively; while 1-octen-3-ol, 2-acetyl-2-thiazoline, and (*E*,*Z*)-2,6-nonadienal were present in higher concentrations in IWG bread crust than WW by 13.7, 1.50, and 1.22-fold, respectively. The noted lower concentration of Maillard reaction products detected in IWG bread sample suggested this well-known flavor generation pathway was suppressed compared to WW bread, possibly due to higher phenolic content of the flour. The lower concentration of the yeast fermentation products identified in IWG compared to WW also suggest the pathway was inhibited likely due to a decrease in the fermentation activity of the yeast. The higher concentrations of 1-octen-3-ol and (*E*,*Z*)-2,6-nonadienal in IWG, but higher concentrations of 1-octen-3-one and (*E*,*E*)-2,4-deacdienal in WW suggest the lipid oxidation pathways in the bread crusts generate different end products, which is likely caused by the flours' unique compositions. Descriptive analysis resulted in the identification of eight aroma attributes in both crusts. WW bread crust was rated significantly higher in roasted and toasted attributes, while IWG was rated significantly higher in green, raisin, and bran attributes. The higher roasted and toasted attributes in WW corresponded to the higher concentration of Maillard reaction compounds in WW. The higher green attribute rating in IWG bread corresponded to the higher concentration of 1-octen-3-ol and (*E*,*Z*)-2,6-nonadienal in IWG bread.

C. Long-term sustainability and impact of the project

Understanding nutritional and flavor attributes as well as the functionality of perennial grains for food applications is vital if the dual use of these crops is to be realized. Farmers will be reluctant to plant the crop if there is not a strong market for these crops, and this market pull cannot be established unless the crop is characterized and developed for food use. Data gathered thus far will provide necessary information to the breeding program for the continued screening and breeding efforts to ultimately develop IWG with good yield, size and quality traits for food applications.

D. Additional funds secured to continue the project:

Additional funds have been secured from Forever Green Initiative Program in Oct 2015, and in 2016, and also from the Minnesota Department of Agriculture (MDA). Funds are being used to

complement and expand our current project. Funds from the MDA are being used to assess the overall safety of the grains by determining presences of chemical residues, such as pesticides and mycotoxins, heavy metal, allergens, and anti-nutrient factors. The shelf-life of IWG with and without heat treatment and the effect of heat treatment on functionality will be determined not only for stored grains but also for stored flour, whole, partially refined and refined. Samples will be stored under various temperatures and analyzed for problematic enzymes, rancidity, functionality, and changes in flavor and taste. Based on the results obtained additional heat treatments will be tested to determine the best heating conditions that will result in elimination of problematic enzymes while maintaining functionality. Information gathered will provide necessary feedback to the breeding program for the continued screening and breeding efforts to ultimately develop IWG with acceptable quality traits including shelf-life stability. Funds are also used to determine the effect of reducing fiber content by refinement and the use of dough conditioners on functionality. While breeders are intensifying efforts to enhance the yield and size of the grain as well as its quality parameters based on characterization data, it is crucial to determine ways to optimize utilization of the grain in order to expand its market potential as a stand-alone flour or as a replacement for wheat in commercial applications.

E. Undergraduate, graduate student and post-doc education and opportunities provided by the grant:

The grant provided several educational opportunities to two post docs, three graduate students and several undergraduate researchers. All were able to present results at national and international conferences.