Cereal Chemistry

RESEARCH ARTICLE OPEN ACCESS

Effect of Nitrogen Treatment on the Physico-Chemical and Functional Properties Intermediate Wheatgrass (*Thinopyrum intermedium*) Grown in Different Locations

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Received: 18 November 2024 | **Revised:** 6 October 2025 | **Accepted:** 20 October 2025

Funding: This study was funded through the Kernza CAP AFRI Sustainable Agricultural Systems Coordinated Agricultural Program (SAS-CAP) grant no. 2020-68012-31934 from the USDA National Institute of Food and Agriculture.

Keywords: functional Properties | Intermediate wheatgrass | nitrogen fertilizer

ABSTRACT

Background and Objectives: Intermediate wheatgrass (IWG), a new perennial crop, is being explored for food applications; however, nitrogen application effects on its properties have not been investigated. This study evaluated the effects of applying 90 kg ha^{-1} of nitrogen in spring or fall seasons on the properties of IWG grown in Minnesota and Wisconsin, USA.

Findings: Nitrogen treatment increased protein and fat content of IWG while reducing total carbohydrates, regardless of location, treatment time, or refinement. Total dietary fiber increased in whole grain samples. Starch hot paste viscosity generally decreased with nitrogen treatment. Farinograph water absorption and dough stability increased with nitrogen fertilization in samples from Wisconsin. IWG kernel size increased with nitrogen application regardless of growing location and timing of treatment.

Conclusion: Nitrogen treatment impacts on IWG properties varied based on growing location, time of application, and whether it was refined or not. While protein and fat contents significantly increased, in the spring season, seed weight was more impacted by fall nitrogen application. Nitrogen treatment also results in strong IWG dough.

Significance and Novelty: This study is the first to show the effect of nitrogen fertilization and timing on the properties of IWG.

1 | Introduction

The dominance of annual cereal grains in both human diets and global agriculture has indeed been significant, constituting approximately 50% of human calorie intake and agricultural land use worldwide. However, this high reliance on annual grains has contributed to environmental degradation such as soil erosion, loss of biodiversity, and greenhouse gas emissions. The Food and Agriculture Organization (FAO 2016) highlighted in 2016 the increasingly alarming impact of climate change on agriculture, emphasizing its detrimental effects on food security. Climate change-related factors such as extreme weather events, shifts in precipitation patterns, and rising temperatures pose significant challenges to crop production systems worldwide. Thus, there is a pressing need to find alternative grains to traditional annual grains. Perennial grains, for instance, have the potential to provide sustainable food production while mitigating some of the negative environmental impacts associated with annual cropping systems (Jungers et al. 2017). Efforts to develop and promote perennial grains as viable alternatives

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to annual grains are underway globally (Wagoner and Schaeffer 1990). By harnessing advancements in agricultural technology, genetics, and agronomic practices, researchers and farmers seek to create cropping systems that are more resilient to climate change, require fewer inputs, and promote ecosystem health (Jungers et al. 2017). The shift towards perennial grain agriculture represents a significant paradigm change in food production systems (Crews et al. 2018). By incorporating perennial crops into agricultural landscapes, we can promote biodiversity, conserve natural resources, and build resilience to climate change (Asbjornsen et al. 2014). Moreover, the ability of perennial grains to provide food directly for human consumption contributes to food security by diversifying our food sources and reducing our reliance on annual crops (Ryan et al. 2018). Intermediate wheatgrass (IWG), scientifically known as *Thinopyrum intermedium*, is a cool-season sod-forming perennial grass. It belongs to the Triticeae tribe of the Pooideae subfamily (Kantarski et al. 2018). Originally native to central Europe, the Balkans, and Asia Minor, IWG was introduced to the United States in 1932 primarily as a forage crop. Since then, it has been cultivated extensively throughout the western regions of North America (Hybner and Jacobs 2012). Trials and research on IWG have demonstrated its potential as a multifunctional crop, capable of yielding various commodities while providing valuable ecosystem services (Muckey 2019; Favre et al. 2019). Unlike annual crops such as wheat, which often deplete soil organic matter and require substantial inputs, IWG offers a more sustainable alternative (Crews et al. 2018; Law et al. 2022). One notable advantage of IWG is its ability to yield crops while simultaneously building soil organic matter, thus enhancing soil health and fertility over time (Culman et al. 2013). This characteristic makes IWG particularly valuable in sustainable agriculture systems, where maintaining soil quality and resilience is important. By promoting soil health and organic matter accumulation, IWG contributes to improved soil structure, water retention, and nutrient cycling, ultimately enhancing the overall productivity and sustainability of agricultural lands (Bajgain et al. 2022). To explore additional uses of IWG as a stand-alone food ingredient, its refinement process has been investigated. Refining IWG involves conditioning the grains with water to toughen the bran and soften the endosperm, thereby enhancing milling efficiency. During milling, the grains are ground into progressively finer particles. The resulting material is then sifted through a screen to separate the bran and germ from the starchy endosperm. This process yields refined flour composed primarily of the endosperm, with the bran (insoluble dietary fiber portion) and germ removed (Tyl et al. 2019). The exploration of nitrogen fertilizer application in IWG cultivation is a significant aspect of efforts to develop it for mainstream food use. Nitrogen plays a crucial role in plant growth and development, and optimizing its application can have substantial impacts on crop yield, quality, and nutritional content (Dobbratz et al. 2023). Since the mid-20th century, farmers have significantly increased the application of nitrogen fertilizer in cereal crops as an agronomic practice aimed at boosting productivity (Hawkesford 2014). The global demand for nitrogen fertilizer utilization is expected to reach 111.6 tons in 2022 (FAO 2019). Despite the agronomic and nutritional benefits of applying nitrogen fertilizer to meet food security needs through increased grain production (Smil 1999; Swarbreck et al. 2019), its use is under growing scrutiny due to greenhouse gas emissions associated with nitrogen fertilizer volatilization (McIsaac et al. 2010). Consequently, there has been a push toward innovation (Jez et al. 2016; Yan et al. 2020) and the establishment of policy (Environment and Climate Change Canada 2020) targets aimed at reducing nitrogen application to crops (Trevisan et al. 2022). To achieve these goals, optimum nitrogen fertilizer application and the time it is applied have been the major strategies to improve crop yield, quality, and nutritional content (Shejbalova et al. 2014). The focus of preceding research on IWG response to nitrogen application has been to investigate how nitrogen treatment can influence both the biomass yield and chemical composition of forage and bioenergy of IWG crops (Vogel et al. 2002; Jungers et al. 2017). However, research into how optimum nitrogen fertilizer application on IWG, and time of application, on the chemical composition and functional properties of IWG as well as kernel characteristics is llacking. This study evaluates the effect of nitrogen fertilizer application in the spring or fall seasons on the chemical composition, functional properties, and kernel physical characteristics of IWG grown in Minnesota and Wisconsin.

2 | Materials and Methods

2.1 | Field Experiment and Treatments

IWG plots were established in fall of 2021 using the variety "MN-Clearwater" (Bajgain et al. 2020). A detailed overview of the establishment methods, site description, and grain crop yields are in Cassani et al. (2025). Grain samples from the 2023 harvest at two sites were used for this study. The study sites were the University of Minnesota's Rosemount Research and Outreach Center in Rosemount, Minnesota, and the Michael Fields Agricultural Research Institute in East Troy, WI. Total soil nitrogen measured in the top 20 cm averaged 6220 kg N ha^{-1} at the WI location and 5586 kg N ha^{-1} at the MN location. The average (from 2006 to 2020) temperature and annual precipitation are 7.9°C and 976 mm at WI and 6.9°C and 860 mm at MN. Field plots of IWG were fertilized with urea (46-0-0) at a rate of 90 kg N ha^{-1} in the fall (September 2022 at WI and October 21, 2022 at MN) and spring April 2022 at WI and April 18, 2022 at MN). Urea was pre-weighed to achieve the specified rate for the size of the research plot and broadcast over the plants by hand. Unfertilized IWG plots were used as control. IWG grain was harvested on October 2022 at WI and on July 23, 2022 at Minnesota when the plants reached physiological maturity. During harvest, seed heads were cut from all plants within two separate 1 m × 1 m quadrats randomly placed in each plot. Seed heads were dried for 7 days at 35°C before being threshed and sieved of non-seed debris for analytical analysis.

2.2 | Sample Preparation

Whole grain samples were milled using UDY Cyclone sample Mill equipped with 0.5 mm screen (UDY, Fort Collins, CO, USA). Refined samples were prepared by tempering grains to a moisture content of 14% at 30°C for 4 h (Bharathi et al. 2022) and milled using the Quadrumat Junior mill equipped with

0.25 mm screen (Brabender GmbH & Co. KG, Duisburg, Germany).

2.3 | Chemical Composition

Moisture content of IWG flour samples was determined in duplicates using Quincy Labs oven (Model 40GC Lab Oven, Hogentogler & Co. Inc. Columbia, MD) at 130°C for 1 h. (AACC 44-15A). Protein was determined in duplicates by using the Dumas nitrogen combustion method (AACCI 46-30.01) using a nitrogen analyzer (LECO TruSpecNTM, St. Joseph, MI, USA) with a conversion factor of 5.70. Fat content was determined in duplicates by the Soxhlet method (AOAC 948.22 [12]). Ash content was determined in duplicates by dry ashing method (AACCI 08-01.01). Total carbohydrate was determined by difference. Total, insoluble, and soluble dietary fiber were determined following the AACC method 32-07.01. Total starch was determined following the AACCI method 76-13.01.

2.4 | Starch Pasting Properties

Starch pasting properties of flour samples were determined in duplicates using the Micro Visco-Amylo-Graph (MVAG) (Brabender GmbH & Co. KG, Duisburg, Germany) (AACCI 22.10). Parameters evaluated were pasting temperature (°C), maximum viscosity (BU), breakdown (BU), and setback (BU).

2.5 | Dough Mixing Rheology

The dough mixing properties of IWG flour samples were evaluated in duplicates using a Brabender Farinograph (C. W. Brabender) equipped with a 10 g bowl according to the AACCI standard method 54-21. Parameters evaluated were water absorption (%), dough development time (s), dough stability (s), and mixing tolerance index (MTI) (s).

2.6 | Protein Aggregation Kinetics

Protein aggregation kinetics of IWG flour samples were evaluated in duplicate using a Brabender GlutoPeak (C. W. Brabender) based on the method reported by Chandi and Seetharaman (2012), where the peak maximum time (s), maximum torque (MT) (BU), and aggregation energy (GPE) were evaluated.

2.7 | Physical Properties

For thousand kernel weight (TKW), thousand kernels were counted manually in duplicates for samples, and subsequently weighed using the Sartorius weighing balance (Sartorius, practum 224–IS, 28906164, Sartorius Weighing Technology GmbH, Germany). The length and width of IWG kernel were measured using a digital Vernier caliper (Altraco Inc., Sausalito, California, USA) with calibration to 0.01 mm.

2.8 | Statistical Analysis

Field replicates were conducted in duplicates. Multifactor analysis of variance (ANOVA) was done using Statgraphics centurion XV to evaluate interactions between nitrogen treatment, refinement, and location. A multiple range test was done to determine significant ($p < 0.05$) difference among the mean values.

3 | Results and Discussion

3.1 | Chemical Composition

The chemical composition of nitrogen treated and untreated IWG is shown in Table 1. Nitrogen application of IWG significantly ($p < 0.05$) increased the protein content of refined and whole grain samples from Minnesota and Wisconsin both in fall and spring seasons (Table 1). Adequate nitrogen fertilization promotes the synthesis and accumulation of proteins in grains, thereby changing their physicochemical properties (Wan et al. 2023). The increase in protein content with nitrogen fertilization was more pronounced during the spring than in the fall season. This result indicates that the effect of nitrogen fertilization also depends on the timing of application. Even though nitrogen fertilization can increase protein content in cereal grains, excessive application can lead to a decline in protein content and negatively affect grain quality (Lan et al. 2021). Excessive nitrogen fertilizer applications can reduce grain nitrate reductase and glutamine synthetase activities, which are considered crucial enzymes for protein synthesis and accumulation (Wan et al. 2023). This result suggests that the application of 90 kg N ha^{-1} of nitrogen fertilizer was sufficient to maintain the high activity of nitrate reductase and glutamine synthetase during protein synthesis and accumulation in IWG. There were some differences in protein content between IWG samples obtained from Minnesota and Wisconsin. This may be attributed to other environmental factors such as precipitation, pH, temperature, and soil type which can affect soil nitrogen availability (Masclaux-Daubresse et al. 2010). The soil type for Minnesota and Wisconsin was Waukegan and Plano silt loam soils, respectively. Both soils have a silt loam texture, meaning they contain a high proportion of silt, with moderate amounts of clay and sand. This texture allows for moderate to high water infiltration rates, especially when soils are not compacted. As water moves downward through the soil profile, the nitrogen fertilizer is easily carried with it beyond the root zone (Cassani et al. 2025). The total precipitation in the fall, August to October, was 202 mm and in the spring, March to May, was 186 mm in Rosemount, Minnesota, and 260.2 mm in the fall, August to October, and 152.4 mm in the spring, March to May, in Madison, Wisconsin, respectively in 2022. This might have accounted for the higher protein content in the spring than in the fall season.

The average total soil nitrogen content measured in Wisconsin (6220 kg N ha^{-1}) was higher than that in Minnesota (5586 kg N ha^{-1}). Additionally, Wisconsin recorded a higher average annual temperature (7.9°C) and precipitation (976 mm) compared to Minnesota (6.9°C and 860 mm, respectively).

TABLE 1 | Chemical composition of nitrogen treated and untreated IWG (dry basis).

Refinement	Location	Nitrogen treatment	Protein (%)	Fat (%)	Ash (%)	Total starch (%)	Total dietary fiber (%)	Insoluble dietary fiber (%)	Soluble dietary fiber (%)	Total carbohydrate (%)
Refined IWG samples	MN	No treatment	20.51^{a}	2.32^{a}	0.62^{a}	63.20^{ef}	6.32^{b}	3.30^{b}	3.02^{ab}	76.55^{f}
		Spring treatment	21.91^{d}	2.37^{a}	0.58^{a}	61.95^{d}	6.47^{b}	3.45^{b}	3.02^{ab}	75.15^{e}
		Fall treatment	21.12^{b}	2.34^{a}	0.55^{a}	63.14^{e}	8.33^{c}	5.00^{c}	3.33^{ab}	76.04^{ef}
	WI	No treatment	21.05^{b}	2.55^{b}	0.79^{b}	64.23^{fg}	5.15^{a}	2.70^{ab}	2.45^{a}	75.66^{ef}
		Spring treatment	22.88^{e}	3.03^{c}	0.52^{a}	64.33^{g}	5.22^{a}	2.28^{a}	2.94^{ab}	73.58^{d}
		Fall treatment	21.10^{b}	2.90^{c}	0.57^{a}	61.32^{d}	6.55^{b}	3.43^{b}	3.12^{ab}	75.43^{e}
Wholegrain IWG samples	MN	No treatment	20.53^{a}	3.62^{e}	2.41^{cd}	46.39^{bc}	14.75^{d}	11.92^{d}	2.84^{ab}	73.44^{d}
		Spring treatment	22.83^{e}	4.35^{g}	2.30^{c}	44.90^{a}	19.36^{f}	14.76^{f}	4.60^{cd}	70.57^{a}
		Fall treatment	21.23^{c}	3.68^{e}	2.36^{c}	47.25^{c}	17.11^{e}	13.35^{e}	3.76^{bc}	72.50^{bc}
	WI	No treatment	21.00^{b}	3.25^{d}	2.55^{d}	45.41^{ab}	15.52^{d}	13.06^{e}	2.46^{a}	73.20^{cd}
		Spring treatment	21.70^{cd}	4.08^{f}	2.33^{c}	45.93^{b}	19.23^{f}	14.24^{f}	5.00^{Bd}	72.17^{b}
		Fall treatment	21.85^{d}	3.67^{e}	2.37^{c}	45.73^{b}	17.95^{e}	14.24^{f}	3.72^{bc}	72.11^{b}

Abbreviations: MN, Minnesota; WI, Wisconsin.

Note: Mean values with superscript lowercase letters in each column indicate significant difference ($p < 0.05$) between refinement, nitrogen treatment and location.

These environmental differences may contribute to the observed variations in protein content between the two locations.

Nitrogen treatment of IWG significantly ($p < 0.05$) increased the fat content in both refined and whole grain samples in the fall and spring seasons at both locations. The fat content in all the whole grain samples was higher than that of the refined samples. Fat content in grains is mostly found in the germ which is lost during refining (Šramková et al. 2009).

There was a significant decrease in ash content with nitrogen treatment for refined samples from Wisconsin and whole grain samples from Wisconsin and Minnesota in both the fall and spring seasons. Refined samples from Minnesota, however, did not show any significant ($p < 0.05$) difference. Different cereal grains respond differently to nitrogen fertilizer application in ash content, with some showing decreased ash content. The effect of nitrogen treatment on ash content can vary depending on factors like soil type, climate, and specific agronomic practices. Lewandowski and Kauter (2003) reported that in grain of rye and triticale, the ash content decreased slightly with nitrogen treatment. The ash content of the whole grain samples was higher than that of the refined samples from both locations. The whole grain samples are made from the entire grain of IWG seeds, including the bran, which contains a higher amount of minerals than the refined samples (Šramková et al. 2009).

The process of grain filling involves the transport of carbohydrates to the kernel and subsequent accumulation of starch (Krishnan and Dayanandan 2003). Nitrogen promotes the synthesis of carbohydrates and their transport to the grain by promoting photosynthesis. Thus, the effect of nitrogen on carbohydrate transport can vary (Liu et al. 2021). However, Lan et al. (2021) showed that the amount of carbohydrates transported to the grain decreased under high nitrogen conditions, owing to the large amount of carbohydrates consumed for the vigorous growth of vegetative organs such as stems and leaves. Therefore, under high nitrogen and high plant density, less carbohydrate accumulates in the grain (Sun et al. 2019). Owing to the reduction in the amount of substrates caused by the decrease in carbohydrate content in the grain, the synthesis of starch is inhibited, and the grain weight decreases (Jiang et al. 2008).

The decrease in total starch content was not significant with nitrogen fertilizer application of IWG samples, except for whole grain samples from Minnesota. Total starch content was lower in the whole grain samples than in refined samples from both locations (Table 1). Starch is mostly found in the endosperm and, therefore, the lower starch content in the whole grain samples may be attributed to dilution from the bran, which was absent from the refined samples (Šramková et al. 2009).

Similarly, nitrogen treatment significantly decreased the total carbohydrate content of IWG in the fall and spring seasons of both refined and whole grain samples from both locations. This may be attributed to the decrease in carbohydrate transport to the grain under high nitrogen conditions, owing to the growth of vegetative organs, such as stem and leaves, which consume many of the carbohydrates, and may reduce their transport to the grain (Sun et al. 2019). Total carbohydrate content was lower in the whole grain samples than in refined samples from both locations (Table 1). This may again be attributed to dilution from the bran as most of the carbohydrate is concentrated in the endosperm, which was removed during refinement (Šramková et al. 2009). Moreover, nitrogen treatment increased the total dietary fiber content of IWG for both refined and whole grain samples in the fall and spring seasons at both locations, some of which were significant ($p < 0.05$) (Table 1).

The total dietary fiber content of the whole grain samples was higher than that of the refined samples. This can be attributed to the bran in the whole grain samples, since most of the dietary fiber is in the bran. The high dietary fiber in whole grain may impact on the functional properties and bread baking qualities of IWG (Rahardjo et al. 2018). Similarly, there was an increase in the insoluble dietary fiber content of IWG samples with nitrogen treatment (Table 1).

The insoluble dietary fiber content of the whole grain samples was higher than that of the refined samples, and this may be attributed to the bran in the whole grain which is absent in the refined samples due to its removal during refinement. The whole grain IWG, like most cereals, is richer in dietary fiber (particularly insoluble dietary fiber) than refined IWG (Dai et al. 2021). The high insoluble dietary fiber content of the whole grain samples may potentially affect the water absorption capacity and gluten network formation (Wang et al. 2002).

Nitrogen treatment did not have any significant effects on the soluble dietary fiber content of the refined samples from both locations. However, the soluble dietary content of the whole grain samples showed a significant ($p < 0.05$) increase with nitrogen fertilizer treatment in the fall and spring seasons at both locations. Soluble dietary fiber, like the insoluble dietary fiber, is concentrated in the outer layers of cereal grains (notably the pericarp and aleurone layer) where it may account for up to half of the dry weight but low (often less than 5% dry weight) in the starchy endosperm (Shewry and Serna-Saldivar 2023). This may account for the higher soluble dietary fiber content in the whole grain samples than the refined samples, in which most of the pericarp and aleurone layer is lost during refinement. The effect of nitrogen fertilizer application on chemical composition of IWG was more pronounced in the spring season than in the fall season. Spring application of nitrogen is generally more effective than fall application for winter wheat. This may be ascribed to the fact that fall applications are more susceptible to nitrogen loss through leaching and denitrification. High precipitation during the fall season favors loss of nitrogen by leaching and denitrification, making it less efficient than the spring application (Welch et al. 1966).

Nitrogen fertilization can enhance the protein, fat, and fiber content of IWG, especially in whole grain samples and with spring application. However, there is a nutrient trade-off, notably with carbohydrate and mineral content, highlighting the importance of balanced fertilization strategies for IWG.

3.2 | Starch Pasting Properties

The starch pasting properties of nitrogen treated and untreated IWG are shown in Table 2. The pasting temperatures of IWG flour samples increased with nitrogen treatment, with that of

TABLE 2 | Starch pasting properties of nitrogen treated and untreated intermediate wheatgrass.

Refinement	Location	Nitrogen treatment	Pasting temperature (°C)	Maximum viscosity (BU)	Breakdown (BU)	Setback (BU)
Refined IWG samples	MN	No treatment	55.75^{b}	335.50^{de}	182.50^{e}	410.50^{ef}
		Spring treatment	56.30^{bc}	246.00^{b}	121.00^{cd}	268.00^{b}
		Fall treatment	56.60^{c}	296.50^{cd}	143.00^{d}	336.00^{cd}
	WI	No treatment	56.75^{c}	515.00^{h}	122.00^{cd}	639.50^{gh}
		Spring treatment	56.90^{cd}	510.00^{h}	122.00^{cd}	649.00^{h}
		Fall treatment	56.50^{bc}	446.00^{g}	117.00^{cd}	578.50^{g}
Wholegrain IWG samples	MN	No treatment	58.70^{f}	257.00^{bc}	109.00^{bc}	272.00^{bc}
		Spring treatment	58.80^{f}	190.50^{a}	83.00^{ab}	176.00^{a}
		Fall treatment	58.75^{f}	198.50^{a}	84.50^{ab}	190.00^{a}
	WI	No treatment	54.70^{a}	404.00^{fg}	108.50^{bc}	450.50^{f}
		Spring treatment	58.30^{ef}	364.50^{ef}	83.00^{ab}	374.00^{de}
		Fall treatment	57.70^{de}	253.50^{bc}	78.00^{a}	450.00^{f}

Note: Mean values with superscript lowercase letters in each column indicate significant difference ($p < 0.05$) between refinement, nitrogen treatment and location. Abbreviations: BU, Brabender Unit; MN, Minnesota; WI, Wisconsin.

the refined samples from Minnesota and whole grain samples from Wisconsin treated in the fall and spring seasons being significant ($p < 0.05$) (Table 2).

The pasting temperature, which represents the temperature at the onset of gelatinization, is strongly related to the ability of starch granules to imbibe water. Thus, factors that affect the ability of starch granules to absorb water, such as the ratio of amylose to amylopectin, the concentration of starch, and the presence of lipids and other components do affect the pasting temperature and other pasting properties of starches (Sasaki et al. 2000). As previously discussed, nitrogen fertilizer treatment increased the protein and fat content of IWG grains (Table 1). The higher pasting temperatures in refined flour samples from Minnesota and the wholegrain samples from Wisconsin may be attributed to the higher fat content which may form starch–lipid complexes. More energy is therefore required to uncoil these starch–lipid complexes resulting in higher pasting temperatures (Kaur and Singh 2000). The pasting temperatures of the wholegrain IWG flour samples were higher than the refined flour samples regardless of the location where IWG grains were harvested and timing of treatment. The fat content in the wholegrain samples was significantly ($p < 0.05$) higher than that of the refined samples. Fat content in grain is mostly found in the germ and bran which is included in the whole grain but lost during refinement (Šramková et al. 2009). The higher pasting temperatures in the wholegrain samples may be attributed to the higher fat content which may form starch–lipid complexes which may result in higher pasting temperature.

Nitrogen treatment significantly ($p < 0.05$) decreased the peak viscosity of IWG in both the whole grain and refined flour samples in the fall and spring seasons irrespective of growing location, except for fall treated refined samples from Minnesota which were not significant (Table 2). This observation could be attributed to the presence of more protein and less starch content in the nitrogen-treated IWG samples (Table 1). Lim et al. (1999) reported a similar protein effect, where protein content negatively correlated with peak viscosity but positively correlated with pasting temperature. Protein has intact disulfide bonds that can form a matrix around and/or within the starch granules, making it harder to break the starch granules (Hamaker and Griffin 1993). Thus, the molecular structure of the protein can prevent swelling, decrease viscosity, and increase pasting temperature (Hamaker and Griffin 1993). Secondly, nitrogen-treated IWG samples have less starch than untreated samples, and therefore the treated samples will be expected to have lower peak viscosity. Gao et al. (2021) also reported that the peak viscosity of common buckwheat starch was significantly reduced after the application of nitrogen fertilizer, indicating that nitrogen fertilizer can reduce the swelling rate of common buckwheat starch granules, causing the starch granules to absorb water slowly, thereby reducing the starch viscosity. The peak viscosity of refined samples was higher than that of whole grain samples. This may be attributed to the higher dietary fiber content of the whole grain samples, which are removed during refinement. Dietary fiber competes with the starch granule for water, which is needed for starch granule swelling and gelatinization. This can interfere with starch granule swelling and gelatinization of the starch slurry (Collar et al. 2006; Marti et al. 2015), decreasing the peak viscosity of the whole grain samples. Breakdown viscosity values of IWG decreased with nitrogen treatment in the fall and spring seasons, that of whole grain samples from Wisconsin being significant ($p < 0.05$) (Table 2).

The breakdown viscosity values demonstrate the stability of the starch granules and indicate the leaching of the amylose and amylopectin out of the starch granule under heat and thus, the

lower the breakdown viscosity value, the more stable the starch granules are (Rahardjo et al. 2018). That is, breakdown viscosity values can reflect the ability of starch granules to resist heating. The lower the breakdown viscosity value, the higher the stability indicating that IWG starches had a higher ability to resist heating under higher nitrogen levels (Zhou et al. 2020; Gao et al. 2021). These results indicate that nitrogen treatment could increase the stability of IWG starches which could inform its food application.

Generally, nitrogen fertilizer treatment decreased the setback viscosity values of the IWG in both the refined and whole grain samples in the fall and spring seasons at both locations (Table 2), some of which were significant ($p < 0.05$). Setback viscosity is an index that measures the stability of starch paste after cooling, and a high setback viscosity indicates a higher tendency of starch pastes to retrograde (Uarrota et al. 2013). This result indicates that nitrogen treatment of IWG samples decreases the tendency of its starch to retrograde. This is consistent with that reported by Gao et al. (2021) in buckwheat at higher nitrogen treatment. Higher amylose content will therefore result in products with firmer gels upon retrogradation (Thomas and Atwell 1999).

These results show how nitrogen treatment influences starch behavior, such as pasting temperature, peak viscosity, and retrogradation, which are critical for texture, stability, and processing. It also shows how refining impact starch behavior, which is important when developing functional whole grain-based foods.

3.3 | Dough Rheological Properties

Water absorption is the amount of water needed for optimum dough consistency, that is, at 500 BU on the Farinograph (Rahardjo et al. 2018). Water absorption significantly ($p < 0.05$) increased with nitrogen treatment of IWG in both refined and whole grain samples in the fall and spring seasons from both locations, except for whole grain samples from Minnesota which showed a decrease with nitrogen treatment (Table 3). Water absorption of flour is affected by factors such as fiber content and gluten protein quality. Generally, flour with a high level of gluten protein quality absorbs more water because water is needed for gluten formation (Rahardjo et al. 2018). The increase in water absorption with nitrogen treatment may be attributed to an increase in the gluten protein quality of the IWG samples with nitrogen treatment.

Apart from wholegrain samples from Minnesota, all the other samples showed a significant ($p < 0.05$) increase in dough development time (DDT) in the fall and spring seasons at both locations. This is consistent with that reported for wheat treated with nitrogen fertilizer (Liu et al. 2022). Since dough development time is regulated by gluten proteins, nitrogen treatment can affect this process (Trevisan et al. 2022). A higher protein content with the application of nitrogen fertilizer has been reported to prolong the DDT for hard winter wheat (Horvat et al. 2022). The increase in DDT was more significant in the whole grain than in the refined samples, at both locations. This was because the bran in the whole grain flour samples caused the dough to take a longer time for the gluten network to form, hence the higher DDT for whole grain samples than the refined samples (Schmiele et al. 2012).

Similarly, nitrogen fertilizer treatment increased the dough stability of both refined and whole grain samples in the fall and the spring seasons at both locations with that of samples from Wisconsin being significant ($p < 0.05$) (Table 3). This is also consistent with that reported for wheat treated with nitrogen fertilizer (Liu et al. 2022). Dough stability depends on levels of high molecular weight glutenin (HMWG) which is lacking in IWG (Rahardjo et al. 2018). The higher the HMWG the higher the dough stability index. Increased dough stability with nitrogen treatment of IWG may be attributed to an increase in HMWG with nitrogen treatment.

Nitrogen treatment decreased the dough MTI of IWG of both refined and whole grain samples in the fall and spring seasons

TABLE 3 | Dough rheological properties of nitrogen treated and untreated Intermediate wheatgrass.

Refinement	Location	Nitrogen treatment	WA (%)	DDT (s)	Dough stability (s)	MTI (s)	AT (s)
Refined IWG samples	MN	No treatment	63.35^{de}	61.50^{a}	43.00^{bc}	234.00^{g}	46.50^{a}
		Spring treatment	62.80^{ab}	82.00^{b}	50.50^{cd}	208.00^{f}	60.50^{ab}
		Fall treatment	64.05^{f}	88.00^{b}	52.00^{cd}	203.00^{f}	57.00^{ab}
	WI	No treatment	61.00^{a}	61.00^{a}	29.50^{a}	184.50^{e}	48.00^{a}
		Spring treatment	67.35^{h}	106.00^{c}	41.00^{abc}	149.00^{d}	77.00^{cd}
		Fall treatment	67.10^{BB}	87.00^{b}	36.50^{ab}	133.00^{c}	72.00^{bc}
Wholegrain IWG samples	MN	No treatment	64.75^{g}	114.00^{c}	57.50^{de}	128.50^{c}	89.00^{de}
		Spring treatment	64.25^{f}	134.50^{d}	74.50^{f}	122.50^{c}	101.00^{e}
		Fall treatment	63.60^{e}	111.00^{c}	66.00^{ef}	134.00^{cd}	85.00^{cd}
	WI	No treatment	61.15^{a}	145.00^{d}	94.00^{g}	60.50^{b}	102.0^{e}
		Spring treatment	62.70^{b}	222.00^{e}	118.00^{h}	43.50^{a}	182.50^{g}
		Fall treatment	63.05^{cd}	227.500^{e}	149.50^{i}	58.00^{ab}	163.00^{f}

Note: Mean values with superscript lowercase letters in each column indicate significant difference ($p < 0.05$) between refinement, nitrogen treatment, and location.
Abbreviations: AT, arrival time; DDT, dough development time; MN, Minnesota; MTI, Mixing Tolerance Index; s, seconds; WA, water absorption; WI, Wisconsin.

at both locations, with that of samples from Wisconsin being significant ($p < 0.05$). MTI is an important parameter when accessing dough quality (Mann et al. 2008). It reflects how quickly the gluten structure breaks down after optimum dough development. Flour with higher MTI, like IWG, indicates less tolerance and more difficulty during mechanical handling and makeup of the dough (Abera et al. 2017). The decrease in MTI may be attributed to the increase in gluten protein quality of IWG treated with nitrogen.

Arrival time (AT) significantly ($p < 0.05$) increased with nitrogen treatment of IWG in both the refined and whole grain samples in the fall and spring seasons at both locations, except for whole grain samples treated in the fall at Minnesota and Wisconsin which showed a decrease. AT refers to the time from the first addition of water until the top of the curve to first intercept the 500BU dough consistency line. This is also referred to as hydration time (Ahmed et al. 2013). Boehm et al. (2004) reported that nitrogen fertilizer treatment increased the AT of spring wheat, which is consistent with what was observed with IWG in this study. Shorter AT is desired to speed up the dough mixing process. The AT of whole grain samples was higher than that of the refined samples. This may be attributed to the high bran content of the whole grain samples which compete for water with gluten proteins which increase the time taken for their hydration. The generally higher values for water absorption, dough development time, and arrival time, alongside lower mixing tolerance index values observed in samples from Wisconsin compared to those from Minnesota, may be ascribed to the higher average total soil nitrogen content recorded in Wisconsin (6220 kg ha^{-1}) relative to Minnesota (5586 kg ha^{-1}). Furthermore, Wisconsin experienced higher average annual temperature (7.9°C) and greater precipitation levels (976 mm) than Minnesota, which had averages of 6.9°C and 860 mm, respectively. These climatic variations may help explain the differences in rheological properties observed between the two locations.

The results indicate that nitrogen fertilization enhances the functional dough properties of IWG, particularly by enhancing water absorption, which supports better dough structure, and modifying hydration time. While whole grain samples tend to show greater variability due to fiber interference, the overall improvements suggest that nitrogen-treated IWG has greater potential for baking applications, particularly when dough strength and tolerance are critical.

3.4 | Protein Aggregation Kinetics

The application of nitrogen fertilizer significantly ($p < 0.05$) increased the peak maximum time (PMT) of the IWG samples in the fall and spring seasons at both locations apart from refined IWG samples from Minnesota (Table 4). PMT indicates the time for complete gluten network formation and corresponds to the time for MT (Grassi et al. 2021). This shows that nitrogen treatment increased the gluten aggregation time in IWG. This agrees with farinograph DDT, which is also a parameter that measures the time to achieve gluten formation. IWG grain samples treated with nitrogen during the fall and spring seasons in Wisconsin exhibited higher value compared to those from Minnesota, except for refined samples treated in the fall. The differences observed in the whole grain samples were significant ($p < 0.05$). This might be attributed to the higher average total soil nitrogen measured in Wisconsin (6220 kg ha^{-1}) than Minnesota (5586 kg ha^{-1}). The higher peak maximum time (PMT) value observed in the whole grain samples, relative to the refined samples, may be attributed to their higher dietary fiber content. Dietary fiber is known to compete with gluten proteins for water, thereby prolonging the hydration and aggregation processes required for gluten network formation (Sun et al. 2023).

Apart from whole grain samples from Wisconsin, MT increased with nitrogen treatment with refined samples treated in the spring from Minnesota and refined samples

TABLE 4 | Protein aggregation kinetics of nitrogen treated and untreated Intermediate wheatgrass.

Refinement	Location	Nitrogen treatment	Peak max. time (s)	Max. torque (BU)	Aggregation energy (GPE)
Refined IWG samples	MN	No treatment	18.00^{a}	38.00^{de}	930.51^{c}
		Spring treatment	22.50^{abcd}	42.00^{f}	1077.89^{d}
		Fall treatment	23.50^{bcd}	37.00^{d}	975.93^{c}
	WI	No treatment	20.50^{ab}	29.00^{b}	759.00^{b}
		Spring treatment	25.50^{cd}	40.50^{ef}	1156.40^{d}
		Fall treatment	21.00^{abc}	37.0^{d}	945.70^{c}
Wholegrain IWG samples	MN	No treatment	24.00^{bcd}	29.50^{b}	805.72^{b}
		Spring treatment	26.50^{de}	32.50^{c}	909.02^{c}
		Fall treatment	21.50^{abc}	28.00^{b}	738.95^{b}
	WI	No treatment	30.50^{e}	22.00^{a}	621.08^{a}
		Spring treatment	35.50^{f}	20.50^{a}	621.00^{a}
		Fall treatment		21.50^{a}	635.00^{a}

Note: Mean values with superscript lowercase letters in each column indicate significant difference ($p < 0.05$) between refinement, nitrogen treatment, and location. Abbreviations: BU, Brabender Unit; GPE, gluten performance energy; MN, Minnesota; s, seconds; WI, Wisconsin.

TABLE 5 | Thousand kernel weight, kernel length, and width of nitrogen treated and untreated IWG.

Location	Nitrogen treatment	Thousand kernel weight (g)	Length of kernel (mm)	Width of kernel (mm)
MN	No treatment	7.32^{ab}	5.29^{a}	1.39^{a}
	Spring treatment	7.45^{abc}	5.78 ^{cd}	1.47^{a}
	Fall treatment	7.57^{bc}	5.56^{bcd}	1.47^{a}
WI	No treatment	7.10^{a}	5.34^{ab}	1.44^{a}
	Spring treatment	7.62^{bc}	5.79 ^{d}	1.51^{a}
	Fall treatment	7.81^{c}	5.52^{abc}	1.51^{a}

Note: Mean values with superscript lowercase letters in each column indicate significant difference ($p < 0.05$) between location and nitrogen treatment in IWG samples. Abbreviations: MN, Minnesota; WI, Wisconsin.

treated in the fall and spring from Wisconsin being significant ($p < 0.05$). MT corresponds to the maximum peak occurring as gluten aggregates (Grassi et al. 2021). Higher MT indicates a stronger gluten network. The higher MT with nitrogen treatment may be attributed to the formation of a stronger gluten network which is desirable in bread baking. Similarly, the application of nitrogen fertilizer significantly ($p < 0.05$) increased the aggregation energy of IWG of both refined and whole grain samples in the fall and spring seasons in Minnesota and Wisconsin. Aggregation energy (AgE) corresponds to the area under the GlutoPeak curve and is an indication of gluten network strength. The positive correlation between MT and AgE with nitrogen application may be ascribed to the increase in gluten protein content in the IWG that was treated with nitrogen. Refined IWG samples exhibited higher values compared to their whole grain counterparts. This difference may be ascribed to the presence of bran in the whole grain samples, which constitutes the insoluble portion of dietary fiber. Bran has been shown to interact with gluten proteins, disrupting the continuity of the gluten network (Zhou et al. 2021). Such disruption weakens gluten strength, thereby reducing parameters associated with gluten aggregation, including MT and AgE (Sun et al. 2023).

These findings highlight the positive effect of nitrogen fertilizer on gluten functionality in IWG, offering improvements in elasticity and structural strength that are advantageous in processing and product quality.

3.5 | Kernel Physical Properties

TKW is the weight of thousand sound grains. It is a quality test applied to wheat and other grains to determine its potential milling yield. TKW of cereal grains depends on its size and density. The application of nitrogen significantly ($p < 0.05$) increased the TKW and length of IWG grains in the fall and spring seasons at both locations (Table 5). The kernel width was however not significantly affected (Table 5). Different studies have reported different responses of TKW of some cereals to nitrogen application. In a Swedish study, it was reported that TKW of different varieties of oats were not affected by nitrogen application (Valkama et al. 2013). Esala and Larpes (1986) also reported that an experiment conducted in clay soil demonstrated an increase in TKW with nitrogen treatment of wheat (50–200 kg ha^{-1}), but in barley, no increase was observed with nitrogen application (100 kg ha^{-1}). Raja (2001) also reported a positive response in kernel weight with nitrogen treatment in maize. Increasing nitrogen rates increased the activity of enzymes in maize which increased the kernel weight (Amanullah et al. 2009). The increase in TKW and length of IWG may be attributed to the increase in the activities of enzymes in IWG which increase the TKW and size of IWG kernel.

The increase in TKW and kernel length in IWG suggests that nitrogen application can improve grain quality and milling yield potential, which holds economic significance for processors and breeders seeking to enhance IWG's commercial viability.

4 | Conclusion

Nitrogen application had varied effects on the physicochemical and functional characteristics of IWG. The chemical composition of IWG was impacted in different ways. While protein and fat contents significantly increased in the whole and refined samples, there were not clear trends in the other chemical parameters. The nitrogen effects on the other parameters depended on whether they were refined or not and the location. The weight of the seeds significantly increased particularly in the fall season for both locations. The MT and energy required to aggregate IWG protein indicated that nitrogen application produced stronger doughs, particularly with spring applications, regardless of location. This observation was supported by increased dough development time and stability on the Farinograph. Starch hot paste viscosity generally decreased with nitrogen fertilization, as did starch breakdown and setback.

These results on the effects of nitrogen on the chemical composition and functional properties of IWG could provide valuable insights for its potential food applications.

Author Contributions

Idea: George Amponsah Annor. Data analysis and original draft preparation: Obed Aduama. Reviewing and editing: George Amponsah Annor.

Conflicts of Interest

The authors declare no conflicts of interest.

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