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Intermediate wheatgrass (*Thinopyrum intermedium*): a promising perennial grain for sustainable food production—a review of multifaceted potential and innovations to address nutritional deficiencies

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Intermediate Wheatgrass (IWG), a perennial grain, is gaining increasing attention as a sustainable alternative to conventional cereals due to its environmental and nutritional benefits. This review explores IWG's cultivation, processing, and nutritional potential, while critically examining both the advantages and limitations of its adoption in food systems. Additionally, it proposes future research directions to advance IWG's role in sustainable food systems. IWG offers significant agricultural, economic, and environmental advantages, making it a promising sustainable crop. Despite nutritional challenges hindering its widespread adoption, IWG is rich in dietary fiber, protein, and essential micronutrients, surpassing traditional grains in nutritional value. Advances in processing, such as flour refinement, extrusion, and bran treatment, have addressed some limitations. Innovations in dough improvement and functional additives further enhance the quality and consumer appeal of IWG-based products, although certain aspects remain understudied. While progress has been made in understanding IWG's nutritional potential, further research is needed to improve flour rheology, optimize value-added product formulations, and develop enzymatic treatments. This review highlights innovative processing methods and the incorporation of diverse functional ingredients as promising strategies to improve the quality of IWG-based foods and promote their broader adoption in sustainable diets.

KEYWORDS
dough improvement, enzymatic treatment, intermediate wheatgrass, Kernza, nutritional value, perennial grain, sustainable agriculture

1 Introduction

The global population is expected to increase by 2 billion by 2050, reaching 9.7 billion individuals (Paul et al., 2024), and is projected to rise to 11.2 billion by 2,100. Given the ongoing rate of population growth, it will be essential to convert an estimated 1 billion additional hectares of land for agricultural use to meet food requirements (Sutherlin et al., 2019). Meeting the nutritional needs of a rapidly increasing global population, boosting productivity, and simultaneously preserving the integrity of the natural resource base through sustainable intensification constitute the primary challenges of 21st-century agriculture (Schlautman et al., 2021; Law et al., 2022b; Law et al., 2022a).

The cultivation of annual cereal grains represents one of the most extensive land uses in global agriculture (Bharathi et al., 2022) and contribute approximately 50% of human caloric consumption. However, large-scale cultivation of these crops has been associated with significant environmental degradation (Jungers et al., 2017). The current agricultural methods for producing these staple foods mostly focus on cultivating annual crops, which depend heavily on synthetic fertilizers and intensive labor inputs, while also contributing to CO_2 emissions and disrupting natural biological processes (Cassani et al., 2024; Tang et al., 2024).

Annual agricultural systems face critical ecosystem challenges stemming from practices such as soil tillage, fertilization, irrigation, pesticide application, and the prolonged absence of vegetative cover. These practices contribute to significant issues, including topsoil degradation, carbon loss, nutrient runoff, volatilization, eutrophication of aquatic systems, and excessive water and energy consumption (Li et al., 2020; Pinto et al., 2021). Therefore, identifying alternatives to traditional agricultural practices is essential. The 2030 Agenda for Sustainable Development, which was adopted by all United Nations Member States in 2015, underscores the importance of minimizing the environmental footprint associated with food production and consumption (De Oliveira et al., 2020).

Unlike annual crops, perennial crops play a vital role in promoting agricultural sustainability by maintaining year-round soil cover and developing extensive root networks. These crops not only reduce soil erosion and nutrient runoff (Pinto et al., 2024) but also prevent groundwater pollution and support carbon fixation. The prevalence of perennial crops ensures consistent productivity over time and enhances the resilience of crop species' diversity in the face of drought (Sakiroglu et al., 2020). Recently, agronomists, plant breeders, and environmentalists have turned their attention to the potential of cultivating perennial grain-producing species as staple crops, recognizing their ability to address ecological challenges commonly linked to annual agriculture (Dick et al., 2018; Becker et al., 1991).

This cool-season perennial grass is now commercially grown and sold under the trade name Kernza, and it is recognized as the first perennial grain crop to enter the market.

Inspired by the concepts proposed by Wes Jackson in 1980, the Rodale Institute (RI) assessed roughly 300 species in the early 1980s to determine their potential for perennial agriculture aligned with natural ecosystems. In 1985, after assessing over 100 grass species, intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey) was identified as the most promising candidate for perennial grain development due to its favorable plant structure and nutritional profile (Crain et al., 2024). This cool-season perennial grass, is now commercially promoted under the trade name Kernza (Pinto et al., 2021) and has gained recognition as the first perennial grain crop to enter the market. Its seeds are relatively large and comparable in size to those of conventional wheat varieties (Lanker et al., 2020) (Figure 1A). Nevertheless, due to the absence of high-yielding edible grains from IWG in nature, efforts to domesticate this species and enhance its productivity for agricultural practices have been underway since the 1980s (Craine and DeHaan, 2024).

Perennial IWG typically reaches heights between 90 cm and 1.2 m and is distinguished by its compact rhizomes and extensive root system. Its seed-bearing spikes typically measure between 10 and 20 cm in length, while the leaves, which are green to blue-green, measure 4 to 8 mm in width. Notably, the root system of IWG can extend more than 3 meters below the soil surface, exceeding the depth and density of annual wheat roots by more than twofold. Additionally, IWG seeds are considerably smaller, measuring approximately one-fifth the size of conventional wheat seeds (de Oliveira et al., 2018; Wills et al., 1998). Figure 1B provides a visual representation of IWG's seed morphology and size in relation to its grain-bearing spikes.

FIGURE 1
Morphology of IWG (*Thinopyrum intermedium*) from field to grain. **(A)** Field of IWG at the Land Institute research farm, Salina, Kansas, showing mature seed heads and foliage (photograph by DeHaan, 2008; Creative Commons BY-SA 3.0). **(B)** Dehulled seeds and unprocessed spikes of IWG (*Thinopyrum intermedium*). (Image: Alicia DeHaan; adapted from Craine and DeHaan, 2024).

1.1 Production areas

IWG, originally native to parts of Eurasia (Paul et al., 2024), has historically been cultivated as a forage crop within Canadian agricultural systems, and is well-suited for temperate climates (Dick et al., 2018; Barriball et al., 2022; DeHaan et al., 2018). Its distribution includes Europe, western Asia, and southern Africa, with successful establishment also reported in regions such as the United States (Marti et al., 2016). Breeding efforts to enhance IWG traits began in 2003 at The Land Institute (TLI) in Salina, Kansas, and have been complemented by collaborative initiatives launched in Minnesota (USA, 2011), Manitoba (Canada, 2011), Utah (USA, 2019), and Uppsala (Sweden, 2019) (Crain et al., 2024; Craine and DeHaan, 2024; Crain et al., 2022; Crain et al., 2020). IWG was initially introduced to the United States as a forage crop in the early 1900s (Culman et al., 2023; Cureton et al., 2023). At present, it is cultivated on 1,600 hectares, with Kernza—its derived grain—being the sole perennial grain available to American farmers (van der Pol et al., 2022). While most scientific studies on IWG originate from North America, global attention in the crop is steadily increasing, especially in in major cereal-producing zones such as Europe, southern South America, and Australia (Ivancic et al., 2021). For instance, Europe has made significant efforts to domesticate IWG, which can provide both forage and grain as a potential alternative to annual wheat, the predominant small grain crop in the region. Research into IWG for grain production began in

France in 2017 through on-farm experiments (Ginot et al., 2024). Given its winter hardiness, seed production has also been successfully established in cold climates in Canada (Ivancic et al., 2021). The global expansion of IWG cultivation and breeding efforts is illustrated in Figure 2 and Table 1, highlighting key production regions and research initiatives discussed in this study.

1.2 Cultivation requirements

Around 1.45 billion hectares of former cropland worldwide have been left unused, with approximately 910 million hectares of this area classified as degraded or marginal for agricultural purposes. Cultivating perennial crops such as IWG on these lands is especially beneficial, as it helps in preventing further degradation (Li et al., 2020). Unlike annual crops, they do not require replanting on a yearly basis, providing several benefits, including decreased requirements for fertilizer and tillage, along with the potential to eliminate irrigation in areas with adequate rainfall (de Oliveira et al., 2018). While IWG demonstrates tolerance to drought, it achieves optimal growth in regions receiving a minimum of 300 mm of seasonal precipitation (Wills et al., 1998). Hillside fields prone to erosion are suggested to provide suitable conditions for this perennial species; however, planting it in wet or lowland areas is not recommended. Moreover, for sustainable growth, high-drainage soils are preferable, while clay soils should be avoided (Paul et al., 2024). Established practices in the United States indicate that in coarse-textured soils, the drilling depth should not exceed 25 mm, while a depth of 10 mm is appropriate for planting in medium to fine-textured soils. The suggested seeding rate for intermediate and pubescent wheatgrasses species ranges from 11 to 14 kilograms per hectare of Pure Live Seed (PLS). It is evident that efficiency would be increased by cultivating in a firm, weed-free seedbed (Wills et al., 1998).

IWG, like other cool-season perennial grasses, requires cool temperatures and/or shortened daylength for its primary induction phase, allowing the initiation of inflorescence primordia. Following this, warmer conditions and/or extended daylight are needed for secondary induction, which promotes culm elongation and the subsequent development of inflorescences (Ivancic et al., 2021; Locatelli et al., 2022a). Exposure to cold during the winter in vegetative stage is crucial for flowering and seed production in the subsequent growing season; therefore, precise timing in planting is essential (Olugbenle et al., 2021). Given the potential applications of Kernza as both grain and forage, three annual harvest periods can be identified. First, an initial forage harvest occurs prior to stem elongation in spring. Second, grains can be harvested at physiological maturity, accompanied by the removal of crop remnants such as straw. Finally, a third harvest of forage can take place in the fall (Favre et al., 2019).

1.3 Production potentials

The literature indicates that the yield potential of IWG varies across regions due to factors such as annual weather conditions and management practices, showing a tendency for gradual decline from the initial year through the fourth year of production (Ginot et al., 2024). IWG production yields are comparatively low relative to those of annual wheat, averaging 460 kg ha^{-1} in major production areas (Pinto et al., 2022). In the first year of harvesting, current yields generally fall between 450 and 1,010 kg ha^{-1} (Curtis et al., 2024). However, experimental plots

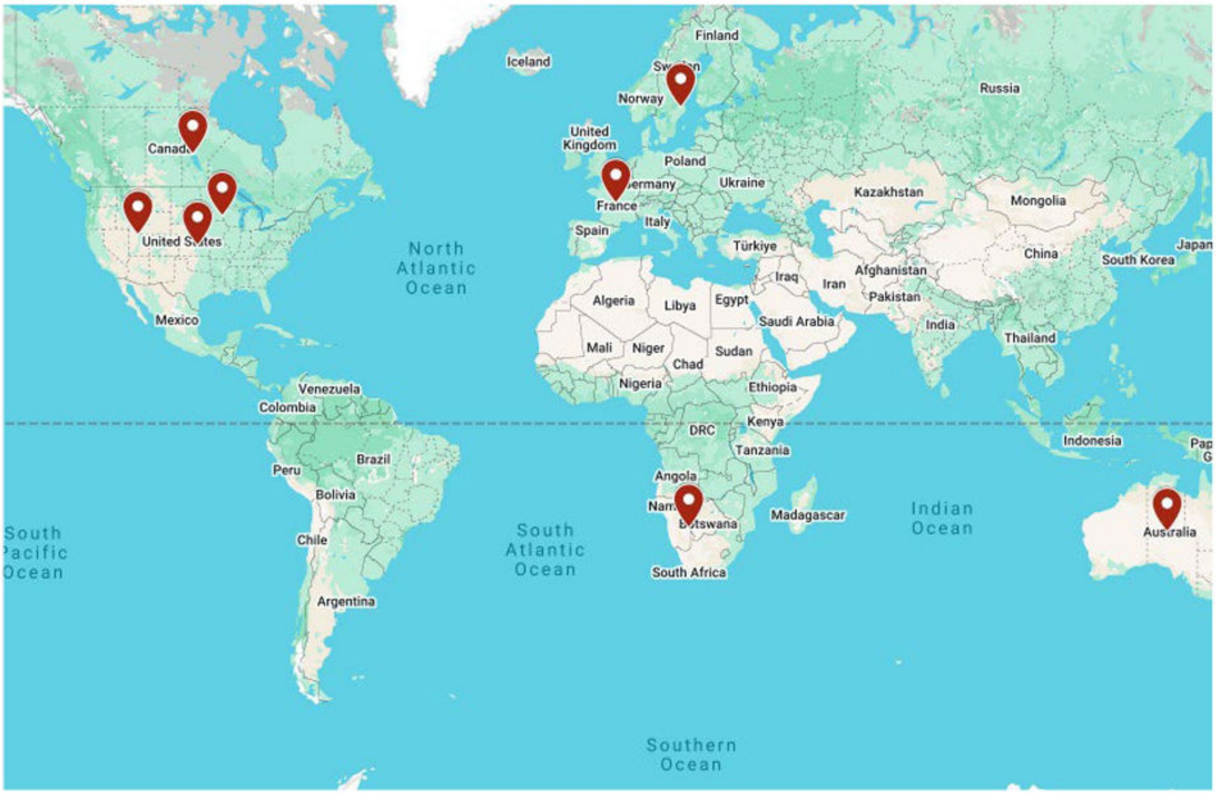

FIGURE 2
Global distribution of IWG cultivation and breeding programs (based upon 17, 19, 25, 26).

TABLE 1 Major breeding and cultivation sites for IWG grain.

Location	Country	Significance	Source
Salina, Kansas	USA	Primary breeding site by The Land Institute (TLI) since 2003	Crain et al. (2020, 2022, 2024)
Minnesota	USA	Breeding program established in 2011	Crain et al. (2020)
Utah	USA	Breeding program established in 2019	Crain et al. (2024)
Manitoba	Canada	Breeding program established in 2011; cold-hardy seed production	Ivancic et al. (2021)
Uppsala	Sweden	Breeding program established in 2019 (European adaptation)	Crain et al. (2024)
France	EU	On-farm grain production experiments since 2017	Ginot et al. (2024)
Southern Africa	Regional	Naturalized IWG distribution (non-native)	Marti et al. (2016)
Australia	Regional	Emerging interest in IWG as a perennial grain	Ivancic et al. (2021)

have shown that grain yields of IWG can attain levels of up to 1,500 kg ha^{-1} (Locatelli et al., 2022b). It is crucial to conduct additional research to enhance crop yields and productivity in the near future.

1.4 End applications

IWG, as a perennial plant, has traditionally been cultivated for forage and has more recently attracted interest for its potential to produce edible seeds (Clément et al., 2022). This dual-purpose cultivation offers benefits to farmers by providing multiple outputs, while also enhancing ecological functions (Pinto et al., 2024). For instance, mixed cropping systems that combine IWG with species such as alfalfa and red clover can compensate for reduced IWG grain yields through increased forage productivity (Pinto et al., 2022). Due to the absence of gluten aggregation in IWG grain, it is not advisable to use whole IWG flour to produce gluten-containing foods such as bread and pasta (Marti et al., 2015). Nevertheless, whole IWG flour and germinated flour provide a variety of culinary applications and nutritional benefits (Oliveira et al., 2024). In cookie production, the crispness and friability largely depend on the ratios of sugar and fat, with gluten contributing minimally to the overall formulation and quality. As a result, cookies made with IWG flour display an appearance and spread comparable to those made with all-purpose flour (Marti et al., 2016). Furthermore, studies have shown that mixing IWG and wheat flour in equal proportions maintains baking quality while also boosting nutritional content (Crain et al., 2024). Products made with IWG flour are richer in dietary fiber and antioxidant compounds, thereby significantly enhancing their health benefits (Pototskaya et al., 2022). In addition to its nutritional applications for food and feed, IWG can also function as a fuel source and various non-food bioproducts (Cui et al., 2018).

1.5 Motivation and objective of the study

However, there has been comparatively limited focus on addressing nutritional shortcomings and improving food-quality attributes. Existing research has predominantly emphasized the agricultural and environmental advantages of IWG, along with cultivation strategies to enhance yield over time. However, there has been comparatively limited focus on addressing its nutritional shortcomings and improving its quality attributes. To ensure the successful market integration of IWG, it is crucial for food processors and consumers to access comprehensive insights into its nutrient profile and functional properties. A thorough assessment of quality characteristics and key parameters such as dough rheology, the impact of dough conditioners, diverse treatment methodologies, and processing techniques for various food applications, is essential for developing methods to optimize the properties of end products (Tyl and Ismail, 2019).

This review begins by providing a comprehensive evaluation of IWG as a perennial grain ingredient, examining its strengths and limitations from agricultural, economic, environmental, and nutritional perspectives. Subsequently, it synthesizes current findings on IWG's use as a food ingredient and explores methods to refine its quality for incorporation into cereal-based products. This review aims to propose a combination of enzymatic treatments and previously studied approaches to address existing gaps, thereby enhancing the properties of IWG flour for industrial applications and positioning it as a wholesome and nutritious dietary ingredient.

Several reviews published over the past decade have examined intermediate wheatgrass (IWG) from multiple vantage points, including agronomic, ecological, and breeding perspectives, with emphasis on perenniality, ecosystem services, soil quality, yield limitations, and domestication progress (Crain et al., 2024; DeHaan et al., 2018). Other syntheses have focused on compositional attributes and introductory food-use applications, particularly in bakery products (Bharathi et al., 2022; Marti et al., 2016). This review paper is designed to complement the existing literature by explicitly centering on food processing and functionality, with particular emphasis on dough performance, flour modifications, and the use of enzymes and functional additives to address known limitations of IWG flour. A distinguishing contribution of this review is its synthesis of processing-based solutions and enzyme-driven improvement strategies, including single and combined enzyme systems. Where direct experimental evidence in IWG is limited, relevant findings from conventional wheat and non-wheat cereal systems are incorporated to establish mechanistic rationale and to identify potential research directions. These extrapolations are presented as hypotheses and opportunities for future study rather than as established functionality in IWG systems.

2 Merits

2.1 Agricultural and economic advantages

IWG is well adapted to semi-arid climates, and its extensive root system promotes water efficiency and resilience to drought and frost (Paul et al., 2024). Its beneficial agronomic characteristics, such as winter hardiness and high biomass yields, render it a valuable resource for wheat genetic enhancement (Loehr et al., 2024). It has also demonstrated strong resistance to several diseases that commonly affect conventional wheat varieties (*Triticum aestivum L.*) (Li and Wang, 2009; Cox et al., 2010). Furthermore, its threshing and harvesting processes exhibit greater efficiency, producing larger seeds and higher grain yields than other perennial species (Zhang et al., 2015). Some other factors, such as less demand for nitrogen fertilizer, fewer tillage operations, reduced farm equipment usage, and lower expenditures on seeds and weed control, lead to diminished overall energy and operational expenses for agricultural producers (Bharathi et al., 2022; Cui et al., 2018). Eliminating annual replanting also substantially reduces labor and maintenance costs, illustrating improved cost efficiency in agricultural production (Oliveira et al., 2024).

2.2 Environmental benefits

IWG's unique qualities enable it to provide significant ecosystem services and environmental advantages, such as enhancing soil integrity and water quality (Law et al., 2021), capturing and storing carbon within the soil to mitigate anthropogenic climate change, reducing soil erosion, and lowering nutrient runoff to groundwater (Bajgain et al., 2020). These plants' deep and dense roots significantly influence the physicochemical and microbial soil characteristics, including soil organic carbon (SOC) and microbial communities (Pugliese et al., 2019). Studies have demonstrated that transitioning from annual cropping systems to perennial grain cultivation can store approximately 1.7 tons SOC per hectare annually, highlighting their long-term potential as a carbon sink (Udawatta et al., 2024). Moreover, their deep root systems significantly reduce nitrate leaching into groundwater beneath the stands, improving water quality compared to annual crops (Bajgain et al., 2020). They also enhance soil regeneration by improving soil structure and fertility (Dimitrova Mårtensson et al., 2021). Research conducted at the plot scale across various soil types in Minnesota has aligned with the mentioned decrease in nitrate leaching into groundwater (Jungers et al., 2023).

2.3 Nutritional value

Nutritionally, IWG grain is notable for its relatively high protein and dietary fiber contents compared to conventional cereals. It comprises approximately 73% carbohydrates, 3% fat, and 2% ash (Oliveira et al., 2024). Research comparing the nutritional profiles of whole grain IWG flour and whole grain wheat flour has reported about 20% protein and 16.4% dietary fiber in IWG flour, compared with 13% protein and 11% fiber in whole grain wheat flour (Pototskaya et al., 2022). Although IWG protein, like that of wheat, is relatively low in lysine, it contains elevated concentrations of all other indispensable amino acids (Craine and DeHaan, 2024). Moreover, this perennial grains is rich in phytochemicals with high antioxidant activity, including carotenoids (the primary yellow pigments) and phenolic compounds such as hydroxycinnamic acids (Trevisan et al., 2024). Given these compositional features, IWG shows potential as an ingredient for enhancing the nutritional profile of bakery products, such as bread. Consumption of whole grain products rich in bran has been associated with a reduced risk of chronic diseases, including obesity, cardiovascular disease, type 2 diabetes, and certain types of cancer (Bharathi et al., 2024; Gómez et al., 2020). Therefore, based on its compelling compositional parallels with other whole grains, IWG consumption may offer similar health benefits, a promising hypothesis that warrants future clinical investigation. Table 2 summarizes key compositional data from various studies comparing IWG and wheat.

3 Challenges and limitations

3.1 Agricultural and economic aspects

While IWG offers several advantages, it is also important to recognize the accompanying shortcomings that require attention. Adopting IWG perennial grain production faces some challenges, such as inconsistent and low grain yields, insufficient agronomic knowledge among producers, and the lack of approved chemical options for managing weeds and pests (Barriball et al., 2022). Current estimates indicate that this perennial crop yields approximately 25 to 30% of annual wheat yields (Crain et al., 2024), resulting in significantly higher production costs. Moreover, its relatively low production rate limits overall availability (Friesen et al., 2024). Another agronomic factor influencing the long-term sustainability of IWG is the decline in grain yields associated with stand age, a trend that varies with environmental conditions (Bergquist et al., 2022). Typically, yields decrease due to soil nutrient depletion after 2 years (Li et al., 2020). Maintaining consistent seed production over the entire lifespan of a IWG stand is essential to ensure its viability, as frequent re-establishment may diminish the environmental benefits these crops provide (Tautges et al., 2018). The reduction in grain productivity over time may be attributed to physiological mechanisms, such as changes in plant density and tillers competition (Bergquist et al., 2022). The significant lack of research on yields in the second and subsequent years of cultivation underscores the pressing need for investigations in this area to address the issue in future studies (Tautges et al., 2018).

3.2 Nutritional aspects

Due to its relatively higher fat content than that of wheat (Rahardjo et al., 2018), IWG is susceptible to rancidity during storage. Lipids and enzymes concentrated mainly in the bran layers contribute to undesirable chemical alterations, notably hydrolytic and oxidative rancidity development. However, the presence of antioxidants reduces oxidation processes (Loehr et al., 2024). IWG bran is rich in insoluble dietary fiber, particularly water unextractable arabinoxylans (WU-AX), which adversely affect both dough structure and baking characteristics. Specifically, WU-AX dilute the gluten matrix, reduce gas retention, and lead to water redistribution, ultimately resulting in the dehydration and disruption of the protein network in IWG dough (Bharathi et al., 2024). Enzymatic treatment with endoxylanase, which converts WU-AX to water-extractable arabinoxylans (WE-AX), has been shown to enhance bread's volume and crumb structure (Dai et al., 2021). In terms of protein composition, IWG predominantly contains α- and γ-type gliadins and low molecular weight (LMW) glutenins. The absence of high molecular weight (HMW) glutenin, which are crucial for strong gluten network development, suggests limited gluten-forming potential (Marti et al., 2016). This results in relatively less extensible dough and reduced mixing ability, leading to inferior baking performance and smaller loaf volume relative to traditional wheat-based breads (Cetiner et al., 2023).

TABLE 2 Comparative nutritional profiles of whole grain and flour samples of IWG and wheat reported in various studies.

Sample/ grain type	Energy (kcal)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Total CHO[a] (%)	Total Starch (%)	Total dietary fiber (%)	Reference
IWG samples									
Flour MN-Clearwater[b]	–	7.31	2.12	21.3	3.69	72.9	54	17.5	Loehr et al. (2024)
Flour MN1603-SYN3[b]	–	5.29	2.09	20.2	3.81	73.9	55	16.9	Loehr et al. (2024)
Flour MN1601-SYN2[b]	–	5.84	2.18	16.9	5.42	75.5	55.1	16.1	Loehr et al. (2024)
Whole grain flour	–	–	–	20	–	–	46.7	16.87	Marti et al. (2015)
Six Grain sample[c]	233–326	–	2.4–3.0	20.1–26.4	1.6–3.1	68.9–75.6	–	17.1–37.46	Craine and DeHaan (2024)
Whole grain sample	–	10.41	2.74	23.18	1.84	–	–	16.96	Friesen et al. (2024)
Wheat samples									
Jagger grains[c]	343	–	1.8	12.7	2	83.5	–	13.08	Craine and DeHaan (2024)
Whole grain flour	–	–	–	13	–	–	72	11	Pototskaya et al. (2022)
Whole grain sample	–	11.33	1.98	14.46	1.82	–	–	10.60	Friesen et al. (2024)

[a]CHO: Carbohydrates.
[b]Values for MN-Clearwater, MN1603-SYN3, and MN1601-SYN2 IWG flours were adapted from Loehr et al. (2024). These data represent untreated (control) samples prior to extrusion or germination. All samples were milled using a 0.5 mm mesh Udy Cyclone Sample Mill and equilibrated to approximately 8% moisture before analysis.
[c]Values for the six IWG grain samples were adapted from studies analyzing IWG lines Rodale1, TLIC1, TLIC3, TLIC4, TLIC5, and EllsworthC5, along with a wheat check (cv. Jagger) (Craine and DeHaan, 2024).

4 Advances in breeding

In recent years, advancements in agricultural science have introduced sophisticated techniques to enhance seed size, grain yield, the suitability of IWG for food applications, and its domestication potential, utilizing bulk breeding and mass selection strategies (Zhong et al., 2019; Culman et al., 2013). Early work to improve spikelet fertility and seed size commenced in 1988 at the Rodale Research Center (Kutztown, PA). Subsequently, in 2001, The Land Institute (TLI; Salina, Kansas), utilized these materials to establish a dedicated breeding program (Stoll et al., 2024). Over two selection cycles, IWG breeders at TLI achieved remarkable progress, increasing grain yields by ~77% and seed size by 23% (Zhong et al., 2019). In 2011, the University of Minnesota (UMN; St. Paul) advanced these efforts by acquiring 2,560 genets sourced from 66 TLI Cycle 3 genets, thus launching its inaugural breeding cycle (UMN-C1) (Stoll et al., 2024). Collaborative initiatives between TLI and UMN yielded a doubling of grain production compared to traditional forage cultivars. A significant milestone was reached in 2020 with the introduction of 'MN-Clearwater,' the initial commercial IWG grain variety (Reilly et al., 2022). By 2023, UMN's breeding program had successfully completed seven cycles of recurrent selection, targeting both enhanced field performance and progressive domestication (Stoll et al., 2024).

5 Processing

Given the aforementioned characteristics of IWG grain outlined in Section 3.2, various processing approaches have been implemented to enhance the appeal of the final product. These processes are applied during the conversion of IWG into flour, in the formulation of flour as an intermediate product, and during the production of final products such as bread and pasta. To mitigate the high levels of insoluble dietary fiber in IWG flour and to address the imbalance in its protein profile, flour refinement is undertaken. Additionally, extrusion serves as an effective method for enhancing the commercial viability of this grain (Boakye et al., 2022; Boakye et al., 2023a). For bran-enriched or whole-grain applications, supplementary strategies are employed, such as the tempering process (Tyl et al., 2019), mixing with wheat flour (Marti et al., 2016; Marti et al., 2015; Trevisan et al., 2024; Cetiner et al., 2023), and incorporating dough conditioners like enzymes, along with other improvers such as vital wheat gluten (VWG) or ascorbic acid (AA) (Banjade et al., 2019a; Banjade et al., 2019b).

6 Processing and functional modification of IWG flour

6.1 IWG flour characterization

To facilitate the commercialization of IWG, it is essential to understand its properties and develop food products enriched with it. Rahardjo et al. (2018) analyzed the chemical composition, functional characteristics, and baking potential of wholegrain flour from 16 IWG breeding lines. Their findings revealed that while IWG grains offer nutritional advantages over conventional wheat, the flour presents challenges for bakery applications that require dough with strong rising properties. IWG wholegrain flour contained higher protein, dietary fiber, and ash content but lower starch levels than wholegrain wheat flour. It was also deficient in high molecular weight glutenins (HMWG). Despite similar amylose-to-amylopectin ratios in both IWG and wheat flours, IWG flours exhibited reduced viscosity during thermal processing. Dough made from IWG flour showed reduced stability, lower extensibility, and less resistance to extension compared to wheat flour dough. While the resulting bread achieved specific volume comparable to wheat-based counterparts, its rising capacity was limited due to weaker gluten network formation. This inferior baking performance was attributed to the lack of HMWG and the high fiber content in IWG flour, which hindered robust gluten structure development (Rahardjo et al., 2018).

Beyond these physicochemical and textural limitations, IWG exhibits a distinctive sensory profile that significantly influences consumer perception. Comparative studies have reported that IWG breads display lower specific volumes than hard red wheat breads, resulting in denser crumb structures, with bran content inversely correlated to loaf volume (Banjade et al., 2019b). Aroma profiling of IWG bread crusts using gas chromatography identified significant differences in volatile composition compared to whole wheat bread, including lower concentrations of Maillard-derived compounds associated with roasted notes. Sensory descriptive analysis further indicated higher intensities of bran and green attributes in IWG breads, which may affect overall flavor perception and consumer acceptance. Given these combined functional and sensory limitations, using IWG wholegrain flours in production is not advisable without blending with other flours, prior processing, or breeding strategies aimed at improving sensory quality alongside functionality (Paravisini et al., 2019).

6.2 Tempering

IWG is primarily used in flour production, as it is a key ingredient in numerous cereal-based applications. Before milling, the tempering process -which involves the addition of water- improves flour extraction efficiency by strengthening the bran layers and softening the endosperm (Kweon et al., 2009). A study by Tyl et al. (2019) examined the effects of tempering parameters on the chemical composition, color characteristics, solvent retention capacity, starch damage, and polyphenol oxidase activity to optimize processing conditions for end-use applications. They assessed how the flour's responses to different tempering moisture levels (control vs. 12 and 14%), tempering times (4, 8, and 24 h), and temperatures (30 °C and 45 °C). The findings revealed that target moisture significantly influenced all studied parameters, although the differences between the 12 and 14% moisture treatments were minimal. Tempering yielded flour with a lighter color, reduced browning, and enhanced yellowness, with increased starch content, higher levels of damaged starch, and only minor protein losses. Additionally, tempering time and temperature had negligible effects on most flour properties. These results suggest that tempering is essential in flour production to achieve desirable properties for final applications (Tyl et al., 2019).

6.3 Mixing with wheat flour

Research on processing and functional modification of IWG has evolved progressively over the past decade. Several studies have explored how blending IWG with varying proportions of wheat flour influences the quality of the resulting products. Early studies primarily assessed the feasibility of blending IWG with wheat flour and its impact on dough rheology. Subsequent investigations focused on elucidating protein network dynamics and structural functionality. More recent research has expanded toward product-level optimization, quality retention, and strategies to mitigate dough weakening at higher substitution levels.

In 2015, Marti et al. investigated blends of IWG and refined wheat flour (HWF) using rheological instruments, testing ratios of 0:100, 50:50, 75:25, and 100:0. The GlutoPeak tester revealed that IWG proteins can aggregate and generate torque. Notably, the torque value for the 50% IWG flour blend (42.9 ± 2.96 BE) was comparable to that of HWF (42.0 ± 0.99 BE), suggesting its viability in baked goods while maintaining gluten network formation during processing. Blending IWG flour with HWF increased consistency and reduced dough development time, likely due to the higher fiber content. Specifically, peak torque values rose with increasing IWG content: from 489 ± 8.5 BU for HWF to 780 ± 5.6 BU, 850.5 ± 4.9 BU, and 862.5 ± 10.6 BU for the 50, 75, and 100% IWG blends, respectively. Moreover, dough development time decreased as a result of the introduction of IWG flour, with times of 5.8 min for HWF compared to 1.66, 1.75, and 1.85 min for the 50, 75, and 100% IWG blends, respectively. It is noteworthy that higher proportions of IWG flour accelerated gluten aggregation kinetics and lowered peak torque, indicating a weakening of wheat gluten strength. A 50% IWG enrichment provided nutritional improvements in cereal-based products without significantly altering starch gelatinization, gluten aggregation kinetics, or overall protein quality (Marti et al., 2015).

Marti et al. (2016) investigated the structural characteristics of proteins in IWG and wheat flour blends to address gaps in understanding IWG protein functionality. Their research examined aggregate dynamics, thiol group accessibility, and changes in secondary structure during the mixing process, utilizing the same blend ratios as in their earlier work. The viscoelastic network of IWG dough differed markedly from those of wheat dough, a phenomenon attributed to IWG's high fiber content as well as differences in protein profile and secondary structure. IWG-enriched doughs showed increased protein solubility and thiol content, which rose with higher IWG proportions, suggesting a protein network primarily governed by non-covalent interactions. The highest levels of SDS-soluble protein (SDS: sodium dodecyl sulfate) were observed in doughs containing 50 and 75% IWG, with values of approximately 720 and 700 mg/g protein, respectively. These results suggest that the combination of IWG and wheat proteins enhances hydrophobic interactions within the system. Analysis of readily accessible thiols revealed no significant variation between samples, with all values consistently falling within a narrow range (5–7 μmol/g protein). However, when thiols were measured in the presence of SDS, a substantial increase was observed, with values of approximately 15, 28, 36, and 52 μmol/g protein for the 0, 50, 75, and 100% IWG formulations, respectively. As expected, protein denaturation increases the accessibility of thiol groups. Moreover, the low thiol concentration and limited protein solubility observed in the wheat-only dough suggest that some thiol groups may be present in protein aggregates that are not readily accessible or soluble. Analysis of secondary structures revealed that β-sheets were predominant in both IWG-enriched and control (wheat) doughs. However, IWG enrichment led to a rise in random structures at the expense of β-sheets, with no significant effect on β-turn structures. These changes in secondary structure could influence dough processing, as β-sheets play a critical role in maintaining elasticity and stability. A 50% IWG enrichment increased random structures, while a 75% blend resulted in a more pronounced reduction in β-sheets and further growth in random structures, indicating a loss of structural order (Marti et al., 2016).

Cetiner et al. (2023) conducted a detailed study to investigate the impact of IWG-incorporated flours (15, 30, 45, and 60%) on gluten properties, dough mixing behavior, loaf quality, and staling properties, comparing them to a control bread made solely from wheat flour. The study also included analyses of yellow pigment content, total phenolic content, and antioxidant activity in the breads. Increasing IWG incorporation resulted in a progressive decline in Farinograph water absorption, dough stability, and quality number, accompanied by a higher softening degree, indicating diminished rheological performance with increasing IWG content. From a technological and industrial perspective, reductions in stability and quality number reflect lower resistance to mechanical mixing and a narrower processing tolerance, while increased softening suggests a weaker gluten network with reduced capacity to withstand mechanical stress during large-scale automated production. Such rheological changes may negatively affect machinability, gas retention, and process robustness at high substitution levels. Despite this general weakening trend, moderate substitution (15%) produced the highest loaf volume (485 mL), representing approximately a 9% increase compared with the control (445 mL), and reduced crumb firmness on day 3 by about 27% (1,046 g-f vs. 1,436 g-f), indicating delayed staling and improved short-term textural quality. Higher levels of IWG led to significant reductions in Zeleny sedimentation and gluten index values, despite increases in both dry and wet gluten content. Additionally, greater IWG inclusion increased yellow pigment intensity and crumb b^* values. Pigment intensity and crumb b*. The enrichment also improved the bread's phenolic and antioxidant properties. Overall, these findings indicate that while high IWG substitution may require formulation adjustments or technological aids to maintain dough handling performance in industrial breadmaking, moderate incorporation levels can simultaneously maintain acceptable processing behavior, improve loaf characteristics, and enhance nutritional quality (Cetiner et al., 2023).

Trevisan et al. (2024) evaluated the pasting and rheological performance of IWG and wheat flour blends (0–60%). Their findings revealed that increasing IWG flour proportions weakened dough properties, as shown by both empirical (Mixograph, Kieffer dough and gluten extensibility, Glutograph) and fundamental (linear oscillatory frequency sweep and creep-recovery) rheological measurements. Higher IWG substitution levels significantly reduced RVA peak, trough, and final viscosity values. Specifically, the peak, trough, and final viscosity values declined from 2,061 ± 9, 1,347 ± 27, and 2,499 ± 8 cP in the control samples (0% IWG flour) to 1,367 ± 6, 786 ± 3, and 1868 ± 4 cP in samples containing 60% IWG flour. Similar weakening effects were observed for mixograph mixing properties (DDT, PH, BW, and WIP values), Kieffer uniaxial extension properties (Rmax and Area), G′ and Jmax values, and Glutograph properties. In formulations containing 60% IWG flour, both dough development and stretch times were reduced to 3.8 min and 6 s, respectively, compared to 6.1 min and 125 s in the control samples. Moreover, doughs composed entirely of wheat or supplemented with 15% IWG flour exhibited significantly lower Jmax values than those containing 30% or 60% IWG, indicating reduced deformation under constant stress. Notably, the dough-weakening effect of IWG flour was manageable at a 15% substitution level. At lower substitution levels, IWG-containing flour blends exhibited a reduced retrogradation tendency compared to bread wheat flour, indicating slower staling during bread storage while preserving overall product quality (Trevisan et al., 2024).

6.4 Extrusion

Extrusion cooking is a versatile thermo-mechanical processing technique used in production of a wide range of edible and feed items. It involves the simultaneous mixing, homogenization, kneading, cooking, shearing, and shaping of raw materials with water under conditions of elevated temperature, pressure, and mechanical stress (Boakye et al., 2023b). An extruder typically consists of a feeding hopper, rotating screws, barrel, die, cutter, and a motorized drive system, and it often includes a preconditioner to adjust material moisture levels (Mironeasa et al., 2023). During the process, raw materials undergo starch gelatinization, protein denaturation, and melting, forming a molten matrix. This material is then forced through a die, where the combination of high temperature and sudden pressure drop causes flash evaporation processing, resulting in product expansion (Boakye et al., 2023b; Mironeasa et al., 2023). This method offers significant potential for enhancing the commercial viability of IWG by enabling the development of various value-added products, including puffed snacks, breakfast cereals, baby foods, and texturized proteins. Nevertheless, optimizing extrusion parameters necessitates a comprehensive understanding of its effects on IWG's chemical composition and functional properties.

Boakye et al. (2022) applied response surface methodology (RSM) to determine optimal processing parameters for extruding IWG, focusing on three variables: feed moisture content (FM) (20–28%), screw speed (200–400 rpm), and barrel temperature (130–170 °C). These parameters were evaluated for their impact on the physicochemical and functional properties of expanded IWG products. Key characteristics evaluated included moisture content, expansion ratio, bulk density, hardness, water absorption index (WAI), and water solubility index (WSI). The findings indicated that higher screw speeds combined with lower extrusion temperatures resulted in extrudates with increased expansion ratios, lower bulk density, and reduced hardness. Screw speed was identified as the most influential factor, with higher speeds significantly decreasing WAI and increasing WSI. The optimal conditions for producing extrudates with a high expansion ratio and desirable WAI were found to be 20% FM, screw speeds between 200 and 356 rpm, and an extrusion temperature ranging from 130 °C to 154 °C (Boakye et al., 2022). A detailed summary of variable effects on extrudate properties is provided in Table 3.

Boakye et al. (2023a) conducted the first investigation into the impact of extrusion on IWG's nutritional and physicochemical characteristics. The findings indicated that while the dietary fiber, fat, starch, and amylose contents in IWG experienced a slight reduction following extrusion, the protein and ash levels remained stable. A notable increase in starch damage was observed after extrusion, which significantly enhanced starch digestibility and improved the hydration properties of IWG. This, in turn, resulted in a substantial reduction in the amounts of slowly digestible and resistant starches. The study also highlighted a rise in antioxidant activity and elevated levels of phenolic acids, predominantly due to ferulic acid, following extrusion. However, carotenoid content experienced a significant decline, with lutein and zeaxanthin decreasing by up to 65.8 and 50.4%, respectively. The research concludes that extrusion cooking may enhance the bioavailability of phenolic acids and enhance IWG's antioxidant potential, but it is less effective in preserving carotenoids. Importantly, essential nutrients such as proteins and dietary fiber were largely maintained after the extrusion process (Boakye et al., 2023a). A comprehensive summary of their findings, including variations in chemical composition, starch properties, hydration characteristics, bioactive compounds, and antioxidant activity under different extrusion conditions (Extrudate I: 20% FM, 200 rpm screw speed, 150 °C extrusion temperature; Extrudate II: 20% FM, 300 rpm screw speed, 150 °C extrusion temperature 200 rpm vs. 300 rpm), is provided in Table 4.

6.5 Refining IWG flour and enhancing dough performance with additives

To enhance the properties of bread produced from IWG flour for commercial viability as a standalone product, specific processing techniques must be employed. Research has explored various methods, including the use of dough conditioners, the reduction of the bran fraction (flour refinement), and the pretreatment of IWG bran, with the goal of enhancing the structural and sensory attributes of the dough and final baked product.

Banjade et al. (2019a) found that adding bran to IWG dough increased β-sheet content and resistance to extension, while reducing β-turns and extensibility. Their study highlighted the significant role of bran reduction in altering IWG's composition, protein secondary structures, and dough rheology. Flour refinement consistently decreased resistance to extension and improved extensibility in some cases. Additionally, reducing bran content led to higher β-turns and lower β-sheet content, suggesting a more hydrated protein network. Notably, in dough containing 100% bran, β-sheet content increased by 24.3 and 11.8%, and resistance to extension rose by 607.4 and 149.6%, compared to fully refined

TABLE 3 Effects of extrusion setting on the physicochemical and functional characteristics of IWG-based extrudates (Boakye et al., 2022).

Key parameters investigated	Observed range	Min value observed at	Max value observed at	Optimal range	Effect on IWG extrudates
Expansion ratio	0.97–2.34	28% FM [1], 400 rpm, 170 °C	20% FM, 400 rpm, 130 °C	Target: >2	Increased with low FM, higher screw speed and lower temperature.
Bulk density (kg/m^3)	100.45–392.95	20% FM, 400 rpm, 170 °C	28% FM, 200 rpm, 130 °C	Target: Minimize	Inversely related to expansion; lower with high temperature and low FM.
Hardness (N)	20.39–57.12	20% FM, 400 rpm, 170 °C	28% FM, 200 rpm, 130 °C	Target: Minimize	Lower FM, higher screw speed and extrusion temperature decreased hardness.
WAI (g/g)	3.7–4.5	28% FM, 400 rpm, 130 °C	28% FM, 200 rpm, 130 °C	Target: >4 g/g	Linked to starch gelatinization; increased with higher screw speed; irrespective of FM and temperature.
WSI (%)	15.91–30.98	28% FM, 200 rpm, 130 °C	20% FM, 400 rpm, 170 °C	Target: Minimize	Decreased with low screw speed and high FM; strongly related to shear intensity.
Moisture content (%)	5.14–6.13	28% FM, 300 rpm, 150 °C	20% FM, 200 rpm, 130 °C	20%	Slightly increased with lower screw speed and FM.
Screw speed	–	–	–	200–356 rpm	Higher speeds (400 rpm) increased expansion ratio and WSI but decreased WAI and hardness.
Extrusion temperature	–	–	–	130–154 °C	Lower temperatures (130 °C) improved expansion ratio and reduced bulk density; high temperatures (170 °C) reduced expansion but increased solubility (WSI).

TABLE 4 Effect of extrusion on the chemical composition, starch functionality, and bioactive compounds of IWG extrudates (Boakye et al., 2023a).

Category	Parameter	% Change extrudate I (200 rpm)	% Change extrudate II (300 rpm)	Explanation
Chemical composition	Protein (g/100 g dwb)	No significant change.		Protein stability under thermal-mechanical stress
	Fat (g/100 g dwb)	−25.7%	−26.6%	Lipid expulsion due to shear forces and high pressure.
	Total starch (g/100 g dwb)	−10.5%	−12.6%	Depolymerization into low molecular weight dextrins
	TDF: total dietary fiber (g/100 g dwb)	−9.4%	−9.6%	Extrusion resulted in a significant ($p < 0.05$) reduction in TDF
	IDF: insoluble dietary fiber (g/100 g dwb)	−21.5%	−28.5%	Mechanical shear leads to breakdown of chemical bonds
	SDF: soluble dietary fiber (g/100 g dwb)	+31.1%	+53.9%	Conversion of IDF to SDF due to shear; higher screw speed enhanced solubilization.
Starch properties	Starch damage (%)	+938%	+1,058%	Granule disruption by thermal-mechanical energy.
	RDS: readily digestible starch (%)	+349%	+359%	Starch gelatinization and damage increased enzymatic hydrolysis.
	SDS: slowly digestible starch (%)	−87.2%	−89.6%	Most starch converted to RDS.
	RS: resistant starch (%)	−78.6%	−81.1%	Reduced retrogradation potential due to extrusion.
Pasting properties	Peak viscosity (BU)	−21%	−45%	Molecular and structural disintegration of starch led to its reduction
	Breakdown viscosity (BU)	+72.8%	+21%	Inversely correlates with the degree of stability
	Final viscosity (BU)	−64%	−72.8%	Reduced retrogradation tendency
Hydration properties	WAI: water absorption index (g/g)	+136%	+124.7%	Starch gelatinization enhances hydration.
	WSI: water solubility index (%)	+89.5%	+141.2%	More small-molecule solubles (dextrins).
Bioactive compounds	Total phenolic acid (µg/g flour)	+1.7%	+4.1%	Ferulic acid accounted for the majority of the phenolic acid increase.
	Total carotenoids (µg/g flour)	−73.9%	−63.3%	Lutein exhibited a higher degradation rate compared with zeaxanthin, consistent with its higher initial concentration.
Antioxidant Activity	DPPH: 2,2-diphenyl-1-picrylhydrazyl (µM TE/g dwb)	+123% in the acetone extracts, +226% in the alkaline hydrolysates		Maillard reaction products and increased phenolic extractability.

(0% bran) samples from the Rosemount (RM) and Roseau (RS) locations, respectively. Conversely, β-turns decreased by 46.1 and 18.4%, and extensibility declined by 46.4 and 41.1%, in RM and RS samples, respectively. The study also investigated the effects of dough conditioners, including wheat protein isolate (WPI), VWG, AA, xylanase, and transglutaminase (TG), on extensibility, resistance to extension, and protein secondary structures of IWG dough. The findings indicated that dough conditioners were most effective when the refined flour contained either no bran or only a partial reintroduction of bran. Across all treatments and locations, fully refined samples (0% bran) consistently exhibited lower resistance to extension than their unrefined counterparts, highlighting that bran content exerted a more substantial influence on rheological properties than the dough conditioners employed. Among the conditioners, VWG, WPI, and TG improved either extensibility or resistance in fully or partially refined samples, whereas AA and PB (PowerBake) showed negligible effects at these refinement levels (Banjade et al., 2019a).

In a subsequent study, Banjade et al. (2019b) examined how flour refinement and the application of dough conditioners influenced IWG dough stickiness and bread structure, utilizing the same dough conditioners as in their earlier research. The findings revealed that TG reduced dough stickiness in both fully and partially refined breads. However, in whole (unrefined) breads, TG led to an excessively dense crumb and a significant reduction in crumb cell counts, decreasing from 237 ± 16 to 93 ± 22 cells/cm^2 in RM, and from 311 ± 38 to 86 ± 7 cells/cm^2 in RS, indicative of protein over-crosslinking. The most notable improvements were observed with AA and PB. For instance, PB increased the specific loaf volume of fully refined RM flour from 2.54 ± 0.20 to 2.85 ± 0.10 mL/g, while AA reduced crumb cell counts in refined flour from 247 ± 3 to 129 ± 6 (RM), and from 356 ± 23 to 104 ± 4 (RS), suggesting the formation of larger, more uniform gas cells. Additionally, AA produced breads with smoother surfaces and greater visual appeal compared to the uneven textures observed in breads made with other conditioners or without additives. These results underscore the importance of tailored processing strategies for optimizing the breadmaking potential of IWG flour (Banjade et al., 2019b).

The key findings from the studies conducted by Banjade et al. (2019a, 2019b) are summarized in Table 5, highlighting the effects of bran content and different dough conditioners on both the rheology of IWG dough and the associated bread quality parameters, as previously discussed. These results emphasize the pivotal role of flour refinement and the distinct effectiveness of TG, VWG, and WPI in augmenting dough functionality, while AA and PB optimize crumb structure and volume, offering practical strategies to enhance IWG's utility in baked goods.

6.6 Bran treatment

Dai et al.'s research demonstrated that pretreating bran with xylanase enables the effective integration of IWG into bread formulations while maintaining quality parameters. The results indicated that while xylanase pretreatment had no impact on dough stickiness, it markedly decreased bread firmness and enhanced specific loaf volumes relative to both untreated controls (lacking xylanase) and positive controls (containing xylanase but without pretreatment). Nevertheless, breads made with enzymatically treated bran displayed uneven surfaces caused by structural collapse during baking, resulting in fewer but larger gas cells. The inclusion of AA modified the effects of xylanase in the pretreated breads, though it did not resolve the issue of uneven surfaces. Additionally, xylanase pretreatment resulted in a slight but significant increase in accessible thiol concentrations, which may be linked to a less compact crumb structure. The study also suggested that endogenous xylanases, with apparent activities of 0.46 and 5.81 XU/g in flour and bran, respectively, may have been activated during the pretreatment process (Dai et al., 2021).

Steam explosion (SE), a hydrothermal processing technique, has gained recognition as a cost-effective and eco-friendly method for enhancing bran stability while minimizing its negative effects on gluten networks. Bharathi et al. explored how varying SE conditions, such as temperature, residence time, and severity factors, influence the physicochemical characteristics of IWG bran. Notably, even under the mildest conditions (130 °C, 5 min), peroxidase, which is known for its high thermal stability in grains, was entirely deactivated. The researchers reported significant increases in free phenolic acids and water-extractable arabinoxylans, alongside a reduction in phytic acid levels following SE treatment. However, severe treatments led to undesirable outcomes, such as heightened starch damage, elevated free fatty acids, and increased browning, all of which compromise product quality. The findings underscore SE as an effective approach for diminishing enzymatic activity and enhancing phenolic compounds and soluble fiber in IWG bran. Nonetheless, maintaining severity factors at or below 2.5 is advised to prevent undesirable color changes, reduce rancidity, and achieve bran with optimal hydration properties for bakery formulations (Bharathi et al., 2024).

6.7 Comparative framework and toward strategic considerations

Based on the literature reviewed above, no single processing intervention can fully overcome the functional limitations of IWG while simultaneously maximizing nutritional integrity and industrial scalability. Flour refinement improves dough rheology by reducing bran interference but compromises whole-grain nutritional value. Enzymatic and dough conditioning approaches provide targeted reinforcement of protein networks while preserving fiber fractions; however, they require precise optimization and increase formulation complexity. Blending with wheat flour remains the most immediately scalable strategy, although it primarily dilutes functional deficiencies rather than fundamentally overcoming them. As summarized in Table 6, optimal processing pathways are product-specific and require balancing functionality, nutritional retention, and economic feasibility. In practice, integrated approaches, such as partial refinement combined with controlled enzymatic conditioning, may provide a more balanced method toward commercial viability while maintaining sustainability attributes. Future research should systematically evaluate such combinatorial strategies using design-of-experiments frameworks to identify synergistic effects and define optimal processing conditions.

TABLE 5 Influence of bran and dough conditioners on IWG dough and bread properties.

Reference	Parameter	Effect of bran addition	Effect of dough conditioner
Banjade et al. (2019a)	Extensibility	Decreased with bran	VWG and WPI improved extensibility.
	Resistance to extension	Increased with bran	TG and VWG significantly increased resistance
Banjade et al. (2019b)	Stickiness (Newtons)	Higher in unrefined samples	TG, WPI, and VWG reduced stickiness at all refinement levels
	Specific volume	Lower in unrefined samples	PB yielded the highest volume improvement
	Crumb firmness	Denser with full bran	AA improved surface appearance and crumb texture

7 Perspective and future prospects

To the best of our knowledge, comprehensive studies specifically examining the enzymatic modification of IWG flour are currently limited. This is particularly evident in the scarcity of studies investigating enzyme combinations aimed at addressing its deficiencies, which may adversely impact quality of the final product. While existing studies have primarily focused on single-enzyme treatments for dough improvement, no research has evaluated their combined impact when used in synergy. The incorporation of diverse enzymes in flour formulation presents significant advantages. These enzymes not only enhance dough workability, extend shelf life, and improve the quality of fresh products, but their protein structures are also denatured during baking, ensuring they become inactive after bread production. They are considered an effective and safer alternative to chemical additives (Palabiyik et al., 2016). However, the effectiveness of enzymatic treatments in fiber-rich dough systems such as IWG may be constrained by diffusion limitations. The high content of IDF and arabinoxylans strongly influences water distribution and dough microstructure (Liu et al., 2025). Arabinoxylans–water interactions dominate system behavior, complicating water adjustment and interpretation of functional outcomes. The strong water-binding capacity and increased viscosity of fiber-rich matrices may restrict enzyme mobility and limit enzyme–substrate accessibility, thereby reducing catalytic efficiency. These structural and mass transfer constraints highlight the need for optimized enzyme combinations (Solomou et al., 2025).

The existing literature also highlights a significant gap in understanding the measurement of α-amylase activity in IWG flour as a critical quality indicator, which is typically assessed using the falling number test. An optimal flour is characterized by a falling number ranging from 200 to 250 s. Flours with low amylase activity, indicated by a high falling number, require supplementation with additional α-amylase, such as fungal amylase, to achieve optimal properties (Shafisoltani et al., 2014). The incorporation of α-amylase (AM) facilitates the production of fermentable sugars, thereby promoting enhanced loaf volume, improved crust coloration, and superior flavor profiles. Furthermore, when used in appropriate amounts, α-amylase promotes the hydrolysis of damaged starch, leading to desirable dough rheological properties (Si and Drost-Lustenberger, 2002).

It is proposed that future studies focus on exploring the synergistic effects of combining different improvers in IWG flour, along with a more comprehensive understanding of α-amylase activity to determine the necessary optimizations during treatment processes.

7.1 Functional additives and enzymatic strategies for dough improvement

Enzyme modification represents a cost-effective approach for upgrading flour quality, as various enzymes have been demonstrated to modify their physicochemical and processing behavior properties (Huang et al., 2024). This section discusses several well-established functional additives and enzymatic treatments that have long been utilized in the baking industry, but whose renewed application in novel grain systems such as IWG may offer opportunities to overcome its weak dough structure and limited baking performance. Although these additives are not new, their mechanistic relevance and potential adaptation to IWG-based formulations represent a promising direction for future research on sustainable, perennial grain utilization.

Fungal AM is extensively utilized in bread production as an anti-stalling agent. It functions by randomly hydrolyzing starch molecules, thereby reducing their water-binding capacity and enhancing gluten hydration (Liu et al., 2023). Moreover, by breaking down damaged starch into smaller dextrins, it ensures continuous CO_2 production by yeast during fermentation, resulting in enhanced loaf volume and refined crumb texture. Additionally, this enzyme facilitates Maillard reactions, crucial for achieving crust browning and fostering the development of desirable flavors in baked bread (Shafisoltani et al., 2014). Complementary findings further support its technological benefits: in a study conducted by Kim and Yoo (2020) on frozen bread dough, AM applied at a 100 ppm level significantly increased the specific volume of baked bread by 24.5% in fresh dough and 21.9% in frozen dough, compared to untreated controls. It also markedly reduced crumb hardness by 63.4 and 58.3% in fresh and frozen doughs, respectively (Kim and Yoo, 2020).

Glucose Oxidase (GOx) is also increasingly recognized in the baking industry as a sustainable alternative to traditional chemical oxidizers, and it may offer a targeted approach to addressing the specific dough-strengthening challenges posed by IWG flour. It catalyzes the enzymatic oxidation of glucose, producing gluconic acid and hydrogen peroxide, which can promote protein crosslinking and reinforce dough structure (Bilal et al., 2023). GOx treatment enhances dough strength and stability, increases loaf volume, and improves crumb texture and softness in the final product (Sarabhai et al., 2021).

TABLE 6 Strategic comparison of processing approaches for IWG.

Product goal	Recommended strategy	Rationale
Immediate market entry, consumer acceptance priority	Blending with wheat flour (15–50% IWG)	Most reliable functionality, familiar product characteristics
100% IWG positioning, premium sustainability narrative	Flour refinement + optimized conditioner combination (e.g., AA + VWG)	Maintains IWG identity while improving functionality
Nutrition-focused products	Fermentation or minimal processing	Preserves bran-associated nutrients; fermentation may enhance bioavailability
Targeted functional improvement	Enzymatic treatment	Offers precision modification without bulk ingredient changes
Novel product formats (snacks, extruded cereals)	Extrusion processing	Leverages unique textural possibilities of extrusion

Studies conducted in rice-based dough systems have reported that GOx addition increases bread specific volume and reduces crumb hardness, with rheological analyses showing higher elastic and viscous moduli relative to untreated controls, suggesting greater resistance to deformation within the linear viscoelastic range. Although such measurements reflect small-deformation behavior and may not directly represent processing conditions, they indicate a strengthening effect on the dough matrix (Renzetti and Rosell, 2016). In another study, the addition of GOx at 1.5 U/g to potato pulp-enriched dough significantly enhanced its viscoelastic properties and strengthened the gluten network. This treatment also improved fermentation performance by increasing dough height and gas retention capacity, ultimately yielding steamed bread with higher specific volume, softer crumb texture, and more favorable sensory attributes (Cao et al., 2021). While direct rheological data for IWG dough are limited, its weak and incohesive structure suggests that oxidative crosslinking enzymes such as GOx could potentially enhance its network formation, elasticity, and gas retention. This hypothesis warrants experimental validation but highlights GOx as a promising and sustainable dough improver for IWG-based formulations.

The incorporation of xylanases (Xyl), a type of hemicellulase, enhances dough properties and improves bread quality while reducing the staling effect during storage (Sarabhai et al., 2021). These enzymes break down the xylan backbones of water-unextractable arabinoxylans, converting them into water-extractable forms (Banjade et al., 2019a; Banjade et al., 2019b). This process decreases the water-binding capacity of the insoluble fiber fraction and releases water, thereby facilitating gluten hydration. Such enzymatic modification contributes to a softer dough texture, increased loaf volume, and the formation of a more uniform and refined crumb structure (Sarabhai et al., 2021). Rheological analyses of xylanase-treated dough, as reported by Ghoshal et al. (2017), demonstrated a notable increase in extensibility (13.5–19.5 mm) and a corresponding decrease in resistance to extension (34–47 gf), compared to control samples with lower extensibility (12.9–13.7 mm) and higher resistance (54–61 gf). These findings confirm that xylanase addition results in a softer, more workable dough, attributes that are highly desirable for enhancing bread texture and overall quality.

Transglutaminase (TG) also serves as an effective dough improve primarily by strengthening protein networks through mechanisms such as fostering gluten aggregation and catalyzing protein polymerization via an acyl-transfer reaction (Banjade et al., 2019a). The incorporation of TG significantly increases dough strength through protein crosslinking (Banjade et al., 2019b). Studies have further demonstrated that TG application in wheat-based baked goods reduces the mechanical energy required for dough mixing, lowers water absorption, enhances dough stability, increases loaf volume, reinforces bread crumb structure, and improves the overall quality of products made with weak wheat flours (Renzetti and Rosell, 2016)

Ascorbic acid (AA), though not an enzyme, possesses comparable functional properties. It is widely used in bread dough systems as an oxidizing agent, akin to the role of cross-linking enzymes. By oxidizing sulfhydryl groups, AA strengthens the gluten network, leading to an improved dough structure (Dai and Tyl, 2021). Notably, research conducted by Amiri et al. (2017) demonstrated that increasing AA concentrations led to a measurable increase in the gluten index, indicating stronger gluten quality suitable for bread-making. Rheological tests further revealed that AA treatment increased resistance to extension and decreased extensibility, suggesting a firmer, more elastic dough. Moreover, AA treatment enhanced the particle size distribution and storage modulus (G') of glutenin macropolymer (GMP) gels, while reducing the gamma (γ) value, indicating a significant improvement in the stiffness and elasticity of the gluten network. These findings confirm AA's efficacy in reinforcing gluten structure, improving dough performance, and contributing to superior final product quality (Amiri et al., 2017).

So far, among the aforementioned additives, the effects of AA, TG, and xylanase on the functional properties of IWG have been assessed in single systems. However, research examining the effects of GOx and AM is lacking. Therefore, evaluating the individual effects of these two enzymes in individual systems is recommended for future research.

7.2 Combined use of additives

It is important to note that while each of the mentioned additives improves specific quality attributes in breadmaking, their individual application can also introduce undesirable effects. For example, the use of GO at higher concentrations tends to excessively strengthen the gluten network through oxidative cross-linking, resulting in increased crumb firmness and chewiness. Similarly, high levels of AM can lead to excessive starch degradation, which contributes to dough softening and overbrowning of the crust due to the accumulation of reducing sugars. Xylanase, although effective in breaking down arabinoxylans and improving loaf volume, may significantly increase dough stickiness when applied alone in high doses, thereby complicating dough handling (Eugenia Steffolani et al., 2012). Utilizing these additives in binary, ternary, or multiple combinations could potentially mitigate these negative effects while optimizing their beneficial contributions.

The adverse effects associated with the use of single dough conditioners, such as Xyl, can be mitigated by employing a combination of multiple bread improvers (Dai and Tyl, 2021). Although a high dosage of pure Xyl may increase bread volume, it also results in excessive dough stickiness, making the dough difficult to process. However, incorporating even a small amount of fungal AM alongside Xyl not only reduces the required dosage of Xyl and enhances volume expansion but also improves overall dough quality while eliminating undesirable stickiness. Studies have also shown that the synergistic use of Xyl and fungal AM refines the crumb structure, resulting in thinner cell walls and a smoother, silkier crumb texture (Si and Drost-Lustenberger, 2002). In their study, Si and Drost-Lustenberger demonstrated that this synergistic enzymatic effect significantly improved both the specific volume index and crumb structure of bread (Figure 3). The most favorable results were achieved using 120 FXU/kg of xylanase and 10 FAU/kg of fungal α-amylase, which yielded the highest volume index and the finest crumb texture. This enzyme combination outperformed individual applications, resulting in markedly improved bread softness, a more uniform crumb structure, and sensory attributes consistent with high-quality baked products.

Response surface methodology (RSM) can be utilized to study the effects of combining different improvers. As a statistical technique, RSM is designed to analyze the interactions between multiple independent variables, or factors, and one or more response variables to identify optimal results (Boakye et al., 2022). Using this method, Eugenia Steffolani et al. (2012) investigated the effects of combining GO, AM, and Xyl on the properties of dough and the quality of bread produced from wheat flour. In their initial optimization study, the optimal levels were identified as 0.0026 g Gox, 0.016 g Xyl, and 0.01 g AM per 100 g of flour. This combination resulted in bread with a specific volume 43% greater than the control (enzyme-free) and a uniform, soft crumb texture. In a subsequent optimization aimed at maximizing specific volume while minimizing crumb firmness, chewiness, and dough stickiness, the optimal formulation was adjusted to 0.0037 g Gox, 0.0089 g Xyl, and 0.0105 g AM per 100 g of flour. While this second combination produced bread with a slightly lower volume (37% above the control), it significantly reduced dough stickiness, thereby improving dough handling and processability. Overall, the study demonstrated that intermediate levels of the three enzymes synergistically reduced stickiness, enhanced volume, and improved crumb uniformity and softness (Eugenia Steffolani et al., 2012). In a similar study conducted by Shafisoltani et al. (2014) the optimal doses of AM, GO, and Xyl enzymes were determined to be 5 ppm, 30 ppm, and 20 ppm, respectively.

Given the significant research gap in this field, a comprehensive investigation is warranted to identify the optimal formulation by analyzing the complete rheological, physicochemical, and textural properties of final products, such as baked goods, pasta, macaroni, and confections made from IWG flour. Indeed, much research throughout the supply chain is required in order to fully realize Kernza's potential in the martketplace, some of which are illustrated in Figure 4.

8 Constraints beyond agronomy and processing

Beyond agronomic performance and food-processing challenges, broader adoption of IWG in grain and food markets is influenced by regulatory, safety, and supply-chain considerations. From a regulatory perspective, IWG has achieved food use acceptance in North America; however, pathways to market differ internationally. In Europe, for example, Kernza has been evaluated under the European Union's Novel Food framework, with a recent scientific opinion issued by the European Food Safety Authority (EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), 2025). Such assessments play a critical role in determining allowable food categories, labeling requirements, and conditions of use, and they directly impact the pace and scale of adoption across not only EU member states, but in many countries. Regulatory clarity will therefore essential for encouraging private-sector investment and for enabling food manufacturers to integrate IWG into commercial product lines with confidence (Cureton et al., 2023).

Supply-chain and production scale constraints further shape the feasibility of widespread adoption. Current Kernza production remains severely limited relative to conventional cereals, with geographically concentrated acreage, variable year-to-year yields, and a slowly developing post-harvest infrastructure (Lanker et al., 2020; van der Pol et al., 2022). Specific challenges include seed availability, grain cleaning and milling capacity, consistency of quality specifications, and the logistics of aggregating relatively small volumes for food manufacturers. These factors contribute to high ingredient costs and limit participation by large-scale processors. Continued expansion will likely require coordinated advances in breeding for yield stability, investments in dedicated supply chains, long-term grower contracts, and alignment between breeders, farmers, processors, and end users (Culman et al., 2023). Addressing these adoption constraints alongside agronomic and processing improvements is critical for transitioning Kernza from a niche perennial grain to a broadly utilized component of sustainable food systems.

9 Conclusion

This review has provided a comprehensive exploration of Intermediate Wheatgrass (*Thinopyrum intermedium*), a perennial

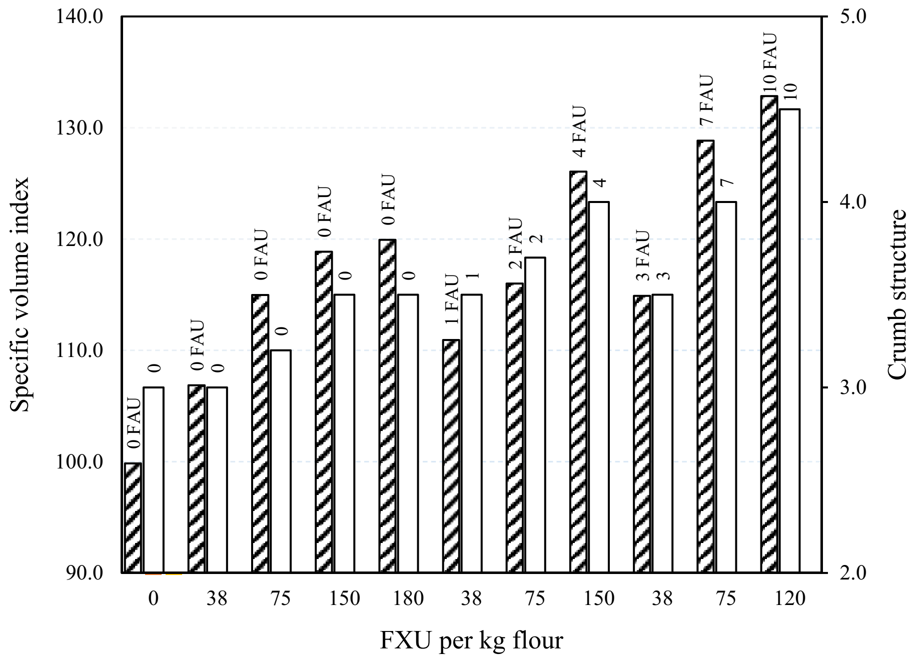

FIGURE 3
Comparative effects of Xyl alone and in combination with AM. ▨ volume index: ☐ crumb structure. FXU, fungal xylanase units per kilogram of flour; FAU, fungal α-amylase units per kilogram of flour (adapted from 77).

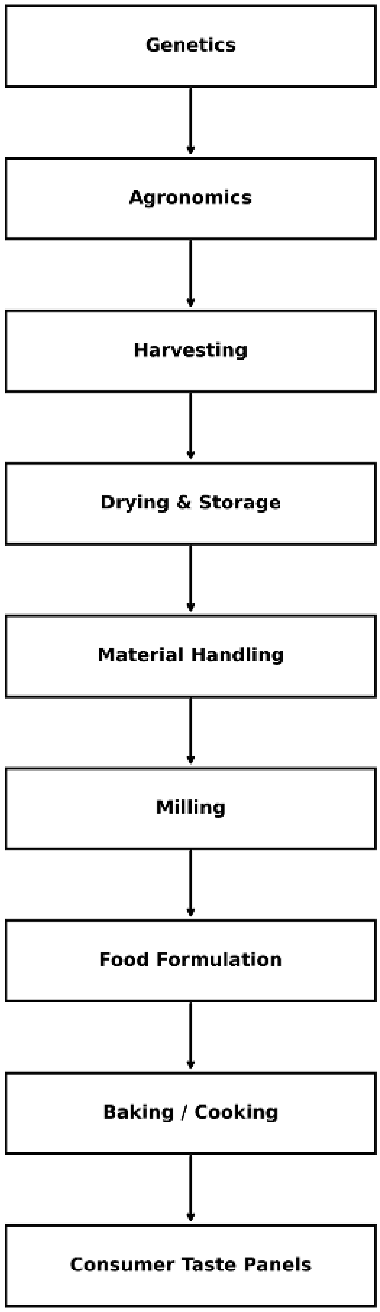

FIGURE 4
Research trials need to be conducted throughout the grain supply chain in order to optimize its use in human foods.

grain that holds significant potential for sustainable food production. The agricultural and environmental benefits it offers, including winter hardiness, high biomass yields, reduced soil erosion, decreased reliance on nitrogen fertilizers, enhanced soil health, and lower nutrient runoff, position this perennial grain as a promising candidate in promoting sustainable farming practices. However, current grain yields remain substantially lower than annual wheat (approximately 25–30%), with additional declines observed in multi-year stands, posing significant challenges for economic competitiveness and large-scale adoption. Nutritionally, IWG flour demonstrates notable advantages compared to annual grains, offering higher protein content (20%) and dietary fiber (16.4%), while also containing beneficial phytochemicals that support health and reduce the risks associated with obesity and chronic diseases. Nevertheless, its deficiency in high-molecular-weight (HMW) glutenins leads to inferior dough extensibility and negatively impacts the quality of final products. These limitations currently constrain its viability as a large-scale commodity crop.

While advancements in breeding and agronomic practices have improved both yield and seed size, there is a critical need for innovative strategies to address its nutritional and functional shortcomings. Current approaches, such as tempering, blending with wheat flour, and the use of dough conditioners, have shown promise but require further optimization. Notably, future research efforts should prioritize innovative methods, including optimizing enzymatic treatments and exploring the synergistic effects of dough conditioners. Moreover, gaining insights into α-amylase activity in IWG flour is crucial for enhancing its functional properties and processing performance. To more fully realize the potential of this perennial grain, extensive studies are necessary to investigate the rheological, physicochemical, and textural properties of IWG-based products. By addressing these gaps, IWG may strengthen its role within sustainable diets and value-added food systems, although its broader commercial integration will depend on long-term improvements in yield stability, cost competitiveness, and supply-chain development.

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