

High-Amylose Wheat Foods: A New Opportunity to Meet Dietary Fiber Targets for Health

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ABSTRACT

Poor diet is recognized as a major risk factor that can be modified to prevent the growing prevalence of noncommunicable diseases globally and the deaths attributed to them. Enhancing the nutritional quality of staple foods such as cereals offers a promising strategy for addressing poor diets. Whole grain wheat is of particular importance in this strategy because of its well-established health-promoting potential and its versatility as an ingredient, which can be used to produce foods that appeal to consumers. With this in mind we utilized wheat breeding strategies to develop a wheat with a high amylose content (>80%) in the starchy endosperm and have shown that this improves indices of glycaemic and digestive health. Testing revealed the high amylose content resulted in significantly more resistant starch (RS) in breads and popped wheat (>200% more RS), udon noodles (60-fold more RS), and ramen noodles (15-fold more RS) than was found in equivalent products made using conventional wheats. These increases in RS were obtained using refined (white) high-amylose wheat (HAW) flour, which did not compromise processing, end-product quality, or sensory properties. Further product development and clinical intervention trials will expand the range of foods that can be made with HAW and provide a deeper understanding of the benefits HAW can provide for improving health and preventing noncommunicable diseases.

The health benefits of plant-based foods—fruits, vegetables, nuts, legumes (e.g., soybeans, peanuts, pulses), and whole grain cereals—are well established and widely acknowledged, yet very few people consume enough of these foods to meet the dietary recommendations established by health authorities (3,21,26). Furthermore, government initiatives to encourage people to eat a health-promoting diet and increase their physical activity have had limited success (13,15). New approaches that tailor the composition of plant-based foods to the eating habits, lifestyles, and nutritional and health requirements of contemporary consumers are required to provide practical approaches to address what is a seemingly intractable public health problem.

Dietary fiber is a component that is strongly associated with the health benefits of plant-based foods (16,22,24,41). In particular, dietary fiber from cereals is more effective in protecting against lifestyle-related, noncommunicable diseases than are fibers from vegetables and fruit (24,28). It is important to note that the evidence primarily comes from large prospective studies of North American and European populations in which wheat was the predominant cereal consumed and, hence, source of fiber in the diet (18). Indeed, wheat-based foods are ubiquitous in Western diets, and although the recent trend toward consumption of gluten-free foods is lessening consumption of wheat-based foods in Western societies, this is not true elsewhere, with the popularity of wheat-based foods continuing to rise globally (20,40). Despite decades of public health campaigns promoting the benefits of consuming whole grains, however, many consumers still prefer and buy foods made from refined (white) wheat flour due to their greater sensory appeal (16,29,39).

Although wheat grain composition is generally comparable to that of other cereals, wheat is one of the best sources of total dietary fiber, containing 30–100% more fiber than other economically important cereals, such as rice, maize, barley, oats, rye, sorghum, and millets (19,27). Cereal fiber diversity is limited, however, comprising mostly insoluble fibers in the form of arabinoxylans. Fructans, galactans, and β -glucans are also present but at much lower levels. The resistant starch (RS) content of mainstream cereals, including wheat, is low because the starches they contain are extensively digested in the small intestine. Starches in popular conventional cereal-based foods are rapidly digested in the upper gut, eliciting a sharp and pronounced rise in blood glucose, which is associated with an increased risk for developing metabolic and cardiovascular disorders (12). Conversely, consumption of starches that are digested slowly or not at all in the small intestine are associated with a variety of health benefits (8,25,37).

Given the versatility of wheat as an ingredient, dietary prominence of this grain, and popularity and appeal of wheat-based foods, targeted changes in the nutritional content of wheat has the potential to significantly improve the health status of individuals and broad populations. Wheat flour is an ideal vehicle for improving the nutritional quality of food supplies, particularly in countries in which the quantities and types of fibers consumed are less than optimal and diet-related health problems are prevalent.

Most of the fiber in grains is lost during milling to produce refined cereal flours. Nutritional manipulation of the wheat endosperm provides an opportunity to greatly expand the range of health-promoting foods that can be produced, in particular from white wheat flour. Elevating the proportion of amylose in the endosperm results in more starch that is less digestible, a greater RS content (32,34,36), and, accordingly, a lower available carbohydrate density and glycemic load. RS also yields less metabolizable energy (23). However, for these benefits to be realized more broadly, beyond a small niche of health-conscious

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consumers, any changes in the physiochemical characteristics of nutritionally improved grains must not compromise the processability or sensory appeal of the new and reformulated end products that are made from them.

Starch Molecular Structure, Digestibility, and RS

Starch, the dominant component of grain, provides a plethora of possibilities for modification, enabling the expansion of wheat grain applications with improved product functionality and nutritional benefits. Amylose and amylopectin, the building blocks of starch, are made up of glucose backbones connected through α -1,4 and α -1,6 linkages. The α -1,4 linkage generates linear chains, whereas branch points are created by α -1,6 linkages. Generally amylose is a mainly linear molecule that has fewer than 1% branches, unlike amylopectin, which is highly branched (almost sixfold more branches). The spatial location of these two polymers, their order, and the arrangement of the chains within starch granules govern the semi-crystalline structure and unique functionality of a starch (9,10). Conventional starches are comprised of about 25% amylose; the remainder is amylopectin. Amylose forms long-chain, double-helical crystallites, as well as single-helical inclusion complexes. The glycosidic bonds in these structures are difficult for digestive enzymes (e.g., α -amylases) to access. Conversely, amylopectin, which forms shorter chains (that also form double helices) and more abundant nonreducing ends, is more susceptible to amylolysis. In addition, linear amylose recrystallizes during and after processing, forming double helices that produce retrograded starch that is resistant to enzymatic hydrolysis.

The amylose component of starch largely accounts for its content of RS, which is starch that is not digested in the small intestine and reaches the colon intact. RS qualifies as dietary fiber, and there is a growing body of evidence supporting the role of this type of fiber in promoting metabolic and digestive health. Increasing the amylose content in wheat grain, therefore, presents an attractive proposition for plant scientists.

Development of High-Amylose Wheat and Potential Health Functionality

Starch synthesis involves multiple metabolic pathways and steps, including chain elongation, branching, and debranching (Fig. 1). Several isoforms of starch synthases (SSs), starch branching enzymes (SBEs), and starch debranching enzymes, along with some other minor enzymes, act collectively in amylopectin synthesis. Amylose synthesis is more straightforward, essentially involving a single major enzyme, granule-bound starch synthase (GBSS). Branching enzymes are also involved to some extent (35), as is a protein targeting to starch gene (38). Regulatory elements controlling amylose and amylopectin synthesis are also being revealed in the literature (14).

Impairment in the function of genes encoding the various starch biosynthetic enzymes causes either subtle or major alterations in the structure of starch. Strategies have been defined to alter (either elevating or depleting) amylose content in cereals, including corn, rice, and wheat. The breeding approaches used to advance these strategies are much simpler for corn and rice, due to their diploid nature (one genome), than for bread wheat, which is a hexaploid comprising three complete genomes. For example, development of waxy cereals (amylose-free starch), involving downregulation of one gene (GBSS) was easier in maize because only two alleles from its genome needed to be down-

regulated. To develop waxy wheat, six alleles (two per genome) needed to be downregulated.

Amylose, at the levels present naturally in wheat, produces ~1% RS. Elevation of RS content in the grain can be achieved by increasing the proportion of amylose or amylose-like molecules using three possible mechanisms: reduced SSIIa activity, enhanced GBSS activity, or hindered SBEII activity. The highest level of amylose attained in wheat involved cosuppression of two isoforms of SBE (SBEIIa and SBEIIb) using a traditional breeding approach. In 2006, CSIRO (Commonwealth Scientific and Industrial Research Organisation, Australia), Limagrain Céréales Ingrédients (France), and GRDC (Grains Research & Development Corporation, Australia) formed a joint venture company (Arista Cereal Technologies) to develop, patent, and commercialize high-amylose wheat (HAW). Combining null alleles of SBEIIa from all three wheat genomes together with one null allele of SBEIIb from one genome resulted in a grain with >80% amylose content (32).

The minimum level of amylose required to produce a significant improvement in health outcomes is ~60% (7). Much higher amylose (and accordingly RS) levels are preferable to allow for greater flexibility when (re)formulating foods and to accommodate RS losses during food manufacture and preparation.

Preliminary feeding trials in animals provided evidence that HAW contained markedly elevated levels of RS and induced meaningful changes in physiological and biochemical markers of digestive health (33). These findings are consistent with other studies demonstrating the benefits of RS, in particular its fermentation by the saccharolytic microflora to create a healthy intracolonic environment, including raised butyrate levels. Subsequent preclinical studies in rats confirmed the results of earlier work (17) and also highlighted the capacity of HAW to improve metabolic health (11).

The potential of HAW to deliver substantial quantities of RS to the colon was confirmed in a study with human ileostomates (D. Belobrajdic, A. Regina, and T. Bird, *unpublished data*). Recent acute clinical studies in healthy men and women have shown that HAW lowers glycemic response. Prototype whole-meal (whole wheat) or refined breads formulated with HAW reduced peak blood glucose concentrations and area under the 2–3 hr glycemic response curves relative to comparable breads made with conventional wheat flours. It is noteworthy that post-

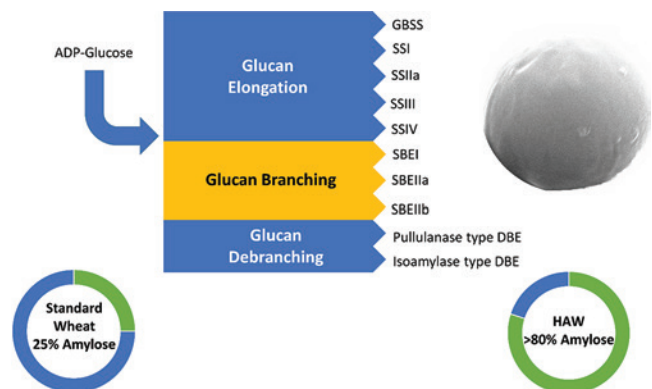


Fig. 1. Diagrammatic representation of enzymes responsible for starch biosynthesis within the amyloplast organelles of wheat endosperm cells. ADP-Glucose: adenosine diphosphate glucose; GBSS: granule-bound starch synthase; SS: starch synthase enzymes; SBE: starch branching enzymes; DBE: starch debranching enzymes; HAW: high-amylose wheat.

prandial insulinemic response to the high-amylose test meals was also attenuated, with the magnitude of the reduction similar to that of glucose (D. Belobrajdic, A. Regina, B. Klingner, I. Zajac, S. Chapron, P. Berbezy, and T. Bird, *unpublished data*). Dampening acute glycemic response without disproportionately increasing gut hormonal responses or increasing demands on the pancreas for insulin is associated with improved glucose homeostasis and reduced risk of developing type 2 diabetes and other chronic diseases.

Clinical trials performed to date have focused on breads made with HAW flour, but a wide range of convenience food products have been developed using HAW flour that are also significantly higher in RS and, predictably, total dietary fiber (TDF).

HAW Food Products

Having successfully developed wheat lines with very high amylose contents, Arista has partnered, for instance, with Limagrain Cereal Seeds (LCS) to breed HAW commercial lines for the North American market (Fig. 2). Once HAW lines are available, it is necessary to determine how well they perform in cereal-based products, in terms of both processing and consumer acceptability. Arista has evaluated HAW performance in a variety of cereal-based foods. The primary focus initially was on the



Fig. 2. Plots of HAW (high-amylose wheat) lines growing in a field nursery in the northwestern United States.



Fig. 3. Top and front views of breads made with different incorporation levels of HAW (high-amylose wheat) flour (left to right: 0, 60, 80, and 100% of total flour content).

bread market, but other cereal-based foods, including rolled wheat flakes, scones, pizza crusts, noodles, and extruded and popped wheat products, have also been investigated.

Breads. Sandwich-style, lidded-pan breads, which constitute the largest component by volume of bread markets in most countries, have been made with refined (white) HAW flour at incorporation levels of 60, 80, and 100% of total flour content. All HAW incorporation levels produced doughs with good handling and normal consistency properties; dough extensibility during shaping increased with HAW incorporation level. The HAW breads were very similar to the control in terms of visual appearance and had a slightly larger volume (Fig. 3). Moreover, despite the significant differences in starch composition, the flavor and texture of the HAW breads were as good as the control.

The fiber content of HAW breads was measured using AOAC Method 2011.25 (AACCI Method 32-50.01) (1,2,31), which measures all types of RS. Breads made with HAW flours showed an increase in fiber content, ranging from 170% (60% incorporation ratio) up to 218% (100% incorporation ratio) (Fig. 4).

Use of white HAW flour in bread formulations allows for the production of white bread with a high fiber content, without the use of modified ingredients and with no detrimental effects compared with conventional white bread with a low fiber content. This suggests that high-fiber HAW breads could have greater consumer acceptance than that of high-fiber wholemeal breads, which are usually perceived as having a more bitter flavor than white breads (5), and have a fiber content between 6 and 8% (AOAC Method 2011.25 [AACCI Method 32-50.01] [1,2,31]) depending on the wheat variety (data not shown). Hence, consumption of HAW white bread could assist in increasing dietary fiber intake in the diet, particularly among consumers who avoid wholemeal (whole wheat) breads.

Noodles. The performance of HAW flour has been investigated in three different types of noodles: Japanese udon, ramen, and fried instant noodles (Fig. 5). The noodles were formulated

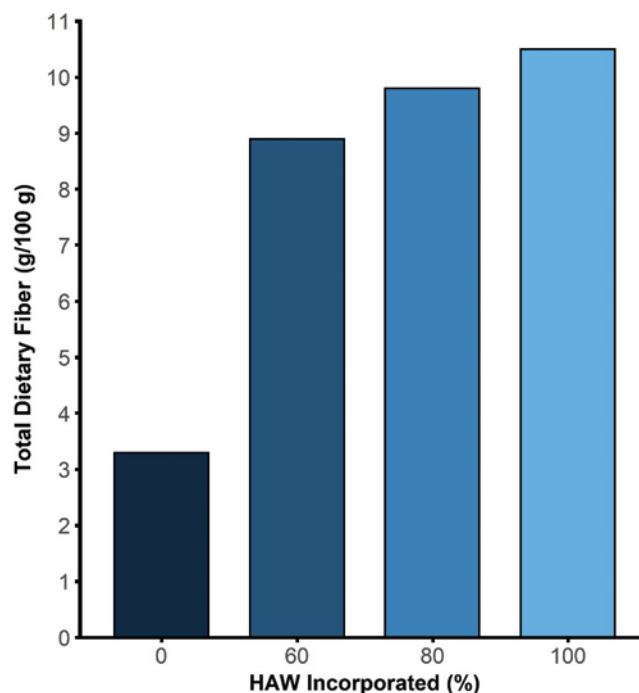


Fig. 4. Dietary fiber content in breads made with different HAW (high-amylose wheat) flour incorporation ratios, as determined using AOAC Method 2011.25 (AACCI Method 32-50.01) (1,2,31).

from refined (white) HAW flour, with an incorporation rate of 60% HAW flour and 40% control (conventional) wheat flour specific to each type of noodle. HAW flour increased the firmness of each style of noodle, which is an advantageous property for ramen and instant noodles. RS levels (as measured by AOAC Method 2002.02 [AACCI Method 32-40.01] [1,2,30]) in control noodles ranged from 0.1 to 0.5% (udon had the lowest RS content and ramen had the highest). In comparison, there was substantially more RS in noodles made with HAW flour. Instant noodles had ~17 times more RS when HAW flour was incorporated compared with the corresponding control product. The increase in RS with HAW flour incorporation was greatest for udon noodles (almost 60-fold) and least for ramen noodles (~15 times).

The TDF values for the control noodles, as measured by AOAC method 2011.25 (AACCI Method 32-50.01) (1,2,31), ranged from 2.1% in udon noodles to 15.3% in ramen noodles. With 60% HAW flour incorporation, TDF content increased by ~3 times in instant noodles, ~7 times in udon noodles, and ~2 times in ramen noodles. Thus, irrespective of the type of noodle, inclusion of HAW flour greatly increased the levels of RS and, not unexpectedly, TDF compared with the respective control products.

Popped Wheat Grain. Popped wheat grain can be used in applications ranging from cereal bars to toppings and can be a simple way of increasing the fiber content of everyday food products. HAW grains were popped by spinning the grains at 240°C for 35 sec using a home corn-popping machine. HAW and standard wheat grains popped during the process acquired a crunchy texture that was more pronounced for the HAW grain. The flavor developed in HAW popped grains was pleasant and similar to that of the popped standard wheat.

The HAW popped grains had a fiber content (as measured by AOAC Method 2011.25 [AACCI Method 32-50.01] [1,2,31]) that was 2 times greater than that of the popped control grains: 30.8 and 16.2 g/100 g, respectively. HAW popped grains constitute another alternative for providing more fiber for consumers and is a good illustration of the versatility of HAW grain.

These investigations of the performance of HAW in a range of cereal food products demonstrated that there were no negative texture or flavor attributes arising from the greatly elevated amylose content of HAW. Furthermore, the ability of HAW to be incorporated into baked products and a range of different

foods shows there are no major technical issues regarding processing performance or end-product quality that would constrain the use of HAW in a variety of everyday foods. In 2017 Bay State Milling (Quincy, MA, U.S.A.) established the HealthSense™ banner (6) to market HAW in North America. Further HAW products are anticipated to reach the consumer market in the near future.

Conclusions

Increasing the nutritional quality of foods people routinely choose to eat is a direct, and likely more effective, approach to improving public health. Cereals are central to the diets of most people (4), and wheat, because it is ubiquitous in diets globally, is a prominent target. Minor changes to the nutritional content of this grain could greatly improve diet quality and, consequently, the overall health of many populations. Increasing the amylose content of the starchy endosperm of wheat to a level that generates a physiologically meaningful increase in RS content has been shown to effectively improve indices of glycemic and digestive health. These benefits were not just confined to whole-meal HAW products but were observed for products made of refined HAW flour as well. Wheat breeding strategies specifically targeting the endosperm permit a greater range of foods to be developed that could help ensure people consume sufficient quantities of dietary fiber in their diets. HAW flours can be readily incorporated into a range of everyday foods, including staples such as breads and noodles, without jeopardizing end-product quality. Further product development and clinical substantiation studies are in the pipeline to extend the range of foods that can be made with HAW and to understand their health benefits.

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Conflicts of Interest

A. Regina, A. Bird, and P. Berbezy are coinventors on patents relating to high-amylose wheat.

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Fig. 5. HAW (high-amylose wheat) Japanese noodles prepared for sensory and instrumental quality evaluation.

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Pierre Berbezy is currently the research and development manager for innovation raw materials with Limagrain Céréales Ingrédients. He is a plant biochemist working on carbohydrate metabolism and starch functionality in cereal-based products.

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Sophie Chapron is currently the bakery applications specialist manager with the Limagrain Céréales Ingrédients' Baked Goods Department in France.

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Ahmed Regina is a principal research scientist at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia. Her primary research goals are directed toward understanding the genetic basis of starch biosynthesis in cereal grain and modifying grain components for enhanced nutritional benefits and processing quality. Regina has made major research and research management contributions to the high-amylose wheat (HAW) program of Arista Cereal Technologies (a joint venture partnership between CSIRO and Limagrain Céréales Ingrédients). Regina's project leadership took the HAW program through various stages of development, from unravelling the genetics, to proof of concept studies in transgenic systems, to biochemical characterization and generation of totally non-GM HAW lines with enhanced levels of resistant starch (RS), to nutritional substantiation, through to establishing processing quality for product development. Regina is an AACCI member and can be reached at ahmed.regina@csiro.au.

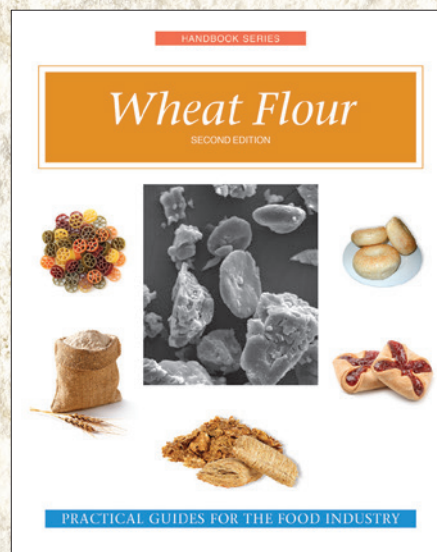
Tony Bird is a principal research scientist with Commonwealth Scientific and Industrial Research Organisation (CSIRO) Health & Biosecurity, where he leads multidisciplinary research teams investigating the role that dietary constituents play in human health and wellbeing. He received B.Agr.Sc. and M.Agr.Sc. degrees from La Trobe University (Victoria, Australia) and earned his doctoral degree in gut physiology from North Carolina State University. His research efforts over the last two decades have centered on determining the nutritional and health benefits of cereals and grain components, their potential for modifying risk of bowel and metabolic diseases, and application of this knowledge to foster development of improved cereal-based foods that deliver substantiated health benefits to consumers.

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