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Linda Malcolmson

Mike Sissons

Advances in the Breeding and Processing of Grains and Pulses

Linda Malcolmson and Mike Sissons
Guest Editors

Global demand for healthier and high-protein, plant-based foods continues to inspire cereal scientists to innovate with grains and pulses. Many consumers are seeking gluten-free, whole grain, and low-glycemic foods, as well as vegetarian and vegan-based diet options, but they don't want to give up taste or variety in their food experiences. Cereal scientists are accepting this challenge by exploring novel solutions from the seed to the finished product. In this issue of *Cereal Foods World* (*CFW*), we explore what's new in the breeding and processing of grains and pulses and how these innovations are delivering new consumer benefits.

In the area of breeding, scientists are using novel tools, as well as conventional methods, to produce new varieties of grains and pulses that deliver improved nutritional benefits, such as higher levels of dietary fiber and lower levels of gluten. In addition, these innovations allow the formulation of foods without the need to add other ingredients during processing, which supports the clean label trend.

With greater detection of celiac disease and greater awareness and incidence of non-celiac gluten sensitivity, there continues to be a demand for gluten-free products. Development of a celiac-safe wheat is a long way off. However, Crispin Howitt at CSIRO and his coauthors have worked on developing a barley variety (Kebari®) with a low gluten content (below the 20 ppm limit) that can be used in a range of gluten-free products. One of these products is a gluten-free barley-based beer (Pionier, Radeberger). The feature article, "Gluten Reduction Strategies for Wheat and Barley," shares their work.

By increasing the amylose content in the wheat endosperm through breeding, Marcus Newberry and colleagues from CSIRO and Limagrain Céréales Ingrédients have enhanced the resistant starch (dietary fiber) level in wheat grain with minimal impact on product taste and acceptability. The feature article, "High-Amylose Wheat Foods: A New Opportunity to Meet Dietary Fiber Targets for Health," illustrates another example of how breeding can be used to tailor a wheat to improve a nutritional component (fiber) of a common food source (wheat).

The feature article, "Glabrous Canary Seed: A Novel Food Ingredient," reports on the development of an annual glabrous (hairless) canary seed that has been approved for food use in Canada and the United States. Carol Anne Patterson from Pathfinders and her coauthors highlight the nutritional properties of this new canary seed, which can be used in a variety of foods with minimal impact on flavor and texture.

Martin Scanlon from the University of Manitoba and his coauthors share their review on pulse flour milling, "The Critical Role of Milling in Pulse Ingredient Functionality." They discuss how different wheat milling techniques can be applied to pulse milling to create value-added pulse ingredients. They also explore factors that affect the milling quality of pulses.

Further innovation in foods and assurance that a new product makes it to the marketplace can come from communicating protein levels to consumers. Unfortunately, current regulatory frameworks in Canada and the United States may limit protein content claims for plant-based protein foods. In the final feature article, "Navigating Protein Claim Regulations in North America for Foods Containing Plant-Based Proteins," Chris Marinangeli from Pulse Canada and his coauthors outline the challenges encountered in navigating the current regulatory frameworks for protein content claims in North America.

Exploring another health trend in their article, "Purple and Blue Wheat—Health-Promoting Grains with Increased Antioxidant Activity," Heinrich Grausgruber and colleagues at the University of Natural Resources and Life Sciences, Vienna, discuss the development of purple and blue pigmented wheat varieties that contain high levels of anthocyanins with beneficial antioxidant properties. These novel wheats are being incorporated into an array of innovative products to boost their health attributes.

Advancements in faba bean breeding have led to the development of new varieties with lower levels of vicine and convicine, which can be potentially toxic for humans who have a specific enzyme deficiency (variants of glucose-6-phosphate dehydrogenase [G6PD]). Constance Chiremba with the Saskatchewan Pulse Growers and her coauthors address how these new faba bean varieties are expected to increase faba bean production and their use in foods in their hot topic article, "New Opportunities for Faba Bean."

In the Spotlight section of this issue, we are excited to profile the International Maize and Wheat Improvement Center (CIMMYT) and long-time AACCI member Julie M. Jones. *CFW* readers may recall the popular series of nutrition articles contributed by CIMMYT staff and Julie over the last few years. Headquartered near Mexico City, CIMMYT is a global leader in publicly funded maize and wheat research. Julie is a distinguished scholar and professor emerita at St. Catherine University in St. Paul, MN; former president of AACCI; and regular contributor to *CFW*.

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Gluten Reduction Strategies for Wheat and Barley

Crispin A. Howitt,[1] Philip J. Larkin,[1] and Michelle L. Colgrave[2]

Celiac disease is a T-cell mediated autoimmune disorder triggered by ingestion of cereal gluten found in wheat (gliadins and glutenins), barley (hordeins), and rye (secalins). Clinical symptoms of celiac disease are diverse and include flatulence, bloating, fatigue, indigestion, diarrhea, abdominal distension/pain, weight loss, low bone mineral density, anemia, irritability, anxiety, depression, and neurological disorders (23). Celiac disease affects approximately 70 million people or approximately 1% of the global population (15), and an additional 5–10% of the population is affected by non-celiac gluten sensitivity (2,22). Although the roles of gluten and the epitopes that trigger celiac disease are becoming well understood (34,37), the cause of non-celiac gluten sensitivity is not yet understood, with some studies suggesting a role for gluten (5,12), while others point to fermentable short-chain carbohydrates (6,38) or α-amylase/trypsin inhibitors (25).

For both conditions strict avoidance of gluten-containing grains is the recommended treatment. In addition to these two conditions there is a growing number of consumers who believe gluten-free diets are healthier for them, despite the fact there is no apparent clinical reason to avoid gluten. Greater awareness of celiac disease and non-celiac gluten sensitivity has led to rapid growth in the gluten-free market segment. In 2013 the global gluten-free market was estimated at approximately US$3.8 billion and anticipated to grow to US$6.9 billion by 2019 (28).

This rapid growth in the market has led to a greater number and more diverse range of offerings entering the market, which gives consumers who avoid gluten greater choice. These foods are still expensive, however, and may be nutritionally inferior to gluten-containing products. A recent study in the United Kingdom compared the cost and nutritional value of 679 gluten-free products and 1,045 comparable regular (gluten-containing) products (16). On average the price premium for the gluten-free foods was 159%, and more of the gluten-free foods were classified as containing medium or high levels of salt, sugar, fat, and saturated fat compared with the regular foods. Additionally the gluten-free items were more likely to be lower in fiber and protein than the regular foods.

These differences in the nutritional properties of gluten-free foods, as well as anecdotal reports of the poor taste and texture of gluten-free foods, have led a number of research groups to investigate ways in which gluten could be reduced or removed from wheat and barley to provide new higher fiber and better tasting options for those who must or who choose to avoid gluten in their diet.

Wheat

In wheat the majority of the known epitopes that are immunogenic for those with celiac disease are present in the gliadins (34,37), and thus, much of the research effort has been focused on strategies to identify or develop lines with lower gliadin contents. Initial studies focused on identification of lines with lower gliadin content. In early studies lines that lacked the locus on chromosome 6A that encoded the α-gliadins were identified (26); however, adverse reactions, similar to those expected upon ingesting gluten, were noted in celiac patients fed bread made from this wheat (8,9). This suggests that loss of this locus alone is insufficient to make wheat suitable for people with celiac disease.

Lines that lacked the locus that encodes α-gliadins on chromosome 6D were shown to have a significantly reduced number of T-cell stimulatory epitopes present. However, it was demonstrated that the reduction in these epitopes resulted in a loss of dough functionality (41). Based on this result it was hypothesized that durum (tetraploid) wheat might contain lower levels of celiac disease-active epitopes. Screening of tetraploid varieties with antibodies to two α-gliadin epitopes, Glia-α9 and Glia-α20, identified three durum varieties with significantly reduced levels of both epitopes (39). The same research group also compared the relative abundance of these two epitopes in modern cultivars and landraces. The modern cultivars tended to have higher levels of these epitopes than did the landraces, with a trend for the Glia-α9 epitope to be present in higher levels in modern cultivars (40).

In addition to the classical approach to identifying and then breeding for lines with reduced celiac disease immunogenic epitope contents, other research groups have focused on the use of gene technology to reduce the gliadin content in wheat. Suppression of α-gliadins by more than 60%, using RNA interference (RNAi), was compensated for by an increase in other gliadin components, low molecular weight glutenin subunits (LMW-GS), albumins, and globulins; the net result was a reduction in total gluten content of approximately 10%. When compared with the controls there was little difference in dough extensibility or resistance, but a slight decrease in loaf volume was observed (3,4).

A similar approach was used to suppress expression of γ-gliadins (24). Suppression of γ-gliadins by approximately 65% was accompanied by a small increase in α-gliadins, a 20–25% increase in LMW-GS, and a 40–50% increase in high molecular weight glutenin subunits (HMW-GS). Small-scale rheological analysis revealed that these changes had no impact on water absorption, and the dough produced was very strong with high stability but was not nearly as extensible as the control lines.

In a series of more comprehensive studies γ-gliadins were suppressed by up to 95% (19,30), which resulted in increased dough strength and enhanced stability. The same research group also developed lines in which total gliadin content was suppressed by up to 90% (20,21). Dough made from these lines was weaker, but more stable, than dough made from the controls, and loaf volume decreased by 20–30% (17,18).

More recently, gene editing has been used to modify the α-gliadins in wheat, with 35 of 45 different α-gliadin genes in one wheat line modified, resulting in a reduction in α-gliadin con-

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tent of up to 82%. In some lines γ- and ω-gliadin expression was also reduced. Analysis of the total gluten content using the R5 and G12 antibodies revealed a three- to fourfold reduction in total gluten content in these lines (31).

Rather than targeting suppression or modification of gliadins and glutenins directly, an alternative strategy is to target genes that control expression of glutenins and gliadins. Use of RNAi to suppress the gene encoding Demeter, a demethylase enzyme involved in the regulation of expression of gliadins and glutenins, resulted in a reduction of these proteins of up to 75% (42). The impact of these changes on functionality was not reported. More recently TILLING (targeting induced local lesions in genomes), a reverse genetics technique, has been used to identify mutations in all three homeoforms of the DNA-binding with one zinc finger (DOF) domain transcription factor that is involved in expression of gliadins and LMW-GS. When mutations in all three homeoforms of the DOF domain were combined in a single wheat line, the gliadin and LMW-GS contents were reduced by 50–60%. The impact on functionality was not determined (29).

Although all of these studies identified strategies to reduce the gluten content of wheat, production of a celiac-safe wheat line remains a challenge that is unlikely to be overcome in the near future. To be safe for consumption by all people with celiac disease, all epitopes that elicit a response need to be removed, and it has been shown that all classes of glutenins and gliadins contain these epitopes (34,37). Bread wheat is hexaploid and contains three related genomes that contain 20–30 glutenin genes and on the order of 100 gliadin genes across 9 loci, which together make up the gluten component of the grain (32). Thus, it is highly improbable that a gluten-free or celiac disease epitope-free wheat can be produced.

Barley

Given the complexity of the gluten-reduction problem in wheat, our research group has focused its efforts on the genetically simpler diploid species barley. The aim is to develop a novel gluten-free cereal that has a higher fiber content than currently available gluten-free cereals and that can be used to produce whole grain foods and malted beverages. In barley there are only four hordein (gluten) protein families: B-, C-, D-, and γ-hordeins. The dominant hordeins comprise two multigene families consisting of at least 13 B-hordein genes (27) and 20–30 C-hordein genes (33). Lines that do not accumulate B-hordeins, Risø 56 (27); C-hordeins, Risø 1508 (13,14); and D-hordeins, Ethiopian-derived landrace R118 (7), were identified. Using a conventional breeding strategy the low-hordein trait from each of these lines was combined into a single line, ULG 3.0 (35), which is now known as Kebari®.

In the Kebari line the gluten content has been reduced to approximately 5 ppm, which is below the 20 ppm level recommended for classification as gluten-free by the Codex Alimentarius Commission (10). Compositional analysis of Kebari flour showed there was little or no change in starch or monosaccharide content, while β-glucan content was reduced approximately threefold. Surprisingly, the protein content remained unchanged despite the loss of the hordeins that normally comprise approximately 50% of the grain protein content. This was partially due to a 10- to 15-fold increase in the level of free amino acids in the grain (35), and subsequent work has shown that the levels of some globulins increased (M. L. Colgrave, *unpublished data*). Levels of α-amylase in the grain were similar or slightly higher than in commercial controls, whereas β-amylase levels were reduced approximately 50-fold.

The initial version of Kebari was in a hulled background, which was suitable for malting and brewing, but Kebari seeds are smaller and thinner than current commercial cultivars, which creates some processing challenges. We have implemented a breeding program to improve seed size and have also developed a hull-less version that can be used as whole grain and flour in gluten-free products, for which barley has an advantage over grains like rice and corn, because it has a much higher fiber content. Small-scale processing trials have shown that Kebari can be rolled, flaked, and extruded (C. A. Howitt, *unpublished data*).

Gluten-free and Reduced-Gluten Beers

The recent rapid growth in the demand for gluten-free products has also resulted in rapid development of the gluten-free beer market. The global gluten-free beer market in 2017 was valued at US$268 million and is anticipated to grow to US$600 million by 2022 (36). There are three strategies for producing gluten-free and reduced-gluten beers. The first strategy is to use inherently gluten-free cereals (e.g., corn, rice, sorghum, millet) or pseudocereals (e.g., buckwheat). However, these products often lack the distinctive flavor and aroma imparted by malted barley. The second strategy is to remove the gluten during the brewing process, either through filtration or enzymatic digestion during fermentation of the beer. In enzymatic digestion, which is more commonly used, enzymes called prolyl endopeptidases (or PEPs) are used to cut proteins at the amino acid proline, which is present at high levels in hordeins, glutenins, and gliadins. The third strategy is to use gluten-free barley. In 2016 German brewer Radeberger launched Pionier, the first gluten-free barley-based beer brewed using Kebari.

Beers brewed using the latter two strategies are tested using a competitive enzyme-linked immunosorbent assay (ELISA) to determine that the gluten content is below the 20 ppm threshold limit recommended by the World Health Organisation (WHO). However, some questions remain as to the accuracy of these readings. In fact, the U.S. Food and Drug Administration (FDA) has recognized that current analytical methods, including competitive ELISA, are not able to accurately quantify gluten in hydrolyzed or fermented products such as beer. They ruled that beer cannot be labeled "gluten-free" unless it is made from materials, like rice, that are inherently gluten-free. As a result of this ruling, brewers instead label their beers as "gluten-reduced" or "crafted to remove gluten."

A recent study showed that some of these gluten-reduced beers triggered an immune reaction when tested against blood samples from some patients with celiac disease (1), suggesting that gluten-reduced beers may not be suitable for all people with celiac disease. To investigate this further we have used liquid chromatography–mass spectrometry to examine the gluten content in 12 gluten-reduced beers and 4 traditional beers (11). Gluten was detected in all 12 gluten-reduced beers, and in some of these beers, levels were similar to those in the traditional beers; however, in the majority of gluten-reduced beers the level of gluten was reduced (Fig. 1). The lowest level of gluten was observed in the beer brewed from Kebari.

Conclusions

The rapid growth of the gluten-free market over the last decade has led to increased efforts by both research groups and

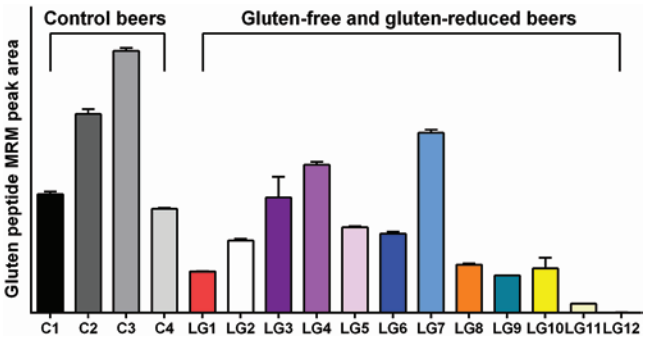

Fig. 1. Liquid chromatography–mass spectrometry analysis of gluten in four commercial gluten-containing beers (C1–C4) and a range of gluten-reduced beers (LG1–LG12). LG12 was brewed from Kebari® barley. The results indicate gluten reduction is variable, and several gluten-reduced beers yielded gluten levels similar to those of traditional barley malt-based beers. MRM = multiple reaction monitoring.

commercial entities to develop palatable, health-promoting dietary options for those who must or who choose to avoid gluten. This has led to improvements in gluten-free diets; however, these products remain expensive and may have lower fiber contents compared with gluten-containing foods. Much effort has been directed toward understanding the epitopes in gluten that cause celiac disease responses and toward strategies for developing epitope- or gluten-free wheat. In our opinion, development of a wheat line that is safe for all people with celiac disease remains an elusive goal that is unlikely to be realized in the near future. Similar strategies in barley have proven more effective, and a barley variety in which the gluten content has been reduced to approximately 5 ppm has been developed. The first product from this barley, a beer, was launched in 2016, and it is hoped that this will be the first of many new products suitable for people with celiac disease and those who avoid gluten in their diets.

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Conflict of Interest Statement

C. A. Howitt and M. L. Colgrave are coinventors on patents relating to Kebari®.

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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 glycemic 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.

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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

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 semicrystalline 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 downregulated. 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 Ingredients (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 wholemeal (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-

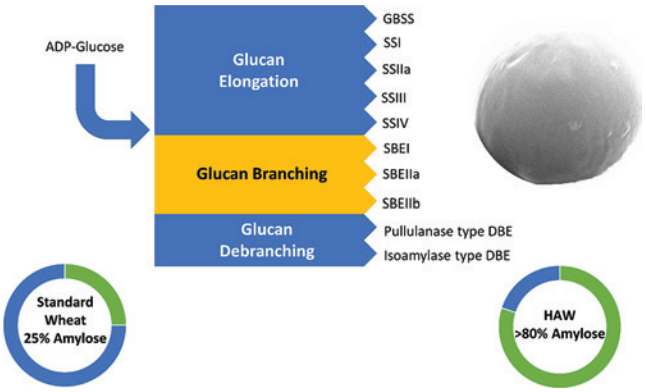

Fig. 1. Diagrammatic representation of enzymes responsible for starch biosynthesis within the amyloplast organelles of wheat endosperm cells. ADP-Glucose: adenosine diphosphate glucose; GBSS: granule-bound starch synthase; SS: starch synthase enzymes; SBE: starch branching enzymes; DBE: starch debranching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

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).

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

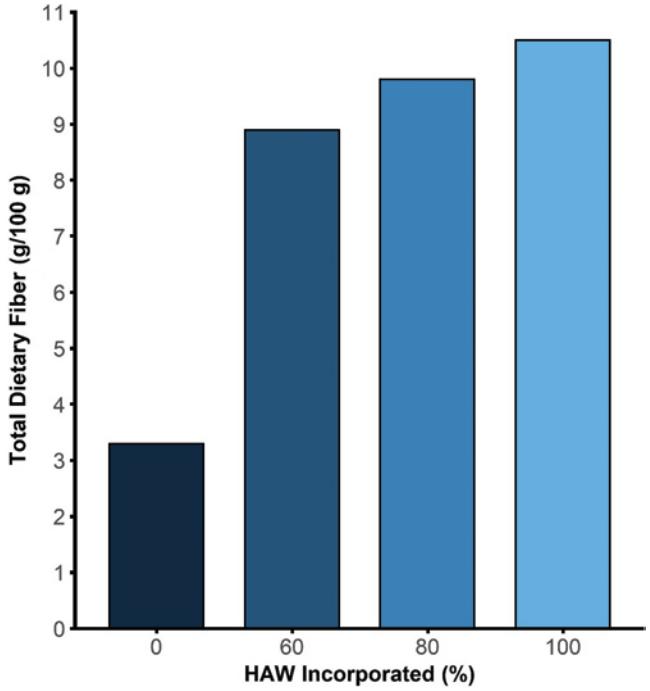

Fig. 4. Dietary fiber content in breads made with different HAW (high-amylose wheat) flour incorporation ratios, as determined using AOAC Method 2011.25 (AACCI Method 32-50.01) (1,2,31).

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.

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

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 wholemeal 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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Glabrous Canary Seed: A Novel Food Ingredient

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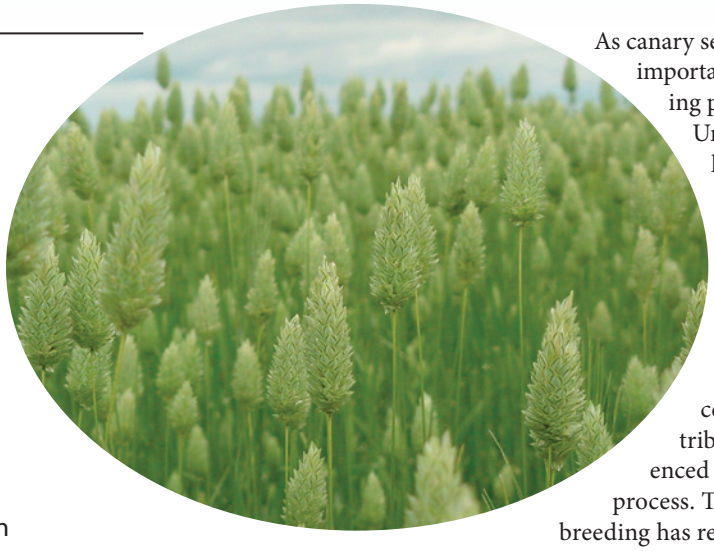

Annual glabrous canary seed is a new whole grain cereal that received novel food approval from Health Canada (11) and generally recognized as safe (GRAS) status (26) from the U.S. Food and Drug Administration (FDA) in 2015. Also known as annual canarygrass, canary seed (*Phalaris canariensis* L.) has gained commercial importance as a specialty grain crop for Canadian producers. Globally, Canada is the world's largest producer of hairy (pubescent) and hairless (glabrous) annual canary seeds. Although it is primarily used as a feed for pets and wild birds, there are opportunities to introduce glabrous canary seed grain to the food industry as a novel, nutritious food ingredient.

Investigations conducted in the 1970s first identified annual canary seed as a potential grain crop in North America (22–24). As canary seed production became more important in western Canada, a breeding program was established at the University of Saskatchewan (Saskatoon, SK, Canada) in the 1990s to eliminate hull pubescence (hairy characteristic) and brown seed color in canary seed. The small silicified hairs (trichomes) or spicules covering the hull surface of commercial pubescent canary seed cultivars contribute to skin irritations experienced by farmers during the harvest process. Two decades later, successful breeding has resulted in a portfolio of five glabrous (hairless hull) varieties of canary seed that are easier to harvest and, with regulatory approval, are now available for use in food applications. Four brown seed varieties (CDC Maria, CDC Togo, CDC Bastia, and CDC Calvi) and one yellow seed variety (CDC Cibo) are registered for commercial production (Fig. 1). Glabrous brown and yellow canary seed varieties with higher yields are also in the development pipeline.

Historical references suggest that canary seed may have originated in the Canary Islands, with some indications that canary seed was used as a food source as far back as the late 1500s, particularly in countries bordering the Mediterranean Sea. Spanish explorers may have introduced the grain to South America and Mexico (13,21,27,29). Pubescent (hairy) canary seed (*P. canariensis*) appears to have been introduced to North America in the mid to late 1800s (27), with the Canadian Ministry of Agriculture growing annual canary seed at its Indian Head Experimental Farm in Saskatchewan in the 1890s (20). Pubescent canary seed was grown commercially as a grain crop in the northern Great Plains in the Red River Valley of North Dakota and Minnesota starting after World War II, whereas commercial production of pubescent canary seed in Canada began in the 1960s in Manitoba and 1970s in Saskatchewan. Commercial production of hairless (glabrous) varieties began in Saskatchewan in the late 1990s.

The growth and development of annual canary seed is quite similar to that of wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.). It can be grown as either a spring-sown crop in regions with severe winter climates or as a winter-sown crop in milder Mediterranean climates (1). Canary seed produces small, elliptical grains with lengths and widths of approximately 4.0–5.1 and 1.5–2.0 mm, respectively (6). The glabrous grain weighs approximately 7 mg, with an average test weight of 70 kg/hL (14). Abdel-Aal et al. (3) have shown that the microstructure (bran, starchy endosperm, and germ) of glabrous canary seed is similar to that of wheat, oats, barley, and rice.

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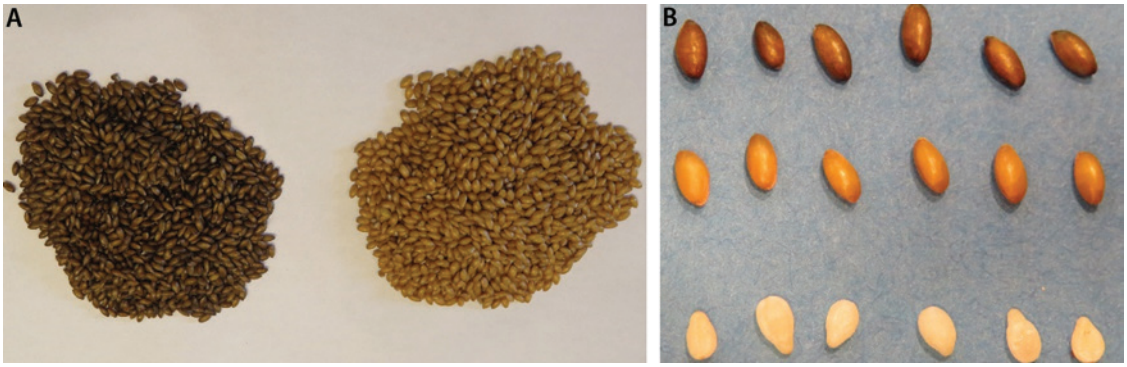

Fig. 1. A, Brown (left: CDC Maria) and yellow (right: CDC Cibo) canary seed groats. **B,** Comparison of brown (top) and yellow (center) canary seed groats and commercial sesame seeds (bottom).

Nutrient Composition

Glabrous canary seed is a highly nutritious cereal grain that contains, on a dry weight basis, 19.3–23.1% protein (higher than other common cereal grains), 55% starch, 5–7% crude fat, 6–8% dietary fiber, and 2–3% total ash in the whole grain (3). No significant difference in nutrient composition between yellow- and brown-colored grain varieties is evident (8).

Similar to other cereal grains, the proteins in canary seed are deficient in lysine; however, compared with other common cereals canary seed contains higher levels of tryptophan, phenylalanine, and cysteine (3). The major proteins found in canary seed are prolamins, which make up approximately 45.5% of the total protein, along with albumins and globulins, both of which are found at levels below those found in wheat (13.1% versus 23.6%) (1,6).

Canary seed crude fat contains about 85% unsaturated fatty acids, of which approximately 32% is monounsaturated and 55% is polyunsaturated. Like other cereals, the predominant fatty acids in glabrous brown and yellow canary seeds are palmitic (11%), oleic (29%), and linoleic (55%). Dehulled canary seeds (also known as groats) contain about 2% linolenic acid.

Canary seed groats contain 56.9–64.4% starch, which is composed of small uniform polygonal granules with A-type starch crystals (2,5). Compared with wheat starch, yellow and brown canary seed starches had higher gelatinization transition temperatures, a broader gelatinization range, and a higher swelling power and water solubility index (15). Differences have been shown between canary seed starches derived from brown and yellow seed varieties, with yellow seed varieties having higher pseudoplasticity and thixotropy properties compared with starch derived from brown seed varieties (17).

Canary seed groats contain lower levels of dietary fiber compared with most common cereals (6–8% versus 13–21% in wheat and 11–25% in oats) (25). Canary seed fiber is primarily composed of insoluble fiber, with <1% of the total fiber being soluble (3).

From a micronutrient perspective, canary seed contains the B vitamins thiamine and riboflavin at levels comparable to other cereals, but its niacin levels are lower than those found in wheat and barley. Total folate content in canary seed is higher than in wheat, barley, and oats. The predominant phenolic acids in glabrous canary seed are ferulic, caffeic, sinapic, and ρ-coumaric (4,19). Glabrous brown and yellow canary seed groats exhibit the same flavonoid profiles and are rich in flavonoid glycosides (18).

Canary seed is gluten-free (7) and can be safely consumed by individuals with celiac disease provided it is produced using methods to avoid cross-contamination with a gluten source (11). Celiac disease or gluten-sensitive enteropathy is a condition triggered by the consumption of cereal grains (e.g., wheat, barley, and rye) that contain gluten (gliadin or glutenin) proteins. The immune system of a person with celiac disease reacts to gluten in the diet, causing inflammatory damage to the inner lining of the small bowel. However, due to a possible cross-reactivity between canary seed proteins and wheat proteins responsible for allergic reactions, canary seed may not be suitable for individuals who are allergic to wheat (12). Wheat-allergic individuals are individuals who have an IgE-mediated allergic reaction to a wheat protein (e.g., albumin, globulin, gliadin, or glutenin). Thus, canary seed could pose a concern for individuals with wheat allergies, as opposed to individuals with celiac disease, because a gluten-free label claim can imply that a product is wheat-free. The cross-reactivity issue between wheat and canary seed proteins continues to be investigated by the Canaryseed Development Commission of Saskatchewan.

Use of Canary Seed Flour and Groats in Foods

Because glabrous canary seed is a novel whole grain cereal, prototype food products were developed to show the potential applications of canary seed ingredients in a variety of products. The results were used to determine proposed maximum usage levels for these novel foods and GRAS dossiers (Table I).

Canary seed groats can be used as whole groats or milled into a whole grain flour that is well suited for the bakery, cereal, pasta, snack, and convenience bar markets. Whole groats can also be used as a low-fat substitute for sesame seeds in bread and snack foods or in combination with other seeds as toppings or ingredients. Whole grain canary seed flour can be used to replace or complement other ingredients (e.g., wheat flour, rolled oats, or sesame seeds) in food formulations. The whole grain flour could also be sold as a stand-alone flour product in the retail market.

For the development of prototype food products, brown CDC Maria and yellow CDC Cibo canary seeds were milled into flours by first dehulling the seeds using an abrasive cone-type dehuller followed by air aspiration. The dehulled groats were then milled into flour using a hammer mill. Whole grain flours were analyzed for color, protein, ash, moisture, total starch, and dietary fiber (Table II). Roasted canary seed groats were prepared by spreading 2 kg of seeds in a single layer on a baking sheet and roasting at 325°F for 33 min. The details of

how canary seed flours were incorporated into baked product, pasta, and snack product formulations are described in the following sections. Products were assessed for their sensory characteristics and overall acceptability by experienced panelists.

Pan Bread. Canary seed flour was substituted for 25% of the wheat flour in a standard commercial no-time bread formulation (quantities expressed in baker's percentage): 75% wheat flour, 25% canary seed flour, 4% yeast (fresh), 4% sugar, 2% salt, 2% canola oil, 2% dough conditioner (S500 Red, Puratos), and 62% water. The use of canary seed flour required minimal ingredient and processing changes to produce a quality pan bread. The addition of canary seed flour did result in a reduction in water absorption and an increase in final proofing time compared with the 100% wheat flour control dough. However, all other baking steps (mixing, dough rounding, and baking) remained the same as for the 100% wheat flour dough. Loaf volume (especially for the yellow canary seed flour), crumb texture, and structure were maintained (Fig. 2). The addition of canary seed flour did result in a change in crumb color, but the crumb color for both breads was considered acceptable. There was minimal effect on the flavor of the bread with the addition of canary seed flour.

Roasted canary seed groats were also evaluated as a seed topping on the bread and as an ingredient in the dough (added at 20%). The appearance and texture of the roasted canary seed groats were comparable to other seeds and grain bits commonly used in breads or as bread toppings (Fig. 3). The flavor of the roasted canary seed groats was mild, especially compared with sesame seeds.

Tortillas. Canary seed flour was substituted for 35 and 50% of the wheat flour in a standard commercial tortilla formulation (quantities expressed in baker's percentage): 65 or 50% wheat flour, 35 or 50% canary seed flour, 10% canola oil, 2% baking powder, 1.5% salt, 0.5% sugar, 0.5% monoglycerides, 0.375% sodium stearoyl lactylate, 0.25% fumaric acid, 35 ppm L-cysteine hydrochloride, and 54% water. Slightly less water was required for the tortillas made with canary seed flour. The canary seed flour tortillas had a similar flavor, texture, and rollability compared with the control tortilla. The color of the tortillas made with yellow canary seed flour was more appealing than that of the tortillas made with brown canary seed flour (Fig. 4).

Snack Crackers. Yellow canary seed flour was substituted for 30% of the wheat flour in a standard commercial snack cracker formulation (quantities expressed in baker's percent-

Table I. Proposed maximum usage levels for glabrous canary seed in novel foods

Food Category	Food Products	Maximum Usage Level (%)
Baked goods and baking mixes	Bagels	25
	Biscuits	20
	Breads and rolls	25
	Cakes	20
	Cookies	50
	Corn breads, corn muffins, and tortillas	25
	Crackers	26
	Croissants and pastries	25
	Doughnuts	25
	Flours and brans (prepackaged)	100
	Muffins	20
	Pancakes and waffles	25
	Pies	10
Breakfast cereals	Instant and regular hot cereals	15
	Ready-to-eat breakfast cereals	15
Grain products and pastas	Energy, meal replacement, and fortified bars	25
	Granola and cereal bars	25
	Macaroni and noodle products	15
	Pasta, rice, and other grain products	15
Snack foods	Savory snacks	25
	Seed-based snacks	40

Table II. Nutrient content (g/100 g, dwb) and color of whole grain brown and yellow canary seed flours

	Brown Canary Seed (CDC Maria)	Yellow Canary Seed (CDC Cibo)
Protein (N × 5.7) (%)	22.5	19.9
Ash (%)	1.93	2.11
Total starch (%)	55.9	56.2
Moisture (%)	8.5	8.3
Total fiber (%)	6.7	7.1
Insoluble fiber (%)	5.6	5.9
Soluble fiber (%)	1.1	1.2
Color		
L^*	66.5	74.5
a^*	0.47	1.56
b^*	10.1	17.3

Fig. 2. Bread made with canary seed flour. Left to right: 100% wheat flour control; 25% yellow canary seed flour; 25% brown canary seed flour.

Fig. 3. Bread made with 25% yellow canary seed flour and topped with roasted yellow canary seed groats.

age): 60% wheat flour (9.8% protein), 30% canary seed flour, 15% roasted canary seed, 0.7% yeast (fresh), 9% shortening, 5.4% corn syrup, 1.4% salt, 0.4% baking soda, 0.4% acid powder, and 32% water. Processing of the crackers followed a straight-dough procedure with bulk fermentation of 4 hr. No changes in processing steps were required with the addition of canary seed flour and roasted canary seeds to the formulation. The crackers had an acceptable appearance (Fig. 5) and mild flavor. The texture was similar to multigrain or whole wheat crackers, with a slightly gritty mouthfeel that was acceptable.

Spaghetti. Canary seed flour was substituted for 25% of the durum wheat semolina in spaghetti. The spaghetti was processed using a pilot-scale pasta extruder and dryer following standard commercial processing conditions. The partial substitution of durum semolina with canary seed flour required minimal ingredient and processing changes. There was a slight increase in the amount of water required compared with the 100% durum semolina spaghetti control. No changes to the extrusion parameters or drying cycle conditions were needed. Figure 6 shows the color of dried spaghetti made with yellow and brown canary seed flours. Compared with the control spaghetti, the spaghetti made with canary seed flour had lower L^* values, higher a^* values, and negative b^* values.

The cooked spaghetti made with yellow canary seed flour had a color similar to the whole grain durum semolina spaghetti, which was more acceptable than the color of the spaghetti made with brown canary seed flour (Fig. 7). Minimal flavor differences were detected in the cooked canary seed spaghetti compared with the durum semolina spaghetti. Spaghetti made with canary seed flour had a firmer cooked texture and lower cooking losses than did the durum semolina spaghetti.

Muffins. Yellow canary seed flour was substituted for 35% of the whole wheat flour in a household-sized muffin recipe: 120 g of whole wheat flour, 65 g of canary seed flour, 90 g of rolled oats; 50 g of brown sugar, 50 g of unsweetened shredded coconut, 50 g of wheat germ, 2.5 g of cinnamon, 1.5 g of allspice, 5 g of baking powder, 5 g of baking soda, 2.5 g of salt, 50 g of canola oil, 360 g of buttermilk, 50 g of whole egg, 7.5 g of vanilla extract, 180 g of liquid honey, 50 g of chopped walnuts, and 100 g of raisins. The muffins made with canary seed flour required 9% less liquid compared with the 100% whole wheat flour control muffins. The muffins made with canary

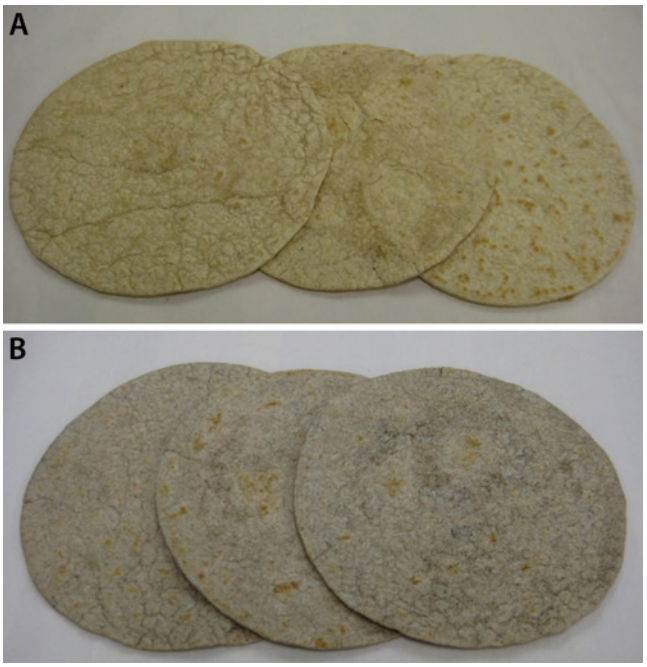

Fig. 4. Tortillas made with canary seed flour: **A,** 50% yellow canary seed flour; **B,** 50% brown canary seed flour.

Fig. 5. Snack crackers made with 30% yellow canary seed flour and topped with roasted yellow canary seed groats.

Fig. 6. Spaghetti made with 25% yellow canary seed flour (top) and 25% brown canary seed flour (bottom).

seed flour had acceptable appearance (Fig. 8), texture, and flavor.

Confections. Roasted canary seed groats were substituted for 100% of the sesame seeds in a sesame seed confection (snap) formulation (quantities expressed in percentage of total weight): 50% sugar, 40% canary seed, 5% honey, 7.5% water, and 0.1% lemon juice. The snaps were made by heating the sugar, honey, lemon juice, and water to 155°C before adding the roasted canary seed groats. The mixture was then spread on a pan, scored into individual serving sizes, and allowed to cool before breaking along the score lines. Roasted yellow canary seed groats produced a more visually appealing product than roasted brown canary seed groats (Fig. 9). Both products had good texture and a mild flavor.

Cereal and Fruit Bars. Roasted yellow canary seed groats were substituted for 100% of the sesame seeds in a cereal and fruit bar formulation (quantities expressed in percentage of total weight): 23% rolled oats, 5.5% roasted canary seed groats, 5.4% unsweetened shredded coconut, 3.6% wheat germ, 0.3% salt, 5% brown sugar, 9.4% slivered almonds, 0.2% cinnamon, 0.5% vanilla, 26.1% liquid honey, 10.4% chunky peanut butter, 11% dried cranberries, and 0.13% xanthan gum. The ingredients were mixed together, pressed into a pan, and cut into individual serving sizes. The bars had an appealing appearance (Fig. 10) and a very acceptable flavor and texture.

Fig. 7. Cooked spaghetti made with yellow and brown canary seed flour compared with spaghetti made with 100% durum semolina, 100% whole grain durum semolina, and 30% chickpea.

Fig. 8. Muffins made with 35% yellow canary seed flour.

Other Opportunities for Canary Seed Applications

Fractionation of canary seed has yielded protein-, starch-, and oil-rich fractions with unique characteristics (2). It has been suggested that canary seed starch may be well suited to applications in the cosmetic industry due to the small and uniform size of the starch granules compared with wheat starch (10). Furthermore, canary seed starch shows unique characteristics compared with wheat starch and may have potential for use in both food and nonfood applications (15–17). Likewise, protein isolated from canary seed groats may have potential as a supplementary or blending protein due to its high tryptophan content (6) and as a health promoter due to the antihypertensive properties of its peptides (9,28). More research on protein structure and functionality needs to be undertaken, particularly in light of the growing interest in plant-based proteins.

Conclusions

Glabrous canary seed provides a novel and nutritious alternative ingredient for use in the food industry, either as a complement to or as a substitute for common grains in food formulations. The examples of food products presented here demonstrate that dehulled glabrous brown and yellow canary seed (groats) can be processed into flour or roasted to produce a wide variety of bakery, pasta, and snack products with few required adjust-

Fig. 9. Confections (snaps) made with 100% roasted brown (left) and yellow (right) canary seed groats.

Fig. 10. Cereal and fruit bars made with 100% roasted yellow canary seed groats.

ments to product formulations and processing conditions. The flavor of canary seed flour and roasted canary seed groats is mild and does not detract from the flavor of the other ingredients in a formulation nor do canary seed flour and groats negatively affect product texture. Products made with yellow canary seed flour and roasted groats are more visually appealing than products made with brown canary seed flour and roasted groats.

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The Critical Role of Milling in Pulse Ingredient Functionality

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ABSTRACT

The milling process is critical for the creation of value-added ingredients from pulses (grain legumes). In this article, we summarize the outcomes of a comprehensive review of the peer-reviewed literature on the milling of pulses. We identify what is already known in wheat milling that could be applied to pulses and point to research issues that should be addressed so that pulses can be consistently milled into high-quality ingredients for the food industry. As in wheat milling, the size and hardness of incoming grain legumes are influential factors affecting pulse flour functionality. However, the relationship between grain hardness and the millability of pulses is not as well understood as it is for wheat flour milling. To allow better comparison of pulse flour functionality from studies in different laboratories, we recommend that wheat flour regulations on maximum particle size be adopted. We also recommend that systematic studies of grain legume microstructure and its relationship to starch damage during milling be conducted. The favorable environmental and nutritional reputation of pulses is an impetus for further development of pulse ingredients for use by the food industry, and understanding the critical role of milling in ingredient functionality is important for the full utilization of pulses.

Pulses, or grain legumes, are a relatively inexpensive source of protein, complex carbohydrates, and fiber (39). The protein content in grain legumes ranges from 22 to 24% compared with the 7 to 15% typically observed in cereals (24). In addition, pulse proteins are a good source of the essential amino acids lysine and leucine, making them highly complementary to cereals from a nutritional perspective (24). Because of these favorable nutrient attributes, and the positive consumer perception of pulses as having a favorable environmental footprint due to their capacity to fix nitrogen (11,17), there has been significant growth in consumer acceptance of pulses. Forecasts for retail utilization of pulses project growth of 9% by 2022 (7).

Transforming pulses into flours that can be utilized as value-added ingredients for a number of cereal-based foods is one means of exploiting the favorable attributes of pulses. Representative products in which pulse flour can be partially substituted for wheat flour include cakes (13,14), cookies (33,50), extruded snack foods (16,19,35), pasta (29), noodles (44), and breads (20,23). The first step in the transformation of grain legumes into pulse ingredients with desired functionalities is the milling process.

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A comprehensive review on the milling of pulses has been completed (36) in which we examined pulse milling from a wheat flour miller's perspective. Because wheat flour milling is a mature topic, with process flows optimized for many decades, we identified what is already known in wheat milling that could be applied to pulses and also identified what additional information is needed for pulses to be consistently milled into high-quality ingredients for the food industry. In conducting the review, there was a bias toward roller milling (versus other mill types) for two reasons: there is a tremendous amount of established infrastructure globally (as a result of the international success of roller milling processes), and roller milling allows flour properties to be precisely manipulated to attain the three main purposes of milling (particle size reduction, separation of components, and mechanochemical changes to components).

Purposes of Milling

As an ingredient, pulse particles must mix well with other ingredient particles. Therefore, the size reduction of grain legumes to particles small enough to blend well with other ingredients is a primary purpose of milling (30).

A second purpose of milling is separation of components (2). In pulse milling, one main separation outcome is removal of the hull (seed coat) from the cotyledons (43,49). This is achieved reasonably easily for pea but not for most other pulses. There may be additional separation objectives as well. For example, sieving and air-classification have been used for many years alongside size-reduction processes to separate protein-rich streams from starch-rich streams (40).

A third purpose of milling is to induce mechanochemical changes to components in an ingredient. In wheat flour milling the main outcome is starch damage (32), which results in flour particles with enhanced water absorption capability (8). Starch damage appears to be important to the functional properties of pulse flours as well (22).

A simplified schematic of wheat flour mill flow is shown in Figure 1. The locations for effecting each of these three milling purposes are highlighted. If roller milling processes are to be used to produce functional ingredients from pulses, the location of each purpose within the mill flow (and its magnitude) needs to be understood.

Grain Legumes Entering the Mill

In a recent review, we identified four principal factors that affect the millability of grain legumes (36). None of these will be unfamiliar to wheat flour millers, but the extent to which each factor dominates the conduct of the milling process likely differs when milling a particular grain legume. These factors are seed characteristics, premilling treatments, drying, and postharvest storage. Only the first two factors will be discussed, although all four were considered in the review (36).

Effects of Seed Characteristics on Milling Performance. Genotype and environment (G × E) influence seed characteris-

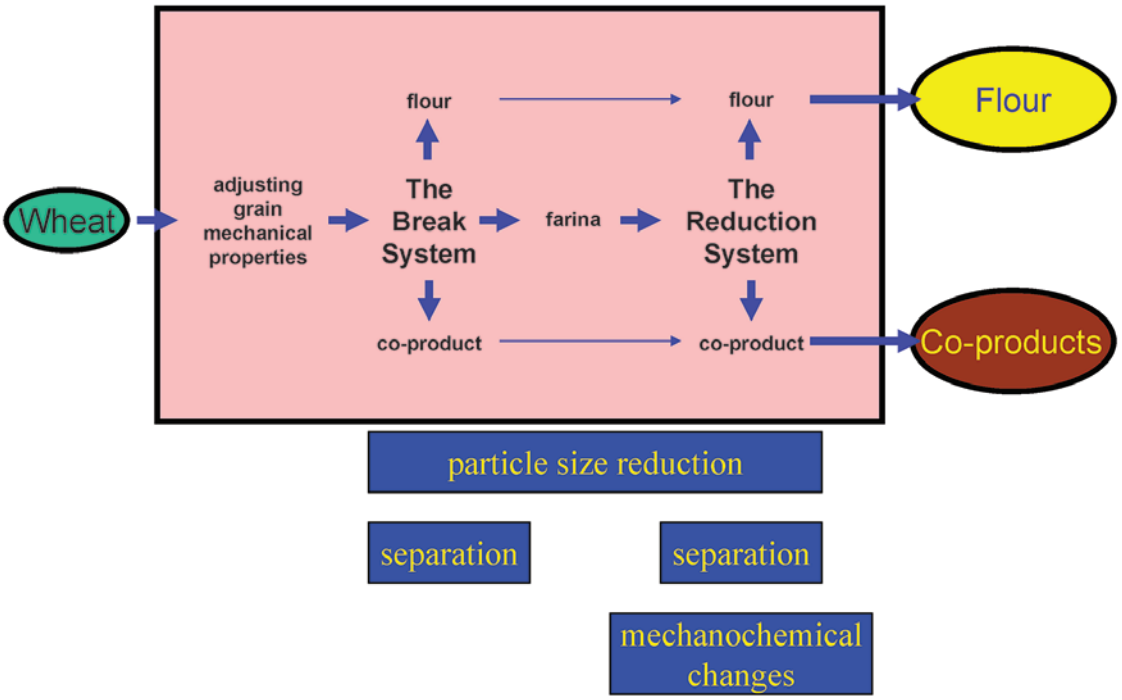

Fig. 1. Schematic of wheat flour mill flow showing where size reduction, separation, and mechanochemical modification occur.

tics, and information on variability in seed characteristics is critical for accurately estimating the milling performance of a particular grain legume entering the mill (46). For millability, two predominant issues related to G × E effects are variability in grain size, which affects how the mill is set up, and variability in grain hardness, which affects the attainment of one or more of the three milling purposes.

Variability in grain legume seed size is greater than the variability in wheat grain size. As a result, screen sizes need to be carefully set up (15) so roll gaps are adjusted appropriately for the incoming material. An alternative strategy is to use fixed roll gaps but employ presizing operations to attain appropriate particle sizes in the incoming pulse stocks (45).

It is likely that the hardness of grain legumes is as important in pulse milling as it is in wheat milling. According to Pasha et al. (26), endosperm texture in wheat is the principal quality parameter because it is used to grade wheat, affects the conduct of milling and baking processes, and governs the quality of the finished baked products. Therefore, changes in grain legume hardness are highly relevant for optimization of pulse milling processes. However, most research on grain legume hardness has targeted evaluation of the hardness of the cooked whole grain legume because cooked hardness determines sensory acceptability (25).

Some research on the hardness of raw grain legumes has been conducted. For example, a single-kernel characterization system has been used to measure the hardness of mung bean seeds (6). Hardness index increased with increases in moisture content, but only up to 16%. In a study to attain protein-rich streams from pulses, lower seed hardness in lentil compared with chickpea, pea, and bean seeds was deemed responsible for the higher protein content in the fine fraction produced from lentil flour (27). Based on these findings, grain hardness is a critical variable that affects the millability of grain legumes and the characteristics of the resulting pulse flour, just as it does for soft and hard wheats.

Investigations on the compositional and structural bases for grain legume hardness have been conducted. Although protein acts as a structural agent (5), G × E effects that alter fiber content and its location in the grain legume likely play the predominant role in hardness (38). In a comprehensive study of chickpea genotypes, differences in the amounts of soluble and insoluble nonstarch polysaccharides in the seed coat significantly affected dehulling performance (47). Structural differences at the junctions between the seed coat and cotyledons also played significant roles in milling differences between chickpea genotypes (48).

A number of agencies have defined tests to measure variability in quality parameters. The USA Dry Pea & Lentil Council lists the U.S. grading standards for pea, chickpea, bean, and lentil (41), while the Canadian Grain Commission (CGC) defines quality parameters using tests that include seed color, cooking time, dehulling characteristics, firmness of cooked seeds, 100 seed weight, protein content, seed size distribution, starch content, and water absorption (3). Internationally, the Codex Alimentarius Commission (CAC) and the International Pulse Quality Committee (IPQC) define pulse quality parameters.

Some of these quality parameters relate directly to pulse milling performance (Table I). For instance, the 100 seed weight is relevant to setting the gap between rolls, whereas the various compositional specifications are relevant for millers striving to meet target specifications for a flour (e.g., seed protein content for flours that will be processed into concentrated protein products, such as concentrates and isolates). Nevertheless, the focus of these national and international standards is on the quality of whole or split pulses. A lack of internationally recognized quality standards and accepted nomenclature for the milling performance of grain legumes has slowed the development of milling and processing of pulses relative to that of wheat (37).

Premilling Treatments. A number of premilling treatments have been used to change the quality attributes of pulse flours.

Table I. National and international pulse quality parameters relevant to milling performance

Quality Parameter	Standard Committee
Seed size/shape/weight	US, CAN, IPQC
Moisture content	US, IPQC, CAC
Crude protein content	CAN, IPQC
Starch content	CAN, IPQC
Water absorption	CAN, IPQC
Purity	US, CAC
Defectiveness	US, CAC
Fiber content	IPQC
Maturity	CAC
Antinutrient factors	IPQC

[a] US: USA Dry Pea & Lentil Council; CAN: Canadian Grain Commission; IPQC: International Pulse Quality Committee; CAC: Codex Alimentarius Commission.

Traditional treatments, such as soaking and conditioning (typically with water, but sometimes with oil), have a long history of use as dehulling aids (43). Other pretreatments, such as hydrothermal treatments, micronization, and partial germination, have more recent origins.

The effect of germination on the nutritional and culinary properties of pulses has been investigated, but there are few studies of its effect on milling performance. Indeed, for fixed milling conditions, no study has established how premilling treatments such as roasting, partial germination, or micronization affect the particle size of the resultant flour. Some quality outcomes are known, however. Water-holding capacity increases in micronized pulses (31), and flavor profiles improve in pregerminated pulses (1), whereas mixed trends have been reported for fat-holding capacity and foaming characteristics. Given the dissonance in the literature, systematic studies are required to understand the effects of premilling treatment on milling performance (36).

Pulse Milling

Various researchers have determined the particle size distribution of pulse flours, and a wide range of values has been reported. A pronounced effect of mill type on particle size has been observed. Jet and pin mills result in very fine flours (<60 μm), whereas hammer, roller, and stone mills produce a variety of particle sizes depending on their specific configuration. It is extremely difficult, therefore, to compare milling effects on pulse flour functionality in studies where mill type and configuration differ. As a first step toward enabling meaningful comparisons between studies in different laboratories, we recommend that the wheat flour granulation specification be used when the term "pulse flour" is used.

Title 21 of the U.S. *Code of Federal Regulations* defines flour as a powder made from wheat grains where "not less than 98 percent of the flour passes through a cloth having openings not larger than those of woven wire cloth designated 212 μm (No. 70)" (42). As a result, comparisons of functional properties between wheat flours are made with particle granularity defined in this manner. This is not the case in the vast majority of the peer-reviewed literature on pulse flours, where particle size in bakery applications has been reported as ranging from 17 μm (13) up to 1,000 μm (23); this size variability significantly impedes comparisons of pulse flour functionality across different studies.

Typical commercially milled pulse ingredients include whole pulse flours; dehulled/decorticated pulse flours; fiber-rich, starch-rich, and protein-rich fractions; and concentrates and isolates made from these fractions (9). All but whole pulse flours require the milling process to not only reduce particle size but also to separate components. Particle size plays a significant role in how effectively components can be separated. Protein or starch enrichment obtained according to differences in the particle size of starch granules and protein bodies is fairly well established (27). However, a wide range of sizes of starch-, protein-, and fiber-enriched particle is evident for different pulses generated by similar mill flows, so any definition of coarse and fine needs qualification. Nevertheless, milling of grain legumes to an appropriate particle size is a prerequisite for the manufacture of pulse ingredients that are enriched in a particular component.

Various milling conditions have been studied with respect to starch damage in flour. In wheat flours, an inverse relationship between particle size and starch damage is observed (8,32). In pulse flours, many studies have shown that starch damage increases when high-speed milling is used to create small particles (e.g., in hammer milling of cowpea) (18). Jet milling of pea at high classifier speeds generated greater starch damage in flours than did impact milling in a study reported by Pelgrom et al. (28). However, assessing the effect of milling process on starch damage alone (and therefore its impact on pulse flour functionality) is difficult because there are no studies that have disentangled the influences of mill type, mill configuration, and particle size from starch damage.

Due to the lack of research on how starch is damaged during pulse milling, it is recommended that systematic studies be conducted on this subject. Roller milling should be employed for at least part of this research because the degree of compression and shear imposed during size reduction can be manipulated independently (32).

Products Prepared from Pulse Flours

Pulse flours have been used as ingredients in many cereal-based products, including cold- and hot-extruded products (e.g., pasta, noodles, and snacks) and various baked products (e.g., cakes, cookies, breads, and gluten-free products). The application of several pulses, and fractions derived from them, in baked, cold-extruded, and other products was recently reviewed (10,34). One of the issues in comparing studies with differently milled pulse flours is that poor miscibility of ingredients (as a result of large particle size) can significantly influence quality assessments of the finished product. In our recent review (36), we focused only on the literature that reported how the milling process itself impacted the quality of the products in which the pulse flour had been incorporated.

Particle size plays a significant role in baked product quality. Some appreciation of this can be gleaned from the bakery application shown in Figure 2, in which milled pea hull flour was added to wheat flour. Reducing the particle size clearly depressed loaf volume, even though the same quantity of pulse hull flour was incorporated into each bread formulation. In contrast, sponge cakes with higher volume have been produced from finer pulse flours (14). A particle size effect was observed also for cookie formulations, in which finely milled pulse flours decreased dough spread and increased cookie hardness (50). Some pulses have been studied extensively in baked product formulations, whereas others (e.g., black bean, pigeon pea, and black-eyed pea) require further research to fully understand their functionality in baked goods.

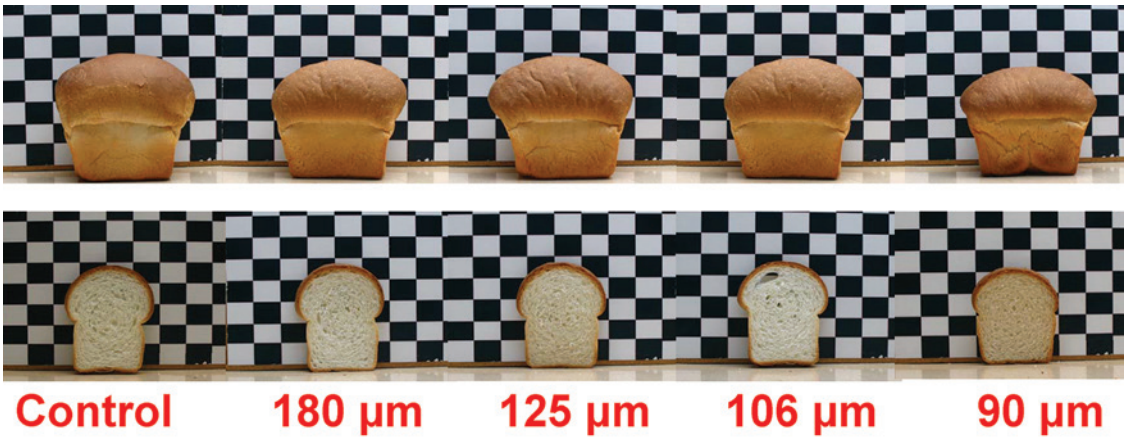

Fig. 2. Changes in loaf volume and crumb structure for a bread formulation with 2 g of milled pea hull (of various particle sizes) per 100 g of wheat flour (from A. L. Shum and M. G. Scanlon, *unpublished results*).

In expanded snack and breakfast foods, blends of air-classified pea starch and field pea flour have produced acceptable extrudates (16). Extrudate quality does decline, however, with an increase in protein content: increased hardness is an outcome of lower expansion indices. A challenge for pulse flours in these applications is their lower starch content. A minimum starch content of 60% has been recommended for expanded cereal products (4), so innovative processes (19) or reformulation strategies (35) may be required to optimize pulse flour functionality in these applications.

Research Gaps

Extensive milling studies on wheat have clearly established a number of millability parameters that govern the quality of the resulting wheat flour (30). However, these parameters have not been well defined for pulse milling. Differences in milling performance arising from variability in seed characteristics have been established for certain pulses, but further investigation is essential to enable breeding programs to develop pulses with high milling efficiency. Although investigations of pulse drying and storage have provided valuable insights into their effects on nutritional and culinary qualities, there is a lack of studies relating drying and storage effects to the millability of pulses. Changes in the physical properties of pulses arising from pretreatment processes, such as partial germination and micronization, also need to be linked systematically to milling behavior.

Understanding how microstructure affects fracture paths in comminuted pulse particles is essential for devising milling processes that will yield pulse flours with functionalities that produce high-quality end products. Some microstructural studies related to starch damage and protein separation arising from the size reduction process do exist. However, a general perspective based on a thorough assessment of the literature is that the relationship of microstructure to grain legume hardness and its effect on the millability of pulses requires systematic study.

A number of questions with respect to milling of grain legumes into pulse flours need to be answered, including the following questions:

- Is there a desired degree of size reduction for good miscibility of ingredients?
- Does the target particle size differ according to pulse type and according to the product being made from the pulse flour?
- How must separation operations in the mill be configured for different pulses?
- Is there a reliable means of defining separation efficacy in pulse flour milling, analogous to control of wheat flour mill extraction rate by ash measurement?
- Are there defined enrichment targets for components in pulse ingredients akin to wheat flour protein specifications?
- If these enrichment targets are met, are costream(s) still valuable as ingredients?
- What is the correct amount of starch damage for pulse flours for specific applications?
- Do starch damage assays for wheat flour correctly measure starch damage in pulse flours?

One important additional research gap relates to the nutritional reputation of pulses. Starch digestibility impacts the nutrient quality of pulse-fortified foods produced using different processing methods (12). Milling is one such processing technique, and it appears that flour particle size effects on nutrient availability cannot be ignored (21). Systematic studies in this area, preferably studies investigating the underpinning microstructural mechanisms, are required.

Conclusions

The literature on the effects of milling on pulse flour functionality is rather fragmented. There is a clear need to evaluate separately the effects of the three primary purposes of milling (particle size reduction, separation of components, and mechanochemical changes to components) on pulse flour properties. There also is a need for fundamental research on the effects of the composition and structure of pulses on their millability, and much of this research should be tailored to the specific end use of the pulse flour as an ingredient.

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Navigating Protein Claim Regulations in North America for Foods Containing Plant-Based Proteins

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Over the last decade, protein has been a major driver of purchasing decisions made by consumers (43). As the appeal of plant-based foods for consumers continues to grow, it is not surprising that foods containing plant-based proteins are becoming increasingly prominent within the North American marketplace (22,31). Simultaneously, given their ties to human health (24) and environmental sustainability (40,47), plant-based proteins are being highlighted within dietary guidelines across jurisdictions, including North America (15,29,32,50). Although plant-based protein sources, including legumes (soybeans, pulses), nuts, and seeds, have always been available, the food industry is responding to dietary guidelines and consumer trends with innovative and reformulated foods that are underpinned by significant levels of plant-based protein per serving (22,31). Due to increased interest in the use of plant-based proteins, in 2018, the government of Canada announced a CA$150 million investment to help develop Canada's plant-based protein sector (1).

Protein content claims are used by consumers to identify foods as a source of dietary protein. Regulatory guidance for "source of protein" claims differs across jurisdictions. Compared with other developed regions, such as Australia and New Zealand (11), China (30), and the European Union (7), the regulatory environments in Canada and the United States are unique, because protein content claims are dependent on protein quality. In Canada and the United States, for persons ≥1 year of age, the protein quality of a food is quantified using the protein efficiency ratio (PER) method to derive a protein rating (17) or a protein digestibility corrected amino acid score (PDCAAS) (53), respectively. Plant-based sources of protein generally have lower levels of indispensable amino acids (mg/g of protein) and lower digestibility coefficients compared animal-based protein sources, which affects their measured protein quality. That being said, in the context of the total diet, these properties of plant-based protein sources do not necessarily devalue their contribution of protein to a healthy diet. From a regulatory perspective, these methodologies can introduce challenges for establishing "source of protein" claims for plant-based foods. The inability to identify for consumers foods with significant levels of plant-based protein could impede the adoption of dietary patterns that align with dietary guidelines.

Moreover, given the similarities and shared food systems between Canada and the United States, many of the same manufactured foods can be found in retail outlets in both countries. However, because of differences in regulatory frameworks concerning protein, some foods that can be identified as a "source of protein" in one country cannot make a similar claim in the other.

The purpose of this review is to discuss the current regulatory landscape for protein content claims in North America and outline possible modifications to the regulatory frameworks that would increase the ability of manufacturers to communicate to consumers in Canada and the United States that foods are a significant source of plant-based protein.

Regulatory Landscape for Protein Content Claims in Canada and the United States

Protein quality is assessed based on the ability of a dietary protein to provide adequate levels of bioavailable essential or indispensable amino acids for metabolic work (27). For humans, nine amino acids are indispensable and are required at variable levels, depending on life stage and state of health (21).

Various methodologies have been developed to assess the protein quality of foods. However, few have been integrated into existing regulatory frameworks to support protein content claims. Canada and the United States are exceptions, however, and have implemented different methodologies for determining protein quality to support protein content claims for foods that target noninfant populations.

Support for Protein Content Claims in Canada. A summary of the protein rating method used to support protein content claims in Canada is provided in Figure 1 (26). To determine the protein rating of a food, a rat bioassay is used to measure its PER. Weanling rats are fed diets containing a test protein (10%) or control protein, casein (10%). After 4 weeks, the protein efficiency (weight gain [g]/protein intake [g]) is determined for each diet. The ratio of the protein efficiencies between the test and control diets is the PER. To account for inter- and intralaboratory variability, the calculated PER is adjusted using the standard average PER for casein of 2.5. For the remainder of this review, PER refers to the adjusted PER value (13). The protein rating is determined by multiplying the PER of the protein source by the Reasonable Daily Intake (RDI) value established for the same food (3). A food with a protein rating ≥ 20 is a "good source" of protein, whereas a food with a protein rating ≥ 40 is an "excellent source" of protein. RDI values for specific foods are available in schedule K of Canada's *Food and Drug Regulations* (3). If an RDI for a food does not exist, the reference amount, which is the regulated serving size of a food, can be used to determine the protein rating (3).

The methodological challenges associated with using the protein rating method to delineate the protein quality of a food and its subsequent use within a regulatory framework are outlined in Table I. First and foremost, the use of a growing rat does not accurately assess the indispensable amino acid requirements of humans. This is particularly true for sulfur-containing amino

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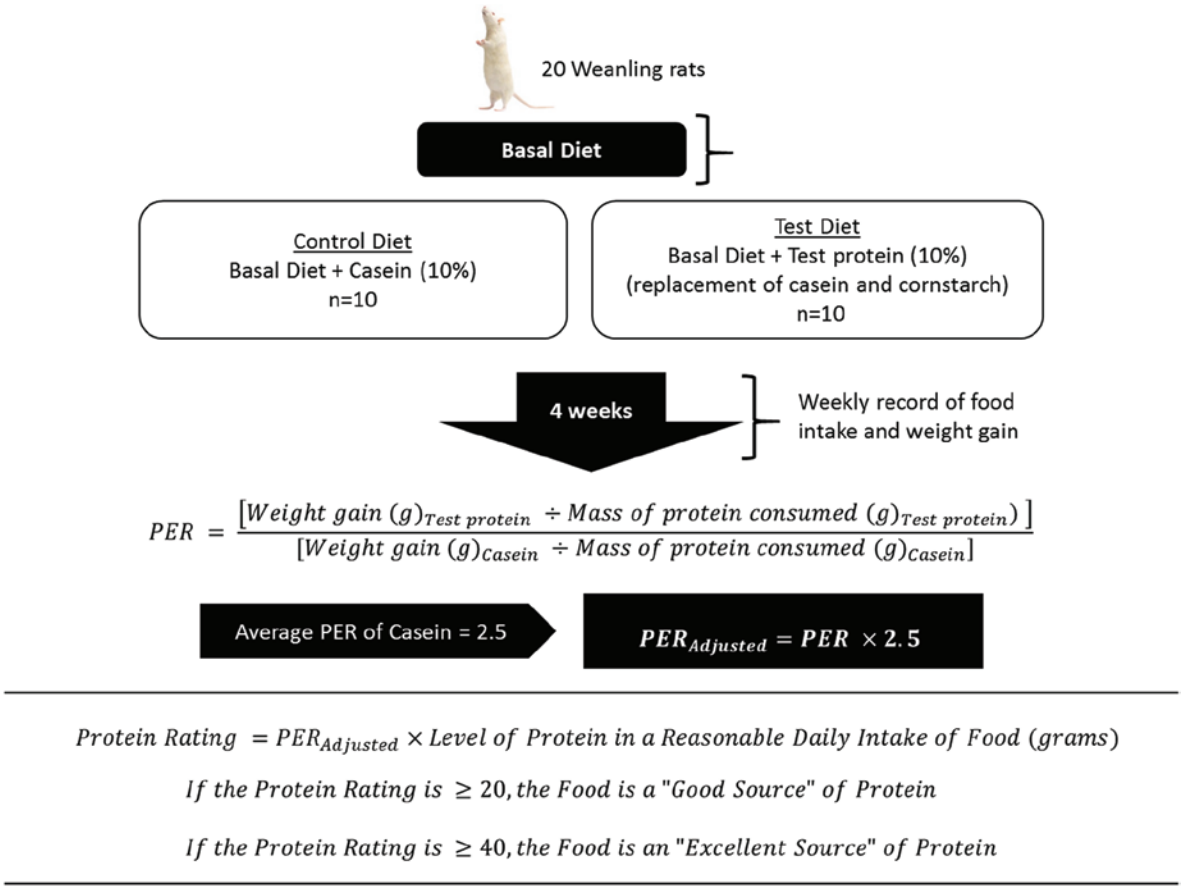

Fig. 1. Summary of protein rating/protein efficiency ratio (PER) method and regulatory framework for protein nutrient content claims in Canada (3,13). (Adapted from Marinangeli and House [26])

Table I. Methodological challenges associated with protein quality methods used in Canada and the United States for establishing protein content claims[a]

Method	Method Description	Region	Methodological Challenges
Protein rating/PER	Rat growth bioassay and protein rating calculation	Canada	Indispensable amino acid requirements of growing rats differ from those of humans. Method does not credit amino acids required for maintenance. PER values are not additive within a food matrix. New food formulations are required to be tested to determine the PER and protein rating. Limited availability of PER values. Hedonic properties of a food can affect consumption of test diets.
PDCAAS	Digestibility assay and amino acid scoring calculation	United States	PDCAAS values are truncated at 1.0, which limits the ability to compare protein sources of high quality. Use of true fecal nitrogen digestibility does not represent and can overestimate the digestibility of indispensable amino acids. Limited availability of digestibility values. The are no considerations for differences in the microbiome.

[a] PER: protein efficiency ratio; PDCAAS: protein digestibility corrected amino acid score.

acid requirements, which are elevated in rats due to their role in fur production and maintenance (10,41). The utility of the protein rating method is further challenged as a growth assay, because indispensable amino acids necessary for maintenance of tissues and biological processes are not credited (10). Additionally, given that the PER is highly dependent on the consumption of test foods by rats, the hedonic properties of a test food could alter consumption and produce an artificially low or high PER. Furthermore, nutrient geometric analyses used to evaluate how mixtures of nutrients affect dietary choices suggest that protein intake in humans is more strongly regulated than carbohydrate and fat intakes (45): dietary intake is about 15% protein and 85% carbohydrate and fat. Although protein content also drives macronutrient selection in rats, Simpson and Raubenheimer (46) found that adult male Sprague-Dawley rats generally selected about 40% protein and 60% carbohydrate and fat. In addition, the protein/energy ratio may change depending on environmental temperatures, e.g., rats will maintain their protein intake but selectively increase energy intake from fat and carbohydrate. These results demonstrate differences in the dietary preferences of humans and rats.

Few PER values exist in the peer-reviewed, private, and regulatory literature that can be used for application within the regulatory frameworks to establish protein content claims. The Canadian Food Inspection Agency (CFIA) provides a limited list of PER values for the food industry (3), and because PER values

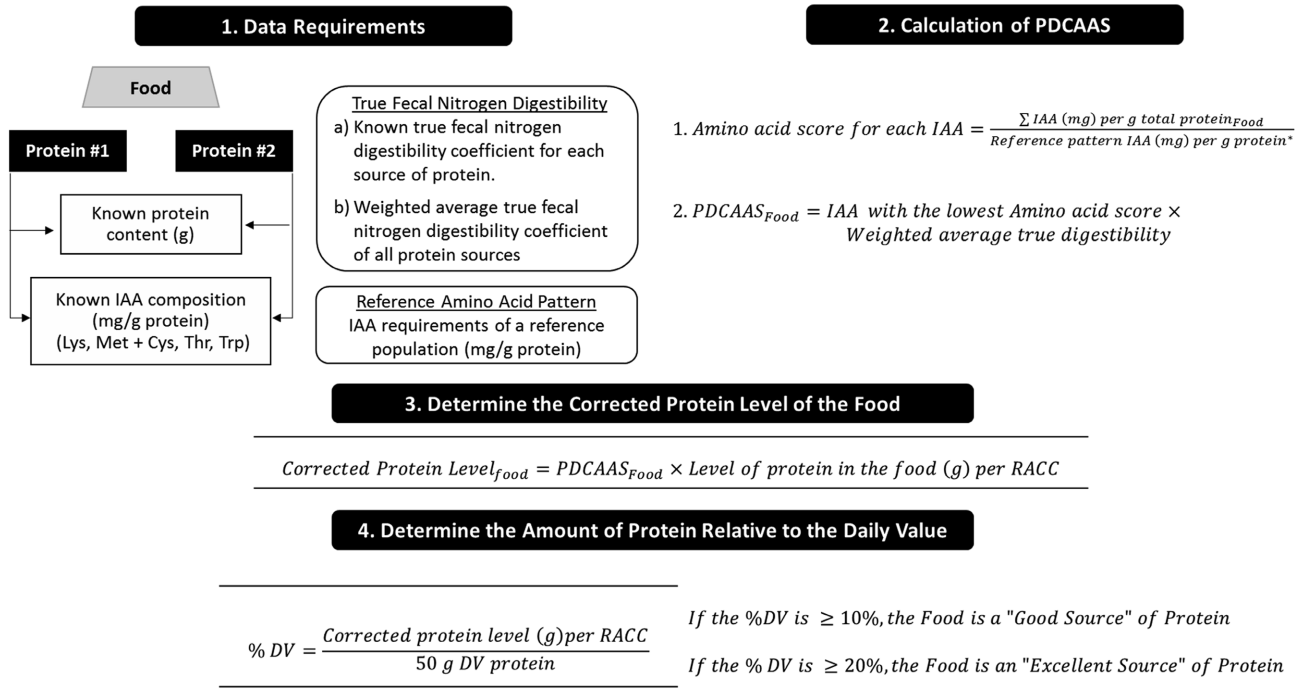

Fig. 2. Summary of the protein digestible corrected amino acid score (PDCAAS) method and regulatory framework for protein nutrient content claims in the United States for individuals who are ≥4 years of age. (Adapted from Marinangeli and House [26]) * Preschool child 2–5 years of age, as per the 1991 Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Expert Consultation on Protein Quality Evaluation (10). DV: daily value; IAA: indispensable amino acid; RACC: reference amount customarily consumed.

are not additive, new food formulations containing a mixture of protein sources require that the PER be determined using the rat bioassay. In our opinion, this requirement can limit food innovation because of the cost and difficulty associated with making a protein content claim for mixed foods. It is noteworthy that in Canada unless a food can be characterized as a "source of" protein (protein rating ≥ 20), the level of protein per serving of food cannot be advertised to consumers other than in the Nutrition Facts table on the label (16). Regulations are similar in the United States, where rules concerning the use of nutrient content claims extend to any statements on labels about the level or range of a nutrient, including protein, in a food (52).

Support for Protein Content Claims in the United States. In the United States, PDCAAS is the regulatory tool used to support protein content claims. The PDCAAS method relies on in vivo true fecal nitrogen digestibility coefficients, the indispensable amino acid requirements of a human reference population, and the level of indispensable amino acids (mg/g) of each protein source in a food formulation (Fig. 2). The level of each indispensable amino acid is divided by the indispensable amino acid requirements of a reference population and is defined as the amino acid score (10). The indispensable amino acid with the lowest score is multiplied by the weighted average for true fecal nitrogen digestibility to derive the PDCAAS of the food. PDCAAS values > 1.0 are truncated at 1.0 (10). The PDCAAS is multiplied by the level of protein in a serving of food, which adjusts the level of protein in the food for digestibility. In the United States, the serving size used for this calculation is the reference amount customarily consumed (RACC), which is outlined in Title 21, Section 101.12 of the U.S. *Code of Federal Regulations* (54). The level of corrected protein per RACC is divided by the daily value (DV) for protein for individuals who are ≥4 years of age (50 g). For foods targeted to children 1–3 years of age, the DV for protein is 13 g/day (53). If the food contains ≥10% of the DV for protein per RACC, the food is a "good source" of protein. If the food contains ≥20% of the DV for protein per RACC, the food is an "excellent source" of protein (51).

The PDCAAS was adopted in the United States as the framework for supporting protein content claims following the publication of the 1991 Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Expert Consultation on Protein Quality Evaluation (10). Although indispensable amino acid requirements in reference populations have changed over time (8,10,56), regulations in the United States stipulate that the indispensable amino acid requirements for children 2–5 years of age, based on the 1991 report, remain in use (53). Growth rate and higher protein requirements are the rationale for using children 2–5 years of age as the reference population.

From a nutritional perspective, the PDCAAS largely addresses the shortfalls of the protein rating method used in Canada to support protein content claims. However, this has not stemmed criticisms. For foods with a PDCAAS > 1.0, the truncation of values to 1.0 does not permit high-quality protein sources to be accurately compared (8). Furthermore, the use of total fecal nitrogen digestibility does not accurately represent the digestibility of each indispensable amino acid or account for microbial assimilation and uptake of endogenous and exogenous nitrogen in the large intestine, which can inflate digestibility values, and, thus, the PDCAAS of a protein source (9,39,42,56).

That being said, in contrast to the protein rating method used in Canada, PDCAAS values can be added together (10,56), eliminating one of the barriers encountered when using the protein rating method. Therefore, if digestibility coefficients and indispensable amino acid levels for each protein source are known, the PDCAAS can be derived for foods with multiple protein sources without the need for an in vivo total fecal nitrogen digestibility study. However, a lack of total fecal nitrogen digest-

ibility values for new ingredients can limit the calculation of the PDCAAS and require assessment based on rat balance studies.

Protein Quality of Plant-Based Protein Sources

In general, regardless of the methodology used, the protein quality of plant-based protein sources is lower than that of animal-based sources. Plant-based protein sources can have lower total fecal nitrogen digestibility coefficients and/or lower levels of one or more indispensable amino acids compared with animal-based proteins. The lower digestibility values of plant-based protein sources can be secondary to the inherent structure of the protein and the presence of nonprotein constituents in plant-based foods such as fiber and antinutritional factors (10). Most antinutritional factors that disrupt protein digestion are inactivated by cooking and/or processing (38). Nonetheless, lower digestibility and/or indispensable amino acids levels can decrease the PER and PDCAAS values for a food. Typically, legumes have higher levels of lysine (mg/g of protein) and lower levels of sulfur-containing amino acids (mg/g of protein), whereas the reverse is true for cereals. Thus, it is recommended, particularly for vegetarians, that diets include plant-based proteins from a variety of sources, such as legumes, cereals, nuts, and seeds, to ensure daily requirements for indispensable amino acids are met. This approach has long been accepted as combining "complementary proteins" and is the backbone of animal nutrition when complementary sources and supplemental amino acids are used in formulating diets to balance the dietary amino acid profile (44).

Some PER and PDCAAS values for a variety of animal- and plant-based protein sources are summarized and ranked (from highest to lowest) in Table II. It should be emphasized that the data presented in Table II are a summary of publicly available data, and PER and PDCAAS values for similar foods listed could be higher or lower because of differences in variety, production, and processing. As expected, for the most part, lower PER values are derived for plant-based protein sources compared with animal-based foods. Exceptions include soybeans, soy protein, and chickpeas, with PER values of 2.0, 2.3, and 2.32, respectively. Despite observing reasonably high true nitrogen digestibility values, the same trends are apparent for PDCAAS, in which plant-based proteins generally have scores that are ≥30% lower than those for animal-based protein. Similar to PER, PDCAAS values for soy proteins are similar to animal-based proteins.

Protein Nutrient Content Claims for Plant-Based Food Sources in Canada and the United States

As dietary guidelines place increased emphasis on the consumption of plant-based protein sources, it is reasonable to suggest that significant utilization of legumes, cereals, nuts, and seeds in food formulations should render those foods a source of protein and, in particular, provide greater guidance for those who choose to eliminate or limit consumption of animal-based proteins. Given that Canada and the United States use two different regulatory frameworks to support protein content claims, it is expected that each framework would yield different results under the context of protein claims.

One of the challenges created by having two very different methods for assessing protein quality across regions with similar food landscapes is demonstrated by lentils and chickpeas. As shown in Table II, the PER for lentils, with the exception of green lentils, is extremely low at 0.3 and would produce a very low protein rating of 2.8 (based on 0.074 g of protein/mL in cooked lentils [U.S. Department of Agriculture (USDA) National Nutrient Database (NDB) ID 16070 (48)] and a 125 mL reference amount [14]), which is 86% below the protein rating (≥20) required to permit a protein claim in Canada. Based on the averaged protein content of cooked lentils (9.02 g/100 g [USDA NDB ID 16070 (48)]), a general PDCAAS for canned lentils of 0.52 (10) and a 90 g RACC (54), the corrected level of protein per RACC is 4.22 g. Although the corrected level of protein falls short of the threshold for a protein claim in the United States (5 g of corrected protein/RACC), the shortfall of 16% is modest and provides a reasonable opportunity to combine lentils with a complimentary protein source or sources to attain levels that would facilitate a claim. Furthermore, as demonstrated in Table III, the PDCAAS for green lentils is 0.628 and is high enough for a protein claim to be made in the United States based on a 90 g RACC, but it is not high enough for a claim to be made in Canada. The reverse is true for chickpeas, for which the PER is 2.32—a ratio which, relative to the casein positive control, could generate a fairly high protein rating for food with moderate-to-high levels of protein. Conversely, the PDCAAS for chickpeas is 0.519, which is 50% lower than that of animal-based protein foods. Thus, the use of chickpeas in a manufactured food product may enable a protein content claim in Canada, but not in the United States.

The application of PER and PDCAAS for protein content claims in Canada and the United States, respectively, for whole foods that dietary guidelines identify as foods with protein is summarized in Table III (20,49). In Canada and the United States, animal-derived protein foods, such as milk, eggs, and chicken, are considered to be "good" or "excellent" sources of protein. In contrast, no legumes, with the exception of tofu made from soybeans, are considered to be a "source" of protein in Canada. In the United States, however, all legumes, with the exception of baked beans, black beans (no sauce and/or not canned in liquid), and split yellow peas, are considered "good" sources of protein.

Flours, protein concentrates, and protein isolates are ingredients that can be utilized to facilitate an increase in the consumption of plant-based protein. Recently, Chaudhary et al. (4) evaluated the effects of reformulation of pan bread, breakfast cereal, and pasta with whole yellow pea flour. Refined high-protein, all-purpose, or semolina wheat flour was replaced with whole yellow pea flour at 15, 53, and 30% total flour, respectively. Using the known amino acid composition of the refined wheat flours and whole yellow pea flour and their known true fecal nitrogen digestibility, the PDCAAS and corrected levels of protein per serving of foods from the Chaudhary et al. (4) study was modeled against the RACC for bread (50 g), breakfast cereal (40 g), and pasta (dry) (55 g). Reformulation with whole yellow pea flour increased protein quality by 41% for pan bread, 111% for breakfast cereal, and 100% for pasta (Fig. 3A). The respective corrected protein levels also increased by 51, 201, and 128% for pan bread, breakfast cereal, and pasta (Fig. 3B). Levels of corrected protein that would permit a protein content claim in the United States (5 g/RACC) were attained for reformulated pasta, but not for reformulated pan bread or breakfast cereal (Fig. 3B). This was true even when reformulation was modeled to a 100% inclusion rate of whole yellow pea flour. These results demonstrate some of the technical and communicative challenges concerning reformulation and innovation with plant-based proteins.

Table II. Examples of protein efficiency ratio (PER) values, total fecal nitrogen digestibility (TFND) percentages, and protein digestibility corrected amino acid score (PDCAAS) values

PER[a]		TFND and PDCAAS			
Food	**PER**	**Food**	**TFND (%)**	**PDCAAS**	**Source**
Animal-based protein sources		Animal-based protein sources			
Egg, whole	3.1	Beef	98	0.92	FAO and WHO (10)
Egg white	3.0	Casein	96.59–99	1	FAO and WHO (10); Nosworthy et al. (37)
Pork, ham	2.7	Egg white	100	1	FAO and WHO (10)
Pork, tenderloin	2.7				
Poultry	2.7	Plant-based protein sources			
Beef or veal, muscle	2.7	Soybean protein concentrate	95	0.99	FAO and WHO (10)
Fish	2.7	Canola protein concentrate	95	0.93	FAO and WHO (10)
Beef, liver	2.7	Soybean protein isolate	98	0.92	FAO and WHO (10)
Shellfish	2.7	Canola protein isolate	95	0.83	FAO and WHO (10)
Muscle meats (bison, lamb, etc.)	2.7	Lentil protein concentrate	91.1	0.681	Nosworthy and House (35)
Dried whey	2.6	Bean, navy	79.96	0.667	Nosworthy et al. (37)
Casein	2.5	Pea, split yellow	87.94	0.643	Nosworthy et al. (37)
Cheese, cheddar	2.5	Lentil, whole green	87.89	0.628	Nosworthy et al. (37)
Milk	2.5	Bean, pinto	76.23	0.59	Nosworthy et al. (37)
Wiener	2.1	Oats, rolled	91	0.57	FAO and WHO (10)
Bologna	2.1	Faba bean isolate	97.2	0.564	Nosworthy and House (35)
Chicken frankfurter	2.1	Bean, red kidney	78.6	0.549	Nosworthy et al. (37)
Macaroni and cheese	2.1	Pea protein concentrate	92.6	0.541	Nosworthy and House (35)
Sausage	1.7	Buckwheat flour	71.13	0.54	Nosworthy et al. (33)
		Lentil, split red	90.6	0.538	Nosworthy et al. (37)
Plant-based protein sources		Bean, black	69.99	0.534	Nosworthy et al. (37)
Chickpea, cooked	2.32	Pea protein isolate	97.1	0.526	Nosworthy and House (35)
Soybean, heated	2.3	Peanut meal	94	0.52	FAO and WHO (10)
Soy protein	2.0	Chickpea	85.02	0.519	Nosworthy et al. (37)
Oats, rolled	1.8	Pea, split green	85.15	0.5	Nosworthy et al. (37)
Barley	1.7	Lentil protein isolate	96.1	0.498	Nosworthy and House (35)
Peanut	1.7	Faba bean concentrate	94	0.433	Nosworthy and House (35)
Bean, pinto	1.6	Wheat, whole	91	0.4	FAO and WHO (10)
Bean, black	1.6	Sunflower protein isolate	94	0.37	FAO and WHO (10)
Bean, kidney	1.6	Rice-wheat gluten	95	0.26	FAO and WHO (10)
Bean, navy (dry)	1.5	Wheat gluten	96	0.25	FAO and WHO (10)
Rice	1.5				
Pea, split yellow	1.4				
Bulgur wheat	1.4				
Corn, whole	1.4				
Lentil, whole green	1.3				
Rye	1.3				
Pea flour	1.2				
Sunflower seed	1.2				
Bread, white	1.0				
Wheat, whole	0.8				
White flour	0.7				
Almonds	0.4				
Lentil, cooked (all others)	0.3				

[a] Source: Canadian Food Inspection Agency (3).

Debating the Future of Protein Content Claim Regulations in North America

Across regions, dietary guidelines and government agencies (2,12,23,29,32), as well as the guiding principles for the next iteration of Canada's *Food Guide* (15), emphasize consumption of plant-based sources of protein to facilitate health and/or decrease the environmental impacts of diets. Lack of harmonization between Canada and the United States could impede the development and marketing of food offerings that align with dietary guidelines to increase consumption of plant-based proteins. Given the heightened awareness concerning the impact of food choices on health and environmental sustainability, there is an interest in exploring regulations that govern protein nutrient content claims in Canada and the United States. Analysis should determine whether current frameworks are sufficient to enable a food landscape that credits the positive nutritional attributes of foods and facilitate healthy food choices by consumers.

The use of protein quality as support for protein content claims in Canada and the United States is intended to facilitate the consumption of healthy diets. However, the frameworks for protein content claims in Canada and the United States assume that a threshold for indispensable amino acids must be met for every food and/or meal and that deficits from one eating occasion cannot be compensated for at other eating occasions or by inclusion of complementary protein sources within a meal. Given the variety of foods available and recommendations to increase the proportion of plant-based foods as part of healthy dietary patterns, there is merit in reexamining protein quality-based

Table III. Comparison of the protein rating/protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS) frameworks for permitting protein content claims in Canada and the United States[a]

Food (NDB ID)[b]	Protein (g/100 g) (g/mL)	Canadian Regulatory System[c]				U.S. Regulatory System[d]			
		RDI[e]	PER	APL (g)[f]	Claim Permitted	RACC[g]	PDCAAS (untruncated)	CPL (g)/ RACC[h]	Claim Permitted[i]
Animal-derived foods									
Milk, whole (01077)	3.15 (0.032)	852 mL	2.5	72.10	Excellent source	240 mL	1 (1.1)	7.68	Good source
Egg, hard-boiled (01129)	12.58	100 g	3.1	39.00	Good source	50 g	1 (1.05)	6.29	Good source
Chicken breast (05064)	32.02	100 g	2.7	83.8	Excellent source	100 g	1 (1.01)	32.02	Excellent source
Legume/pulse foods									
Bean, baked (16006)	4.75	250 g	1.51	17.90	No claim	130 g	0.6	3.71	No claim
Bean, black (16015)	8.86 (0.064)	125 mL[j]	1.61	12.88	No claim	130 g (sauce, canned, refried)	0.534	6.15	Good source
						90 g (other preparations)	0.534	4.26	No claim
Chickpea (16057)	8.86 (0.061)	125 mL[j]	2.32	17.69	No claim	90 g	0.74	5.90	Good source
Lentil, green (16070)	9.02 (0.074)	125 mL[j]	1.3	12.03	No claim	90 g	0.628	5.10	Good source
Pea, split yellow (16086)	8.34 (0.068)	125 mL[j]	1.42	12.07	No claim	90 g	0.643	4.83	No claim
Soy-based tofu (16426)	17.27	85 g	2.3	33.80	Good source	85 g	0.56	8.22	Good source

[a] Table adapted from Marinangeli and House (26). APL: adjusted protein level; CPL: corrected protein level; NDB: U.S. Department of Agriculture (USDA) National Nutrient Database; RACC: reference amount customarily consumed; RDI: Reasonable Daily Intake.
[b] NDB ID is the identification number used in the USDA Nutrient Database (48).
[c] Based on protein rating—Approved method FO-1 (13).
[d] Based on U.S. Food and Drug Administration (FDA) 21CFR101.9 (53).
[e] Unless otherwise indicated, calculations are based on RDI, as per schedule K of the Canadian *Food and Drug Regulations* (19).
[f] Protein rating of 20–39.9 = good source and ≥40 = excellent source (18).
[g] RACC based on FDA 21CFR101.12 (54).
[h] CPL = crude protein content (g)/RACC × PDCAAS (53).
[i] A CPL between 5 and 9.9 g/RACC (≥10% of the daily value for protein) = good source of protein; a CPL ≥ 10 g/RACC (≥20% of the daily value for protein) = excellent source of protein (51,53). The daily value for protein in the United States is 50 g/day for individuals ≥4 years of age.
[j] When an RDI is not available, the reference amount for a food is used (3,14).

regulatory frameworks for protein nutrient content claims in Canada and the United States to ensure they align with dietary guidance and do not confuse consumers.

Some disadvantages of the protein rating/PER method have been discussed in this review, and it would seem that Canada's transition to PDCAAS as support for protein content claims would be, at least in the interim, a logical approach for modernizing protein claim regulations. The CFIA has outlined that, when PER values are not available, or when foods contain multiple protein ingredients, the PDCAAS methodology can be used. Subsequent to deriving a PDCAAS for the food, the CFIA suggests that the PDCAAS be multiplied by 2.5 (the PER for casein) to derive an adjusted PER for the food, from which the protein rating can be calculated (3). However, the conversion of PDCAAS to PER outlined above has not been validated and does not necessarily address the fundamental challenges inherent within Canadian regulations (25).

PDCAAS is also not without its challenges. As demonstrated in Table III, some foods, such as black beans (no sauce and/or not canned in liquid) and peas, which are suggested sources of plant-based protein in the USDA *Dietary Guidelines for Americans* (49), may not meet thresholds for protein content claims in the United States. The use of rat balance studies to determine true fecal nitrogen digestibility is also time-consuming and costly. The acceptance of in vitro methods to determine true fecal nitrogen digestibility could be a solution to expedite the determination of digestibility coefficients of new foods. Studies by Nosworthy et al. (33–36) have demonstrated that, whereas in vitro digestibility analysis underestimates nitrogen digestibility compared with in vivo methods, coefficients of determination between in vivo PDCAAS and theoretical PDCAAS are >0.9. This relationship is illustrated in Figure 4 for pinto bean and buckwheat flours subjected to various cooking and processing methods (33). Results from Nosworthy et al. (33–36) suggest that in vitro digestibility coefficients and regression analysis can be used to derive the PDCAAS for foods to determine whether they would qualify for a protein content claim in the United States.

Moving forward, it is important to acknowledge recent discussions regarding the best approaches for determining the protein quality of foods. In 2013, the FAO and WHO introduced the digestible indispensable amino acid score (DIAAS) (8), which addresses the shortcomings of the PDCAAS (Table I). Similar to the PDCAAS, the DIAAS uses the indispensable amino composition of a food and the indispensable amino acid requirements of a reference population. However, rather than total fecal nitro-

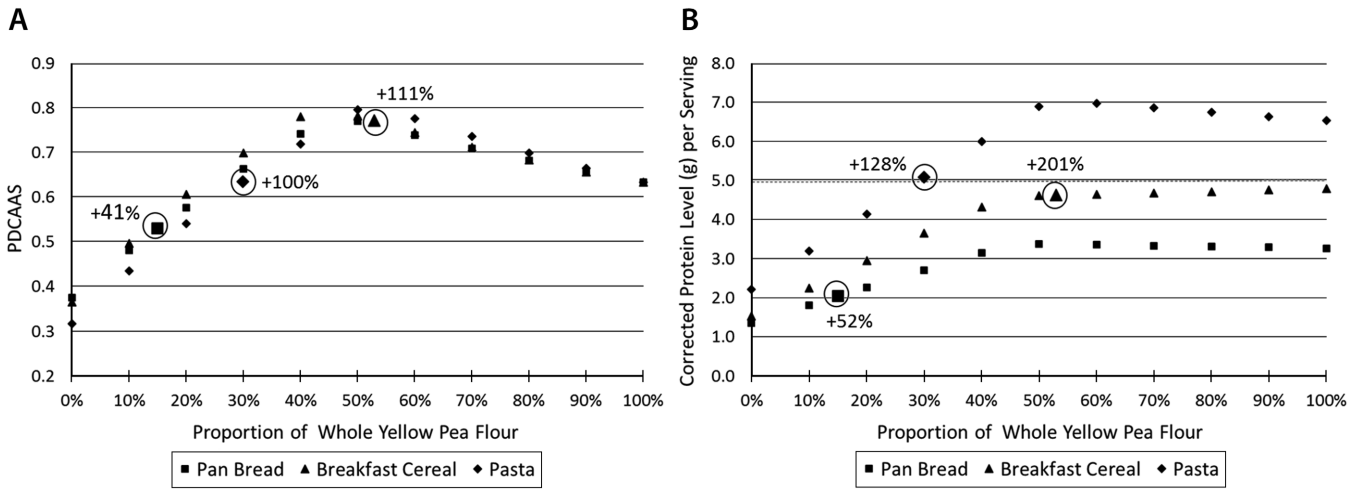

Fig. 3. A, Protein digestible corrected amino acid scores (PDCAASs) for pan bread, breakfast cereal, and pasta reformulated with whole yellow pea flour. **B,** Corrected protein level per reference amount customarily consumed (RACC) for pan bread, breakfast cereal, and pasta reformulated with whole yellow pea flour. Enlarged and circled markers represent tested reformulated foods for which a proportion of the total refined wheat flour in the formulation was replaced with whole yellow pea flour (15% for pan bread, 53% for breakfast cereal, and 80% for dry pasta). Percentages next to these markers indicate the change from baseline. Formulations were adapted from Chaudhary et al. (4). The dashed line in panel **B** is the corrected protein threshold for making a protein nutrient content claim in the United States (5 g/RACC) (51,53) (Fig. 2). PDCAAS was calculated based on requirements for protein content claims in Title 21, Section 101.09 of the U.S. *Code of Federal Regulations* (53) and methods outlined in the Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Expert Consultation on Protein Quality Evaluation (10). The true fecal nitrogen digestibility (TFND) for whole wheat was used as a proxy for refined wheat (0.96) (10). The TFND for split peas (0.8794) (37) was used as a proxy for whole yellow pea flour. Indispensable amino acid (IAA) concentrations for refined wheat flours used in each formulation were taken from the U.S. Department of Agriculture National Nutrient Database for Standard Reference (48) per Chaudhary et al. (4). The ratio of each IAA to total protein (25.26 g of protein/100 g) in split pea flour (37) was used to determine levels of IAAs per gram of whole yellow pea flour (18.66 g of protein/100 g) (4). RACC per eating occasion for individuals ≥4 years of age: bread (50 g), breakfast cereal (40 g), and dry pasta (55 g) (54).

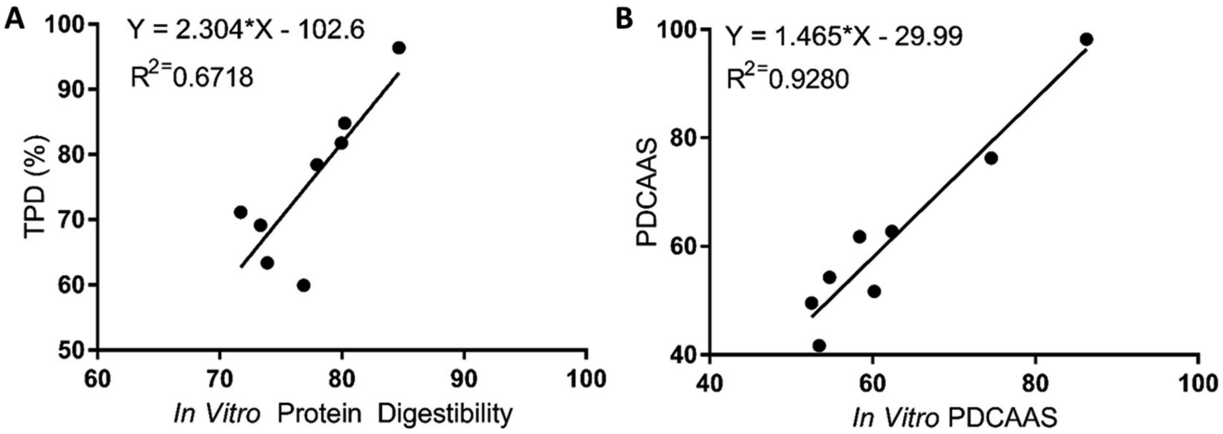

Fig. 4. A, Relationship between the nitrogen digestibility of buckwheat and pinto bean food products, as determined by in vivo and in vitro methods. **B,** Relationship between the protein digestibility corrected amino acid scores (PDCAASs) for buckwheat and pinto bean food products, as determined by in vivo and in vitro nitrogen digestibility. TPD: total protein digestibility. (Reprinted with permission from Nosworthy et al. (33): © 2017)

gen digestibility, ileal indispensable amino acid digestibility coefficients are used, and DIAAS values for single foods are not truncated at 1.0 (8,55). DIAAS values for mixed diets and sole-source foods are truncated to prevent levels of protein in diets or sole-source foods to appear higher than absolute levels. Further, as a regulatory framework, it was suggested that foods would only be eligible for a "source of protein" claim when the DIAAS for a food is ≥0.75 (8). However, this threshold could further limit the ability for high-protein plant-based foods to be identified as a source of protein.

Although the DIAAS is a more accurate reflection of protein quality, its utility within national regulatory frameworks requires further assessment. Albeit limited in scope, a recent comparison of the proposed DIAAS regulatory framework with current regulations for protein content claims in Canada and the United States showed that plant-based foods currently able to be claimed as a source of protein would not be permitted using the proposed DIAAS framework (26). Thus, adoption of the DIAAS into regulatory frameworks could antagonize efforts to increase consumption of plant-based protein. Sources of analytical error, limited availability of data, the use of animals to substantiate product claims, and upfront costs required to derive ileal digestibility coefficients should also be considered (26,35). Possible effects on national nutrition policies and public health would also require further assessment (26).

Other jurisdictions, as well as the Codex Alimentarius Commission, use absolute levels of protein as support for identifying foods as a source of protein (Table IV). In Australia and New

Zealand, 5 g and 10 g per serving of food are thresholds for a "general" and "good" source of protein claims, respectively (11), whereas at least 12% energy/serving supports the lowest tier protein content claim in the EU (7) (Table IV). Codex standards (5), China (30), and South Korea (28) recognize multiple qualifiers for protein content claims when absolute levels of protein represent a proportion of the daily reference value for protein per 100 g, 100 mL, 100 kcal, or serving.

In a recent commentary, the challenges associated with Canada's use of the protein rating method were discussed, and adoption of a regulatory framework for protein claims underpinned by absolute levels of protein was positioned as an option for regulatory modernization (25). Although there is no evidence to suggest that protein claims supported by absolute levels of protein in a food have had negative effects on food choices and quality of diets, a risk assessment would be warranted before any regulatory change took effect. Furthermore, regulations that prevent the addition of ingredients that contain single or a substantial imbalance in amino acids may require consideration (25).

Conclusions

Dietary protein has received substantial attention over the last decade. Given the linkages to health and environmental sustainability, plant-based proteins are increasingly emphasized in dietary guidelines and are resonating with consumers. Although industry stakeholders have responded with an influx of innovative and reformulated foods that incorporate proteins from legumes, seeds, nuts, and cereal grains, the regulatory environment in North America can be a hurdle for communicating protein content claims to consumers. Despite other jurisdictions that use absolute protein levels to support protein content claims, "source of protein" claims in Canada and the United States are based on protein quality. Use of the protein rating/PER method for determining the protein quality of foods marketed in Canada can be more restrictive than the PDCAAS-based system used in the United States. Thus, adoption of PDCAAS in Canada could help address challenges associated with the protein rating/PER method and further efforts to harmonize regulations. Moreover, the acceptance of in vitro methods for determining the true fecal nitrogen digestibility of protein, which is used to calculate the PDCAAS, would also expedite innovation in both countries and increase options for consumers by reducing the costly and time-consuming testing required for in vivo methods. Although the DIAAS has been put forward as a more accurate framework for assessing the protein quality of foods, a comprehensive review of its use in regulatory frameworks is required (26). The same is true for replacing protein quality with protein content to support protein claims, which is currently practiced in various regions with highly developed food systems (25). As dietary patterns, health, and environmental well-being continue to be highlighted, regulatory frameworks must be adapted to help drive changes in consumer behavior.

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Conflicts of Interest

C. P. F. Marinangeli is an employee of Pulse Canada and former employee of Kellogg Canada. A. K. Shoveller and W. D. Mansilla have no conflicts to declare.

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Table IV. Summary of alternative regulatory frameworks and international foods standards (Codex Alimentarius) for protein content claims[a]

Australia and New Zealand[b]	Europe[c]	Codex Alimentarius,[d] China,[e] and South Korea[f]
General Protein Claim ≥5 g of protein/serving	"Source" of Protein ≥12% of energy/serving	"Source" of Protein ≥10% of NRV[g]/100 g (solids), or ≥5% of NRV[g]/100 mL (liquids), or ≥5% of NRV[g]/100 kcal (Codex and South Korea) or 420 kJ (China), or ≥10% of NRV[g]/serving (Codex and South Korea)
"Good Source" of Protein ≥10 g of protein/serving	"High Source" of Protein ≥20% of energy/serving	"High Source" of Protein At least 2× the level of protein that qualifies for a "source" claim

[a] Reprinted from Marinangeli et al. (25). NRV: nutrient reference value.
[b] Food Standards Australia New Zealand (11).
[c] European Commission (7).
[d] Codex Alimentarius Commission (5).
[e] Ministry of Health People's Republic of China (30).
[f] Ministry of Food and Drug Safety (28).
[g] NRV for protein: Codex Alimentarius: 50 g/day (6); China: 60 g/day (30); South Korea: 55 g/day (28).

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Purple and Blue Wheat—Health-Promoting Grains with Increased Antioxidant Activity

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ABSTRACT

Anthocyanins are flavonoid pigments that are responsible for red, purple, and blue colors in diverse organs in a wide array of plants. Anthocyanins also act as antioxidants, for example by scavenging free radicals. In wheat, anthocyanins can be present in the pericarp (purple anthocyanins) or aleurone (blue anthocyanins) layer of the grain. Purple and blue wheat grains, therefore, can be processed into innovative whole wheat (wholemeal) products that are rich in both dietary fiber and antioxidants. Combining the genetic components that produce purple pericarp and blue aleurone traits significantly increases the total concentration of anthocyanins and, as a result, the total antioxidant activity.

Anthocyanins are the most abundant and widely occurring flavonoid pigments and are responsible for most of the blue to blue-black and red to purple colors found in a wide variety of fruits, vegetables, flowers, leaves, roots, and other plant storage organs (9). The first anthocyanin was identified from the blue cornflower (*Centaurea cyanus* L.) in the early 20th century (39). Today, several hundred different anthocyanins have been identified and defined structurally. Interest in anthocyanins has increased recently because they represent natural alternatives to artificial food colorants, and research suggests they have potential health benefits due to their antioxidant properties (11,16, 21,34). In wheat (*Triticum* spp.), pigmentation by anthocyanins can appear in almost all plant parts (Fig. 1)

Purple Pericarp and Blue Aleurone Traits

Purple wheat grains were first introduced to the scientific community by Wittmack in the late 1800s (40). The grains were originally collected in Abyssinia (northern Ethiopia) in 1872 and 1873 (40,41). In his compendium on cereal varieties, Körnicke (19) described two tetraploid, purple-grained Ethiopian wheat varieties, *T. aethiopicum* var. *arraseita* and *T. aethiopicum* var. *schimperi*. In 1905, a German expedition to Abyssinia collected seeds from purple-colored wheat, which were further distributed to researchers in Europe by Wittmack (42). At the same time, two samples of purple wheat from Abyssinia designated as "*frumento eloboni*" were displayed at an agricultural exhibition in Italy (42). It is clear from these publications that purple wheat from Abyssinia was brought to Europe at the end of the 19th century and was widely distributed across Europe by the beginning of the 20th century, either through further distribution by botanists or repeated introduction from East Africa (47).

At the same time, plant scientists also carried out interspecific crosses between wheat and wheat relatives and wheat and rye to transfer genes for disease resistance, winter hardiness, perennial habit, forage traits, and yield components into wheat. Breeding activities in Central Europe gave rise to various European 'Blaukorn' germplasm (17,33,37,46). The source of the blue aleurone trait in this material originates from einkorn wheat (46). Simultaneously, hybridization with *Agropyron* spp. (wheatgrass) in North America resulted in various blue-aleurone genetic wheat stocks (30,31,38,47,48).

From Genetic Studies to Breeding

Purple and blue wheat strains were widely used during the first half of the 20th century to elucidate the inheritance of grain pigmentation (7,17,47). Finally, the purple grain color was transferred into advanced bread wheat material (8). In the 1960s and 1970s, purple wheat germplasm was developed worldwide for purposes such as the demarcation of feed wheat quality (15), development of hybrid wheat systems (5), and determination of outcrossing rates in the self-pollinating wheat crop (10). In the early 1980s, the first commercial purple wheat variety was released in New Zealand, and novel, eye-catching kibbled and whole grain products appeared on the market (44). Since then, commercial purple wheat varieties have also been released in Australia, Canada, China, and many European countries. In contrast, to the best of our best knowledge, no commercial varieties of blue wheat have been released to date except in Austria (29) and China (14).

Black-grained wheat germplasm has been reported by Chinese researchers (26,35,45). The dark or "black" grain color found in wheat is not due to melanin-like pigments, as is the case in barley (43), but results from a combination of purple pericarp and blue aleurone traits (6,35,36). The abundance of wheat with dark colored grains in China is most likely due to the frequent implementation of wide crosses with wild relatives in Chinese wheat breeding programs.

Anthocyanin Profile, Antioxidant Activity, and Health

Separation of grain anthocyanins by different chromatographic methods revealed distinct anthocyanin profiles for blue and purple wheats. In blue wheat, delphinidin was identified as the predominant anthocyanin aglycon, whereas cyanidin is the main aglycon in purple wheat. Generally, the anthocyanin profile is more complex in purple wheat (2,4,6,13,18,20,28,36). The results with regard to profile and total anthocyanin content are somewhat contradictory and point to interactions between genotypes and environments.

Various studies have demonstrated higher antioxidant properties for purple and blue wheat varieties compared with red or white varieties. The composition of anthocyanins, such as type

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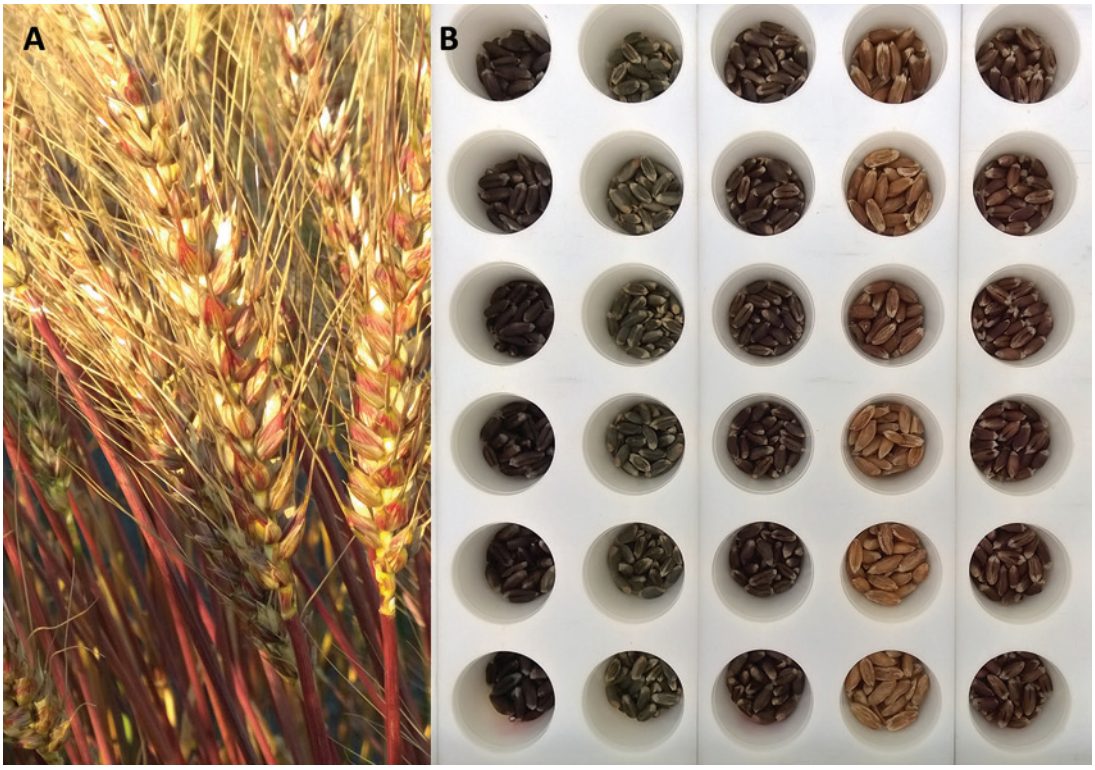

Fig. 1. A, Anthocyanin pigmentation of wheat (purple culm and glume) during ripening. **B,** Pigmented grains of selected single spikes from offspring of purple pericarp × blue aleurone wheat variety crosses prepared for sowing. Left to right: black, blue, deep purple, red, and purple grains. Due to the differences in inheritance of blue aleurone and purple pericarp traits, red or white grain types with no anthocyanin pigmentation can segregate as well as "black" grain types.

of aglycon and sugar moiety, seems to have a significant impact on antioxidant properties (1,16). The highest radical-scavenging activity was reported for a black grain genotype (25) and can be confirmed in our breeding material (Fig. 2). Although other compounds, such as phenolic acids, influence antioxidant capacity, anthocyanins play a major role in the overall free radical-scavenging capacity of colored wheat varieties (14). Therefore, breeding for high anthocyanin content by combining the genetic components of purple pericarp and blue aleurone traits is a promising approach to achieve wheat germplasm with high antioxidant content.

Products and Effects of Processing

A wide range of innovative products incorporating anthocyanin-pigmented wheat varieties has been experimentally and commercially developed (22). In addition to New Zealand, where specialty bread types made from purple wheat were first marketed (27,44), purple wheat products, especially whole grain breads (Fig. 3) and breakfast cereals, have found a niche market in Central Europe and Canada, where they are marketed under trademarked brands (e.g., PurPur® [backaldrin International The Kornspitz Company GmbH] or AnthoGrain™ [InfraReady Products Ltd.]). In China, many food manufacturing enterprises have developed products made with black wheat, such as soy sauce, cakes, and (instant) noodles (22).

Foods made with anthocyanin-rich wheat grains may offer health benefits due to the antioxidant activity of the pigments (12,22); however, processing does have a significant impact on anthocyanins and their antioxidant properties. Fractionation can significantly increase the concentration of anthocyanins (1,32), whereas heat and light can degrade anthocyanins during drying, processing, and storage (23,24). In addition to the use of purple and blue wheat grains for food, extraction of anthocyanins from the bran (3) may also enable their use in nonfood industries. With respect to health benefits, further research on the bioavailability and degradation of anthocyanins after and during processing is needed. However, purple and blue wheat grains definitely increase the diversity of potential cereal products, and by consuming whole grain purple wheat products, consumers can also benefit from a fiber-rich diet.

Conflicts of Interest

The authors declare no conflict of interest with respect to the mentioned companies.

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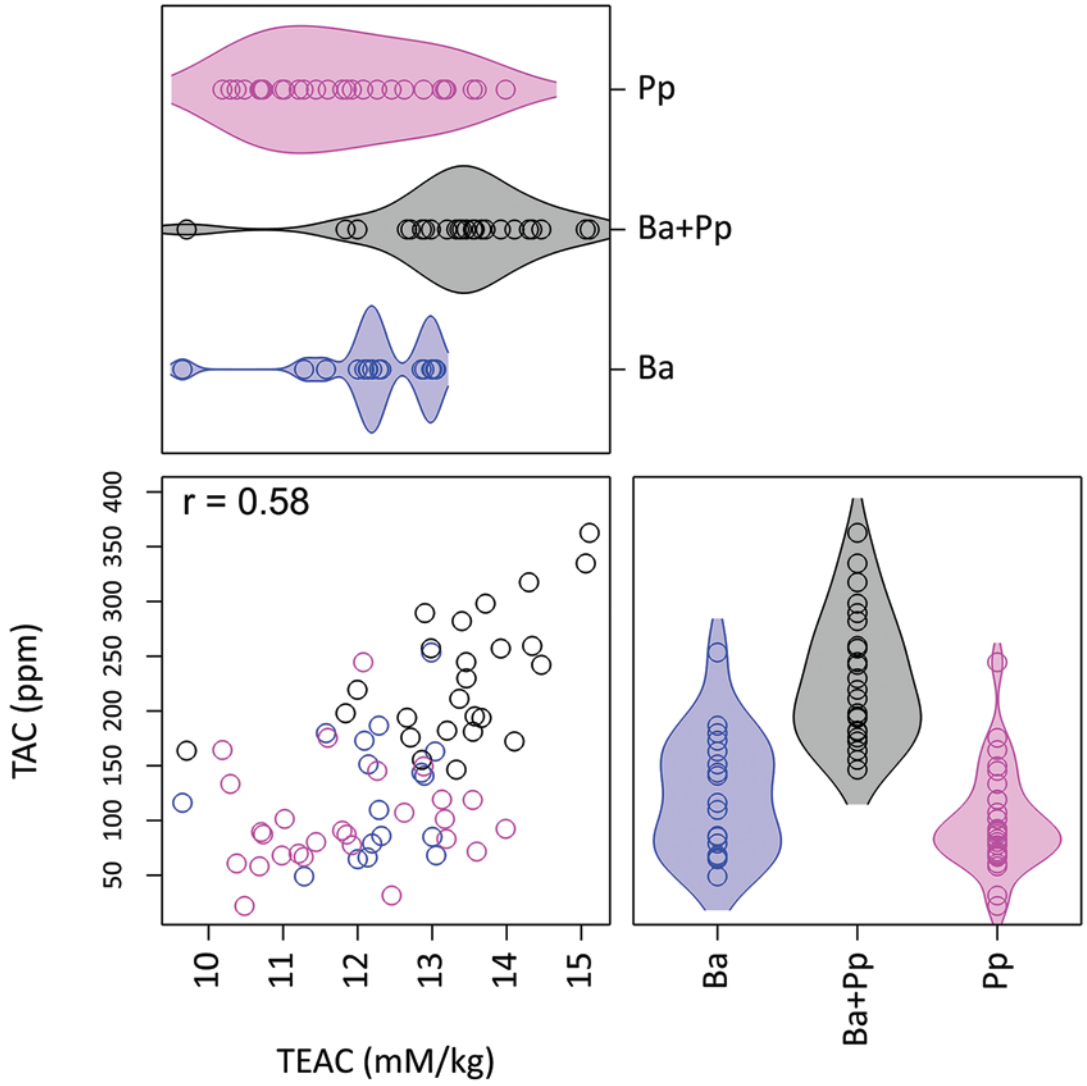

Fig. 2. Relationship between total anthocyanin content (TAC) and Trolox equivalent antioxidant capacity (TEAC) and density plots of purple (Pp), blue (Ba), and "black" (Ba+Pp) breeding lines derived from purple pericarp × blue aleurone wheat crosses.

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Fig. 3. Bread made from whole grain purple wheat. (Photo courtesy of backaldrin International The Kornspitz Company, Asten, Austria)

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New Opportunities for Faba Bean

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Nitrogen fixation by faba bean, which leads to a series of ecosystem benefits, is unparalleled in annual seed crops adapted to cool-season agricultural systems. Cropping systems that include faba bean benefit from lower energy input, which results in a smaller atmospheric carbon footprint; improved soil structure, resulting from deeper roots; and improved soil microbial activity, stemming from residual fixed nitrogen. In addition, faba bean is resistant to a root rot that commonly plagues pea and lentil, thus extending crop rotation options. In northern temperate ecosystems, faba bean seeds contain about 30% protein and produce more protein per unit of land cropped than all other annual legumes, including soybean. In spite of its benefits, faba bean production has been stagnant globally for 40 years. One of the barriers to its wider use has been the presence of vicine and convicine (V-C) in faba bean seeds.

The Importance of V-C

V-C are compounds that are potentially toxic for humans who have a specific enzyme deficiency (variants of glucose-6-phosphate dehydrogenase [G6PD]). For the small proportion of people with a genetically inherited G6PD enzyme deficiency in their red blood cells, V-C–related compounds in faba bean cause the destruction of red blood cells, which presents as an acute hemolytic anemia known as favism. This inherited blood enzyme deficiency is most common in people in the peri-Mediterranean region and affects populations that have the highest faba bean consumption. V-C compounds pose no health risk for the vast majority (>95%) of individuals who do not have this enzyme deficiency.

Traditional means of estimating V-C contents are expensive and require highly sophisticated equipment. Recent efforts have led to the successful development of a new biological test (bioassay) and laboratory methods to detect V-C content in faba bean (2). These methods are relatively inexpensive and can have a turn-around time of as little as one week. The results of the newly developed bioassay are in agreement with the "gold standard" analytical methods for measuring V-C, which are performed by specialized analytical chemists using sophisticated equipment (5). Using the new bioassay ingredient suppliers and food manufacturers will be able to test faba bean-based products, which will support public acceptance of use of this nutritious pulse crop in everyday food products.

Development of New Faba Bean Varieties

Faba bean breeders now have the targets and tools to improve the benefits of faba bean while reducing its potential toxicity. A naturally occurring faba bean variety that produces seeds with 99% less V-C was discovered about 20 years ago. Breeding pure varieties of faba bean is complicated, however, because bees readily cross-pollinate faba beans, making it difficult to maintain pure seed. In addition, the traditional biochemical methods available for measuring V-C, gas or liquid chromatography, are expensive and difficult to use. In 2017, plant breeders discovered a molecular marker for the low V-C gene (vc^-) that makes it easier to quickly develop new varieties with this trait (4).

Further advancements in breeding include reducing seed size to facilitate the use of current agricultural equipment for seeding and harvesting faba bean, making it easier to harvest compared to seeding and harvesting problems associated with large-seed varieties. Breeders are also developing zero-tannin faba bean varieties to decrease antinutritional compounds associated with tannins. When ground, the light-colored seed coats can be used as a source of dietary fiber in food products. Genetic research on faba bean is accelerating due to the imminent availability of a sequenced genome and the development of coordinated international research.

Dry and Wet Fractionation of Faba Bean for Protein and Starch Isolation

Similar to other plants belonging to the *Leguminosae* family, faba bean is a starchy legume that accumulates protein during seed development; typically about 25–28% of the whole seed consists of protein. The faba bean seed coat (hull), which contains the majority of the fiber, color, and flavor and most of the antinutritive compounds, must be removed prior to extraction and purification of the protein and starch fractions for food use. The efficiency of dehulling depends on the seed variety, maturity, moisture content, age, and storage conditions. The dehulled beans can be milled into flour for use in subsequent food applications or downstream processing by employing an impact-milling technique, such as hammer or pin milling.

Dry fractionation of protein and starch using air-classification requires the majority of dehulled flours to be milled to a finer (50–75 μm) particle size. Usually optimal separation can be obtained when the particle size distribution curve of the flour and starch granules overlaps with the protein bodies, which are smaller particles compared with starch granules. Faba bean contains large starch granules compared with protein bodies and, therefore, is easier to fractionate using air-classification (6). The fine protein fraction has a protein purity of 65–75%, and an 18–22% yield can be achieved using select faba bean varieties (internal data). The coarse starch fraction (which also contains protein and fiber) produced as a coproduct from air-classification can be used in various food formulations.

A trial was conducted to extract protein concentrates and isolates from Snowdrop, a small-seed, low-tannin faba bean variety. Generally, the method chosen for protein extraction depends on the quality (color, particle size, functionality) of the protein powder needed for the target product application. Faba bean crude protein concentrate (71% protein purity) and acid-precipitated (86% protein purity) and membrane-separated (90% protein purity) protein isolates have demonstrated higher degrees of solubility and emulsifying properties compared with commercial

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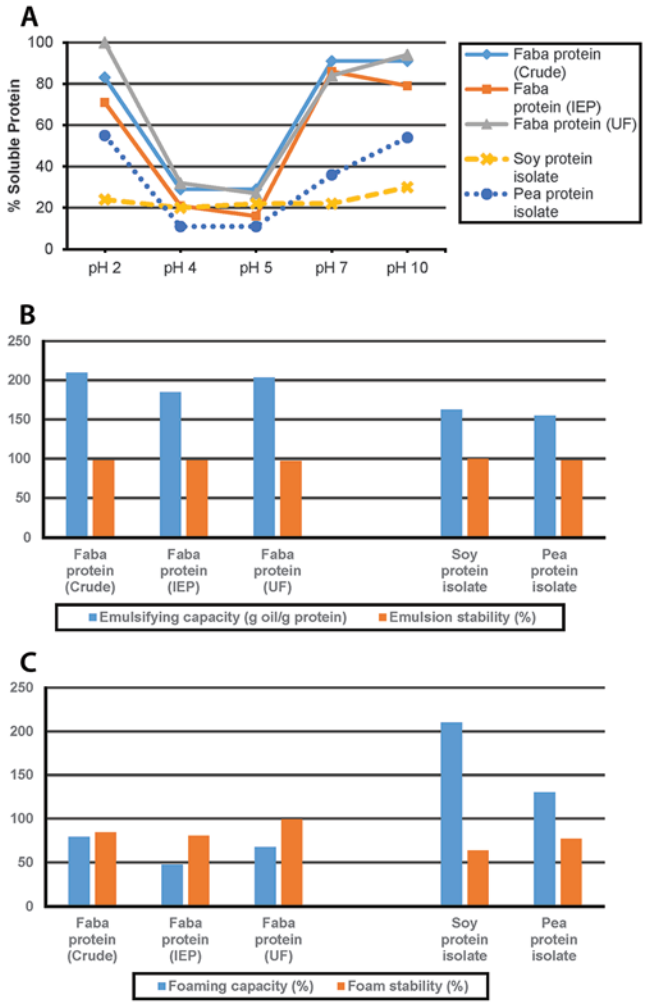

Fig. 1. Solubility (**A**), emulsification (**B**), and foaming (**C**) properties of a protein concentrate and isolates produced through wet fractionation of faba bean flour. Commercial soy protein (79% protein purity) and pea protein (82% protein purity) were used as control samples. IEP: isoelectric precipitated, UF: ultrafiltration membrane separated.

soy and pea proteins tested as control samples (Fig. 1A and B). However, the foaming capacity and stability of these proteins were lower than for the soy and pea protein isolates tested (Fig. 1C). Among the proteins produced during the trial, the membrane-separated faba bean protein contained the lowest amount of residual V-C (Table I). Although wet extraction uses more water and energy compared with dry fractionation, protein isolates produced through wet fractionation have higher purity and improved flavor properties compared with protein concentrates produced through dry fractionation.

Applications

One of the reasons faba bean has the potential to be favored as an ingredient compared with other pulses is its overall high protein content, which enables food manufacturers to readily use the flour and protein fractions to enhance the protein content in products. In applications such as breads and pastas, faba bean flour have worked well as a protein enhancer. Replacing wheat flour with 30% faba bean flour in a bread formulation increased protein content from 11.6 to 16.5% (1). Similarly, for pasta, substituting bread flour with 30% faba bean flour significantly improved the protein quality and fiber content of spaghetti noodles without affecting sensory attributes or physicochemical properties (3).

Table I. Vicine and convicine levels in faba bean flour and proteins produced through wet fractionation[a]

Sample[b]	Vicine (mg/g, dw)	Convicine (mg/g, dw)
Faba bean IEP-extracted protein	7.28	1.60
Faba bean UF-extracted protein	0.89	0.08
Faba bean crude protein extract	22.5	4.78
Snowdrop faba bean flour	5.64	0.94

[a] Method: Purves et al. (5).
[b] EIP: isoelectric precipitation; UF: ultrafiltration membrane separated.

Observations from an extensive ongoing study on faba bean product development suggest that protein concentrates produced by wet fractionation can successfully be applied in the production of shakes and smoothies, while faba bean starches are promising ingredients for use in gluten-free baked products such as cookies and cakes. Faba bean starches also produced viscous pastes when cooked, which would be suitable for soup and sauce applications.

Faba bean ingredients have been tested in extrusion applications as well and were found to work well in products such as breakfast cereals, crisps, and puffs due to their excellent expansion properties. The varying levels of protein in faba bean fractions and ingredients allows for the production of extruded products with a wide range of protein contents. Faba bean has the additional advantage of being naturally neutral in flavor and color. When extruded, these neutral characteristics of faba bean products enable the application of a variety of colors and flavors to suit consumer preferences.

Conclusions

Advancements in faba bean breeding to develop low V-C varieties and development of new food applications are expected to enhance the growth of faba bean production and processing sectors for human consumption. Nutritionally, the high protein content of faba bean makes this pulse crop an attractive choice for incorporation as a protein alternative or protein enhancer in various food products. Promising applications of faba bean ingredients in processes such as extrusion and baking are being tested. It is important to continue focusing on research and product innovation that can help drive increased production and consumption of faba bean.

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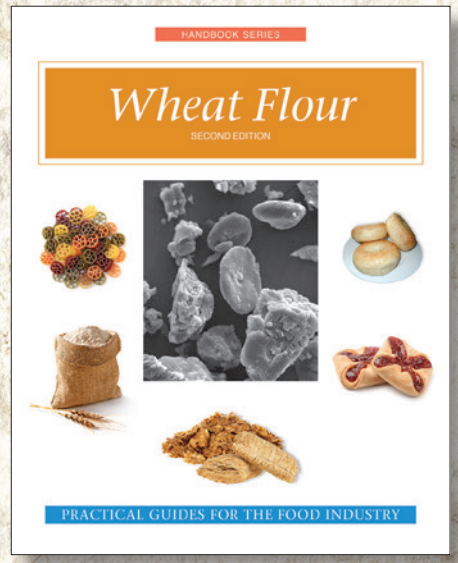

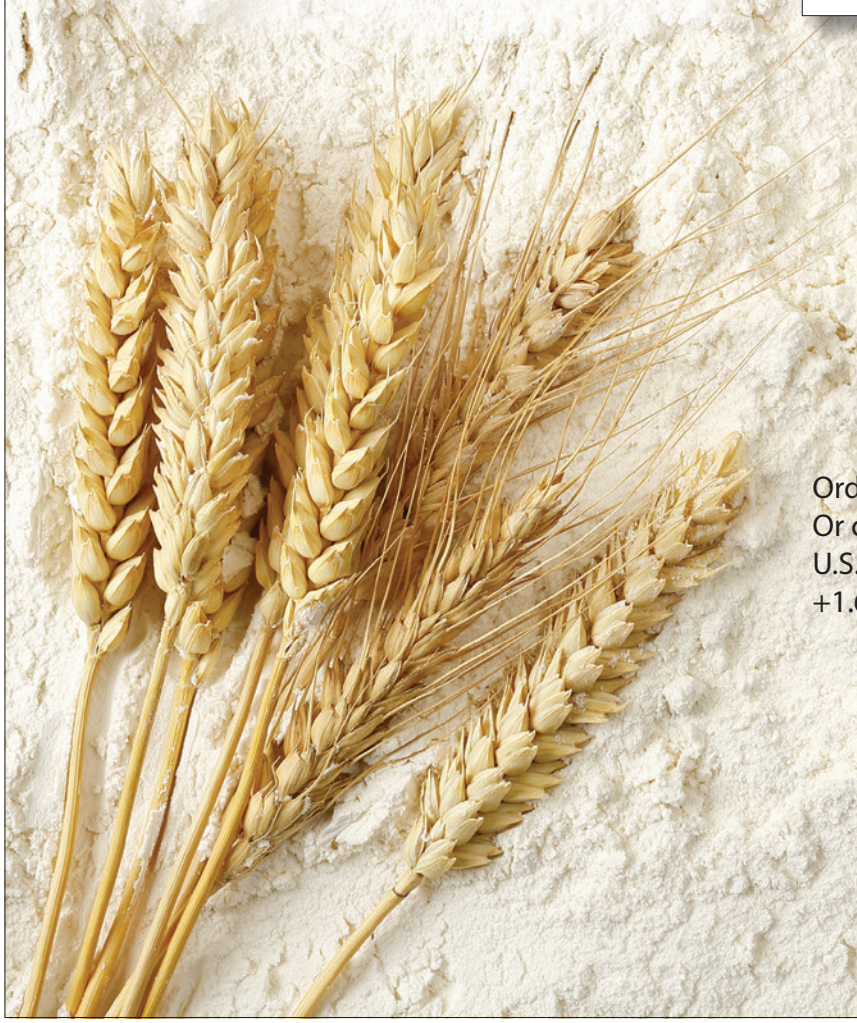

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AACC International Capacity Building on Sampling and Detection Methods for Living Modified Organism Plants Have Been a Key Resource for Implementation of the Convention on Biodiversity Biosafety Protocol

Raymond Shillito
BASF LP, Morrisville, NC, U.S.A.

Background

The Cartagena Protocol on Biosafety (https://bch.cbd.int/protocol/default.shtml) is a supplementary agreement to the Convention on Biological Diversity (CBD; www.cbd.int) that was entered into force 15 years ago on September 11, 2003. The CBD is an international treaty governing the movements from one country to another of living modified organisms (LMOs) resulting from modern biotechnology that "may have adverse effect on the conservation and sustainable use of biological diversity taking also into account risks to human health." These provisions, officially known as the "Biosafety Protocol" (BSP), are intended to provide uniform international requirements for ensuring the safe transport and use of products of "Modern Biotechnology." Modern biotechnology is defined under the BSP as the modification of organisms by recombinant DNA technology (genetic engineering) or by fusion of cells beyond taxonomic families. Like any other technology, modern biotechnology is not intrinsically good or bad. The way the technology is used is the defining factor, and the biosafety system is designed so as to allow one to harness useful applications while avoiding harmful ones.

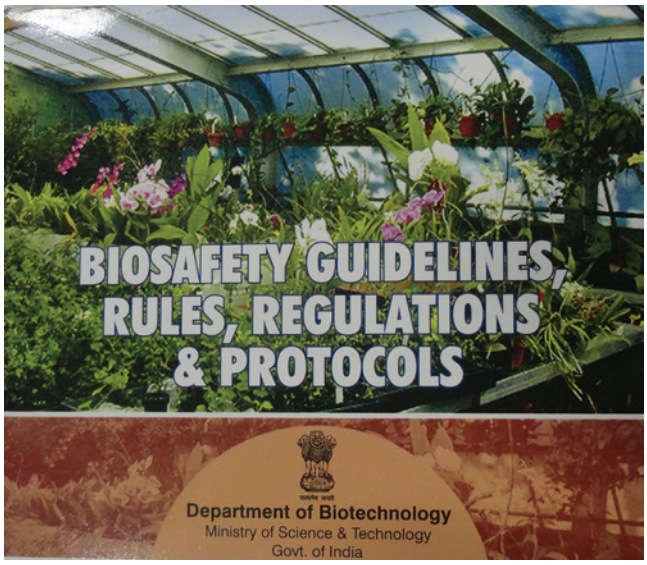
The main requirements of the protocol focus on risk assessment, risk management, and risk communication. Signatories to the BSP are required to have biosafety regulations, which may consist of, for example, a regulatory system for permitting release of LMOs into the environment. It has been stated by some that the BSP and CBD require the implementation of a testing regime; however, this is not the case—what is required is a policy on testing. For countries to develop such policies, it is critical that they understand the nature of modern biotechnology, how the BSP must be applied by signatories, and any analytical methods and other approaches that are available if they decide to include them in their guidelines. To help with this understanding, in 2010 the BSP created a network of laboratories involved in the detection and identification of LMOs (https://bch.cbd.int/onlineconferences/portal_detection/lab_network.shtml). This network is developing a manual and has provided a few capacity-building workshops.

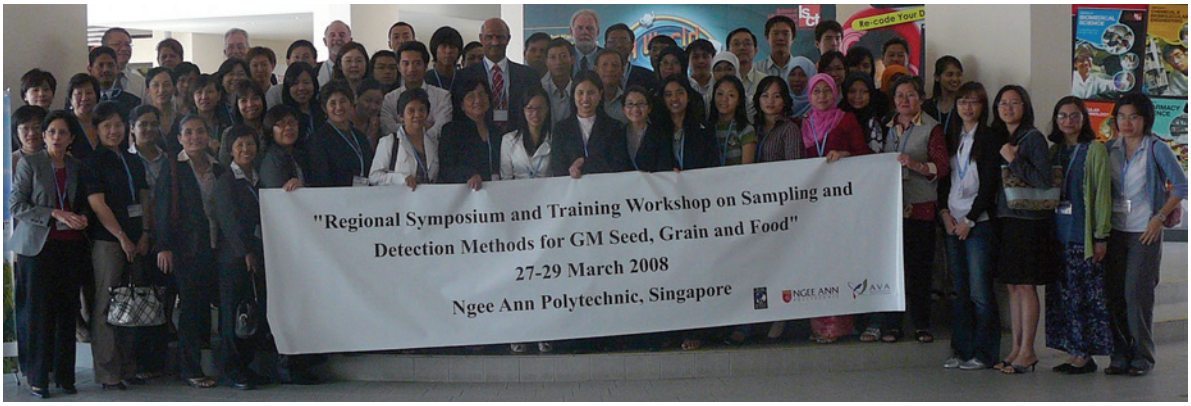
Since 2001, AACC International has been a leading sponsor and organizer of a series of capacity-building workshops on detection methods and sampling for LMOs, particularly plants, and for foods derived from those plants. AACCI worked with local organizations and governments to identify training needs and then delivered workshops that met those needs. The first of the workshops date to before the BSP came into force, although the topics covered were essentially the same as those covered in later workshops. Between 2002 and 2013, the workshops were organized in collaboration with the International Life Sciences Institute (ILSI). These workshops are now organized by the AACCI Molecular Biomarkers for Grain Technical Committee.

Anatomy of a Workshop

Each workshop consists of plenary sessions led by local and AACCI experts and hands-on laboratory exercises. A typical workshop takes place over three to three and a half days.

Both protein-based testing (ELISA and lateral flow strips [LFS]) and DNA-based methods (PCR) are discussed and performed. A typical workshop, like those recently performed in the Caribbean, consists of presentations on the BSP and framework and the local and global status of LMO crops approved for planting and for use in food and feed. This section is followed by a discussion of the supply chains, from seed to grain, for feed and food and the implications and challenges of testing at each stage. These introductory sections are followed by the technical section of the workshop. This technical section includes a discussion of sampling of grain from bulk lots and in the lab and of





plants in the field, as well as the design of sampling plans—good sampling is the basis for all analytical work. The next section of the workshop covers the development and application of biomolecular methods for detection of LMOs. These include PCR, ELISA, and LFS. To date there has not been any interest in detection methods for products of fusion.

Standardization and validation are critically important components of analytical methods. The workshop includes sessions on standardization by internationally recognized organizations such as AACCI, ISO, Codex, and other bodies. Discussions on the importance of reference materials, proficiency testing as a quality management tool, and the design of a control laboratory are also included. Future opportunities for detection technologies are discussed. In all sections of the workshop the presentations allow plenty of time for participant discussions. The goal is for the participants to have their questions answered fully.

Interspersed between the presentations are half-day, hands-on laboratory sessions during which participants perform DNA (PCR) and protein-based testing and hands-on use of sampling.

The workshops finish with a review and discussion of participant results from the laboratory sessions and a round-table discussion on how to select the most appropriate tools for the local situation.

In some cases, countries have requested workshops be focused on specific aspects: for example, in 2013 AACCI provided workshops in Brazil and Peru that were focused specifically on sampling.

Participants

The speakers are chosen from both local experts and experts drawn from AACCI and other organizations, including from the European Union. Participants are sometimes from a single country (e.g., workshops for India and China) or, more often, from a region, as when workshops were held in Singapore, South America, and the Caribbean. The workshops, including travel grants for participants, have been funded by a combination of local governments, biotechnology organizations, ILSI, AACCI, and the United Nations Global Environment fund. They have usually been located at local universities or government facilities.

Outcomes

These activities have established AACCI as an authoritative voice on sampling and detection methods for LM (GM) plants and foods. The workshops facilitate the exchange of knowledge on the latest technologies and approaches for sampling and detecting biotechnology products in seed, grain, food, and feed. Such knowledge is critical tool for parties working to implement the BSP. These activities provide capacity building for governments and local in-country organizations responsible for BSP-related activities by instilling an understanding of the scientific principles of sampling and methods of analysis.

Through this workshop series, we have reached thousands of decision makers who are working to improve harmonization of testing approaches. The programs have created a cadre of people who now have knowledge of the issues and can act as local and regional resources to ensure science-based decision-making processes. The workshops continue to provide capacity building in conjunction with the network of laboratories for the detection and identification of LMOs in the context of the BSP.

"Spotlights" is a series of individual and institutional interviews capturing the unique stories of our many volunteers and their journeys with AACCI.

Improving Livelihoods through Maize and Wheat Science

The International Maize and Wheat Improvement Center

Maize, wheat, and rice together provide 44% of calories in the human diet and 37% of the protein.

The International Maize and Wheat Improvement Center (CIMMYT, www.cimmyt.org) is a global leader in publicly funded maize and wheat research and related farming systems. Headquartered near Mexico City, with 1,500 staff from more than 50 countries in 15 offices throughout the developing world, CIMMYT works with hundreds of partners to sustainably increase the productivity of maize and wheat cropping systems, thereby improving global food security and reducing poverty. CIMMYT receives funding from national governments, foundations, development banks, and other public and private agencies.

The First and Only Nobel Prize for Agriculture

CIMMYT grew out of a pilot program sponsored by the Mexican government and the Rockefeller Foundation in the 1940s and 1950s that aimed to increase farm productivity in Mexico. The program's wheat specialist, Norman Borlaug, worked with Mexican researchers and farmers to develop hardier, short-stemmed wheat varieties that were resistant to devastating rust diseases and that could yield significantly more grain than traditional varieties. The new wheat lines were bred and selected at various locations in Mexico under diverse conditions, which meant they were adaptable to a range of farm environments.

The higher yielding varieties that were developed helped Mexico to attain self-sufficiency in wheat production in the 1950s. Additionally, the varieties were imported by India and Pakistan in the 1960s to stave off famine and soon brought those countries record harvests. This led to the widespread adoption of improved varieties and farming practices, which became known as the "Green Revolution." CIMMYT was formally launched as an international organization in 1966, and Norman Borlaug received the 1970 Nobel Peace Prize for his contributions to reducing world hunger.

50 Years of Global Impact

Started in the 1950s by Borlaug, the CIMMYT-led global wheat improvement pipeline is the main source of public breeding programs for new genetic variation focused on wheat yield, adaptation to climate change, resistance to crop pests and dis-

Dr. Norman E. Borlaug, Nobel Peace Prize laureate, worked at CIMMYT as a wheat scientist and research leader until 1979 and as a special consultant at the center until his death in 2009. (Photo: CIMMYT)

Rapidly emerging and evolving races of wheat stem rust and stripe rust disease—the crop's deadliest scourges worldwide—drove large-scale seed replacement by Ethiopia's farmers during 2009–2014, as the genetic resistance of widely grown wheat varieties no longer proved effective against novel pathogen strains. (Photo: CIMMYT/Apollo Habtamu)

eases, and grain quality. As documented in Impacts of International Wheat Improvement Research: 1994-2014, nearly half of the wheat varieties grown around the world, as well as 70–80% of all varieties grown in South Asia, Central and West Asia, and North Africa, are derived from CIMMYT breeding research. The CIMMYT Global Maize Program develops and delivers germplasm to public and private institutions in 108 tropical and subtropical countries, whose inhabitants include 98% of the poor (i.e., individuals who live on less than US$1.25 a day) who live in maize growing areas.

Grain and Food Quality Research for Better Diets

CIMMYT grain quality research dates back to the center's earliest days and during the 1980s contributed to the development of quality protein maize (QPM), that features grain with enhanced levels of lysine and tryptophan, fostering enhanced health and development in humans and farm animals. CIMMYT cereal chemist Evangelina Villegas was a corecipient of the 2000 World Food Prize for her role in creating QPM.

As part of its focus on agri-food systems, CIMMYT creates maize and wheat breeding lines that are nutritionally enriched and feature good end-use quality for its partners to refine and spread as productive and nutritious varieties. The CIMMYT maize and wheat quality labs explore and use genetic resources to develop healthier and more nutritious value-added foods and feeds. They also analyze physical and chemical grain characteristics to improve processing, provide strategic advice and messaging on nutritious and healthy diets, offer a shared platform for research with the food industry, and help foster sustainable value chains. Finally, CIMMYT researchers develop and refine high-throughput methodologies to include additional quality traits in breeding by studying environmental and genetic effects on grain quality.

Biofortifying Maize and Wheat

In conjunction with HarvestPlus (www.harvestplus.org) and to improve the nutrition and health of the poor who cannot afford dietary supplements or diverse foods, CIMMYT develops high-yielding, biofortified versions of maize and wheat, drawing upon landraces and other sources in the crop gene pools and applying innovative breeding. In recent years, the national research programs of Bangladesh, India, and Pakistan have released six zinc-biofortified wheat varieties derived from this research. Similarly, pro-vitamin A–enhanced maize varieties are available in Africa and South America, and QPM is grown by farmers on 1.2 million ha in Africa, Asia, and Latin America.

Raising Awareness about Cereal Grains and Health

With gracious permission from AACC International, in 2017 CIMMYT published "The Wheat and Nutrition Series: A Compilation of Studies on Wheat and Health," which includes 12 papers from a special series published in *Cereal Foods World* on wheat and health. The detailed reviews cite the best scientific knowledge to show that consumption of whole grains is associated with a lower risk of coronary disease, diabetes, hypertension, obesity, and overall mortality and that eating whole and refined grains is beneficial for brain health and associated with reduced risk for certain cancers. The publication has been widely promoted in CIMMYT efforts to raise awareness about the importance of wheat as part of a nutritious and healthy diet.

Maize is the preferred staple food for 900 million people worldwide who live on less than US$2 a day, is the number one food crop in sub-Saharan Africa, and is in rising demand in Asia as a feed crop. A farmer in Nepal is shown preparing to shell ears of corn. (Photo: CIMMYT/Peter Lowe)

In March 2018, CIMMYT hosted the 4th Latin American Cereals Conference, which was co-organized with the International Association for Cereal Science and Technology (ICC, www.icc.or.at), and the 13th International Gluten Workshop. The events drew more than 250 participants from 46 countries, including professionals in agricultural science and production, the food industry, regulatory agencies, and trade associations.

For more information or to inquire about working with CIMMYT, please contact Natalia Palacios for maize and Carlos Guzmán for wheat.

Spotlight on Julie M. Jones

AACC International members each have their own story, and we want to highlight all of their amazing accomplishments. "Spotlights" is a series of individual and institutional member interviews capturing the unique stories of our many volunteers and their journeys with AACCI.

Julie M. Jones
St. Catherine University
Member for 44 years

Q: What is your current position and what type of work do you do?

A: I am a distinguished scholar and professor emerita at St. Catherine University (SCU) in St. Paul, MN. I started as an instructor at the University of Minnesota (UMN), Department of Food Science and Nutrition, and finished nearly 40 years later as a full professor at SCU. I was also an adjunct professor at UMN. In addition, I do some writing and speaking and sit on a variety of advisory boards that deal with nutrition, grains, carbohydrate, dietary fiber, glycemic response, and diets.

Q: When and how did you first decide you wanted to work in cereal grain science?

A: I didn't really decide to work in the field. It just evolved as a result of my participation in AACC International and IFT local sections and attendance at some national meetings. Because AACCI headquarters were in the Twin Cities and it was a small, focused organization, it was easy to get to know people and to get involved. The organization offered both opportunities and mentors who encouraged my increased participation.

Q: How have you been involved with AACCI? How has your involvement with AACCI enriched your career?

A: I started as treasurer of the AACCI Minnesota Section and held many roles at the local level, including chair. At the national level, I started working on AACCI short courses, writing a monthly column for *Cereal Foods World*, and organizing meeting sessions and workshops and continued on with many activities until I served as AACCI president and chair of the AACCI Board of Directors. I have continued to contribute to the association in many ways. Every time I said "yes" to a request, my career was enriched by learning new things, traveling to new places, and meeting wonderful people who helped me in my career and became friends. I loved the annual meetings, as they were a manageable size, offered good science, and created wonderful interactions that resulted in connections with important colleagues and good friends.

Q: In what ways do you see health and nutrition affecting advancements in cereal- and pulse-based foods? How are health issues affecting cereal science and the cereal grain industry overall?

A: Health and nutrition should play an important role in grain-based foods. Although these foods form the base of dietary guidance in many countries , their position is being challenged as high-carbohydrate, processed foods with little nutritive value. This message seems to contrast the actual data, which show that intake of whole grains, dietary fiber from grains, and the right balance of enriched/fortified of grains is associated with better health. The latter messages about the healthfulness of grains are not being understood or actualized in changing consumer behavior in most parts of the world.

The industry must do everything possible to ensure that grain-based foods are rich in dietary fiber and nutrients and get the "microphone" back to tell consumers how important these foods are in the diet. Further, the industry needs to own the fact that grain-based desserts and snacks are overconsumed by many and that these foods must be eaten in moderation. It must work actively with public health groups to find ways for these foods to be available but not overconsumed. The industry also needs to help consumers understand how additives are used to preserve nutrients, keep costs low, and enhance nutritional value through enrichment and fortification. Changes in breeding of grains, varieties chosen, new processes for developing foods with slower glycemic responses, and techniques such as biofortification are just a few ways in which the industry can help to improve foods for better health.

Q: This issue of *Cereal Foods World* focuses on breeding and processing of grains and pulses to deliver health benefits. Do you have any perspectives on this topic?

A: Breeding can be used to improve grains and pulses in a number of ways, including 1) grains with more resistant starch and fiber in the endosperm, both to increase fiber intake and to lower glycemic response; 2) increased proteins in both grains and pulses that have higher biological value and easier digestibility; 3) grains and pulses with lower phytate and other antinutritional factors to improve iron and zinc absorbability; 4) grains with better translocation of minerals from the soil (regular and biofortified); and 5) grains with gluten that is functional but easier to digest.

Q: What's next for you?

A: I will continue to write and speak on the importance of dietary fiber; consuming the right balance of whole and refined, enriched, and fortified grain-based staple foods; and overall dietary balance, such as DASH- or Mediterranean-style diets. I will continue to defend the judicious use of sugar to improve nutrient intakes from foods such as bran cereals and yogurts: the right/balanced choice of processed foods (not the damning of processed foods) is important for diets that meet today's lifestyles, enable women in the workforce, reduce food waste and loss, and keep costs low. Consumers need no instructions to "avoid processed and ultraprocessed foods," rather they need help choosing the right mix of foods from all levels of processing to create diets that meet their calorie and nutrient needs. I will also try to change the language we use to characterize dietary patterns from the "white bread, potato, and meat pattern" to the "pattern that does not contain the right amount of fruits, vegetables, and whole grains and lower fat meats and dairy." Finally, we need to help consumers see that there are foods to be chosen daily and foods to be enjoyed infrequently.

Thank You AACC International Corporate Members

Thank you to all our corporate members, who contribute their knowledge, expertise, and professional involvement to ensure the continued strength of the association and to promote excellence in cereal grain science worldwide. We appreciate their support and encourage you to contact them directly for detailed information on their products. Visit the AACCI Corporate Member web page for comprehensive company and contact information.

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News

People

Unity/CHOPIN Technologies has announced the addition of Jayne Bock as a collaborator in the area of cereal science and technology. In this role, she will be supporting the North American team in answering all technical and scientific questions concerning CHOPIN Technologies devices. This will enhance the company's ability to adapt its offerings and develop new applications to meet customer needs in the North American market. Bock has a broad range of experience in laboratory equipment, dough rheology, and bakery science. She is an adjunct professor of the University of Guelph, ON, Canada; has authored many publications relating to cereal chemistry and processing; and is an active member of several professional organizations, including AACC International.

AMETEK Brookfield has named Vicki Case as global marketing director. She previously spent 12 years at Sealed Air. For the past 3 years, she served as vice president of marketing, global e-commerce, and fulfillment solutions. Case graduated from the University of Michigan with a B.S. degree in chemical engineering. Hitesh Shah has been appointed to the position of global vice president sales. Shah has more than 25 years of experience in sales and executive positions and has worked for General Electric, Meggitt Sensing Systems, and, most recently, for Novanta. Hitesh has a graduate degree in mechanical engineering. Shah has also served on the Engineering Faculty at the Maharaja Sayajirao University of Baroda. For additional information on AMETEK Brookfield visit www.brookfieldengineering.com.

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Volume 63, Number 5

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LINWOODS is a health-foods company based in Northern Ireland, U.K. From humble beginnings our range of healthy seeds, nuts, and berries has grown to include more than 15 different premium blends and healthy seed snacks. Today, we produce a range of healthy superfood combinations, which are a convenient, easy, and quick way to gain a wide range of essential fatty acids, vitamins, and minerals in your diet. What started as an idea in rural Ireland quickly exploded across the globe, with our products now sold in more than 25 countries around the world. With product development being a key driver within our business, we are moving into the ingredients sector, supplying a range of sprouted ancient grains and seeds and stone-milled flours.

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Important AACCI Dates

October 2018

19–20. Cereals & Grains 18: Pre-meeting Chemical Leavening Short Course, London, U.K.

20. Cereals & Grains 18: Pre-meeting Enzymes in Cereal Grains and Cereal-Based Foods Short Course, London, U.K.

20. Cereals & Grains 18: Pre-meeting Methods in Action—Practical Baking Quality Workshop, London, U.K.

21–23. Cereals & Grains 18 – AACCI Annual Meeting, London, U.K.

24. Rothamsted Research Post-meeting Tour, Harpenden, U.K.

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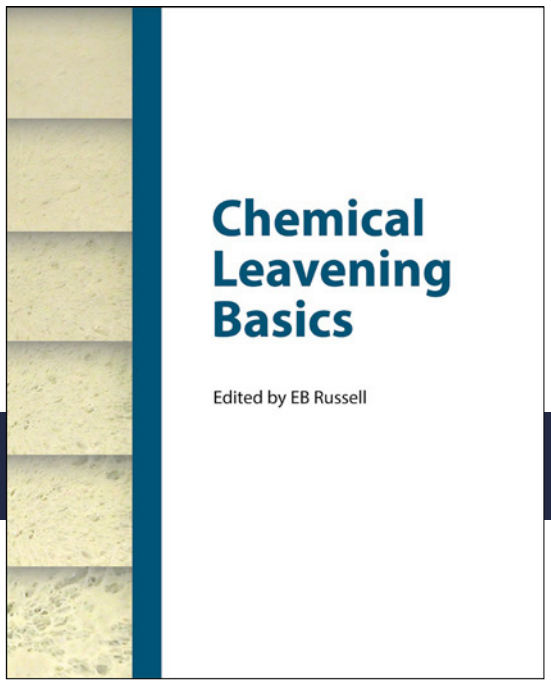

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