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A Grounded Guide to Gluten: How Modern Genotypes and Processing Impact Wheat Sensitivity

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Abstract: The role of wheat, and particularly of gluten protein, in our diet has recently been scrutinized. This article provides a summary of the main pathologies related to wheat in the human body, including celiac disease, wheat allergy, nonceliac wheat sensitivity, fructose malabsorption, and irritable bowel syndrome. Differences in reactivity are discussed for ancient, heritage, and modern wheats. Due to large variability among species and genotypes, it might be feasible to select wheat varieties with lower amounts and fewer types of reactive prolamins and fructans. Einkorn is promising for producing fewer immunotoxic effects in a number of celiac research studies. Additionally, the impact of wheat processing methods on wheat sensitivity is reviewed. Research indicates that germination and fermentation technologies can effectively alter certain immunoreactive components. For individuals with wheat sensitivity, less-reactive wheat products can slow down disease development and improve quality of life. While research has not proven causation in the increase in wheat sensitivity over the last decades, modern wheat processing may have increased exposure to immunoreactive compounds. More research is necessary to understand the influence of modern wheat cultivars on epidemiological change.

Keywords: celiac disease, food allergy, food processing, gluten, inulin, wheat

Introduction

Wheat (*Triticum* spp.) has been consumed by humans for over 8500 y, and currently supplies about 20% of global dietary protein (Braun and others 2010). Recently, the role that wheat, and particularly gluten proteins, should play in our diet has been scrutinized. The public discourse, however, often vacillates between extreme viewpoints on the basic question, "Is gluten good or bad for human health?" The facts are often muddled and incomplete on both sides. Gluten-free diet promoters have described modern wheat as a "perfect, chronic poison" (Davis 2011), while commodity groups have countered that "wheat gluten isn't bad" (National Association of Wheat Growers 2013).

Divided viewpoints also exist when interpreting epidemiological trends. Although studies have suggested that celiac disease has increased 2- to 4-fold over the last 50 y (Lohi and others 2007; Rubio-Tapia and others 2009), causes have not been fully determined. Several authors have questioned whether the last 60 y of breeding produced wheat varieties with more reactivity (Davis 2011; Junker and others 2012), while others consider modern wheat processing to be implicated in epidemiological changes (Di Cagno and others 2010). Without understanding why wheat sensitivity has increased in the population, policy makers cannot effectively address the problem. To help inform consumers, researchers, and policy makers, this article provides a comprehensive summary of (1) the compounds in wheat that can cause wheat sensitivity; (2) the pathologies associated with wheat components in the human body; (3) the differences in reactivity among ancient, heritage, and modern wheats; and (4) the impact of processing methods on wheat components and wheat sensitivity.

Components in Wheat That Can Cause Sensitivity

A grain of wheat is mostly composed of carbohydrates, proteins, lipids, and minerals (Figure 1). While these components can provide basic dietary sustenance for most people, consuming wheat causes negative responses in a small subset of the population. Not all components of the wheat kernel, however, are equally causative of sensitivity to wheat. The compounds implicated in wheat sensitivity, which are labeled in gray in Figure 1, tend to have structures that are difficult for digestion to break apart. This section introduces wheat proteins and fructans, which are most commonly implicated in various types of wheat sensitivity.

Wheat proteins can be classified into 3 main types called gluten, globulin, and albumin. While glutens mainly supply nitrogen to growing seedlings, globulin and albumin proteins serve other specific functions, such as for enzymes, enzyme inhibitors, and structural elongation. The term gluten defines a very diverse and complex group of 2 water-insoluble wheat proteins: gliadin and glutenin. Gliadins are prolamin proteins which are rich in proline and glutamine. The hydrophobic proline is relatively bulky, and thus provides viscosity to dough, allowing it to flow and

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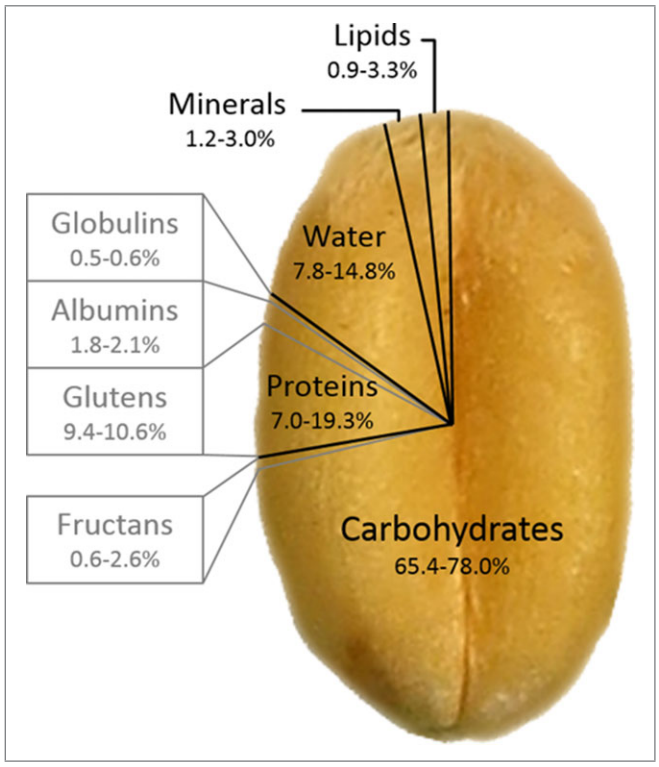

Figure 1–Components of a wheat kernel with variability reported in the literature (Davis and others 1980, 1981; Wadhawan and Bushuk 1989; Wieser and others 1998; Posner 2000; Gafurova and others 2002; Huynh and others 2008; Veenstra 2014). Major components are labeled in black and specific compounds implicated in gluten sensitivity are labeled in gray. Protein fractions reported in the literature were converted to proportion of total grain by multiplying by an average protein content of 12.6%.

rise. With a classification system based on repetitive amino acid sequence patterns, gliadins can be grouped into α-, β-, γ-, and ω-types. Glutenins are polymeric proteins that provide the elasticity and strength to dough, allowing bread to hold its shape. Glutenins can be classified based on electrophoretic mobility at acidic pH into high molecular weight (HMW) and low molecular weight (LMW) types. Similar storage proteins in barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.) are termed hordein and secalin, respectively.

During gastrointestinal digestion, each type of wheat protein breaks down into a wide array of peptides of varying lengths. The rich proline residues in glutens create tight and compact structures that can be difficult to digest (Arentz-Hansen and others 2002). Certain types of these digestion-resistant gluten peptides are found to mediate adverse immune reactions in predisposed individuals.

Amylase-trypsin inhibitors (ATIs), which are albumin proteins, are also implicated in wheat sensitivity. As plant defense proteins, ATIs can block animal enzymes from digesting starch and glycogen in the grain. ATIs have diverse conformational structures that are specific to the enzymes of different animal species, leading some ATIs to affect insect pests without acting strongly against human enzymes (Franco and others 2000). ATI fractions 0.19 and 0.38, which are classified based on fractionation in chloroform and electrophoretic mobility, were found to be active against α-amylase in human saliva and pancreas, respectively (Choudhury and others 1996). ATIs are also found in wheat, rye, triticale, and barley.

In addition to seed proteins, wheat also contains clinically relevant carbohydrates known as fructans. Fructans are fructose polymers with, or without, one glucose conjoined by β-glycosidic linkages (Haskå and others 2008). Fructans can be classified based on their β-glycosidic bond pattern (linear of branched) and the degree of polymerization (short or long). Linear fructans include inulin and levan/phlein which contain β(2–1) and β(2–6) bonds, respectively. Branched fructans are graminan-type and contain a mixture of β(2–1) and β(2–6) bonds. In wheat, these polymers serve the purpose of increasing tolerance to cold and drought (Calderon and Pontis 1985; Hendry 1993).

Fructans are considered dietary fiber as humans are unable to hydrolyze the β-glycosidic bonds. Fructans pass through the upper gastrointestinal tract without undergoing digestion and arrive in the large intestine, where *Bifidobacteria* and other probiotics can utilize and cleave the β-linkages (Playne and Crittenden 1996). Fructans are generally beneficial for most individuals by promoting the growth of healthy gut probiotics, improving stool frequency, and adding fecal bulk (Den Hond and others 2000; Roberfroid 2005; Kleessen and others 2007). Evidence indicates that fructans may reduce fasting insulin levels and thus regulate satiety (Jackson and others 1999; Maziarz 2013) as well as increase absorption of minerals and trace elements (Scholz-Ahrens and Schrezenmeir 2007). However, consumption of high levels of fructans (>15 g/d) may increase bloating, flatulence, and abdominal discomfort (Grabitske and Slavin 2009). While the United States population consumes an average of 3.91 g fructan/d, which is well below the 15 g/d threshold, other global populations consume up to 20 g/d (Moshfegh and others 1999; Shepherd and Gibson 2006).

Although wheat is the major source of fructans in American diets, fructans are also found in 15% of all flowering plants, including artichoke, banana, broccoli, garlic, leek bulb, melon, onions, white peach, and rye (Nelson and Smith 1986; Roberfroid 2005; Muir and others 2007; Fedewa and Rao 2014). Recently, fructans are grouped into a large family of dietary carbohydrates called fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), which can be fermented by bacteria in the large intestinal tract. In addition to fructans, FODMAPs includes sorbitol (stone fruits), raffinose (legumes, lentils, cabbage, Brussels sprouts), and lactose (dairy; Shepherd and others 2008).

Disease Pathologies Associated with Wheat

This chapter and Table 1 review the various sensitivities and intolerances that are found to relate to wheat components including wheat allergy, celiac disease, and nonceliac wheat sensitivity (NCWS), fructose malabsorption, and irritable bowel syndrome (IBS).

Celiac disease

Celiac disease is defined as a chronic, immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals with human leukocyte antigens (HLAs) DQ2 and/or DQ8 (Ludvigsson and others 2013). During digestion, some wheat proteins that are resistant to proteolytic degradation create relatively large peptides. In individuals with celiac disease, some gluten peptides behave like stress-inducing agents that modulate intestinal epithelia and immune cells (Tuckova and others 2002; Londei and others 2005; Thomas and others 2006; Cinova and others 2007), while a few gluten peptides mediate increased intestinal epithelial permeability and increase peptide contact with reactive immune cells (Fasano and others 2000; Clemente and others 2003; Lammers and others 2008; Tripathi and

Table 1–Wheat-related sensitivities, prevalence, and wheat components responsible for the disease pathologies.

Disorder	Prevalence in various populations	Commonly potent reactive components in wheat	Less commonly reactive components in wheat	References
Celiac disease	0.5%–2%	α- and ω-gliadins, CM3 and 0.19 ATIs	γ-gliadins, HMW and LMW-glutenins	Rewers 2005; Tye-Din and others 2010b
Wheat allergy	0.2%–0.5%	–	–	Zuidmeer and others 2008; Vu and others 2014
Baker's asthma	–	ATIs, LTPs, and serpins, α- and ω-gliadins	γ-gliadins, peroxidase, LMW and HMW glutenins	Sanchez-Monge and others 1997; Sandiford and others 1997
Atopic dermatitis	–	LTPs, CM3 ATIs, gliadins and glutenins	–	Kusaba-Nakayama and others 2000; Battais and others 2005b
Urticaria	–	ω-5 gliadin	ω-1,2 gliadin, LMW glutenins, ATIs	Battais and others 2005b
Anaphylaxis	–	ω-5 gliadin, LMW glutenin	ATIs, α-,γ-, and ω-gliadins	Battais and others 2005a; 2005b; Morita and others 2009
Fructose malabsorption	11%–38%[a]	Fructans	–	Truswell and others; Born and others 1995; Ladas and others 2000; Barrett and others 2009
Nonceliac wheat sensitivity	0.55%[a]	ATIs, Fructans (more research is needed to confirm the impact of fructans)	–	Biesiekierski and others 2013; Digiacomo and others 2013; Junker and others 2012
Irritable bowel syndrome	11.5%–14.1%	Fructans with low degrees of polymerization	Fructans with high degrees of polymerization	Roberfroid 1993; Brighenti and others 1995; Rumessen and Gudmand-Høyer 1998; Hungin and others 2005

[a] A large-scale epidemiological study has not been conducted.
LTPs, lipid transfer proteins; ATIs, amylase-trypsin inhibitors.

others 2009). The digestion-resistant gluten peptides are translocated or absorbed to lamina propria (Terpend and others 1998; Schumann and others 2008) where the peptides bind to HLA DQ2 or DQ8 on antigen presenting cells. Due to presence of glutamine and proline in the amino acid sequence, a number of gluten peptides directly bind DQ2 or DQ8 in the binding groove while other peptides require prior modification to enhance binding. The HLA DQ2 and DQ8 receptors preferentially bind peptides with negatively charged amino acids and bulky amino acids at certain anchor residues (Tjon and others 2010). Moreover, tissue-bound transglutaminase selectively deamidates glutamine to create glutamic acid, which allows certain gluten peptides to fit in the binding pockets of HLA DQ2 and DQ8 (van de Wal and others 1998; Arentz-Hansen and others 2000; Vader and others 2002a; Kim and others 2004; Stepniak and others 2005). Once bound to HLA, antigen presenting cells deliver gluten peptides, called T cell epitopes, to T cells. The gluten-restricted T cells proliferate and differentiate into effector Th1 cells that mediate intestinal inflammation through secretion of proinflammatory cytokine interferon-gamma (IFN-γ). The T cells reactive to the tissue transglutaminase could also lead to destruction of the epithelia through generation of autoreactive antibody (Salmi and others 2006; Lindfors and Kaukinen 2012).

Glutens are the major causative antigens in celiac disease. Gluten peptides recognized by T cells in the context of DQ2 and DQ8 have been identified in gliadins, glutenins, hordeins, and secalins. Tye-Din and others (2010b) reported that α- and ω-gliadins appear to harbor most T-cell-recognized epitopes, while fewer T-cell epitopes are found in γ-gliadins and glutenins. A digestion-resistant α-gliadin peptide, LQLQPFPQPQLPYP QPQLPYPQPQLPYPQPQPF, referred to as 33-mer, is one of the highly immunogenic peptides that is often used as a marker for celiac immunoreactivity (Arentz-Hansen and others 2000). In addition to glutens, some wheat ATIs are also considered causative agents that mediate intestinal inflammation by binding to toll-like receptor 4 (TLR4; Junker and others 2012).

Celiac disease is diagnosed through villous atrophy in the small intestine, which is associated with symptoms of poor nutrient absorption, diarrhea, pain, and weight loss. Celiac disease can also be manifested though specific skin symptoms, called dermatitis herpetiformis, and neurological symptoms, called gluten ataxia (Fabbri and others 2012; Jackson and others 2012). Individuals with celiac disease are more likely to develop other autoimmune disorders during their lifetimes, such as type 1 diabetes and thyroid disease (Ventura and others 1999). Although the vast majority of cases are likely undiagnosed, celiac disease is considered the most common genetically related autoimmune disease in the world, affecting 0.5% to 2% of global populations (Rewers 2005). It has been estimated that the safe threshold of gluten consumption for individuals with celiac disease ranges from 10 to 100 mg/d (Hischenhuber and others 2006).

Wheat allergy

Individuals with food allergies have elevated amounts of immunoglobulin E (IgE) antibodies specific to certain food allergens. Upon exposure, allergens bind to IgEs on mast cells or basophils and trigger the release of histamine and other chemicals that mediate the immediate allergic reactions. Nearly all food allergens are proteins that tend to resist degradation from heat, proteases, or acid hydrolysis. Peanut, milk, egg, tree nuts, wheat, crustaceans, fish, and soybeans are the most common allergenic foods (Taylor and Hefle 2001). Symptoms of wheat allergy encompass baker's asthma and rhinitis, which results from inhaled flour; atopic dermatitis, which relates to skin exposure; urticaria, which forms hives after contact with wheat; and anaphylaxis, which is the most life-threatening type of wheat allergy and affects many body systems (Battais and others 2008).

An estimated 0.4% of the world's population is allergic to wheat proteins (Zuidmeer and others 2008; Vu and others 2014). Various types of wheat allergies differentially affect subsets of the population. The majority of wheat allergy cases are found in children,

which are dominated by atopic dermatitis and digestive symptoms (Hischenhuber and others 2006). On average, sufferers outgrow their wheat allergy by age 6 (Keet and others 2009). Wheat-dependent exercise-induced anaphylaxis (WDEIA), results when wheat is consumed before vigorous physical activity and most commonly manifests in teenagers. Baker's asthma and rhinitis are predominantly occupational hazards for bakers and millers, who are exposed to large amounts of airborne flour particles (Walusiak and others 2004).

Wheat allergens are found in a number of glutens, albumins, and globulins (see Table 1 and reviews by Battais and others 2008; Tatham and Shewry 2008). Omega-5 gliadins are the major allergens in WDEIA and urticaria (Battais and others 2005a, 2005b; Morita and others 2009). ATI CM3, α-, and γ-gliadins were linked to atopic dermatitis (Kusaba-Nakayama and others 2000; Tanabe 2004). While a number of albumins and globulins, including ATIs, lipid transfer proteins, and serpins were found to be the strongest triggers of baker's asthma, α- and ω-gliadins and LMW glutenins were also causative (Weiss and others 1993; Sandiford and others 1997). It has been reported that, in general, wheat allergy sufferers can tolerate larger amounts of wheat than celiac patients. More than 1 g was necessary to induce symptoms in most adults with wheat allergy, although a minority of children experienced reactions after less than 10 mg of exposure (Hischenhuber and others 2006).

NCWS, fructose malabsorption, and IBS

Apart from celiac disease and wheat allergy, various ill-defined adverse reactions to wheat are grouped as NCWS (Sapone and others 2012). However, the role(s) of gluten and other wheat components in mediating NCWS remains unclear. Some individuals with NCWS suffer an innate immune reaction that is mechanistically similar to, but less severe than celiac disease (see reviews by Verdu and others 2009; Catassi and others, 2013). In comparison to the general population, NCWS patients appear to have a higher incidence of the HLA DQ2 or DQ8 genetic disposition (Wahnschaffe and others 2001, 2007), and high levels of gluten-specific antibodies (Carroccio and others 2012; Volta and others 2012). Individuals with NCWS, however, lack the villous atrophy characteristic of celiac disease. Certain ATIs appear to implicate NCWS as well. Junker and others (2012) reported that ATI fractions CM3 and 0.19 activated a TLR4-dependent pathway leading to the release of proinflammatory cytokines from monocytes, macrophages, and dendritic cells derived from both celiac and nonceliac patients. The authors hypothesized that individuals with poorly regulated TLR4 could experience inflammation induced by wheat ATIs.

Carroccio and others (2012) reported that 70 of 276 NCWS patients exhibited disease pathology similar to celiac disease. The remaining majority of patients (206 of 276), however, demonstrated allergy-like hypersensitivity to wheat in addition to a broad array of other foods. However, a double-blind placebo-controlled challenge on 920 total NCWS patients revealed that 644 patients (70%) did not react to wheat in the diet. Another double-blind placebo-controlled cross-over study compared 22 NCWS individuals consuming a gluten-containing diet and a nondairy whey protein-containing diet. There was no significant difference in pain, bloating, bowel movements, tiredness, gas, or nausea between the 2 diet types. Patients only experienced reduced gastrointestinal discomfort when placed on a low-FODMAP diet (Biesiekierski and others 2011, 2013). As Carroccio and others (2012) did not control for FODMAPs in study diets, it is possible that FODMAPs could have contributed to symptoms of the 70% of NCWS patients who did not respond to wheat alone. The prevalence of NCWS is not known, although one study from 2010 estimated that 0.55% of individuals in the United States follow a gluten-free diet and do not have celiac disease (Digiacomo and others 2013).

Some individuals with NCWS may suffer from fructose malabsorption rather than gluten sensitivity (see review by Gibson and others 2007 and Fedewa and Rao 2014). In such cases, consumption of wheat fructans can provoke symptoms via fructose malabsorption (Shepherd and Gibson 2006; Ong and others 2010). Individuals with fructose malabsorption are unable to absorb free fructose present in the digestive tract. The unabsorbed fructose undergoes bacterial fermentation and induces abdominal symptoms, such as pain, bloating, and altered bowel habit. Fructose malabsorption can be easily diagnosed through standard testing of hydrogen and methane in the breath following fructose consumption (Fedewa and Rao 2014). The diagnosis of fructan intolerance is less straightforward than fructose malabsorption; however, a protocol for a diagnosis of fructan intolerance was under development (Fedewa and Rao 2014). Although many studies have examined fructose malabsorption in individuals, the lack of standardization in testing and doses have resulted in no firm estimates of the prevalence of fructose malabsorption in the population (Latulippe and Skoog 2011). Between 11% and 38% of healthy individuals have experienced fructose malabsorption when consuming fructose levels that are typical of daily consumption rates (Truswell and others 1988; Born and others 1995; Ladas and others 2000; Barrett and others 2009). The prevalence of malabsorption rises with higher consumption rates of fructose (Gibson and others 2007).

Individuals with IBS may also react to wheat in the diet. Fructan ingestion likely causes discomfort for all IBS individuals because only 5% to 15% of ingested fructans are absorbed in the small intestine (Fedewa and Rao 2014). The low absorption rate results in large quantities of fructans entering, and undergoing fermentation in the large intestine of IBS-affected individuals. This fermentation can further aggravate symptoms in all IBS individuals, regardless of whether or not individuals are fructose intolerant. Patients with diahrrea-predominant IBS exhibited significantly higher small bowel permeability, reduced expression of tight-junction proteins regulating intestinal permeability, and increased frequency of bowel movements on a wheat-containing diet (Vazquez-Roque and others 2013). The consumption of low FODMAP diets is associated with declines in symptom severity for individuals with IBS (de Roest and others 2013; Halmos and others 2014). Nevertheless, low levels of fructan consumption may help manage IBS by stimulating *Bifidobacterium* growth in the gut (Roberfroid and others 2010). Further research is needed to determine the daily fructan intake level that best minimizes symptoms in IBS-affected individuals. IBS is a very prevalent disease that affects an estimated 14.1% of the United States population (Hungin and others 2005) and 11.5% of Europeans (Hungin and others 2003).

Misdiagnosis is common among NCWS, fructose malabsorption, and IBS. For example, it is estimated that up to one-third of patients with suspected IBS, particularly IBS with diarrhea-predominant symptoms, actually suffer from fructose malabsorption (Choi and others 2008). IBS, NCWS, and fructose malabsorption share a broad array of symptoms (Muir and others 2007; Verdu and others 2009). To further challenge correct diagnosis, the mechanisms of disease pathology remain unknown for NCWS and IBS. Regardless of uncertain diagnosis, consumption of fructans by individuals with fructose malabsorption, NCWS, or IBS is not

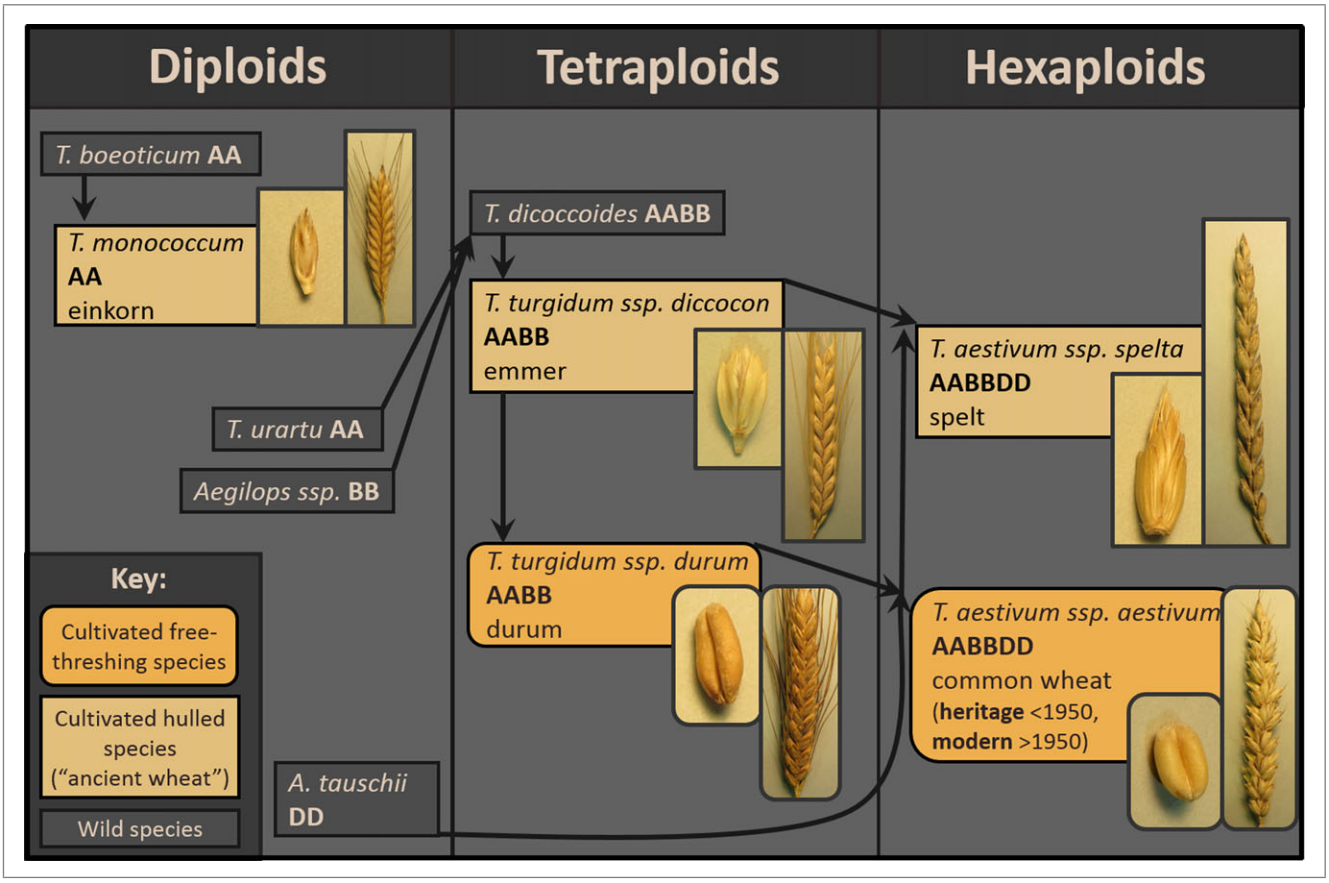

Figure 2–The genealogy of cultivated members of the *Triticum* family, including various cultivated ancient wheat species, durum wheat, and common wheat (adapted from Dawson and others 2013).

recommended due to potential aggravation of symptoms (Roberfroid and others 2010).

Given that all fructan types are predominantly composed of fructose, small amounts of any ingested fructan will be digested to fructose and likely aggravate symptoms in fructose-intolerant individuals (Roberfroid 2005; Jenkins and others 2011). Shepherd and Gibson (2006) indicate that the chain length of fructans, rather than structure type, is an important factor in the amount of discomfort as a result of ingestion. Fructans with a low degree of polymerization induce IBS-like symptoms, have a greater osmotic effect, and are more rapidly fermented than fructans with higher degrees of polymerization (Roberfroid 1993; Brighenti and others 1995; Rumessen and Gudmand-Høyer 1998).

Variation in Reactivity among Species and Varieties of Wheat

"Wheat" is a term loosely used to include a diverse array of cultivated species and genotypes in the *Triticum* genus (Figure 2). As coding regions for wheat storage proteins are highly polymorphic (Payne 1987; Metakovsky and others 1991; Salentijn and others 2013), each genotype produces unique types and quantities of glutens, ATIs, and fructans (Nakamura and others 2005; Veenstra 2014). Consequently, wheat varieties can be assigned a "*reactivity profile*," which indicates the potency and amount of reactive epitopes created after digesting that specific wheat variety. However, the protein and fructan expression of one genotype can change depending on the environment where it was grown. Moreover, reactivity profiles are not universal, as patients differentially react to glutens, ATIs, and fructans. This section reviews the types of cultivated wheat, and evaluates their relative reactivity for celiac disease, wheat allergy, NCWS, fructose malabsorption, and IBS.

Types of wheat

The world's most widely grown species of crop is common wheat (*T. aestivum* L.), which is otherwise known as hexaploid wheat, or bread wheat. Tens of thousands of varieties of common wheat are grown around the world. Modern wheat generally refers to varieties that were developed after the use of dwarfing genes in the 1950s, while heritage wheat varieties were developed before that time period. Landraces, which can be a mixture of genotypes, are categorized as heritage varieties in this review.

Common wheat contains 3 genomes (A, B, and D) that were derived from different ancestors. Ancient wheat refers to the hulled relatives of common wheat, which are separate species that contain different combinations of the three wheat genomes. The oldest cultivated ancient wheat is einkorn (*T. monococcum* L. ssp. *monococcum*), which is a diploid with only the A genome. Tetraploid species that share the A and B genomes with common wheat are emmer (*T. turgidum* L. ssp. *dicoccum* Schrank ex Schübl.), durum [*T. turgidum* L. ssp. *durum* (Desf.) Husn.], rivet (*T. turgidum* L. ssp. *turgidum*), and Khorasan wheat [*Triticum turgidum* L. ssp. *turanicum* (Jakubz.) Á. Löve & D. Löve], for which one variety is marketed under the Kamut® trademark. Although durum wheat is not an ancient grain, but a free-threshing grain primarily used for pasta, it will also be included in this discussion. Spelt [*T. aestivum ssp. spelta*

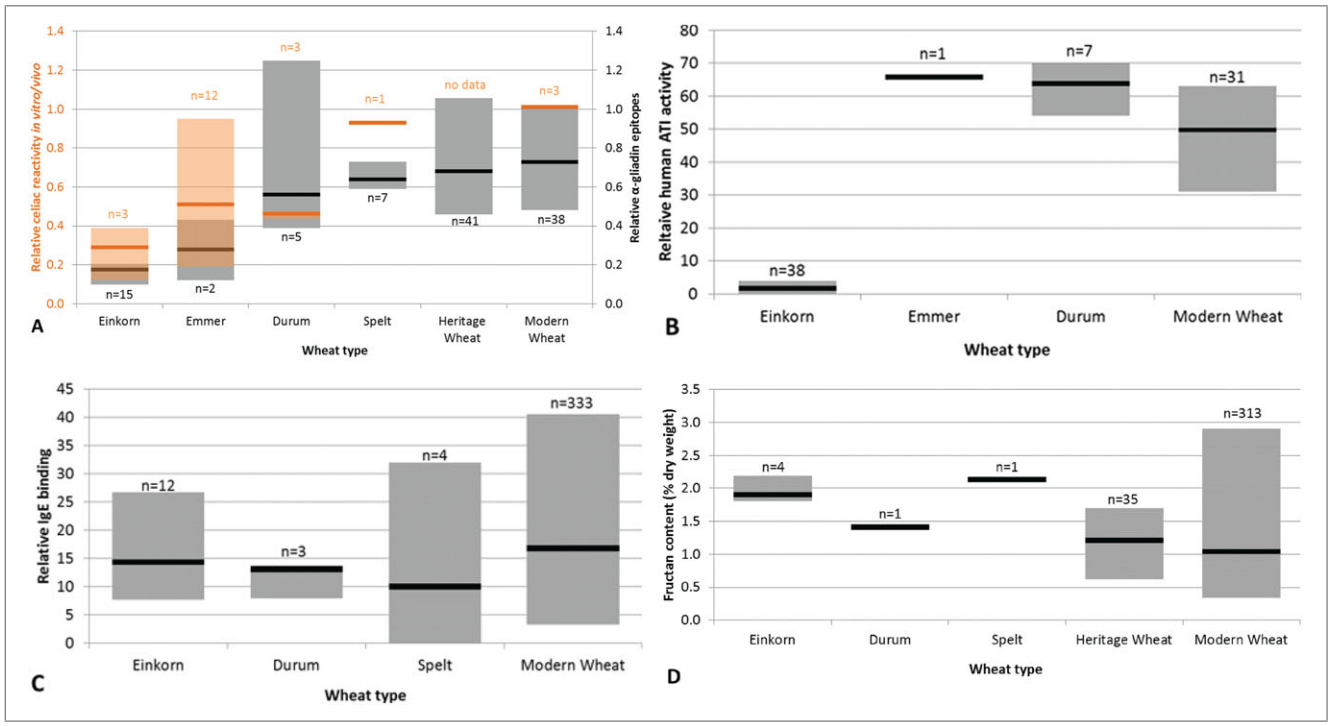

Figure 3–Values reported in the literature within and among wheat types for (A) celiac reactivity (Molberg and others 2005; Pizzuti and others 2006; Vincentini and others 2007, 2009; van den Broeck and others 2010a, 2010b), (B) human α-amylase inhibitor activity (Bedetti and others 1974; Vittozzi and Silano 1976; Sánchez-Monge and others 1996; Wang and others 2007; Zoccatelli and others 2012), (C) allergenicity (Weiss and others 1993; Sánchez-Monge and others 1996; Klockenbring and others 2001; Nakamura and others 2005; Larré and others 2011; Vu and others 2014), and (D) fructan content (De Gara and others 2003; Huynh and others 2008; Brandolini and others 2011; Hammed 2014; Veenstra 2014). Gray boxes show values of intraspecific variation (maximum to minimum values). Black lines represent means for each wheat type. Labels "$n =$ " refer to the number of unique varieties evaluated. Values in A, B, and C were normalized to a relative scale by converting reported average values for modern wheat in each study to a shared value. Modern wheat includes varieties of common wheat that were developed after 1950, while heritage wheat includes varieties and landraces that were developed before 1950.

(L.) Thell.] is a hulled species that shares A, B, and D genomes with common wheat.

Celiac disease: Ancient wheat species

Genome composition can partially explain the variation in celiac immunoreactivity among species of wheat. Several highly immunogenic α-gliadins are encoded by the D genome of wheat (Molberg and others 2005; Spaenij-Dekking and others 2005; van Herpen and others 2006). Consequently, species that lack the D genome of wheat, such as einkorn, emmer, and durum, appear to exhibit average lower reactivity than common wheat (Figure 3A). The B genome of wheat encodes the fewest α-gliadin epitopes implicated in celiac disease (van Herpen and others 2006). However, diploid species with genomes similar to the B genome of wheat are not cultivated or normally consumed by humans.

Since spelt has a D genome, its cytotoxicity was found to be similar to common wheat. Six spelt landraces produced levels of celiac α-9 T-cell epitopes that were similar to 80 common wheat genotypes (van den Broeck and others 2010b). When comparing spelt and common wheat, Vincentini and others (2007) measured similar inhibition of cell growth, activation of apoptosis, release of nitric oxide, release of tissue transglutaminase, and alteration of transepithelial electrical resistance on Caco-2/Tc7 and K562 (S) cell agglutination.

Einkorn, which has only the A genome of wheat, expressed the least celiac disease epitopes among cultivated species. Vincentini and others (2007) reported no cytotoxicity in one einkorn genotype, measured as inhibition of cell growth, activation of apoptosis, release of nitric oxide, release of tissue transglutaminase, and alteration of transepithelial electrical resistance in human colon cancer Caco-2/TC7 and human myelogenous leukemia K562(S) cells. After exposure to gliadin extracted from einkorn, intestinal biopsies of 8 individuals with celiac disease showed no reduction in intestinal villi height or production of IFN-γ (Pizzuti and others 2006). In a rare *in vivo* study, 12 celiac patients experienced no difference in gastrointestinal complaints after 28 d consuming either 2.5 g of rice or the einkorn cultivar "Monlis" (Zanini and others 2009). Nevertheless, einkorn still expressed T-cell immunogenic α- and γ-gliadin epitopes (Molberg and others 2005; van Herpen and others 2006). Fifteen different einkorn genotypes produced substantially different amounts of these celiac disease epitopes (Molberg and others 2005). The amino acid sequences of toxic peptides were also found in one einkorn genotype (Vaccino and others 2009).

Emmer and durum, which have the A and B genomes of wheat, generally appear to be less immunoreactive than common wheat, but more immunoreactive than einkorn. Gliadin derived from 2 durum varieties were less cytotoxic than those from a common wheat, when exposed *in vitro* to biopsies of children with celiac disease. Five times the concentration of durum was necessary to match the intestinal villi damage caused by the common wheat (Auricchio and others 1982). Although the average reactivity of emmer and durum is lower than common wheat, there is a wide range of celiac response depending on genotype. While 3 emmer landraces induced negligible T-cell proliferation and release of IFN-γ, other landraces induced levels that were not significantly

different than common wheat (Vincentini and others 2009). Similarly, van den Broeck and others (2010a) identified 2 emmer and 3 durum accessions with low amounts of the commonly reactive α-9 epitope (PFPQPQLPY). However, other durum accessions expressed amounts similar to those of common wheat.

Individuals with celiac disease differentially react to the gluten profiles of ancient wheat (Vader and others 2002b). T-cell activity from 4 children with celiac disease differed widely after exposure to 9 landraces of emmer (Vincentini and others 2009). Despite lower reactivity overall, einkorn, emmer, and durum still produced reactions in 25% to 38% of tested patients' T cells (Molberg and others, 2005). Such variability underscores the fact that no varieties or species of wheat have been determined to be safe for individuals with celiac disease.

Unfortunately, the D genome, which is associated with celiac epitope expression, is also responsible for expressing most of the HMW proteins that are essential for bread-making quality. Consequently, ancient and durum wheat are not equipped with the gluten profile for bread baking. Moreover, emmer lines lacked HMW 7+8, LMW-2, and γ-45/ω-35 proteins which are important for pasta quality (Vincentini and others 2009).

Celiac disease: A comparison of modern and heritage varieties

Broad diversity in celiac immunoreactivity also exists among varieties of common wheat. Varieties that express more Gli-2 genes from the A or B genomes, rather than the D genome, will produce fewer α-gliadin celiac T-cell epitopes (Salentijn and others 2009). Other varieties have mutations in α-gliadin coding sequences that alter expression of celiac disease T-cell epitopes.

Data compiled from a limited number of studies indicate that heritage genotypes, on average, express lower levels of celiac immunoreactive compounds (Figure 3A). Van den Broeck and others (2010b) compared European heritage and modern varieties for the production of α-9 epitopes implicated in celiac disease. Twelve of 44 heritage collections produced low levels of the epitope, compared to only 1 of 36 modern varieties. In another study, 2 modern genotypes had lower frequency of α-gliadin expression from the A genome (15%), when compared to 5 landraces (29%; Salentijn and others 2009). Among 61 durum accessions, the genotypes expressing the lowest amounts of 3 α-gliadin epitopes (DQ2.5-Glia-α1 (PFPQPELPY), DQ2.5-Glia-α2 (PQPELPYPQ), and DQ2.5-Glia-α3 (FRPEQPYPQ)) were a mix of landraces, old varieties, and modern breeding lines (Salentijn and others 2013). However, modern durum varieties tended to fall in the highest categories of epitope expression. Modern breeding lines constituted 91% of varieties in the most immunodominant category, while old varieties and landraces only represented 9% (Salentijn and others 2013). Genetic linkages between loci for α-gliadins and HMW glutenins may explain why some modern varieties contain more celiac T-cell epitopes. Many modern varieties have been bred for increased HMW glutenin content, which improves bread baking quality when using common wheat and pasta quality when using durum.

Not all heritage genotypes, however, had low T-cell immunoreactivity. Although average intensity of α-9 epitopes was higher in modern varieties, the most immunodominant variety identified by van den Broeck and others (2010b) was a heritage wheat. As Vincentini and others (2009) concluded, old varieties and landraces exist with potent celiac epitopes, indicating that humans have long been exposed to immunoreactive genotypes of wheat. Conversely, studies have identified modern varieties with low expression of α-gliadin epitopes (van den Broeck and others 2010b; Salentijn and others 2013) and IgA reactivity (Constantin and others 2009). Certain varieties of modern wheat have also shown less immunoreactivity than ancient wheat species. In an evaluation of 16 ancient and modern wheats, one line of modern club wheat [*T. aestivum* L. ssp. *compactum* (Host) MacKey] induced the second lowest *in vitro T-cell* response and IFN-γ release (Spaenij-Dekking and others 2005).

Efforts have been made to create modern wheat genotypes with lower celiac immunoreactivity. Varieties devoid of any immunoreactive glutens would not be functional, as a portion of celiac patients react with HMW glutenins, which are essential for baking quality (Molberg and others 2003; Dewar and others 2006; van den Broeck and others 2009). Due to linkage with some immunoreactive gliadins and HMW glutenins (van den Broeck and others 2009), traditional breeding methods have not been able to develop celiac-safe bread wheat. Wheat lines which lacked portions of the short arms of chromosomes 1 and 6 expressed fewer celiac T-cell epitopes, although most had reduced baking quality (Molberg and others 2005; van den Broeck and others 2009). One deletion line, lacking part of the short arm of chromosome 6D (6DS-2), had reduced celiac T-cell epitopes and improved bread quality, but demonstrated poor kernel size and milling yield (van den Broeck and others 2011). Lafiandra and others (1987) developed a mutant line with good baking quality by limiting α-, γ-, and ω-gliadins encoded by the Gli-A2, Gli-D1, Glu-D3 loci. When tested with an *in vitro* organ culture, the mutant line did not cause damage to villi (Frisoni and others 1995), but it did induce IFN-γ and cytokine IL-2 production (Carroccio and others 2011).

Transgenic approaches have been successful at reducing celiac T-cell epitopes while maintaining bread quality. Gil-Humanes and others (2010) used ribonucleic acid (RNA) interference to down-regulate α-, γ-, and ω-gliadins. The transgenic lines produced up to 91% fewer α-gliadins, 81% fewer ω-gliadins, and no γ-gliadins. When tested against T cells derived from celiac patients, several lines were able to substantially reduce T-cell responses. As an added benefit, the transgenic lines compensated for gliadin reductions by increasing HMW-glutenins, which resulted in medium to high bread quality. As these lines still induced low levels of T-cell responses, celiac patients would need to limit consumption of these wheat varieties in their diets.

ATIs

Different species and genotypes of wheat produce varying types and amounts of ATIs implicated in celiac disease, wheat allergy, and NCWS (Figure 3B). ATIs are encoded by the B and D genomes of common wheat, suggesting that diploid and tetraploid species lacking one or both of these genomes might produce fewer ATIs (Figure 3B). Specifically, Wang and others (2006) mapped the problematic 0.19 fraction to the D genome of wheat. As it lacks both B and D genomes, einkorn contained no coding regions and produced no proteins for ATIs (Wang and others 2006; Larré and others 2011; Zoccatelli and others 2012) and no human α-amylase inhibition was detected in various einkorn genotypes (Bedetti and others 1974; Vittozzi and Silano 1976; Sánchez-Monge and others 1996).

Varieties of durum and emmer inhibited total α-amylase activity in human saliva at levels equal to (Vittozzi and Silano 1976) or higher than common wheat (Bedetti and others 1974; Sánchez-Monge and others 1996). While ATIs of durum and emmer differ from those of common wheat, they did contain the

CM3 ATI that was implicated in celiac disease, wheat allergy, and NCWS (Capocchi and others 2013). Although significant varietal differences were found among 3 durum genotypes by Prandi and others (2013), environment had a stronger influence on CM3 ATI content than genotype. Locations that yielded more protein content consistently produced lower amounts of CM3 ATI (Prandi and others 2013).

Types and quantities of ATIs also vary among genotypes within a species. Two-fold intraspecific differences were recorded in α-amylase inhibition among 7 durum lines and 113 common wheat lines (Bedetti and others 1974; Baker and others 1991; Sánchez-Monge and others 1996). Although Junker and others (2012) indicated that ATIs may have been increased through modern wheat breeding programs, no studies that directly compared heritage and modern wheat genotypes for inhibitory activity against human enzymes were found. ATI activity for only 1 variety that was released before 1950 was reported in the literature. The heritage variety, "Clarkan," induced the 5th highest ATI activity out of 104 common wheat varieties studied (Baker and others 1991). Hypoallergenic rice has been developed by downregulating ATIs (Tada and others 1996), but no such varieties have been developed in wheat.

Wheat allergy

As mentioned above, ATIs and glutens implicated in allergic reactions also vary by wheat type (Figure 3C). Intraspecific variation may be most influential in determining allergenicity, rather than differences between species. The ω-gliadins are primarily encoded by the B genome of wheat (Altenbach and Kothari 2007; Denery-Papini and others 2007). However, all cultivated species of wheat, including einkorn, express ω-5 gliadins implicated in WDEIA (Seilmeier and others 2001). For baker's asthma, Sánchez-Monge and others (1996) found no significant difference in IgE binding capacity between einkorn, durum, and common wheat. In a general screening of 324 wheat varieties, 1 variety of einkorn, 1 rivet, and 8 common wheats were the least allergenic (Nakamura and others 2005).

Among 10 einkorn lines, there was a 2-fold difference in ATI-IgE binding between the least and most allergenic genotypes (Sánchez-Monge and others 1996). Allergenicity also varies by patient. Larré and others (2011) exposed sera from individuals with contact urticaria and dermatitis to albumin and globulin proteins from einkorn and common wheat. While the majority of sera demonstrated lower IgE activity with einkorn, a minority of patients' sera (5 out of 18) exhibited similar IgE activity from each species, and serum from 1 patient bound with more intensity to the einkorn wheat protein extract. Einkorn and emmer contained higher proportions of ω-5 than common wheat. Emmer wheat exhibited a distinct ω-5 amino acid sequence from that of common wheat (Seilmeier and others 2001), but the impact of this sequence on allergic reactions has not been determined.

Durum wheat showed slightly lower average IgE binding reactivity for ATIs and other albumin/globulin proteins than common wheat (Lupi and others 2014). However, some durum genotypes exhibited allergenicity equal to common wheat. Among 10 varieties of durum, 1 genotype exhibited 57% less IgE binding reactivity for ATI proteins than the most allergenic genotype (Lupi and others 2014). When tested against sera of patients with food allergy, Khorasan wheat produced similar IgE binding profiles and skin-prick test reactions as durum wheat (Simonato and others 2002).

Some, but not all, varieties of spelt have lower allergenicity than common wheat. Spelt produced lower proportions of ω-5 gliadins implicated in WDEIA than common wheat (Seilmeier and others 2001). In an *in vivo* study of 64 patients with baker's asthma and other types of wheat allergy, only 30% of patients reacted to a variety of spelt (Armentia and others 2012). In both cases, reactions to spelt corresponded to patients who had a more severe manifestation of wheat allergy. Vu and others (2014) isolated sera antibodies obtained from 73 individuals with wheat allergy, and then exposed the sera to 2 varieties of spelt and 1 common wheat. Only 57% to 88% of sera that reacted strongly to common wheat also reacted to the spelt varieties (Vu and others 2014). One hypoallergenic spelt variety, "GWF," has a mutation that alters albumin/globulin proteins (Vu 2014). Other spelt genotypes, however, are not hypoallergenic. Sotkovský and others (2008) found one variety of spelt to elicit more IgE reactivity than 5 of 6 tested common wheat varieties.

Among genotypes of common wheat, allele variants (for example, *Gli-B1c*) exist that greatly reduce ω-5 expression and immunoreactivity. Twenty-nine different cultivars expressed highly variable amounts of ω-5 gliadins, with 1 variety producing 10 times the amount of ω-5 gliadin of the lowest cultivar (Wieser and others 1994). Immunoreactivity of these wheat cultivars to sera from 9 individuals with WDEIA and urticaria generally corresponded to the amount of ω-5 gliadins present (Denery-Papini and others 2007). Nakamura and others (2005) screened 321 wheat varieties for broad allergenicity, and recorded 6 times more IgE binding in the most reactive genotype when compared to the least. For baker's asthma, the least allergenic of 10 common wheat varieties bound only 44% to 63% of the IgE when compared to the most allergenic lines (Weiss and others 1993; Sánchez-Monge and others 1996). After comparing 7 different common wheat varieties, Weiss and others (1993, 1997) found an 8-fold difference in IgE binding to a highly reactive 27 kDa albumin/globulin fraction. No studies were found that directly compared allergic reactions incited by heritage and modern wheats. The least allergenic line was a modern cultivar ("CM32859" CIMMYT) in the large screening conducted by Nakamura and others (2005).

Varieties of wheat have been developed with lower allergenicity. Low ω-5 gliadin expression occurred in wheat lines containing chromosome arm 1RS from rye (Wieser and others 1994) and corresponded to very low allergenicity among subjects with WDEIA and urticaria (Denery-Papini and others 2007). Waga and Skoczowski (2014) developed varieties that were 30% less immunoreactive across a panel of allergic individuals. Through RNA interference technology, lines were developed that partially or completely suppressed the expression ω-gliadins (Altenbach and Allen 2011; Altenbach and others 2014). Wheat genotypes developed to express fewer allergens may have suitable baking quality, because downregulating ω-gliadin expression improved bread quality (Waga and Skoczowski 2014). Downregulation of ω-gliadins sometimes corresponded to reduced amounts of other allergens, such as ATIs, lipid transfer proteins, and serpins (Altenbach and others 2014). However, wheat lines compensated for the removal of ω-gliadins by upregulating α-gliadins (Altenbach and others 2014; Waga and Skoczowski 2014). So, while these varieties may lessen the suffering of individuals with exercise-induced anaphylaxis, they could be worse for individuals with celiac disease.

To complicate the development of hypoallergenic varieties, individual patients differ in the intensity and specificity of their allergic reactions to common wheat genotypes. Lupi and others

(2014) found a significant interaction for genotype and patient serum, indicating that cultivars with lower reactivity for 1 individual were not necessarily less reactive for another individual. Moreover, hypoallergenic varieties for 1 type of allergy may not be hypoallergenic for another type. Constantin and others (2009) found only 1 variety out of 13 that bound with less intensity to the serum antibodies obtained from both individuals with baker's asthma and food allergy.

NCWS, fructose malabsorption, and IBS

The content of fructans, which can impact individuals with fructose malabsorption, IBS, and some cases of NCWS, varies among species and genotypes (Figure 3D). Like gluten proteins, fructans are present in detectable amounts in all species and varieties of wheat. To date, the general ranking of wheat species by fructan content, from highest to lowest, is spelt, einkorn, durum, and common wheat (De Gara and others 2003; Huynh and others 2008; Brandolini and others 2011; Hammed 2014; Veenstra 2014). No studies regarding the fructan content of emmer could be located. As studies have evaluated very few genotypes of einkorn, spelt, and durum, the range of fructan content found in each species is not well known.

In an evaluation of 62 common wheat varieties, fructan content ranged from 0.7% to 2.9% dry weight (Huynh and others 2008). Preliminary measurements of total fructan content in 286 common winter wheat varieties grown in one environment ranged from 0.3% to 1.5% (Veenstra 2014). The 35 heritage wheat varieties studied had an average fructan content of 1.2% dry weight, compared to an average of 1.0% for 313 modern wheats (Huynh and others 2008; Veenstra 2014). As drought and temperature influence wheat stem fructan content and remobilization, both environment and genotype influence the fructan composition of wheat grain (Bancal and Triboi 1993; Ehdaie and others 2006). Further research is needed to understand how the type of fructan structures differs among varieties of common wheat. Two popular Swedish winter wheat varieties contained similar types of fructan structures (Haskå and others 2008).

Summary of immunoreactivity among ancient, heritage, and modern wheats

Although the popular press (for example, Davis 2011) has indicated that consuming ancient or heritage wheat prevents sensitivity, the scientific literature does not support this claim. No wheat species or varieties are currently approved for diagnosed celiac and allergic individuals to consume. Nevertheless, some varieties of ancient, heritage, and modern wheat produce fewer amounts and types of reactive prolamins and fructans. Einkorn is particularly promising for producing fewer immunotoxic effects in celiac research studies. Modern breeding efforts have also produced varieties that are hypoallergenic or less dangerous for celiac individuals. As many celiac and allergic individuals do not always adhere to wheat-free diets (see the review by Hall and others 2009), these less-immunoreactive wheat products may improve their quality of life. Furthermore, these varieties may be good targets for slowing development of disease in populations genetically predisposed to celiac disease and other wheat sensitivities.

The limited data available on common wheat indicate only a slight increase in average expression of components causing celiac disease in modern compared to heritage varieties (Figure 3A). Preliminary data show that modern varieties did not contain higher fructan content than heritage varieties (Figure 3D). These data suggest that the introduction of modern wheat varieties may not fully explain the rise in wheat sensitivity over the last 50 y. To understand how modern wheat breeding has impacted wheat sensitivity, a broader array of modern and heritage genotypes must be screened. In particular, published data have not evaluated the differences in ATI activity and allergenicity between heritage and modern varieties.

As a challenge to interpreting data from the literature, many studies have not controlled for variability in growth environments. Nakamura and others (2005) concluded that allergenicity of a single wheat variety could vary according to where the variety was grown. Omega-5 gliadin content increases with fertilization and temperature during maturity (Wieser and Seilmeier 1998; Altenbach and Kothari 2007; Hurkman and others 2013). A similar less pronounced trend was observed for α-gliadins (Hurkman and others 2013). Many studies included in this review evaluated gliadin content in kernels from plants that were grown under different nitrogen and weather conditions. Consequently, apparent immunoreactive gliadin content may not reflect an inherent genetic difference between varieties, but rather the environmental conditions of the field in which they were grown. The effects of fertilization also suggest that higher nitrogen inputs characteristic of modern production systems could be directly increasing the amounts of reactive ω- and α-gliadins in wheat products.

As most wheat breeding programs do not screen for celiac, allergy, or fructan reactivity in their lines, scientists and the public have little information on reactivity of modern wheat genotypes used in agriculture. To generate meaningful information for wheat breeders and consumers, a first step would be to standardize screening procedures. No standard screening protocol exists for identifying "reactivity" of a wheat variety. Due to the cost and ethical barriers of *in vivo* testing, the full impact of wheat genotypes on a large number of patients is rarely studied. Instead, research has inferred reactivity via genomic information, prolamin sequences, or *in vitro* models. The cumbersome nature of evaluations prevents all potentially reactive prolamins from being evaluated. Similarly, *in vitro* models are often relevant to only a portion of patients. Standardized screening methods could incorporate the most reactive T-cell epitopes in celiac disease, and/or apply varieties to sera from an array of allergenic individuals. Screening of all varieties for fructan content would be beneficial for individuals wishing to control intake levels of fructan. However, the lack of severe health impacts may not justify the cost and time required for widespread fructan screening of varieties.

Even if potential reactivity were determined for all wheat varieties, the variety identity is rarely tracked from farm to mill to bakery to storefront. Moreover, flour is commonly mixed from a variety of wheat genotypes in the milling or baking process. In contrast to potatoes, which are sold under their variety name, such as "Russet" or "Yukon Gold," a wheat variety is rarely labeled in retail products, and most wheat products are sold as combinations of many wheats. Consequently, consumers are not able to distinguish what variety they are purchasing. An informed consumer would require a processing chain that tracks the reactivity of wheat in marketed products.

The Impacts of Food Processing on Wheat Sensitivity

Processing techniques can also impact the nature of reactive components in wheat products. In particular, certain modern processing practices used over the last century may have increased consumer exposure to components implicated in wheat sensitivity. Modern processing can differ from traditional methods by (1) using ungerminated grain, (2) replacing long and diverse fer-

mentation with fast-acting baker's yeast (*Saccharomyces cerevisiae*), (3) using nonacidic dough, (4) adding extracted wheat proteins and inulin to food products, and (5) focusing on refined white flour. The following section reviews the effects of these modern processing practices on wheat reactivity.

Malting and germination enzymes

Wheat, rye, and barley kernels contain their own enzymes that can break down difficult to digest proteins. When the seed imbibes water, proteases in the seed break ATIs into peptides and amino acids to be used in seedling growth. Consequently, ATIs rapidly degrade after germination (Buonocore and others 1977). During germination, endoproteases also cleave the gliadin and glutenin storage proteins into available amino acids for seedling growth (Hartmann and others 2006). Germination strongly induces cysteine proteases, which are responsible for breaking down gliadin (Loponen and others 2007). Five or 6 d after germination begins, ω-gliadins are the first to be degraded (Bigiarini and others 1995). Schwalb and others (2012) documented nearly complete degradation of the immunodominant ω-5 gliadin after 7 d of durum germination. Prolonged incubation of wheat and durum fully degraded gliadins (Bigiarini and others 1995; Stenman and others 2009; Schwalb and others 2012). Germinated and fermented rye and wheat sourdough effectively degraded 99.5% and 95% of prolamins, respectively (Loponen and others 2007, 2009). Specifically, the toxic 12-mer of α-gliadin QLQPFPQPQLPY was hydrolyzed in gliadin treated with germination enzymes (Stenman and others 2009, 2010). In addition to reducing the 33-mer by 83% (Stenman and others 2009), germination and low pH treatment degraded $\geq$99% of the alpha-gliadin T-cell epitope PQPQLPYPQPQLPY in wheat, emmer, and einkorn (Schwalb and others 2012). In the study by Luoto and others (2012), the addition of *Aspergillus niger* prolyl endoprotease was necessary to bring germinated wheat products below the threshold for gluten-free labeling of 20 ppm.

Fewer immunoreactive peptides in germinated products translated into lower celiac disease epitope expression. However, geminated wheat products are generally not safe for individuals with celiac disease. Although *in vitro* Caco-2 cells exposed to endoprotease-treated gluten did not show aggravated barrier function, membrane ruffles, and tight junctions (Stenman and others 2009, 2010), the degraded gluten still induced T-cell proliferation at levels significantly higher than nongluten controls (Stenman and others 2009).

The most vigorous enzyme activity and protein breakdown takes place on the 8th day of germination (Bigiarini and others 1995; Stenman and others 2010), at which point the seed has nearly transformed into a seedling. Industrial use of such extensively sprouted grain would be a challenge. Moreover, germinating the grain greatly reduces the shelf-life of products made from it. As a positive marketing aspect, germinated grain can improve flavor. Loponen and others (2009) reported that rye sourdough made from germinated grain had enhanced flavor compared to bread made from nongerminated grain.

To improve applicability for large-scale production, the enzyme supernatants from a small stock of germinated seed have been used to effectively reduce prolamins in large quantities of flour (Stenman and others 2009, 2010; Schwalb and others 2012). Enzymes from germinated barley may create the most efficient supernatants, as they degraded the largest amount of gliadins (Stenman and others 2010). Optimal germination temperature to cleave gliadins varied by species, with 25 °C functioning best in wheat and one emmer variety and 15 °C facilitating more degradation in einkorn, rye, barley, and another emmer variety (Schwalb and others 2012). Endogenous enzymes from sprouted grain have also been tested as an oral supplement taken during mealtimes (Tye-Din and others 2010a; Siegel and others 2012; Lähdeaho and others 2014).

Given that fructans are carbohydrates, rather than proteins, these compounds face a different fate than gluten and ATI proteins in the processes of malting and germination. The exact role and fate of fructans in wheat germination is unclear. Wheat kernels may have hydrolases similar to onion seed, which degrade fructans during the germination process (Pollock and Cairns 1991; Pollock and Lloyd 1994). In barley, fructans are relatively unaffected by the malting process, but >90% of fructans initially present in wort are fermented by yeast (Krahl and others 2009).

Fermentation and microbial enzymes

Microorganisms involved in fermentation can also contribute to hydrolysis of reactive proteins. A combination of microbial prolyl endopeptidases (PEPs) can be used during wheat processing or during ingestion to break down prolamins (see reviews by Arendt and others 2007; Gobbetti and others 2007; M'hir and others 2012). *Aspergillus niger* PEPs showed promise as an oral supplement for celiac patients to consume with wheat products (Mitea and others 2008). The traditional sourdough baking practice also employs a diversity of microbial proteases. Single strains of lactic acid bacteria degraded some storage proteins, releasing more amino nitrogen than uninoculated dough after 24 h (Wieser and others 2008). Although single strains were able to degrade 23% to 45% of γ-gliadins, only 11% of ω-5 gliadins were affected. Duar and others (2014) reported that each set of PEPs derived from *Lactobacillus ruminis*, *L. johnsonii*, *L. amylovorus*, *and L. salivarius* isolated from the small intestine of pigs fed with gluten-containing diets demonstrated distinct capacity to degrade, yet not completely remove, 3 gliadin peptides harboring T-cell epitopes (the 33-mer, QPQQPFPQPQQPFPWQP, and QLQPFPQPQLPYPQPQ). A combination of enzymes from a diversity of microbes is necessary to effectively break down peptides. The αG-33mer fragment, for example, did not show complete degradation with *Flavobacterium meningosepticum* or *Lactobacillus sanfranciscensis* alone (Gallo and others 2005; Matysiak-Budnik and others 2005), but was successfully degraded with a diverse mixture of *Lactobacillus alimentarius*, *L. brevis*, *L. sanfranciscensis*, and *L. hilgardii* (De Angelis and others 2010). Fermenting durum pasta dough with the diverse microbial mixture decreased gluten concentration by 83% (Di Cagno and others 2005). Microbes also degraded 97% of gluten in bread dough after 48 h and 70% of the 33-mer after only 6 h (Rizzello and others 2007; Greco and others 2011).

In testing the dough developed by Rizzello and others (2007), T-cell proliferation and IFN-γ production was equivalent to nongluten controls. In a double-blind trial using bread made from 30% highly fermented wheat flour, celiac patients did not experience increased intestinal permeability (Di Cagno and others 2004). There is intriguing evidence that sourdough fermentation alone can reduce celiac disease immunoreactivity, whether or not a grain product contains gluten. Amaranth, corn, and rice products that had been fermented with sourdough bacteria generated less inflammation ($P = 0.045$) in celiac patient biopsies (Calasso and others 2012).

Fermented wheat products, however, have not been determined as safe for individuals with celiac disease. Although extensive fermentation degraded 97% of gluten, 2 celiac subjects consuming the fermented products still experienced villous atrophy at levels higher than nongluten controls (Greco and others 2011).

Full gluten hydrolysis with sourdough bacteria and fungal proteases was necessary to eliminate elevated intestinal permeability, cytokine expression, and gliadin antibody levels as shown by Di Cagno and others (2010) and Greco and others (2011). When 98% of prolamins were degraded using extensive germination and fermentation, the remaining 27 mg/kg of secalin in the rye bread still induced duodenitis, cytokinine secretion, small bowel inflammation, and weight loss in celiac mouse models (Freitag and others 2014). Due to remnant amounts of reactive peptides, the authors have encouraged gluten-free baking products that incorporate only small amounts of wheat dough that was highly fermented and/or made from germinated grain. In such products, hydrolyzed wheat dough was mixed with flour from nongluten species, such as millet and buckwheat flours (Di Cagno and others 2004, 2005).

Fermentation often enhances the flavor and shelf-life of baked products. Di Cagno and others (2006) found that certain sourdough cultures increased bread volume and crumb firmness, eliminating the need for baking texture additives. Furthermore, the sensory qualities of foods made with hydrolyzed-gluten wheat flour are often superior to products made from nonwheat flours (Rizzello and others 2007). Unfortunately, there is often a tradeoff between degradation of reactive gluten and retention of gluten for basic baking properties. Large amounts of time and heat may be needed for microbial enzymes to break down problematic peptides. To fully degrade the 33-mer α-gliadin peptide in wheat required 24 h at 30 °C (Gallo and others 2005), while durum required 72 h of fermentation at 37 °C to meet gluten-free labeling standards (De Angelis and others, 2010). HMW glutenins, which are important for baking and pasta integrity, are degraded prior to and more extensively than reactive prolamins during sourdough fermentation (Gänzle and others 2008; Wieser and others 2008). Extensively fermented dough has a high ratio of gliadins to glutenins, which is very undesirable for bakers. The disulfide bonds holding together the gluten macropolymer (GMP), an integral component of baking quality, begin to degrade long before glutens. Only 5 h of fermentation with *Lactobacilli* or acidic chemicals degraded GMP by up to 46% (Wieser and others 2008). Pentosans, an important component for baking rye bread, were also hydrolyzed in germinated sourdough (Loponen and others 2009). Consequently, the long and hot sourdough fermentation to hydrolyze prolamins compromises functional baking properties of the dough. Pasta made with highly fermented durum also had lower stickiness and firmness than unfermented pasta (Di Cagno and others 2005; De Angelis and others 2010).

While microbial cultures in the sourdough fermentation process also impact fructan content, the mechanisms differ from protein degradation. Only a small portion of lactic acid bacteria, 16 of 712 screened strains, were able to degrade various fructans of forage grasses (Muller and Lier 1994). Some strains of *Lactobacilli* from sourdough cultures, such as *L. plantarum*, *L. brevis*, and some *L. sanfranciscensis* strains, actually synthesized their own fructan structures (Dal Bello and others 2001; Di Cagno and others 2006; Bounaix and others 2009), and subsequently stimulated bifidobacterial growth (Dal Bello and others 2001; Korakli and others 2002). Such strains did not, however, synthesize fructans over the relatively short timeframe used for sourdough baking (Di Cagno and others 2006).

Yeast, on the other hand, produces inulinase and invertase enzymes which work together to effectively hydrolyze fructans (Nilsson and others 1987). Fermentation with *S. cerevisiae* for 1.7 h reduced fructan content of whole wheat and white flour by 33% and 48%, respectively (Knez and others 2014). Once baked, the leavened bread contained about half the fructan content of unleavened bread. Although the role of nonyeast microbes in degrading fructans is not fully understood, a diverse sourdough rye culture was most effective at degrading fructans (1.9% remaining), when compared to yeast-fermented bread (3.4%), and air-leavened bread (4.7%; Andersson and others 2009). Similarly, fermented sourdough contained about half the fructosan, a polysaccharide of fructose, content when compared to unfermented dough (Escriva and Martinez-Anaya 2000). Lactic acid bacteria are likely most influential in the fructan degradation process by creating acidic conditions for yeast enzyme activity.

Acidity

Authors have argued that the most important contribution of sourdough fermentation is not the microbial protease activity, but lowering of the pH to levels optimal for wheat endoprotease activity (Hartmann and others 2006; Gänzle and others 2008; Loponen and others 2009). Cysteine proteases operate in a pH range of 3 to 6, with optimal gliadin hydrolysis at 4.25 (Bottari and others 1996). A pH of 4.0 allowed more of the 33-mer degradation in wheat, emmer, einkorn, and rye, although degradation in barley was more efficient at pH 6.5. Similarly, the optimal pH for yeast enzymatic activity in degrading wheat fructose was 4.5 to 5 (Nilsson and others 1987). Escriva and Martinez-Anaya (2000) demonstrated that the fructosan degradation in 2 sourdough cultures was related to the culture's acidification ability.

Acidic conditions alone can help degrade prolamins in wheat and rye (Kanerva 2011). However, chemical acidification has proven less effective than microbial or endoprotease degradation. In celiac patients, chemically acidified bread triggered more intestinal permeability than bread fermented with diverse microbial cultures (Di Cagno and others 2004). In addition, it has been demonstrated that the acidic environment promoted nonenzymatic deamidation of gluten peptides leading to more immunogenicity (Arentz-Hansen and others 2000).

Industrial food products

Since the last half of the 20th century, the food industry has increased its use of wheat proteins (Day and others 2006). Gluten can be separated from wheat (as in "vital wheat gluten"), or modified for specific uses (referred to as "isolated wheat proteins"). Vital wheat gluten not only improves the structural integrity of industrial bakery products, but it costs less per ton of protein than soy, whey, or casein. In Europe and elsewhere, low-protein flours are often fortified with vital wheat gluten to improve baking characteristics (Day and others 2006). For the United States market, vital wheat gluten is often added to bind multigrain breads (Atchison and others 2010). Wheat proteins also act as a binder and protein booster in processed meat, reconstituted seafood, and vegetarian meat substitutes (Day and others 2006). Commonly used as thickeners, emulsifiers, and gelling agents, wheat compounds were found in 86% of packet soups, 65% of canned soups, 63% of candies, 61% of ice cream, 46% of marinades, 26% of vinegars and dressings, 23% of jams, and 21% of baby food, according to a survey by Atchison and others (2010). Such extensive food industry uses of gluten contribute to its nearly ubiquitous nature in the marketplace. The authors estimated that wheat is found in 29.5% of supermarket food products.

Neither vital wheat gluten nor isolated wheat proteins contain most endogenous wheat enzymes that assist in the degradation of persistent prolamins. Isolated wheat proteins might also produce *de novo* allergens. Leduc and others (2003) documented the case

of a patient who did not have an allergy to wheat/gluten, but experienced anaphylaxis after consuming a wheat isolate used by the meat industry. Isolated wheat proteins in hair and skin care products could also provoke contact urticaria in a small subset of patients who are not allergic to gluten (Laurière and others 2006). Isolated wheat proteins can be deamidated by chemical acid or enzyme treatment to increase emulsifying applications (Wu and others 1976). While the impact of industrial deamidation on celiac reactivity remains uncertain, gastrointestinal deamidation from the tissue transglutamase increases the binding of peptides to HLA DQ2/8 and aggravates celiac immune responses (Arentz-Hansen and others 2000). Deamidated prolamins can also evade detection from commercial gluten screening methods like enzyme-linked immunosorbent assay (Kanerva 2011). Consequently, there is a possibility that products labeled as "gluten-free" contain deamidated gluten above the labeling threshold. The increased prevalence of isolated, and particularly deamidated, gluten in food and other products poses an obvious threat to individuals with wheat sensitivity and increases exposure of the general population to reactive glutens.

The food industry has also increased its use of compounds implicated in fructose malabsorption, IBS, and NCWS. Fructose consumption has risen in the last 30 y, largely due to a 60.8% increase in high-fructose corn syrup sweetener availability since 1978 (Gibson and others 2007; Marriott and others 2009). Consumers can also encounter inulin-type fructans in the marketplace. Inulin is added to food products for the purpose of fiber supplementation or fat replacement in low-fat products (Kleessen and others 2007). Such inulin-type fructans are not derived from wheat, but rather extracted from chicory root and Jerusalem artichoke (Kolida and Gibson 2007). Cereals, muffins, cake mixes, instant oatmeal, granola bars, cookies, and bread are often supplemented with inulin-type fructans (Gibson and others 2000; Grabitske and Slavin 2008). Although inulin can benefit most consumers when eaten in moderate amounts, inulin may aggravate symptoms of fructose malabsorption, IBS, and NCWS. Of particular interest to individuals with wheat sensitivity, inulin is often used to improve structure, color, taste, and fiber content in gluten-free breads (Capriles and Arêas 2014). Such food products highlight the need for patients with NCWS to understand the true causative agents of their symptoms. For individuals with fructose malabsorption, IBS, and certain cases of NCWS, gluten-free products with added inulin may be a poor dietary choice.

Flour processing

Modern flour processing can also impact wheat sensitivity. Fungal enzymes are commonly added to wheat flour to improve baking properties. Various fungal enzymatic additives, including α-amylase derived from *Aspergillus oryzae*, xylanase, glucoamylase, cellulase, and β-xylosidase, have been associated with allergies, such as bakers' asthma and contact dermatitis (Quirce and others 1992; Morren and others 1993; Baur and others 1998; Sander and others 1998; Quirce and others 2002). These additives provide an additional exposure risk to bakers (Tatham and Shewry 2008). Although limited research has been conducted, wheat flour treated with γ-irriadiation and microwave radiation were found to elicit more responses from allergic individuals (Leszczynska and others 2003a, 2003b).

The amount of reactive glutens may change with the level of flour refinement. Most endopeptidase activity was found in the bran rather than the endosperm (Hartmann and others 2006; Schwalb and others 2012). This distribution is not surprising, as cysteine proteases are synthesized in the aleurone layer of barley (Hammerton and Ho 1986). Because the bran is removed in the process of making white flour, subsequent products would have fewer enzymes available for prolamin degradation. The total amount of bran also varies by species and variety of wheat, and can impact the amount of endoproteases present.

The content of reactive wheat components is different in various layers of the wheat kernel. ATIs surround starch molecules in the endosperm, protecting them from digestion by insects and mammals. Many of the celiac-reactive α-gliadins are located in the subaleurone layer of the wheat kernel, which can be partially removed by roller-milling. However, the γ-gliadins and the HMW glutenins, which are reactive in a lower number of celiac patients, are concentrated in the endosperm, and will therefore appear in high concentrations in white flour. Omega-gliadins, which are found throughout the grain, will likely not change with the level of flour refinement (Tosi and others 2011). Wheat bran elicited about twice the IgE activity for bakers' asthma than white flour (Armentia and others 2012). The level of flour refinement on celiac immonoreactivity responses has not been directly assessed.

Fructans are not evenly distributed throughout the wheat grain. In terms of wheat milling fractions, bran, and shorts contain more fructan than the flour (Knudsen 1997; Haskå and others 2008). The inclusion of bran in whole wheat flour likely increases the total fructan content of whole wheat flour relative to white wheat flour. Whole wheat flour also contains fructans with a higher degree of polymerization than white flour. The lower degree of polymerization in white flour makes fructans more available for fermentation in the gut, which can aggravate symptoms in individuals with IBS. On the other hand, as fructans with lower degrees of polymerization were more easily degraded by yeast (Nilsson and others 1987; Praznik and others 2002), fructans in white bread were broken down more extensively than those in whole wheat bread (Knez and others 2014).

Summary of the impacts of food processing on wheat sensitivity

Patients with celiac disease, wheat allergy, and some forms of NCWS should avoid products with added gluten and isolated wheat proteins. Individuals with fructose malabsorption, IBS, and NCWS should limit consumption of inulin and high fructose corn syrup. In seeking wheat products with less immunoreactivity, consumers would most benefit from products made with germinated grain, and to a lesser extent fermented products. Free amino acid content (a measure of protein breakdown) in germinated wheat sourdough was 10 times the concentration of nongerminated sourdough (Loponen and others 2007, 2009). Similarly, only 6 h of fermentation were necessary to break down almost all prolamins in germinated sourdough, but prolamins were still present after 24 h of fermentation if the grain had not undergone germination (Loponen and others 2007). Fermented and germinated wheat, however, has not been determined as safe for individuals with celiac disease.

No epidemiological studies have evaluated the impact of wheat processing on the prevalence in wheat sensitivity over the last 50 y. Nevertheless, increases in disease diagnoses correlate with food industry uses of compounds that can trigger sensitivity, such as gluten, inulin, and high fructose corn syrup. Furthermore, modern baking practices used over the last century have focused on short, nonacidic fermentation techniques. Further research is needed to determine how modern wheat processing has influenced epidemiology.

Conclusion

No wheat species or varieties are currently safe for individuals with celiac disease, wheat allergies, or fructose malabsorption. Individuals or populations who are not symptomatic, but seek to lower the amount of reactive wheat components in their diets, have many options: (1) supporting research efforts to identify, develop, and label less-reactive wheat genotypes; (2) finding varieties of wheat and ancient grains that are known to have lower reactivity for the condition in question; (3) eating products made with the processes of germination and/or diverse microbial fermentation; and (4) avoiding vital wheat gluten, isolated wheat protein, and, in certain cases, inulin. As a first step to making meaningful diet change, patients need to understand what compounds are causing their symptoms. When correctly matched to disease pathology, less-reactive wheat products can improve the quality of life for individuals with diagnosed wheat sensitivity. Moreover, such products can slow disease development in populations that are genetically predisposed to celiac disease and wheat allergy. Although the cause of increased prevalence of wheat sensitivity over the last several decades remains unknown, modern wheat processing techniques may have increased consumer exposure to immunoreactive compounds.

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Author Contributions

L. Kissing Kucek designed the study and wrote the article under the supervision of M. Sorrells, who is a wheat breeder and geneticist. L. Veenstra, a researcher of the genetics of wheat fructans, contributed content on fructans. P. Amnuaycheewa, a food immunologist, provided planning, writing, and editing support in regards to gluten and immunological aspects of celiac disease and wheat allergy.

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