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REVIEW

Who is to blame for the increasing prevalence of dietary sensitivity to wheat?

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Abstract

This paper provides an overview of the related scientific literature, with some of our own targeted research, to investigate the possible causes of the recently increased prevalence of various forms of dietary cereal sensitivities. Detailed scientific investigations do not support the controversial idea that human practices, particularly modern wheat breeding, may have contributed to the increase in celiac disease (CD) prevalence during the latter half of the twentieth century. Each of the primitive wheat relatives and each historic or modern bread and durum wheat variety contains more or less amounts of toxic/allergenic epitopes. In the last 120 years, health-related quality attributes have not been considered in pre-breeding or breeding, but the yield- and functional quality-oriented selection procedures have resulted in unintended spinoff effects on the amounts of harmful compounds in new lines. Because of the trend of decreases in overall protein content, as well as the alteration of the glutenin-to-gliadin content to improve dough strength, older varieties are higher in gliadin content with consequent higher CD antigenicity. Meanwhile practices, introduced during the last 50 years in utilizing wheat in the food industry, have significantly increased the consumption of untreated prolamin proteins, including gluten proteins. Other factors for consideration are the incorporation of vital gluten as a cheap protein supplement in some food products and the reduction of fermentation time during bread making. Beyond the obvious effects of improved and more widely used diagnostic tests in medical practice, the increased incorporation of untreated gluten proteins and residual FODMAPs might be major reasons for the increasing prevalence of wheat sensitivity.

Keywords CD · NCGS · Gliadin degradation · ATIs · FODMAP · Sour dough · NTD

Abbreviations

CD	Coliac disease
NCGS	Non-coliac gluten sensitivity
NCWS	Non-coliac wheat sensitivity
IBS	Irritable bowel syndrome
HMW GS	High molecular weight glutenin subunits
LMW GS	Low molecular weight glutenin subunits
ATIs	Amylase-tripsin inhibitors
FODMAP	Fermentable oligo-, di-, mono-saccharides and polyols
GFD	Gluten-free diet
LAB	Lactic acid bacteria
NTD	No-time dough procedure
LC–MS	Liquid chromatography–mass spectrometry

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Introduction

More than 80% of the protein content of wheat has the essential biological role of storing nitrogen for use by the germinating seed. Meanwhile, this highly complex mixture of proteins is the major determinant of the processing (techno-functional) properties of the grain (Shewry 2019). Based on the generally accepted definition of Islam et al. (2011), wheat gluten is a protein–lipid–carbohydrate complex formed as a result of specific covalent and non-covalent interactions among flour components during dough making, during which the components are hydrated and energy from mechanical input from the mixing process is provided. Gluten proteins are encoded by multigene families at multiple loci on the three genomes of bread wheat, with a high degree of polymorphism between genotypes. The individual proteins have characteristic, unusual structure with glutamine- and proline-rich repetitive sequences.

Nowadays, the terms "gluten" and "gluten-free" are used more widely (and wrongly) in everyday practice, the latter

loosely referring to food products not containing cereal prolamin proteins, i.e., wheat gliadin and glutenin analogues.

Although gluten was identified as the trigger for celiac disease (CD) almost 70 years ago, interest in this role of gluten has been limited outside of the scientific community. However, the last 20 years have seen an explosion of interest in gluten, particularly in the popular press and social media. Shewry (2019) reported that a "Google" search, carried out in December 2018, gave almost 400 million hits in less than a minute, all referring to the role of gluten in triggering a range of adverse reactions. Consequently, there have been increasing proportions of the population in many countries choosing to adopt a gluten-free, or low-gluten, diet.

Consumption of "gluten-containing" food is supposed to cause disease for a significant minority of people who consume foods derived from wheat, rye, and barley. Until a few years ago, celiac disease was the major (if not the only) well-known gluten-related disorder. However, in recent years it has become clear that gluten proteins may activate different pathological mechanisms, leading to a wide spectrum of human diseases, including non-celiac gluten sensitivity (NCGS), gluten ataxia, neuro-psychiatric disorders, and many others.

Cereal-related health disorders

The different gluten-related disorders share a trigger (namely gluten) and treatment (namely the gluten-free diet). However, these disorders show specific physiological mechanisms and clinical aspects. For a very long time, awareness of these disorders has been limited and, therefore, the epidemiology of gluten-related disorders is still a "work in progress." Current research strives to clarify the boundaries between these entities, their disease mechanisms, and how a proper diagnosis can be implemented (Catassi and Fasano 2018). One of the most remarkable changes in our knowledge in this area is the realization that in several types of disorders related to the consumption of "gluten-containing" cereals, the trigger compounds are not components of the gluten but specific soluble proteins, such as amylase trypsin inhibitors (Kusaba-Nakayama et al. 2000; Junker et al. 2012; Zevallos et al. 2017; Bose et al. 2020) and fermentable oligosaccharides (FODMAPs), especially fructans (Hungin et al. 2003; Biesiekierski et al. 2013). Scientific literature published in the last 5 years differentiates non-celiac gluten sensitivity (NCGS) and non-celiac wheat sensitivity (NCWS) (Guandalini and Polanco 2015; Kucek et al. 2015; Molina-Infante and Carroccio 2017; Al-Toma et al. 2019). Furthermore, Uhde et al. (2016) reported that NCGS could be differentiated from a CD reaction if there were a reaction to native gliadin IgA but no reaction to deamidated gliadin. Another recently observed characteristic that seems to serve as a differential diagnostic tool is that the anti-gluten IgG antibody in NCGS is significantly different from that of CD in subclass distribution (Uhde et al. 2020).

Despite the numerous valuable recent research publications available in the area covering all aspects of the cereal-related health disorders (Kucek et al. 2015; Rybalka 2017; Brouns et al. 2019; Al-Toma et al. 2019; Rustgi et al. 2019; Caio et al. 2019; Tye-Din et al. 2018; Bose et al. 2020), there is some confusion and a lack of knowledge and understanding in the minds not only of consumers but also of medical practitioners (Branchi et al. 2015; Castillo et al. 2015).

To satisfy the specific health-related demands of specific consumer groups, the challenge is for cereal breeding and the food industry to develop new, "healthier" germplasm and/or to produce food products suitable for those with cereal sensitivities. To define these requirements, a better understanding of the different health-related disorders, their prevalence and the causal compounds is required, not only in the relevant basic research, but also in plant breeding and in the grain industry.

The general public in most Western countries is now aware of the potential adverse effects of cereals containing gluten with reports appearing in the lay press (Braly and Hogganm 2002; Ford 2008; Wangen 2009; Davis 2011) promoting gluten-free diets. Close to 10% of the population is electively following a gluten-free (GF) diet despite having no evidence of relevant disease (Mardini et al. 2015). As a result, the gluten-free market is expanding as GF foods are perceived as a therapeutic food as well as a valid lifestyle choice. The global market for gluten-free products was valued at 4.63 billion USD in 2015 and is projected to reach 7.59 billion USD by 2020, at a compound annual growth rate (CAGR) of 10.4% from 2015 to 2020. In Australia, where more than 23% of new bakery products were marketed as gluten-free in 2014, it is expected that gluten-free retail sales will exceed $100 million in the next 5 years (Jargon 2014). In 2017, Hester et al. (2017) reported that 20% of Australian consumers were avoiding gluten and purchasing gluten-free products, with long-lasting implications for commercial cereal breeding and the grain industry.

Many of these popular reports about the noxiousness of cereal-based food products fail to draw attention to the importance of appropriate diagnosis to define the nature of the presumed gluten "intolerance" that an individual may experience. This lack of appropriate advice poses a significant threat and challenge to the grain industry. Without any scientific or even practical evidence, the public draw the conclusion that the increasing prevalence of cereal-related health disorders are due to modern plant breeding, claiming that ancient relatives of wheat, such as spelt and heritage wheat cultivars, are harmless compared to modern wheats (Braly and Hogganm 2002; Ford 2008; Wangen 2009; Davis 2011; de Lorgeril and Salen 2014).

This report aims to give an overview of some aspects of recent developments in this booming area, providing information about the trigger compounds and prevalence of different cereal-related health disorders, about the scientific views concerning the overall healthiness of a gluten-free diet and of the status of celiac-safe cereal development. Based on the published scientific literature and some directly targeted aspects of our own research, we discuss the answer to the obvious question: What is behind the significantly larger number of cases of cereal-related health disorders in recent years; is it true that our breads today are different from those that our grandparents consumed?

Prevalence

Wheat-related sensitivities, prevalence, and wheat components responsible for these disease pathologies, based on the publication of Kucek et al. (2015), are shown in Table 1. Due to its rapidly increasing prevalence (Boukid et al. 2017; Manti et al. 2017), CD has gained much attention in recent years. Based on the report of Ludvigsson et al. (2013), the incidence of CD in the USA is about 1%. However, a survey conducted from 2009 to 2012 showed that potentially 0.79% of the general U.S. population demonstrates serologic evidence of CD autoimmunity (Mardini et al. 2015). Similar surveys report 2.4% in Finland, 0.3% in Germany, 0.7% in Italy (Mustalahti et al. 2010), and 0.76% north China. (Yuan et al. 2017). Based on the work of Catassi et al. (2014, 2015), the reports of low prevalence (or absence) in sub-Saharan Africa and in the Asia–Pacific region are related to the very low diagnostic rates, mostly due to lack of diagnostic facilities and poor disease reporting.

The most recently published data on the prevalence of CD is a systematic review and meta-analysis by Singh et al. (2018), carried out on global and regional data, underlining that a large proportion of patients with genetic disorders do not show physiological symptoms. The pooled global prevalence of CD was 1.4% in 275,818 individuals, based on positive results from tests for anti–tissue transglutaminase, while the biopsy-confirmed prevalence of CD was 0.7% (95% confidence interval in 138,792 individuals). The prevalence was higher in females vs male individuals (0.6% vs 0.4%; $P < 0.001$). The prevalence of celiac disease was significantly greater in children than adults (0.9% vs 0.5%; $P < 0.001$).

Results from a work carried out on a large set of blood samples from randomly selected individuals ($n = 1145$) (Pasco et al. 2012), analyzed by the IgE RAST method against wheat and milk antigens and food allergy questionnaires, indicated that the prevalence of wheat allergy was 2.5% where both a positive IgE immune response and symptoms against wheat were observed (Vu et al. 2014). It was postulated that the remaining 12.8% ($n = 125$) of individuals who showed raised IgE antibody levels without symptoms might have a latent wheat sensitivity with the potential of developing symptoms sooner or later. It was postulated that the large proportion (12.9%) of the investigated population, who have symptoms associated with the consumption of wheat products but who did not have raised IgE, may suffer from other wheat-related disorders (i.e., not IgE-mediated), such as celiac disease, non-celiac gluten sensitivity (NCGS), or a reaction to fructans (FODMAPs) for those with (irritable bowel syndrome IBS) (Vu et al. 2014). These results are in full agreement with similar investigations on wheat where the prevalence of wheat IgE sensitization in European

Table 1 Wheat-related sensitivities, prevalence, and wheat components responsible for disease pathologies, based on Kucek et al. (2015)

Disorder	Prevalence	Reactive components in wheat	References
Celiac disease	0.5–2%	α, γ, and ω-gliadins, HMW and LMW GS, CM3 and O. l9 ATI	Rewers (2005), Tye-Din et al. (2018)
Allergies	0.2–0.5%		
Wheat allergy		α, γ, and ω-gliadins, HMW and LMW GS, CM3 and O. l9 ATI	Zuidmeer et al. (2008), Vu et al. (2014)
Baker's asthma		ATis, LTPs, serpins, peroxidase α, γ, and ω gliadins, HMW and LMW GS	Sanchez-Monge et al. (1997), Sandiford et al. (1997)
Atopic dermatitis		LTPs, CM3 ATis, gliadins, and glutenins	Kusaba-Nakayama et al. (2000), Battais et al. (2005b)
Urticaria		ω-5 ω-1,2 g liadin, LMW GS	Battais et al. (2008)
Anaphylaxis		α, γ, and ω-gliadins, LMW GS, ATI	Battais et al. (2005a), Morita et al. (2009)
Non-celiac wheat sensitivity	1.1–6.5%	ATI, Fructans	Biesiekierski et al. (2013), Fasano et al. (2015), Junker et al. (2012)
Irritable bowel syndrome	11.5–14.1%	Fructans	Roberfroid (1993), Brighenti et al. (1995), Rumessen and Gudmand-Hoyer (1998), Hungin et al. (2003)

countries is 2.9% (Zuidmeer et al. 2008; Siles and Hsieh 2013).

Gluten-free versus low-FODMAP diet

In a survey carried out in 2012 in the USA (Gluten-Free Foods in the U.S., 5th Edition, 2012) of those who eat gluten-free foods, only 5.7% claimed a formal medical diagnosis. From the rest of the consumers on a gluten-free diet, 36% do so for reasons other than sensitivity, 65% because they think it is healthier, 27% because they believe that it aids weight loss, 7% do so to help reduce inflammation, and 4% do so to combat depression (Digiacomo et al. 2013).

Consumers, food manufacturers, and health professionals are uniquely influenced by the growing popularity of the gluten-free diet. Consumer expectations have urged the food industry to continuously adjust and improve the formulations and processing techniques used in gluten-free product manufacture. Health experts have been interested in the nutritional adequacy of the GF diet, as well as its effectiveness in managing gluten-related disorders and other conditions. Several excellent text books and review articles (Arendt and Dal Bello 2008; El Khoury et al. 2018) provide a clear picture of the current motivations behind the use of gluten-free diets, as well as the technological and nutritional challenges of the diet as a whole. Alternative starches and flours, hydrocolloids, and fiber sources were found to play a complex role in mimicking the functional and sensory effects of gluten in gluten-free products. However, the quality of gluten-free alternatives is often still inferior to the respective gluten-containing products.

The gluten-free diet (GFD) has demonstrated benefits in managing some gluten-related disorders, though consequent nutritional imbalances have been reported (Di Nardo et al. 2019; Melini and Melini 2019).

Comino et al. (2013) provide a good overview on the social and economic repercussions generated by a GFD. Meanwhile, it cannot be regarded as a healthy diet (Balakireva and Zamyatnin 2016; Melini and Melin 2019). As is reviewed by Penagini et al. (2013), a GFD may lead to possible nutrient deficiencies in fiber as well as deficiencies in phytochemicals, trace elements, plus a high glycemic response resulting in consequent diseases. Alternatively, several studies suggest pseudo-cereal sources of fiber instead of gluten-free products to maintain the necessary fiber content levels (Saturni, et al. 2010; Békés et al. 2016). A GFD also leads to a deficiency in Vitamins C, B12, D, and folic acid (Hallert et al. 2002), as well as a lack of microelements, most importantly calcium, magnesium, and zinc (Caruso et al. 2013). At the same time, the gluten-free diet contains high amounts of sugar and hydrogenated fats, which could result in the occurrence of hyperinsulinemia and an increased obesity risk (Lamacchia et al. 2014). Thus, a GFD appears to be an unbalanced diet, being inadequate regarding both macro- and micronutrients. Recent intensive efforts of food manufacturers to improve the nutritional (and functional) characteristics of a GFD show good progress; however, these new-generation GFD products are significantly more expensive and therefore not available to a large proportion of customers.

The current view of medical experts is that, excluding people suffering from celiac disease, the majority of individuals who are feeling better on the "wheat-free" or "gluten-free" diet are automatically selecting a low-FODMAP diet, because by choosing "gluten-free" products they are generally also low in FODMAP (Halmos et al. 2014, 2015).

Realization of the crucial importance of certain fermentable carbohydrate components (FODMAPs, Gibson and Shepherd 2005) for food-related health disorders initiated active research and development in analyzing FODMAP contents, as well as recommending food materials with low-FODMAP content. Compared to the "gluten-free" phenomena, where the problematic food sources are a relatively small group of certain cereals, the establishment of a low-FODMAP diet involves considering a much wider range of plant-origin foodstuffs. Publications (Muir et al. 2007, 2009; Biesiekierski et al. 2011), and even computer and mobile phone applications (http://www.med.monash.edu.au/cecs/gastro/fodmap/iphone-app.html), are available to help to develop custom-designed low-FODMAP diets.

Traditional cereals (wheat, barley, and in particular rye) are relatively rich in FODMAPs, and therefore, food products made from them are not recommended for low-FODMAP diets. Compared to bread wheat, spelt (*Triticum aestivum* subsp. *spelta*) contains significantly lower amounts of fructose and fructans. But even more important is the finding that there is around a fivefold larger inter-varietal difference among spelt varieties compared to those found among bread-wheat genotypes (Pauk et al. 2019). This observation provides a basis for screening spelt cultivars for low-FODMAP content.

Consumers are urged to be mindful of the sensorial limitations and nutritional inadequacies of the GF diet, despite ongoing strategies to improve them.

The effect of breeding on the amount of harmful components in wheats

A large variation can be observed comparing the amounts of harmful compounds found in different cereal species and cultivars (Spaenij-Dekking et al. 2005; Carroccio et al. 2011). Variations in gliadin expression could contribute to the degree of CD antigenicity (Salentijn et al. 2009). Similarly, cereals containing diverse amounts of fructans (relevant to FODMAP) contribute differently to the development

for IBS. If the cause of wheat-related health disorders is the result of spontaneous and later conscious selection activity in wheat breeding, there should be no harmful components (toxic/allergen epitopes) in the "old" wheat cultivars, landraces, even, those primitive wheat relatives from which evolution has built up the current hexaploid bread wheats. In the last 20 years, this working hypothesis was the basis for initiating intensive research activity to analyze the genetics/protein composition and health-related quality attributes of various primitive wheat species and also to re-investigate historic wheat cultivars, comparing them to modern varieties. The depth of this research was significantly improved recently by the availability of the complete wheat genome (Appels 2018), resulting in detailed and precise information on genes and products related to health-related issues (Juhász et al. 2018; Bose et al. 2020).

Comparison of primitive wheat relatives and present bread wheats

Gluten proteins

The triggering responses for wheat-sensitive individuals are specific amino acid sequences in proteins, *toxic/allergenic epitopes*. Intensive research on comparing amino acid sequences of proteins in wheat relatives (Molberg et al. 2005; Spaenij-Dekking et al. 2005; van Herpen et al. 2006) indicated large variations in the distribution among and between different groups of gluten proteins, resulting in variations in the epitope content in different species. Kucek et al. (2015) in their review explain the variation in celiac immune-reactivity among species of wheats, based on their genome composition. Species that lack the D genome of wheat, such as einkorn, emmer, and durum, appear to exhibit on average lower immunogenicity than common wheat, because the most harmful α-gliadins are coded by the D genome of wheat, which is present in common wheat, but absent in most of the "old" wheat species.

Since spelt is hexaploid, containing a D genome, its harmfulness was found to be similar to that of common wheat. In agreement with the data published by Vincentini et al. (2007), and van den Broeck et al. (2010b), Pauk et al. (2019) found similar levels of immune-reactivity when comparing spelt and common wheat. Einkorn, which has only the A genome of wheat, expressed the least number of celiac disease epitopes among cultivated species (Vincentini et al. 2007) with low immune-reactivity (Pizzuti et al. 2006; Zanini et al. 2009). Nevertheless, einkorn still expressed T cell immunogenic α- and γ-gliadin epitopes (Molberg et al. 2005; van Herpen et al. 2006). Although the average reactivity of emmer and durum (containing only the A and B genomes) is lower than that of common wheat (also having the D genome), there is a wide range of celiac responses depending on genotype (Auricchio et al. 1982; Molberg et al. 2005; Vincentini et al. 2009; van den Broeck et al. 2010a; Vader et al. 2002). The B genome of wheat encodes the smallest number of α-gliadin epitopes (van Herpen et al. 2006). Diploid species with genomes similar to the B genome of wheat are not cultivated or normally consumed by humans.

Relative quantitation of CD epitopes using immune techniques (Gell et al. 2015), mass spectroscopy, or proliferic assays with T cell lines from patients with CD when exposed to different diploid, tetraploid, and hexaploid wheats, showed (Suligoj et al. 2013) that all wheat species investigated contain varying levels of CD epitopes and that each of them is antigenic to some degree. So, while some diploid monococcum lines can be found with lower CD antigenicity (Pizzuti et al. 2006), neither diploid wheat relatives (Vaccino et al. 2009; Gianfrani et al. 2012) nor ancient durum relatives, such as Kamut (Gregorini et al. 2009; Colomba and Gregorini 2012), are safe for CD subjects.

FODMAP content

While there is a great amount of information about the FODMAP content and its nutritive importance in bread wheat, reviewed recently by Grausgruber et al. (2019), similar published data on wheat relatives is rather limited. FODMAP content in whole-grain flours and breads made of different varieties of bread wheat, spelt, durum, emmer, and einkorn were determined by Ziegler et al. (2016). Fructans and raffinose were the only FODMAP components detected in wheat flour. Total FODMAP contents ranged from 1.24 ± 0.38 to 2.01 ± 0.42 g/100 g DM in emmer and einkorn flours, respectively. By contrast, Brandolini et al. (2011) reported higher fructan concentrations in four einkorn accessions, compared to a check bread wheat variety; this conclusion was confirmed by Ziegler et al. (2016).

Gibson and Shepherd (2010) recommended consuming spelt products instead of bread wheat. 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 or wheat-sensitive and allergic individuals to consume.

Compared to bread wheat, Escarnot et al. (2015) and Ziegler et al. (2016) reported lower fructan concentrations for spelt wheat, but the ranges of concentrations in spelt and bread wheat were largely overlapping. However, certain spelt lines show significantly lower fructan levels. For example, E3 spelt, grown in Australia, contains almost half of the usual levels of fructans in bread wheat and over 20% less than any other spelt wheat cultivar grown under the same conditions (Békés et al. 2016, 2017). Using E3 spelt as control, 105 spelt lines grown in Hungary have been screened

for FODMAP content and seven lines showed even lower FODMAP levels (< 0.9%) than the control (Békés et al. 2016, 2017; Pauk et al. 2019). The FODMAP content of bread made from E3 spelt, using a typical no time dough (NTD) formulation and process, was considerably lower than the threshold defined for low-FODMAP products, showing significantly better responses to IBS patients than the control in a pilot scale single-blind, crossover intervention trial (Muir et al. 2014).

ATIs content

The genes coding for human α-amylase inhibitors (ATIs) are located on chromosomes 3BS and 3DS of wheat, while there is no evidence of their existence in the A genome. In previous studies, ATI sequences were identified in the A genome of wheat (both in bread and durum wheats), but these genes are silent or expressed at a low level (Zoccatelli et al. 2012; Capocchi et al. 2013). In addition, no activity against heterologous α-amylase has been found in extracts from diploid wheat species with the A genome (Garcia-Maroto et al. 1990, 1991; Dupont et al. 2011) and no inhibition of human α-amylases has been detected in einkorn wheat (Reig-Otero et al. 2017). Lower bioactivity was observed in older wheat variants, including spelt (a hexaploid wheat), emmer (tetraploid), and einkorn (diploid) (Zevallos et al. 2017).

In bread wheat, ATIs represent up to 4% of total wheat proteins and consist of at least 14 types of subunit proteins (Altenbach et al. 2011). As is discussed in depth by Huang et al. (2020), the nomenclature of ATI subunits is inconsistent, including the wheat ATI monomer with a molecular weight (MW) of 12 kDa, often referred to as the 0.28 inhibitor, based on its electrophoretic mobility; there is also the ATI homo-dimer with a MW 24 kDa, referred to as the 0.19 and 0.53 inhibitors, and the ATI hetero-tetramers with MW 60 kDa, also referred to as CM proteins. CM proteins, termed for their solubility in chloroform/methanol mixtures, include subunits CM1, CM2, CM3, CM16, and CM17. Each ATI subunit ranges from about 11 to 16 kDa in MW and contains 10 cysteine residues forming five intramolecular disulfide bonds while dimers and tetramers associate in a non-covalent manner (Altenbach et al. 2011). The multimeric forms of ATIs determine their biological activity; the ATI tetramer is five times more active than the monomer (Zevallos et al. 2017).

Until recently, contents of ATIs in different wheat species were not available. Geisslitz et al. (2018) quantitated the predominant ATI components (ATI 0.19 + 0.53, 0.28, CM2, CM3, and CM16) in eight cultivars, each identified from common wheat, durum wheat, spelt, emmer, and einkorn grown under the same environmental conditions by targeted liquid chromatography-tandem mass spectrometry (LC–MS/MS). The distribution of ATI types was characteristic for hexaploid, tetraploid, and diploid wheat species and suitable as species-specific fingerprints. Spelt and emmer had higher ATI contents than common wheat, with durum wheat in between. Only three of eight einkorn cultivars contained ATIs in very low concentrations.

Comparing historic and modern wheat varieties

Gluten proteins

The primary aim of wheat breeding since its beginning has been to develop new varieties with higher yields to satisfy the need of the growing population in the world. Modern agriculture (since the 1950s) has applied crossbreeding to different wheat and grass species, thus to generate new genetic varieties of increased yield. During the Green Revolution, the introduction of the reduced-height semi-dwarfing genes ((Rht)-B1b and Rht-D1b) led to impressive increases in wheat yields (Hedden 2003).

The impact of breeding on grain yields of wheat varieties released during the twentieth century has been extensively studied, and a general genetically determined relationship has been observed: There is a reciprocal relationship between protein concentration and grain yield (Mohler et al. 2011 and Sherman et al. 2014). Analyzing the results of the German official variety trials and on-farm during the 1983–2014 period, a yield increase of 1 dt/ha resulted in an absolute loss of − 0.071% protein concentration (Laidig et al. 2017). Similar results have been found by Simmonds (1995), Oury and Godin (2007), Oberforster and Werteker (2011), and Riaz et al. (2019b).

The lost protein content had to be compensated by altering protein composition to keep or even to improve the functional properties of new wheat varieties, while the yield–protein relationship is well documented, less information is available on the changes in gluten quality associated with effects on the amount and composition of glutenins and gliadins. Based on the data available, a general trend of two main alterations in protein composition can be observed: to improve dough strength and extensibility (a) glutenin-to-gliadin ratio increased and (b) lines containing superior HMW glutenin alleles have been selected in modern cultivars. Gliadin composition, the relative distribution of alpha-/beta-, gamma-, and omega-gliadins, plays a limited role in determining functional properties, and therefore, there is no consistent trend in the alteration in gliadin composition when comparing historic and modern varieties. In summary, the overall protein content and specifically the gliadin content of the flour have superior roles in determining the amounts of harmful epitopes in the flour.

Because of the considerable sequence variation in alpha-, gamma-, and omega-gliadins, both within wheat cultivars and among different cultivars, detailed knowledge about

the composition of gliadin genes and proteins in individual wheat cultivars is critical for understanding how these proteins contribute to both the functional properties and the immunogenic potential of the flour (Wang et al. 2017; Cho et al. 2018).

RP-HPLC-based quantitative analysis of gliadin types has been carried out on numerous modern bread wheat and durum varieties and landraces as well as spelt samples by Ribeiro et al. (2016). It was observed that the *Triticum aestivum* spp. and *T. vulgare* landraces, which have not been subject to breeding practices, presented greater amounts of potential celiac disease immuno-stimulatory epitopes when compared to modern varieties. The LC–MS-based comparison of thirty wheat cultivars released in North Dakota from 1910 to 2013 showed that immunogenic peptides are present in both historical and modern spring wheat cultivars, irrespective of year release (Malalgoda 2016).

The effects of breeding during the twentieth century on the gluten quality of durum wheat for processing and health were investigated by De Santis et al. (2017), comparing a set of old and modern Italian wheats. The better technological performance observed for the modern varieties was found to be due not only to the introgression of the superior alleles of high molecular weight (HMW GS) and low molecular weight (LMW GS) glutenin subunits encoded at the Glu-B1 and Glu-B3 loci, but also to differential expression of specific storage proteins. The higher gluten index observed in modern genotypes was correlated with an increased glutenin/gliadin ratio. Another marked difference between the old and modern cultivars was the significantly higher expression level of B-type LMW GS in the modern varieties. No significant differences were found among durum wheat genotypes in relation to the expression of the most harmful α-type and γ-type gliadins, while in the modern genotypes a significant decrease was observed in the expression of ω-5 gliadins, a major allergen in wheat-dependent exercise-induced anaphylaxis (WDEIA).

Comparing old and modern Iranian wheat cultivars, similar alterations in Glu/Gli ratio and the changes in the HMW GS alleles have been reported by Izadi-Darbandi et al. (2010).

A set of 78 varieties released between 1860 and 2015 were selected and studied by Riaz et al. (2019a, b) and Florides et al. (2019) in terms of grain quality, dough rheology plus protein composition and FODMAP content, as well as the extent of allergen epitopes in the gliadin proteins. Grain-protein contents have been found to be significantly lower in modern varieties compared to historical varieties and are negatively correlated to increased grain yield data. Over the years, significant changes in quality parameters of grains have been found. Compared to historical varieties, modern cultivars were found to provide stronger dough properties, as observed by micro-Doughlab and Mixograph results, including increases in dough development time, dough stability, peak mixing time, midline peak width and decreases in softening after 5 min of mixing, as well as a weakening slope. The improvement in protein quality, compensating for the decrease in protein content, was found to be associated with the increased glutenin-to-gliadin ratio and with a shift of size distribution for the polymeric proteins (%UPP). The higher %UPP is derived from the systematic alteration of HMW glutenin and LMW glutenin alleles in the modern cultivars. The two most significant examples in this alteration are the increases in the numbers of cultivars containing the *Glu1Dd* and *Glu1Bal* alleles (HMW glutenin subunits 5 + 10 and overexpressed 7) and also the effects of LMW-glutenin alleles determining functional properties.

Different separation methodologies, including reversed and size-exclusion high-performance liquid chromatography, MALDI-TOF, liquid chromatography, MS MS (qTOF), followed by the application of different bioinformatics procedures, and databases have all been used by Florides et al. (2019) to develop a breeder's tool, to quantify the gliadin content and composition of wheat cultivars and to determine their immunoreactive epitope content. Using this tool, a small highly toxic ω-gliadin group was discovered, and the immunoreactive epitopes of its members were mapped. The allergenicity of 170 wheat cultivars was estimated, using this tool, and it was clearly shown that historic wheat varieties are potentially as immunoreactive as the more recently released cultivars.

Fructan content

No significant trend in the alteration of fructan content of wheat varieties released in Australia over the last 150 years has been observed by Riaz et al. (2019b). Fructan levels in flours varied between 1.01 to 2.27% on a dry basis, showing slight variations among the cultivars and significant effects of the harvest years (mean values for 2015 and 2016 samples are 1.38 and 1.74, respectively). Similar results have been reported on the fructan levels of historic and modern Austrian wheat varieties by Fretzdorff and Welge (2003a, b), while extremely high or extremely low fructan contents were not found, it was possible to select a few cultivars with consistently high or low fructan contents; thus, the latter offers the potential to re-utilize these historic wheats with low fructan content in present breeding programs (Riaz et al. 2019b).

ATIs content

The most detailed analysis on the ATI content and diversity across wheat cultivars has been published recently by Bose et al. (2020). The level of 18 ATI isoforms (63 peptides) grouped into four subtypes were monitored across 15

commercial wheat cultivars and the 8 parental lines from a multiparent advanced generation intercross (MAGIC) population (Cavanagh et al. 2008) using liquid chromatography–mass spectrometry (LC–MS) technique. Large variation of ATI content was observed among the of wheat samples, with significantly higher average ATI levels found in cultivars Baxter and Xiaoyan and significantly lower values in cultivars in Pastor and Longreach Scout. This work establishes a reference map for wheat ATIs that presents an opportunity for selecting low ATI wheat lines for use in breeding programs or the development of assays to monitor ATIs in clinical studies.

Breeding for "healthy" wheats

Values reported in the literature within and among wheat types for celiac reactivity, human α-amylase inhibitor (ATI) activity, allergenicity, and fructan content are shown in Fig. 1. These detailed scientific investigations do not support the controversial idea that human practices, i.e., modern wheat breeding may have contributed to the increase in celiac disease or NCWS prevalence during the latter half of the twentieth century. Each of the primitive wheat relatives and each historic or modern bread or durum wheat variety contain more or less amounts of toxic/allergenic epitopes with a declining level trend caused by the altered protein content and composition.

In the light of these results, it can be concluded that while in the last 120 years, health-related quality attributes have not been considered in pre-breeding and breeding, selection has focused on yield and functional quality which has inadvertently resulted in a reduction of the amounts of harmful compounds in new lines. This is because of the decreasing trend in overall protein content as well as the alteration of glutenin to gliadin content to improve dough strength. Older varieties are higher in gliadin content and have higher CD antigenicity (Izadi-Darbandi et al. 2010; Prandi et al. 2014, 2017; Ribeiro et al. 2016; De Santis et al. 2017; Malalgoda 2016; Florides et al. 2019; Riaz et al. 2019a), while there is no alteration in the amounts of fructan (Fretzdorff and Welge 2003a, b; Riaz et al. 2019b).

Screening primitive wheat relatives and revisiting historic wheat cultivars in various studies have revealed some genotypes and old varieties containing low levels of certain gliadin classes and FODMAPs. These findings point to an important wheat genetic pool that can be further exploited

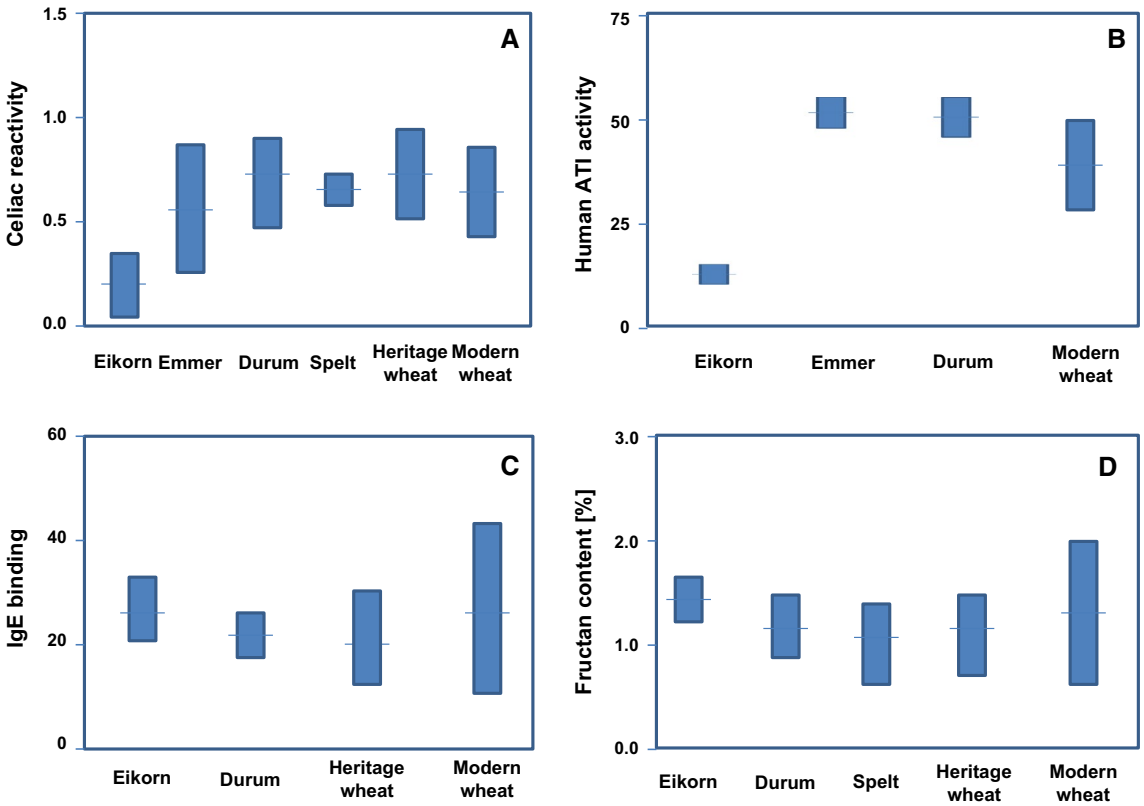

Fig. 1 Values reported in the literature within and among wheat types for **a** celiac reactivity, **b** human α-amylase inhibitor (ATI) activity, **c** allergenicity, and **d** fructan content. Horizontal lines indicate the median value for each of the value ranges. Modern wheat includes varieties of common wheat that were developed after 1950, while heritage wheat includes varieties and landraces that were developed before 1950

for the development of celiac-safe wheat products, while also suggesting great potential in future conventional breeding practices. It would be difficult with this genetic diversity alone to obtain a wheat variety without toxicity while retaining the unique viscoelastic properties of gluten. However, this natural potential can be further exploited using cutting edge molecular breeding techniques encompassing mutagenesis, transgenesis, and genome editing (Shewry and Tatham 2016; Boukid et al. 2017; Jouanin et al. 2018a; Malalgoda et al. 2018; Rustgi et al. 2019).

The expression levels of compounds triggering wheat sensitivities are dependent of environmental factors and growing conditions. The related information, covering the effects of climate changes as well as the different forms of abiotic and biotic stresses on the allergen content of the wheat grain, is discussed in the overview of Juhász et al. (2020).

Aside from the development of such celiac-safe lines, there are several issues to be solved for the future commercial production of "safe" genotypes. As it is stated by García-Molina et al. (2019), low-gliadin wheat lines are being developed as an alternative cereal with a better nutritional profile and organoleptic properties, as well as improved bread making properties, when compared to current gluten-free products. On the other hand, the requirement of fertilization, especially nitrogen, for low-gliadin wheat is of concern for the farmers, as it is well known that protein accumulation during grain filling is strongly influenced by nitrogen fertilization, and it can modify grain–protein composition and yield. Therefore, fertilization strategies for such lines are undoubtedly important to keep gluten levels as low as possible with no compromise to yield and plant growth. Jouanin et al. (2018b) discuss political and economic issues which strongly define the commercial use of genetically modified wheats, including gene-edited genotypes in several countries.

Effects of processing on wheat sensitivity

If primitive wheats, as well as all historic and modern bread wheat cultivars, contain harmful amounts of toxic epitopes, why do we have increased numbers of reported cases of wheat sensitivity nowadays? Our grandparents produced breads from the same kind of "harmful" wheat as we have, today. Is the bread we consume today also the same as that of our grandparents?

To answer for this question, we need today's processes of making bread to be investigated and compared with those in our grandparents' era. This investigation must be extended beyond the baking industry to monitor the changes in processing and formulation across the entire food industry.

No epidemiological studies have evaluated the impact of wheat processing on the prevalence of wheat sensitivity over the last 50 years. Nevertheless, as it is stated by Kucek et al. (2015) in their review, increases in disease diagnoses correlate with food industry uses of compounds that have been reported to trigger sensitivity, such as gluten, inulin, and high-fructose corn syrup.

Industrial products supplied to the food industry

Since Basil Regan and Leonard Winch of Fielders Mills developed the first commercial method for drying unfermented gluten in Tamworth (NSW, Australia) in 1938 (Day et al. 2006), the by-product of wheat starch isolation, "vital wheat gluten," is utilized all around the food industry (Hesser 1987). By the 1970s and 1980s, dried gluten as a cheap source of protein was readily included in many foodstuffs (Hesser 1987; Day 2011; Ortolan and Steel 2017).

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. Low-protein flours are often fortified with vital wheat gluten to improve baking characteristics (Day et al. 2006; Ortolan and Steel 2017). Vital wheat gluten is often used in multigrain bread formulations as a binding agent (Atchison et al. 2010).

Kasarda (2013) was the first to point out that, while the overall gluten content of modern wheats has declined, dietary exposure to gluten has indeed increased in the last 50 years caused by the increased application of vital gluten in many processed foods. Gluten is commonly used as thickeners, emulsifiers, and gelling agents. According to a survey by Atchison et al. (2010), an estimated 29.5% of supermarket food products contain wheat proteins (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). Beyond the baking industry, the largest amount of gluten is used in processed meat, reconstituted seafood, and vegetarian meat substitutes (Day et al. 2006).

It is important to note that isolated wheat gluten does not contain the endogenous wheat enzymes that assist in the degradation of gluten proteins. Leduc et al. (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 (Lauriere et al. 2006).

Other potentially harmful, often-used food additives are high-fructose corn syrup and inulin, both implicated in fructose malabsorption, IBS, and NCWS. Fructose consumption has risen in the last 30 years, largely due to a 60.8% increase in sweetener availability since 1978 (Gibson et al. 2007;

Marriott et al. 2009). Cereals, muffins, cake mixes, instant oatmeal, granola bars, cookies, and bread are often supplemented with inulin-type fructans for the purpose of fiber supplementation or fat replacement in low-fat products (Gibson et al. 2000; Kleessen et al. 2007; Grabitske and Slavin 2009). Inulin-type fructans are not derived from wheat, but rather are extracted from chicory root and Jerusalem artichoke (Kolida and Gibson 2007). Although inulin can benefit most consumers when eaten in moderate amounts, inulin may aggravate symptoms of fructose malabsorption for those with 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 Areas 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.

The effect of wheat milling

The chemical composition of wheat is different in various layers of the wheat kernel (Jones et al. 2015). So, depending on the milling process, flours containing different amounts of harmful components are produced. Cysteine-type endoproteases are synthesized in the aleurone layer (Hammerton and Ho 1986), the removal of bran to produce white flour results in lower enzyme levels available for protein degradation (Hartmann et al. 2006; Schwalb et al. 2012).

The distribution of gliadin proteins from the three main protein classes shows characteristic differences among the morphological parts of the seed. Many of the celiac-reactive α-gliadins are located in the sub-aleurone layer of the wheat kernel, which can be partially removed by roller milling. However, the γ-gliadins and the HMW glutenins are concentrated in the endosperm, therefore appearing in high concentrations in white flour. Omega-gliadins are found throughout the grain (Tosi et al. 2011), so their amount seems not to be altered by the level of flour refinement.

ATIs surround starch molecules in the endosperm, protecting them from digestion by insects and mammals. Wheat bran elicited about twice the IgE activity for bakers' asthma than white flour (Armentia et al. 2012).

Fructans, also, are not evenly distributed throughout the wheat grain. In terms of wheat-milling fractions, bran, and shorts contain more fructan than the related white flour (Knudsen 1997; Haska et al. 2008). The inclusion of bran in whole-wheat flour likely increases the total fructan content of whole-wheat flour, relative to white flour. Whole-wheat flour also contains fructans with a higher degree of polymerization than those in white flour. The lower degree of polymerization of fructans 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 are more easily degraded by yeast (Nilsson et al. 1987; Praznik et al. 2002), fructans in white bread are broken down more extensively than those in whole-wheat bread (Knez et al. 2014).

The use of microbial enzymes in baking formulation

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 et al. 1992; Morren et al. 1993; Baur et al. 1998; Sander et al. 1998; Quirce et al. 2002). These additives provide an additional exposure risk to bakers (Tatham and Shewry 2008). These enzymes do not represent any health-related risks for the consumer, while strict guidelines on the safe handling of enzymes in the bakery supply chain protect employees to avoid contamination (De Vos et al. 2018).

The effects of the dough-making process

Effects on gluten proteins

Yeast-mediated dough fermentation is a critical phase in the bread making process, determining the rheology of the dough and for the final bread product—its texture, volume, and taste. The production of CO_2 and other metabolites by yeast cells involves a large number of reactions related to the enzymatic degradation of the carbohydrate and protein components of the dough. Different factors affect the fermentative performance of yeast cells during dough fermentation, including dough ingredients, fermentation conditions, the type of yeast strain used, and yeast pre-growth conditions. Traditionally most bread involving yeast leavening used to be made with long dough fermentation times of up to 6 h before baking (Kulp 1993; Struyf et al. 2017a, b; Parapouli et al. 2019).

Gluten degradation by proteolysis during dough making has been studied for more than 50 years (Frazer et al. 1959; Messer et al. 1964). Unlike human gastrointestinal proteases, microbial proteases can cleave the peptide bonds next to proline residues, which frequently occur in gluten proteins (10–15% proline). Based on the studies of Lyons (1982), Stepniak et al. (2006) and Walter et al. (2015), gluten proteins could be degraded to small peptides containing less than nine amino acid residues.

Sour dough fermentation for bread making plays a crucial role in the development of sensory properties such as taste,

aroma, texture, and the overall quality of baked goods. This is due to the acidification, proteolysis, and activation of a number of enzymes (Melim-Miguel et al. 2013; Gobbetti et al. 2014). Sour dough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB).

Typical sour dough bread is a staple food contributing to cultural identity in sundry diets, mostly in Central and Eastern Europe (Moroni et al. 2009). Beyond sour dough bread, traditionally, there are some sour dough sweet-leavened baked goods obtained by microbial protease fermentation from lactic acid bacteria, such as the Genoese dry biscuit, called lagaccio, and a soft cake from north Italy, panettone. All of them involve very long-fermentation processes, using lactic acid bacteria (LAB), which provide a sour taste to the product (De Vuyst and Neysens 2005). Sour dough bread, made by traditional techniques, is now becoming increasingly popular through artisan bakeries in many Western countries (Ross 2018).

In the last 20 years, attempts to reduce the harmful components of wheat have produced numerous strategies. The application of lactobacillus-based proteases during the sour dough process or supplementing these enzymes such as especially prolyl-oligopeptidases in yeasted-dough fermentation (Rizzello et al. 2007; Walter et al. 2015; Di Cagno et al. 2010; Greco et al. 2011) have been investigated in great detail (Rizzello et al. 2007, 2014; Ganzle et al. 2008; Gerez et al. 2006, 2012; Di Cagno et al. 2002, 2004, 2010; Rollan et al. 2005; Caputo et al. 2010; Walter et al. 2015; Rizzello et al. 2014; Deora et al. 2014; Guilliani 2012; Engstrom et al., 2015; Heredia-Sandoval et al. 2016; Kristensen 2016).

In most case, these treatments have been effective in reducing gluten immunogenicity, not only for making bread but in cases of other leavened products such as pizza (Pepe et al. 2003) and even pasta (De Angelis et al. 2010). It is also reported that these treatments have marginal effects on functional properties caused by partial depolymerization of polymeric glutenin and proteolysis of the HMW glutenin subunits (Loponen et al. 2003, 2007).

Importantly, several publications underline the significance of the fermentation time used either in cases of yeasted or sour dough fermentation (Thiele 2003; Thiele et al. 2004; Zotta et al. 2006, Siddiqi et al. 2016; Couch 2016; Sakandar et al. 2019): Significant proteolysis can be observed only after a minimum four hours of fermentation.

The effect on FODMAP content

Nilsson et al. (1987) were first to show that dough mixing can reduce the fructan concentration of various wheat flours from 40 to 75%, due to the flour-oxygenation action of the invertase enzyme in baker's yeast (*Saccharomyces cerevisiae*). Fermentation without yeast did not affect the fructan concentration after mixing (Nilsson et al. 1987; Verspreet et al. 2013; Knez et al. 2014; Gelinas et al. 2016). Therefore, it was recommended that individuals sensitive to fructans should consume long-fermentation breads rather than products arising from short mixed and fermented industrial processes. A reduction or complete prevention of fructan degradation can be achieved also by using mutant yeast strains with lower sucrose degradation activity or lacking invertase (Verspreet et al. 2013). Yeast has been shown to have a preference for fructans with a low degree of polymerization (Nilsson et al. 1987; Rakha et al. 2011). Praznik et al. (2002) confirmed that fructan molecules with a higher degree of polymerization or higher average chain length are more resistant to degradation during the baking process. This might explain why fructan degradation during baking can be higher in wheat than in rye breads (Nilsson et al. 1987; Fretzdorff and Welge 2003b; Andersson et al. 2009).

The importance of proofing (fermentation) time, regarding the fructan content of baked products irrespective of the variety used, was demonstrated by Ziegler et al. (2016). Short proofing of only 1 h slightly decreased the raffinose and fructan level and significantly increased "excess fructose" due to the almost complete hydrolysis of sucrose. Longer proofing times of 2.5 h or 4.5 h also reduced the excess fructose levels significantly, resulting in a final FODMAP load of only 29–33% and 10–23% of the initial concentrations for bread and spelt wheats, respectively. Since spelt wheat is often processed by artisan bakeries using traditional recipes and long fermentation times, partly to improve the rather poor technological performance of spelt (Schober et al. 2002; Frakolaki et al. 2018), observations of individuals better tolerating spelt than bread wheat products might rather be related to different processing than to species-related causes. Similar results were obtained in Australia when commercial bread samples, made from wheat and spelt flour and baked with different baking technologies, have been collected and analyzed (Suter et al. 2018).

According to recently published scientific literature, simply monitoring the fructan levels in baked goods does not provide the full picture in relation to the physiological effects on IBS patients. Struyf et al. (2017a, b, 2018) described the alteration of FODMAP levels and the changes in the carbohydrate profile in whole-meal bread produced with yeast fermentation. The effect of yeast strain, fermentation parameters, such as yeast dosage and fermentation time, is discussed including the application of enzyme-based technologies. Recently, Benítez et al. (2018) demonstrated that dough preparation and baking significantly reduced fructan content, whereas no effect was observed on the concentrations of fructose, glucose, and raffinose, and a significant increase was recorded for sucrose and specifically for maltose.

Compared to the yeasted-dough process, sour dough technology results in an even greater drop in FODMAP

content. Acidic conditions are advantageous for the invertase enzyme, playing a crucial role in fructan degradation (Nilsson et al. 1987, Kucek et al. 2015). Costabile et al. (2014) concluded that breads fermented by the traditional long-fermentation and sour dough processes are less likely to lead to IBS symptoms compared to bread made by short- or no-time fermentation processes. Loponen and Ganzle (2018) gave an overview of the biochemical processes during sour dough fermentation, providing details about the roles of different microorganisms in the culture and the enzymes involved. Yeast or sour dough culture plays a crucial role in fructan degradation. Selection of the correct strain and the optimal fermentation parameters are of major importance to steer fructan degradation. Conventional sour dough baking reduces and converts FODMAP in rye and wheat flour; however, the extent of FODMAP reduction is dependent on the fermentation organisms, the fermentation process, the grain raw material, and the sour dough starter dosage in the final bread dough. The production of low-FODMAP bread requires extracellular fructanase activity. Sour dough fermentation with lactobacilli expressing fructanases or the use of fructanase-positive yeasts provide breads with a low-FODMAP content.

Effects on ATI content

Food processing alters the conformation of allergenic wheat proteins and may abolish, and in rarer cases, increase their allergenicity (Pasini et al., 2001). IgE antibodies of wheat-allergic patients reacted with in vitro digested bread crumb and crust, but not with unheated bread dough (Loponen et al. 2007). The TLR4-stimulating bioactivity of ATI was reduced by 20–50% after bread and biscuit making, compared to unprocessed wheat flour (Zevallos et al. 2017).

The current data of Huang et al. (2020) suggest that sour dough fermentation can degrade ATI structure and bioactivity and point to strategies to improve product development for wheat-sensitive patients. ATI tetramers were isolated, fluorescein-labeled, and added to a mini-dough bread making system by Huang et al. (2020). In the case of sour dough fermentation, below pH 4, the ATI tetramers were degraded due to the activation of aspartic proteases, while in yeast fermentation, ATI tetramers remained intact. The amylase inhibitory activity after sour dough fermentation decreased significantly, while the concentration of free thiol groups increased. Compared to unfermented wheat, sour dough fermentation was able to decrease the release of pro-inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor.

Comparing different mixing and fermentation procedures

Up until the early 1980s, most bread was made using yeast with a long dough fermentation of up to 6 h before the breads were baked. In 1961 in the UK, the British Bakers Industry Research Association (BBIRA) developed a new method of making bread called the Chorleywood Bread Process (Chanberlain et al. 1961). This process used high energy Tweedy mixers together with a range of bread improver agents; but in particular, fermentation time was reduced to only one hour (Cauvain and Young 2006). Obviously, this method of making bread was very fast and significantly cheaper than previous methods. This bread making method, also known as the "no-time dough" (NTD) process, was rapidly taken up in a lot of countries.

The consequent alterations in gliadin and fructan levels in bread wheat and spelt flour, in doughs during fermentation, and in the final products have been monitored, comparing the effects of the NTD process to long-fermentation yeasted doughs and sour dough methods (Suter et al. 2019a, b). With minor changes observed for the NTD process, long fermentation in both yeasted- and sour doughs dramatically changed the composition and structure of the gliadin proteins (Fig. 2a) and significantly reduced the fructan content of the baked bread (Fig. 2b). The relative amounts of total gliadin (protein extracted with 70% ethanol) expressed as the percentage of total gliadin in the flour was determined to be 87% and 85% in NTD breads made from wheat and spelt flours, respectively. Fructan levels in the same NTD breads (1.10% and 0.96%, for NTD_W and NTD_S, respectively) showed less than 5% decrease compared to those in the corresponding flours. These fructan content data are in a good agreement with those resulted in from a survey of commercial NTD bread samples carried out by Suter et al. (2018). Compared to these marginal changes, significant reductions have also been observed in the total gliadin and fructan levels in long-fermentation yeasted dough (LFYD) and sour dough (LFSD) breads made from wheat. Decreases in gliadin content were as follows: in wheat breads LFYD = 65.1%, LFSD = 88.6% and spelt LFYD = 59.4%, LFSD = 90.4%. Similar comparisons of fructan contents also showed significant decreases in wheat breads: LFYD = 85.1%, LFSD = 88.9% and in spelt breads: LFYD = 93.0%, LFSD = 98.4%.

The obvious conclusion from these data is that fermentation time is a major factor in reducing gliadin and fructan levels both in wheat and spelt breads, caused either by yeast or sour dough activities. The sour dough process seems to be more effective in reducing harmful component levels, but it does not produce gliadin-free bread.

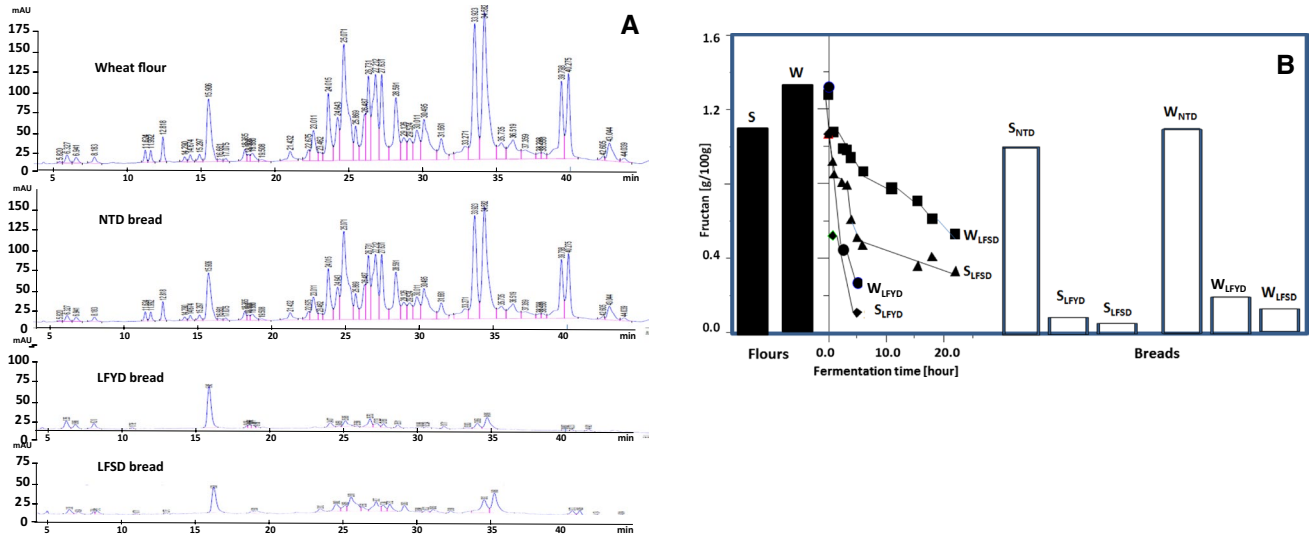

Fig. 2 The alterations in gliadin (**a**) and fructan contents (**b**) during different bread making processes (*NTD* no-time dough, *LFYD* long-fermentation yeasted and *LFSD* long-fermentation sour dough), using wheat (*W*) and spelt (*S*) flours

Producing "healthy" bread

Numerous individuals have reported a series of health difficulties after consuming bread wheat, whereas they claimed spelt products to be easily digestible (Stallknecht et al. 1996; Vu et al. 2014). Biesiekierski et al. (2011) suggested that lower FODMAP contents in spelt products might be the reason for these observations. There has been anecdotal clinical evidence for a long time that a large proportion of non-celiac patients, suffering wheat-related health disorders, can tolerate products made from certain spelt varieties. The first research paper with robust experimental and statistical results also demonstrated this important observation (Armentia et al. 2012).

Bread wheat and spelt flours are often processed differently due to their differing techno-functional characteristics (Schober et al. 2002). Most spelt products in the market are made using artisan-type technologies (Ross 2018) with long yeasted or lactobacillus fermentation. These processing differences may be the main factor responsible for the acceptability by sensitive individuals rather than the grain species alone.

Detailed analysis on the individual peaks in the RP-HPLC analysis of gliadins extracted from long-fermentation yeasted and sour dough breads (Fig. 2a) showed that the proteases of the two types of microorganism have different preference among gliadin polypeptides, so the composition of the remaining gliadins in the breads are different. It suggests there is an opportunity to optimize the fermentation process with the combination of different yeast and lactobacillus strains or using different proteases of lactobacillus origin during yeasted fermentation which may produce products with significantly less harm for sensitive consumers.

Natural primitive wheat relatives or wheat cultivars with low gliadin and/or fructan content have a great potential in traditional breeding in combination with modern gene-modifying technologies to develop healthier wheat cultivars. The utilization of these grains, using proper processing technologies where the harmful components of wheat are partially and hopefully fully eliminated by enzymatic hydrolysis during fermentation, could produce harmless products for sensitive individuals. These breads could be even "healthier" than those that our grandparents consumed.

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