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Modern aspects of wheat grain proteins

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Summary

The unique baking properties of wheat have contributed to the large variety of food products made of wheat. Wheat products are immensely popular, which is reflected in their ubiquitous consumption. Concerning wheat quality, a main challenge for intense growing strategies is to adapt wheat plants of unaltered yield and baking quality to decreased nitrogen input, which will limit unwanted nitrogen leaching into drinking water and safe resources. A probably more important challenge for wheat adaptation will be caused by global climate change. For a relative small percentage of the human population wheat grain proteins can cause a number of serious diseases including coeliac disease. Susceptible persons often have to completely avoid wheat, as well as rye and barley products. Methods for detection of gluten protein are well advanced, increasing safety for patients. Wheat breeding using traditional breeding and modern genome editing approaches are seen to be necessary to develop new wheat cultivars for adaptation to new environmental conditions caused by climate change, reduced nitrogen input and increased production efficiency, as well as reduction of disease potential. Wheat grain protein analytical methods are important, *e.g.* for determination of quality parameters and for decision making in breeding programmes. Aspects of protein extraction, proteomic analysis and database coverage of wheat protein sequences are discussed.

Keywords: baking quality; celiac disease; protein analysis; storage proteins; *Triticum aestivum*; wheat; wheat allergy, wheat sensitivity, gluten

Introduction

Wheat grain proteins are classically categorized and separated according to solubility in albumin, globulin, gliadin and glutenin (Osborne, 1907), with the latter two fractions together corresponding to gluten proteins. In more recent times, gliadin and glutenin, making up between 60-80% of total grain protein, have also been classified as prolamins either being poor or rich in sulphur or of high molecular weight (Shewry et al., 2009; Uthayakumaran and Wrigley, 2017). Based on comparison of amino acid sequences proteins like puroindolines, α-amylase/trypsin inhibitors and lipid transfer proteins are also regarded to be members of the group of small sulphur rich prolamins (Shewry and Tatham, 1999; Shewry et al., 2009). During dough formation the visco-elastic properties of gluten proteins are chiefly responsible for excellent processing and baking quality of wheat. Considering the negative, some proteins of wheat and some closely related proteins of barley and rye can cause a range of health problems in humans (Shewry et al., 2009). The aim of this review is to highlight modern and future aspects of wheat grain proteins concerning baking quality, health problems and corresponding analytical strategies.

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1. Modern aspects of proteins contributing to baking quality

Baking quality of bread wheat is determined by the wheat cultivar, as well as the interplay of the natural grain constituents' proteins, carbohydrates and lipids, each of which are dependent on external conditions of the environment (*e.g.* water, soil, weather, climate) as well as type, amount and timing of fertilisation (Békés et al., 2004; Xue et al., 2016; Rekowski et al., 2019; Xue et al., 2019) (Fig. 1). The considerable impact of milling and baking technology on baking quality (Fig. 1) is not subject of the discussion here; for recent reviews of these topics see Bock et al. (2016); Miskelly and Suter (2017). This chapter will focus on the role of diverse storage proteins in bread making quality. By far the largest part of total protein, approximately 60-80%, is deposited in form of gliadin and glutenin in the starchy endosperm tissue of the wheat grain (Shewry et al., 2009; Uthayakumaran and Wrigley, 2017). Research on the role of protein in baking quality of wheat has been focused on various aspects such as total protein concentration, ratio of gliadin and glutenin concentration, occurrence of disulphide bonds mainly in glutenin, extractable mass of SDS-insoluble glutenin, the so-called glutenin macropolymer, and occurrence of different forms of glutenin and gliadin proteins in a range of cultivars. As members of the prolamin superfamily, puroindolines are largely determining hardness of the wheat grain, having an important effect on processing properties (Shewry et al., 2009; Uthayakumaran and Wrigley, 2017).

Many properties of proteins contributing to baking quality have been established using protein-biochemical methods. The physical perspective of baking quality is investigated employing rheological methods, which are mostly performed in dough formulations.

Fig. 1: Baking quality of wheat is determined by wheat cultivar, the interplay of natural grain constituents' proteins, carbohydrates and lipids, growing conditions of wheat as well as milling and baking technology.

Rheological methods can broadly be distinguished in empirical and fundamental assay formats, the latter of which can determine structure-function properties (DOBRASZCZYK, 2016; TIETZE et al., 2016). Further elucidation of wheat gluten polymer structures and their functionality in response to cultivar, environment and processing is seen as important to improve of gluten-based products, such as bread (JOHANSSON et al., 2013).

Incentives and challenges for improving baking quality of wheat

Currently, price finding for wheat is strongly influenced by high crude protein content with the debateable rationale that more protein leads to higher baking quality, *i.e.* higher loaf volume. Wheat with high crude protein content (generally ≥ 13%), is achieved in intense cultivation systems, using high levels of nitrogen-based fertiliser. Excess nitrogen-fertilisation has often the consequence of leaching of various chemical forms of nitrogen, which in turn creates multiple problems, *e.g.* NO_{3^-} is contributing to eutrophication and pollution of drinking water resources, whereas nitrous oxide released into the atmosphere is a very potent greenhouse gas by far exceeding the effect of CO_2 on a molecule basis (BUTTERBACH-BAHL et al., 2013). In the face of evident climate change, dwindling energy resources and growing demand for food it is imperative to make wheat production more efficient in terms of fertiliser input (ZÖRB et al., 2018).

One option to reach this goal is wheat breeding. Analysis of winter wheat cultivars grown between 1983 and 2014 in Germany has shown some success in this respect: 31,6% increased grain yield was accompanied by 1,5% rise in protein levels and in these 31 years the baking quality parameters sedimentation value (+45,4%) and loaf volume (+8,3%) increased (LAIDIG et al., 2017). While normally grain yield and protein content are negatively correlated, the authors are pointing out that results above were possible due to newly bred wheat cultivars in combination with agricultural practices.

Advancing this positive development, in the future "ideal" wheat needs less fertiliser to maintain grain yield at a lower crude protein content and at the same time to feature proteins (and possibly other grain constituents) conferring improved baking quality. Ways to achieve these aims are seen in wheat breeding for improved grain yield stability and nitrogen use efficiency, and possibly in targeted deletion of genes coding for storage proteins not needed for baking quality, which in summary are predicted to reduce nitrogen input in wheat cultivation (ZÖRB et al., 2018). Wheat breeding might also succeed to improve end-use quality by introducing specific genes into commercial cultivars. As one example, using transcriptomic analysis, the gene wheat bread making (*wbm*) was shown to occur in some wheat genotypes, where a high expression of *wbm* was correlated with improved baking quality (FURTADO et al., 2015; GUZMÁN et al., 2016). The authors are recommending introduction of *wbm* into wheat breeding programmes, which presently has got only a frequency of 14% in CIMMYT wheat germplasm.

Probably having more complex consequences than decreasing fertilization input, climate change will alter numerous environmental conditions (*e.g.* heat and drought stress, rise of atmospheric CO_2 concentration) inevitably leading to changed levels of natural grain constituents (HALFORD et al., 2015). Specifically, the same authors note that temperature > 35 °C during grain filling and drought stress causes a decrease in the proportion of glutenin to gliadin, which impacts negatively on baking quality. To meet the challenges of climate change concerning baking quality, a combination of measures will be required, including agricultural practices, choice of existing species and cultivars suitable for altered climate conditions. Besides traditional breeding, modern tools like genomic selection and gene editing together with comprehensive transcriptomic and genomic data of wheat are holding promise that new cultivars better adapted to changing growing conditions can be developed in a future world (HENRY et al., 2016; INTERNATIONAL WHEAT GENOME SEQUENCING CONSORTIUM, 2018).

2. Wheat related disorders

Consumption of wheat products is ubiquitous. While for a large proportion of humans wheat products are nutritious and healthy, there is a rising number of individuals, which experience health problems when being exposed to products from all *Triticum* species, as well as to products from barley and rye. These wheat-related disorders are connected to the human immune system, where autoimmune, allergic and possibly innate immunity mediated responses are distinguished (Fig. 2). This review is giving a brief summary of wheat-related disorders. For an in depth overview of this topic including disease definitions, clinical symptoms, diagnosis and treatment the reader is referred to SCHERF et al. (2016). Coeliac disease (CD), affecting approximately 1% of the worldwide population, is an immune-mediated inflammatory disease of the upper small intestine caused by gluten ingestion. Patients classically suffer from abdominal pain, diarrhoea and vomiting and often from malabsorption of minerals and vitamins, leading to various forms of malnutrition (SCHERF et al., 2016). Both, dermatitis herpetiformis and gluten ataxia are related to CD, but affecting skin and neurological functions and having a much lower prevalence than CD, respectively (Fig. 2). Wheat allergy (WA) is caused by immunologic reactions to wheat proteins involving IgE antibodies (SAPONE et al., 2012). Expressions of WA are food allergy, wheat-dependent exercise-induced anaphylaxis (WDEIA), respiratory allergy and contact urticaria (Fig. 2). Afflicted

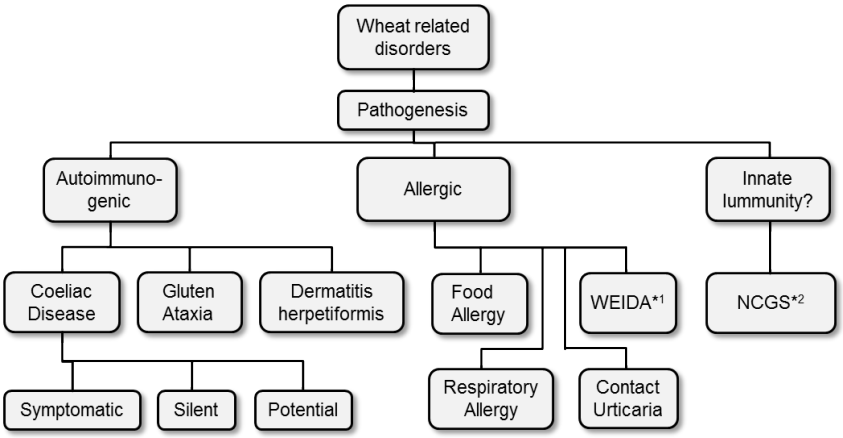

Fig. 2: Categories of wheat related disorders (adapted from SAPONE et al., 2012). *[1]Wheat-dependent exercise-induced anaphylaxis, *[2]Non-celiac gluten sensitivity

are skin (swelling, itching), gastrointestinal tract (abdominal pain, diarrhoea) or respiratory tract (baker's asthma), where a number of wheat proteins have been identified as triggers, *e.g.* α-amylase inhibitors, lipid transfer proteins, as well as various gliadins and glutenins. A severe reaction is anaphylaxis (WDEIA) in response to exposure to ω5-gliadins or high molecular weight glutenins in combination with physical exercise (SCHERF et al., 2016). Non-coeliac gluten sensitivity (NCGS) is a recently defined disorder with symptoms comparable to CD, but leading neither to histological changes of the small intestine nor to production of anti-TG2 antibodies. α-amylase trypsin inhibitors possibly in combination with certain carbohydrates are suspected to play a role in causing NCGS (ZEVALLOS et al., 2017; DIETERICH et al., 2018).

Detection and quantification of gluten peptides

At present, strict avoidance of wheat, rye and barley inclusively products thereof is mandatory for persons susceptible to most of the described disorders. In International Food Standards, it is regulated that food labelled gluten free must not contain more than 20 mg/kg gluten in total (CODEX STANDARD 118-1979, 2015). Determination of gluten in food is routinely performed with ELISAs based on antibodies that target different epitopes in various gluten proteins (LEXHALLER et al., 2017). When using these ELSIA assays it is important to consider variation in commercial test kits as well as to consider influences of wheat cultivars and species (SCHERF, 2017; SCHOPF and SCHERF, 2018). Recent work has identified novel CD-active target sequences in gliadin and glutenin proteins, which are suitable for generation of new antibodies (RÖCKENDORF et al., 2017). The authors are envisaging that the combination of existing and new antibodies will improve detection of gluten due to better coverage of harmful gluten epitopes. In turn, this will increase safety for patients suffering from coeliac disease.

Advancements in LC-MS/MS techniques together with increased database coverage of wheat protein sequences have spurred efforts to use proteomics based methods for detection and quantification of peptides causing CD (VAN DEN BROECK et al., 2015; HUSCHEK et al., 2016; BROMILOW et al., 2017; SCHALK et al., 2018b; a). Due to high selectivity, sensitivity, versatility and applicability also for processed gluten, LC-MS/MS methods are seen as the most promising approach, besides immunological assays, for detection and quantification of gluten (SCHERF and POMS, 2016). Both, LC-MS/MS and immunological methods will profit substantially using well characterised reference proteins from several cereal species that became available recently (SCHALK et al., 2017). Other methods to detect gluten or gluten genes comprise PCR, next generation sequencing, aptamers, magnetic beads, microarrays and multianalyte profiling. These methods have conceptual disadvantages or are technically not as mature and thus are up to date not widely applied (SCHERF and POMS, 2016).

Approaches to reduce or eliminate gluten protein

Approaches to eliminate gluten protein from cereals and cereal products are followed with the aim to make these cereals available mainly for CD patients, increasing the choice of food available to them. In this direction, one option is to use enzymatic modification and, more effectively, digestion of problematic gluten proteins. These treatments can lead to sourdough based wheat bread, pasta, wheat starch and bran, beer and rye products below the threshold of 20 mg/kg gluten tolerable for CD patients (SCHERF et al., 2018). A further option is to eliminate genes from the wheat genome, coding for health related proteins. An example for such an approach is the strong reduction of α-gliadins in wheat obtained either after application of an RNA_i technique (BECKER et al., 2012) or a genome editing technique using CRISPR/Cas9 (SÁNCHEZ-LEÓN et al., 2018). The recent sequencing of the wheat genome has facilitated a very detailed mapping of an allergy/immune-stimulatory set of genes as potential targets for breeding programmes to further reduce health related proteins (JUHÁSZ et al., 2018). While molecular breeding and mutagenic approaches appear very promising for reducing individual genes, two main challenges in this area are seen, *i.e.*: the complex multigenic control of gluten proteins and to retain processing and product functionality in the modified wheat (SHEWRY and TATHAM, 2016).

3. Recent challenges of analysis of wheat storage proteins

The analysis of storage proteins in seeds is an important issue since "pre-modern" laboratories started to evolve knowledge about aspects of nutritive value, seed ingredients, technological function of proteins, effects of genotypes or fertilization or other environmental factors on grain proteins, or pure basic science of storage protein knowledge. These analyses were initiated early 20th century (SHEWRY et al., 2009), especially in countries with important wheat production such as UK, Germany, Denmark, France and the USA. Starting point for analysis of storage proteins was a fractionation based on different solubility where fractions were called albumin, globulin, gliadin and glutenin (OSBORNE, 1907). The terminology in the first half of the 20th century unfortunately is non-regular and up to date, there is some confusion with these terms, (see Introduction for a different classification system of wheat proteins). The so-called 'Osborne fractionation' was a great progress in these former times and it still applied today, *e.g.* for extraction of reference material (see Chapter 2) followed by HPLC analysis (WIESER et al., 1998; SCHALK et al., 2017) and to investigate the wheat grain proteome using one- or two-dimensional gel-electrophoresis (SKYLAS et al., 2000; HURKMAN and TANAKA, 2004). However, the Osborne fractionation has got several disadvantages: a considerable overlap between protein fractions can easily occur, many variations of the procedure lead to widely varying results in the literature, a part of the total protein remains as an extractable residue, see SHEWRY et al. (2009) for an in depth discussion. To overcome the problems of fractionation, especially for proteomic analyses, there have been attempts to develop methods that can extract as many grain proteins as possible in one solution only. One example is the extraction using trichloracetic acid in cold acetone, where the protein extracts are dissolved in urea containing solution and subsequently analysed in 2D-GE (DAMERVAL et al., 1986; MECHIN et al., 2007; ZÖRB et al., 2009). Using an extraction method with an SDS based solution, also followed by 2D-GE and mass spectrometry, DUPONT et al. (2011) were able to identify the majority of wheat storage proteins and to estimate their relative levels. Currently, proteomics approaches aim to advance LC-MS/MS methods. BROMILOW et al. (2016) applied a bottom-up LC-MS/MS technique on wheat grain protein and obtained a protein identification rate comparable to the work of DUPONT et al. (2011). Depending on individual questions researchers are pursuing, they have to decide which method is most appropriate. While analysis of high abundant proteins like gliadins and glutenins can be done with one of the methods discussed above, it will be necessary to use more specific protein extraction techniques when membrane and low abundant proteins, such as regulating proteins, are focussed. These might be interesting to analyse in terms of finding evidence for differences in wheat genotypes and their behaviour in different environments for instance in climate change or different fertilization strategies (JUHÁSZ et al., 2016; DIER et al., 2018).

Challenges for proteomic approaches

Besides establishing and improving methods for protein extraction there are at least two further interconnected challenges for proteomic approaches, *i.e.* to improve identification rates through better database coverage of expressed genes (cDNAs) and quantification of

peptides/proteins when using LC-MS/MS techniques. Protein mass based identification rates specifically of gluten proteins are hampered by very high levels of similarity combined with large numbers of these similar protein types. For instance, based on in depth sequence analysis, there are 47 genes for α-gliadins with 26 encoding intact full-length proteins in hexaploid wheat, cv. Chinese Spring (Huo et al., 2018), and these proteins feature very few amino acids difference. For this reason, both works on proteomics mentioned above (Dupont et al., 2011; Bromilow et al., 2016) stress the importance of carefully curated and comprehensive cDNA databases for wheat storage proteins. Since it has been found that protein sequences are cultivar specific, Dupont et al. (2011) recommend that research on storage proteins of a particular wheat cultivar should comprise a thorough cDNA sequencing project. This suggestion appears feasible because cDNA sequencing has become straight forward and inexpensive. Based on available gluten protein sequences Bromilow et al. (2017), constructed a manually curated database (GluPro) containing 630 discrete protein sequences, which will greatly assist in identification of gluten proteins. The recent completion of the wheat genome sequencing project in combination with RNA sequencing (International Wheat Genome Sequencing Consortium, 2018) will be a further asset for protein identification in wheat proteomics projects.

In bottom-up LC-MS/MS based proteomics methods it is generally required to select unique marker peptides if quantification is required. These marker peptides can then be used for quantification in high resolution mass spectrometers after LC separation and often electrospray ionisation (Juhász et al., 2016). Obviously, the pool of available unique peptides becomes larger with increasing knowledge about protein sequences from diverse wheat cultivars, which refers back to the discussion above and underlines the significance of databases. LC-MS/MS based quantification of a smaller number of targeted proteins or peptides might be technically easier to achieve, when standard proteins or labelled peptides for calibration are available (Scherf and Poms, 2016; Schalk et al., 2017). In summary, a fast progress in availability of wheat protein sequences will promote proteomic approaches, which in turn will help to enhance knowledge about wheat grain proteins.

Conclusions

It has been argued that wheat growing needs to use less nitrogen based fertilizers in order to reduce nitrogen pollution of drinking water and to safe resources. A further challenge to wheat growing and quality will arise because of climate change. Regarding human health, it is important to consider that a rising number of individuals are suffering from wheat related disorders connected to various wheat proteins. Long established grain protein analytical procedures together with modern proteomics methods are important for identification and quantification of proteins connected to both, human disease and changed wheat baking quality parameters. Detailed knowledge about grain proteins will facilitate wheat breeding directed at developing wheat cultivars better adapted to changed environmental conditions caused by climate change, reduced nitrogen input and increased production efficiency, as well as reduction of disease potential.

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Author contributions

Both authors conceptualised and wrote this review. The final version of the manuscript was approved by both authors.

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