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REVIEW

Chemistry of wheat gluten proteins: Qualitative composition

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Abstract

Background and Objectives: Wheat gluten proteins make up one of the most complex protein aggregates in nature. Their qualitative and quantitative composition is determined by genetic and environmental factors as well as technological processes.

Findings: Gluten proteins comprise ω5-, ω1,2-, α-, and γ-gliadins as well as high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight (LMW) GS. About 50% of gluten proteins are monomeric gliadins with MWs from 28,000 to 55,000, while about 15% are present as disulfide-linked oligomeric proteins with MWs between 70,000 and 700,000, called HMW-gliadins. The remaining 35% are disulfide-linked polymeric glutenins with MWs from 700,000 to more than 10 million. Intrachain disulfide bonds, present in all types except ω-gliadins, stabilize the three-dimensional structure, while interchain disulfide bonds, mainly linking HMW-GS and LMW-GS, generate oligomers and polymers.

Conclusions: In this review, we provide an updated and detailed insight into the chemistry of wheat gluten proteins with a focus on the qualitative composition.

Significance and Novelty: An enhanced understanding of gluten protein structure and how it is affected will be essential to select and breed more resilient wheat varieties with favorable processing properties to help ensure nutrition and food security worldwide.

KEYWORDS
amino acid sequences, bread making, disulfide bonds, gliadin, glutenin, polymer

1 | INTRODUCTION

Wheat is among the top three of the world's most important crops cultivated by mankind. Wheat-based products such as bread, other baked goods, pasta, and noodles have been staple foods for thousands of years and are essential pillars for food security worldwide. Today, wheat is grown on about 220 million hectares of arable land, particularly in temperate regions, and yields around 770 million tons of grains per year (FAOSTAT, 2022). Common wheat (*Triticum aestivum* L.), also known as bread wheat, is the most

widespread wheat species and represents around 95% of cultivated wheat. Most of the remaining 5% are durum wheat (*Triticum durum* L.), well-known as pasta wheat. The other wheat species, einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* (Schrank) Schübler), and spelt (*Triticum spelta* L.) only play a minor role in terms of utilization and are processed into specialty products. The success of wheat is largely due to the special chemical and physical properties of its gluten proteins that make up the essence of wheat uniqueness. They correspond to the storage proteins of wheat and represent around 70%–80% of total grain proteins (P. Shewry, 2019; Wieser et al., 2020).

Gluten proteins make up one of the most complex protein aggregates in nature. Sophisticated separation procedures and analytical methods now allow investigations of the structure of single proteins and their intra- and intermolecular linkages. Both genetic and environmental factors as well as technological processes determine the qualitative and quantitative composition of gluten proteins. In this review, we provide an updated and detailed insight into the chemistry of wheat gluten proteins with a focus on the qualitative composition. Different factors determining the quantitative composition of gluten are available in the associated review by Wieser et al. (2022).

2 | CLASSIFICATION OF WHEAT PROTEINS

Proteomic separation of wheat grain proteins by two-dimensional gel electrophoresis (2D-GE) resolved up to 1300 proteins (Skylas et al., 2000). Many of these are closely related due to protein polymorphism, the presence of multigene families, and posttranslational modifications. Corresponding to the number of sub-genomes, hexaploid common wheat and spelt (genome AABBDD, 42 chromosomes) contain the highest number of proteins, followed by tetraploid durum wheat and emmer (genome AABB, 28 chromosomes). Diploid einkorn (genome AA, 14 chromosomes) has the lowest number of proteins. For example, 476 protein spots were detected in a total protein extract from common wheat flour by 2D-GE. Of the 233 proteins subsequently identified by liquid chromatography with tandem mass spectrometry (LC-MS/MS), 122 were gluten proteins (Dupont et al., 2011). 2D-GE analysis of 10 durum wheat cultivars revealed 51 to 75 gluten proteins (de Angelis et al., 2008), whereas einkorn contained about 2030 gluten proteins (Alvarez et al., 2006).

2.1 | Protein fractions

Wheat grain proteins have been traditionally classified into four so-called Osborne fractions according to different solubility: albumins, globulins, gliadins, and glutenins (Osborne, 1907), along with residual proteins (Figure 1). Albumins are soluble in water and dilute salt solutions, while globulins are soluble in dilute salt solutions, but not in water. The albumin/globulin fraction mainly contains regulatory, metabolic, and protective proteins such as enzymes and enzyme inhibitors. Gliadins are soluble in aqueous alcohols, for example, 60% ethanol or 50% propanol, but insoluble in water and salt solutions. They can be subdivided into monomeric proteins and oligomeric proteins, so-called high-molecular-weight (HMW)-gliadins (Schmid et al., 2016). Native polymeric glutenins are insoluble in the three solvents mentioned, but they can be solubilized as monomeric glutenin subunits (GS) after reduction of disulfide (SS) bonds, in, for example, 50% propanol containing a reducing agent such as dithiothreitol. A small portion of proteins including membrane proteins and lipoproteins does not belong to any of the Osborne fractions. Together with starch, they remain in the insoluble residue, after the Osborne fractions have been extracted (Wieser, 2007).

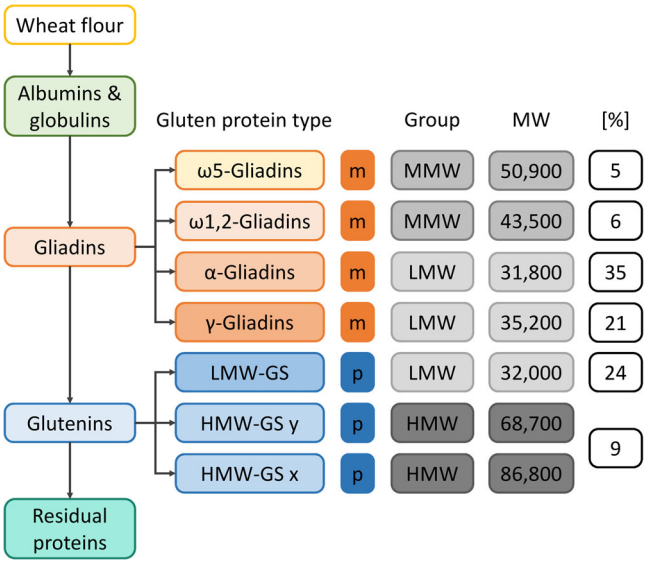

FIGURE 1 Classification of wheat proteins into different fractions and types. The percentages are typical values for each gluten protein type relative to the total amount of gluten determined by modified Osborne fractionation and reversed-phase high-performance liquid chromatography as reported by Lexhaller et al. (2017). GS, glutenin subunits; HMW, high molecular weight; LMW, low molecular weight; m, monomeric; MMW, medium molecular weight; MW, molecular weight; p, polymeric. [Color figure can be viewed at wileyonlinelibrary.com]

Gliadins and glutenins are the storage proteins of the grain, also known as gluten proteins. Exclusively located in the starchy endosperm, they represent around 70% to 80% of total grain protein. Their synthesis starts around 10 days after the flowering of the plants and ends around 20 days later. They are deposited in discrete protein bodies within the starchy endosperm cells. After coalescence of the protein bodies during grain maturation, gliadins and glutenins form a continuous gluten network, in which the starch granules are embedded (Tosi, 2012). Analysis of wheat pearling fractions demonstrated that there is a quantitative and qualitative protein gradient in the starchy endosperm, with HMW-GS mainly located in the central endosperm (He et al., 2013). The main biological function of gluten proteins is to supply the wheat seedling with nitrogen, amino acids, and energy during germination. From a processing point of view, gluten proteins are what make wheat unique in bread making and multiple other applications. When water is added to wheat flour, gliadins and glutenins develop a cohesive viscoelastic gluten network during mixing that retains the gas generated during dough fermentation and leads to the formation of a leavened, fluffy bread crumb (Delcour et al., 2012).

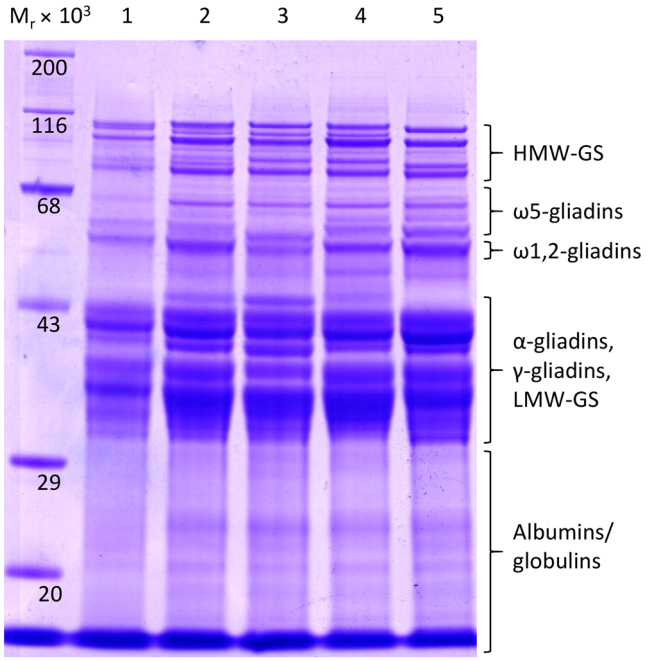

FIGURE 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of wheat flour proteins illustrating the molecular weight distribution of the proteins under reducing conditions. 1, variety Tuareg; 2, wheat flour blend of the varieties Soissons, Bezostaya-1, Glenlea, Hereward, and Mv Magvas; 3, variety Soissons; 4, variety Glenlea; 5, variety Hereward. GS, glutenin subunits; HMW, high molecular weight; LMW, low molecular weight. [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | Gluten protein types

Gluten proteins are classified into six different types according to similarities and differences in the amino acid sequences, respectively (Figure 1). Gliadins are subdivided into α-, γ-, ω1,2-, and ω5-gliadins according to different mobility upon acid PAGE, whereas glutenins consist of HMW-GS and low-molecular-weight (LMW)-GS based on the molecular weights (MWs) observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 2). α-Gliadins, γ-gliadins, and LMW-GS belong to the LMW-group of gluten protein types with a length of 260 to 330 amino acid residues, which corresponds to MWs from 28,000 to 35,000. The medium-molecular-weight (MMW) group comprises ω1,2-gliadins with about 370 amino acid residues and MWs of 39,000 to 44,000 as well as ω5-gliadins with about 420 amino acid residues and MWs of 49,000 to 55,000. A small portion of ω-type gliadins, called glutenin-bound gliadins (ωb-gliadins) is modified by substitution of a single amino acid residue for a cysteine residue that is linked to other gluten proteins by an SS bond. This is the reason why ωb-gliadins appear in the glutenin fraction and can be solubilized only after reduction of SS bonds. HMW-GS belong to the HMW group and are further subdivided into x-type and y-type with about 800 and 600 amino acid residues equivalent to MWs of 83,000 to 88,000 and 67,000 to 74,000, respectively (Juhász & Gianibelli, 2006; Metakovsky et al., 2006; P. R. Shewry et al., 2006).

Gliadin types are mainly encoded on the short arms of chromosomes 1A, 1B, 1D, 6A, and 6B, but some γ-gliadins are also on the long arms of chromosomes 3B and 3D. LMW-GS genes are located on the short arms of chromosomes 1A, 1B, and 1D, whereas HMW-GS genes are on the long arms of the same group 1 chromosome (Juhász et al., 2018). Due to their particular importance for dough and bread quality, single HMW-GS are named after their gene loci (1A, 1B, or 1D chromosome), their size (x-type or y-type), and their mobility upon SDS-PAGE (numbered 1–12) in many cases. HMW-GS 1Dx5, for example, is encoded on chromosome 1D, belongs to the x-type, and appears at position 5 on SDS-PAGE. The criterion of SDS-PAGE mobility always needs to be verified for each specific gel and buffer combination, because mobility can be altered. The elution order of HMW-GS in a Bis-Tris SDS-PAGE system with a neutral running buffer was $5 > 2 \approx 3 > 1 > 6 \approx 2^* > 7 > 8 > 9 > 12 > 10$ (Lagrain et al., 2012), which is different to the Tris-glycine system originally reported by Laemmli (1970). In 6% Tris-glycine

gels with an alkaline glycine buffer the HMW-GS appeared in the following order: 1 > 2 ≈ 2* ≈ 5 > 3 > 6 > 7 > 14 ≈ 17 > 15 ≈ 18 > 8 > 10 ≈ 9 > 12 (Geisslitz et al., 2020).

Gluten protein types can be isolated from wheat flour using modified Osborne fractionation to extract the gliadin and glutenin fractions, followed by reversed-phase high-performance liquid chromatography to separate and enrich the different types according to hydrophobicity (Figure 3) (Schalk et al., 2017). Discovery proteomics experiments of the separated HMW-GS, LMW-GS, α-, γ-, ω1,2-, and ω5-gliadin isolates from common wheat flours showed that this two-step strategy was suitable to obtain the specific types, with gluten proteins making up 58% to 87% of total proteins (Lexhaller et al., 2019). Depending on the type, other protein groups, such as 2% to 26% of amylase/trypsin-inhibitors, <2% to 6% of avenin-like proteins, and <2% to 5% globulins were also present in the respective isolates. A closer look at the gluten proteins revealed that co-mingling of different gluten protein types occurred, for example, with substantial relative amounts of LMW-GS in both the α- and γ-gliadin isolates. These results highlighted the intricate complexity of gluten proteins and their ability to bind other proteins and other flour constituents.

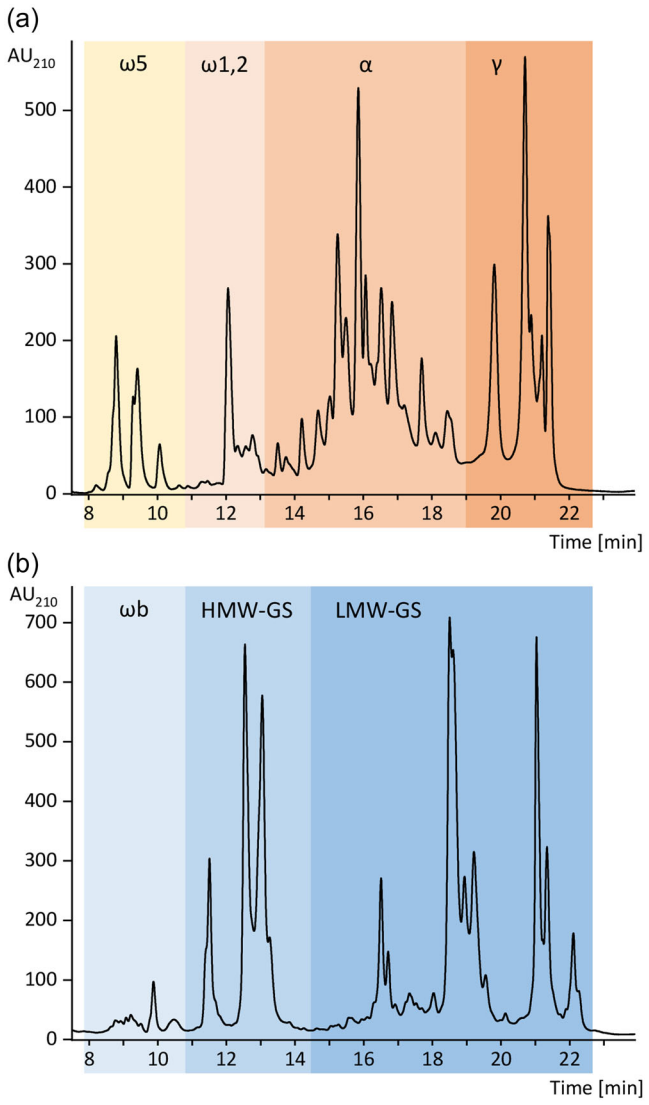

FIGURE 3 RP-HPLC chromatograms of the gliadin (a) and glutenin (b) fractions extracted from the German common wheat variety Akteur. α, γ, ω, gliadin types; GS, glutenin subunits; HMW, high molecular weight; LMW, low molecular weight; RP-HPLC, reversed-phase high-performance liquid chromatography. [Color figure can be viewed at wileyonlinelibrary.com]

3 | PRIMARY STRUCTURE OF GLUTEN PROTEINS

3.1 | Amino acid composition

The amino acid composition of gluten proteins is generally characterized by high proportions of glutamine (32% to 53%) and proline (11% to 29%) (Supporting Information: Figure S1). Both amino acids are important for the biological function of gluten as storage proteins. In contrast to most other amino acids, glutamine contains two nitrogen atoms that supply sufficient nitrogen to the germinating grain. Proline with its secondary amino group causes kinks within the secondary protein structure and, therefore, allows a dense packing of the protein strands in the starchy endosperm (Tosi, 2012). Moreover, peptide bonds that contain a proline residue are resistant to most peptidases and prevent the storage proteins from extensive degradation by external enzymatic attacks (Simpson, 2001).

Next to glutamine and proline, hydrophobic amino acid residues such as valine, leucine, isoleucine, and phenylalanine show relatively high proportions (as a sum up to 28%). These amino acids are responsible for the hydrophobicity of gluten proteins that prevent the leakage of proteins out of the grain during germination. Although cysteine belongs to the minor amino acids (≈2%), it is very important for the formation of intra- and interchain SS bonds and the resulting functionality of gluten (Köhler et al., 1997). Amino acid residues with positive (lysine and arginine) or negative (aspartic acid and glutamic acid) charges are rare (1.0% to 3.8% and 1.4% to 3.3%, respectively). Further common features of gluten proteins are their low content of the essential amino acids lysine (0.3% to 1.1%), methionine (0.0% to 1.8%), and tryptophan (0.0% to 1.0%). Altogether, the amino acid composition of gluten proteins is well-suited to fulfill their function as storage proteins: a source of nitrogen and amino acids for the germinating grain, tightly packed in the starchy endosperm, resistant to degradation by external enzymes, and insoluble in water.

HMW-GS are characterized by a high content of glutamine (32% to 36%), proline (11% to 13%), and

additionally glycine (18% to 20%), which together account for approximately 65% of their total composition. Moreover, HMW-GS have more tyrosine (5.2% to 5.7%) and less phenylalanine (0.3%) compared to the other gluten protein types. Differences between x- and y-type HMW-GS are small and mainly present in the content of arginine (1.2% vs. 2.7%) and histidine (0.5% vs. 2.4%). ω5- and ω1,2-gliadins have extremely unbalanced amino acid compositions marked by the highest content of glutamine, proline, and phenylalanine among gluten proteins, accounting for around 80% of the total composition. ω5-Gliadins show a higher glutamine content (53% vs. 42%), a lower proline content (20% vs. 29%), and a similar content of phenylalanine (9% vs. 8%) compared to ω1,2-gliadins. Isoleucine (4.3% vs.1.6%), leucine (3.1% vs. 4.0%), and serine (2.9% vs. 5.9%) present further significant differences between ω5- and ω1,2-gliadins. All other amino acids are below 2.5% or even missing (cysteine and methionine). The amino compositions of α-gliadins, γ-gliadins, and LMW-GS are related and have a relatively high content of hydrophobic amino acids (phenylalanine, tyrosine, leucine, and valine) apart from glutamine (32% to 36%) and proline (13% to 18%). The higher serine content of LMW-GS (8.9%), the higher asparagine content of α-gliadins (2.6%), and the low tyrosine content of γ-gliadins (0.3%) compared to the other proteins of the LMW group are characteristic features, respectively.

3.2 | Amino acid sequences

Numerous modifications of the ancestral genes during wheat evolution resulted in substitutions, insertions, and deletions of single or multiple amino acid residues of gluten proteins that can be used to trace the origins of modern-day wheat (Pont et al., 2019). Nevertheless, gluten proteins mostly show 75% to 97% of homology within each protein type. The proteome of *Triticum aestivum* currently contains 143,219 amino acid sequences in total, but only 379 (less than 0.3%) manually annotated and reviewed sequences (UniProtKB; August 02, 2021).

A schematic overview of the different sequence domains of gluten protein types was provided by Wieser et al. (2020). The amino acid sequences of HMW-GS can be divided into three structural domains (Figure 4): a nonrepetitive N-terminal domain A comprising 80 to 105 residues, a repetitive central domain B of 480 to 700 residues, and a nonrepetitive domain C of 40 residues (P. R. Shewry et al., 1992). Domains A and C are characterized by relatively balanced amino acid compositions, including most or all cysteine residues and charged amino acids (glutamic acid and arginine). Domain B contains repetitive hexapeptides such as PGQGQQ as a backbone with distinct differences between x- and y-types (Supporting Information: Figure S2). These repeats are frequently modified by single amino acid residues and separated by nonapeptides such as GYYPTSPQQ or GYYPTSLQQ and the tripeptide GQQ (x-type), or the hexapeptide HYPASQ (y-type). In comparison to the x-type, y-type HMW-GS shows a longer domain A, including two neighboring cysteine residues, and a shorter more frequently modified domain B (about 50 repeats vs. 70 repeats). Geisslitz et al. (2020) used a proteomics workflow with high-resolution LC-MS/MS to study differences in the amino acid sequences of Bx6 and Bx7 HMW-GS from common wheat, spelt, and emmer. One peptide (WQPGQGQQGY) was specific for Bx7 from common wheat and it allowed a differentiation to Bx6 from common wheat as well as Bx6 and Bx7 from emmer and spelt.

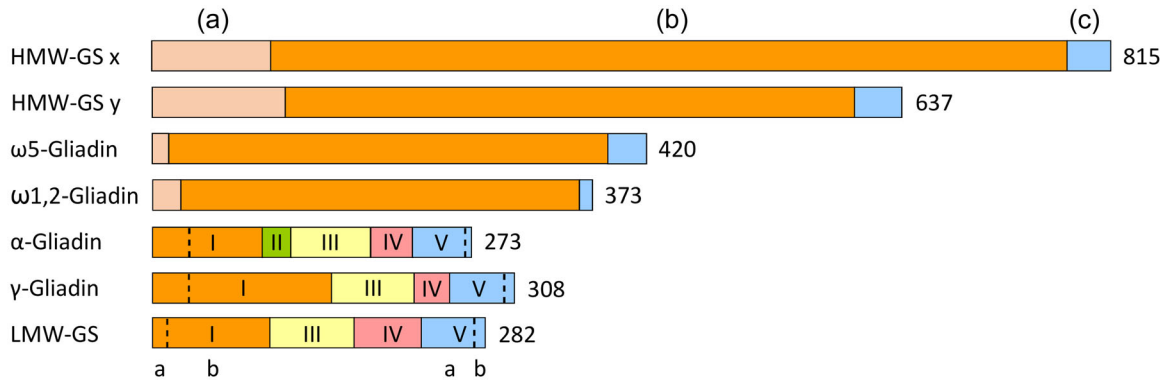

FIGURE 4 Schematic representation of different domains (a–c) and segments (I–V) of gluten protein types adapted from Wieser et al. (2020). The following amino acid sequences were used without signal peptide (UniProtKB identifier in parentheses): HMW-GS x (Q6R2V1), HMW-GS y (Q52JL3), ω5-gliadin (Q402I5), ω1,2-gliadin (Q6DLC7), α-gliadin (Q9M4M5), γ-gliadin (Q94G91), and LMW-GS (Q52NZ4). GS, glutenin subunit; HMW, high molecular weight; LMW, low molecular weight. The numbers at the C-terminal side of the proteins show the numbers of amino acids per protein. [Color figure can be viewed at wileyonlinelibrary.com]

ω5- and ω1,2-gliadins are composed of sequences that almost entirely consist of repetitive units (domain B), with only short nonrepetitive N-terminal and C-terminal domains A and C (Figure 4). Domains A and C have a balanced amino acid composition, while the central domain B mainly consists of glutamine, proline, and phenylalanine. Typical repetitive units of ω5-gliadins are relatively short (e.g., QQQFP) and repeated up to 50 times, while ω1,2-gliadins differ due to a decreased number of up to 30 repetitive units (e.g. QQPQQPFP) (Supporting Information: Figure S3).

The amino acid sequences of α-gliadins, γ-gliadins, and LMW-GS are partly homologous and can be divided into an N-terminal domain containing segments I and II and a C-terminal domain containing segments III–V (Figure 4) (Kasarda et al., 1984). The N-terminal domains (40% to 50% of the total sequence) start with short nonrepetitive sequences (segments Ia), which are characteristic of each type. Segment Ib exclusively consists of unique repetitive units such as QPQPFP and PQQPYP repeated up to five times (α-gliadins), QQPQQPFP repeated up to 16 times (γ- gliadins), and QQQPPFS repeated up to 13 times (LMW-GS) (Supporting Information: Figure S4). Segment II entirely consists of glutamine (up to 18 residues) and occurs only in α-gliadins. The C-terminal domain (segments III–V) presents sequences that are nonrepetitive, have less glutamine and proline than the N-terminal domain, and possess a more balanced amino acid composition. Segment III presents the highest degree of homology in both length and composition, while segment IV is partly homologous and partly unique for each type. Segment V includes homologous sequences (Va) and short unique sequences (Vb).

4 | THREE-DIMENSIONAL STRUCTURE OF GLUTEN PROTEINS

Three-dimensional structural elements (secondary and tertiary structures) of gluten proteins have been predicted from amino acid sequences or determined directly by different spectroscopic measurements of the whole polymer, gluten protein types, single proteins, or synthetic peptides. Examples are shown in Figure 5. The models suggest a stretched structure of HMW-GS, in particular of the x-type, while gliadins and LMW-GS have a more compact shape. Regarding HMW-GS, domains A and C show globular steric structures with α-helices and β-sheets (P. R. Shewry et al., 1992). For example, the N-terminal domain of HMW-GS 1Dx5 has been predicted to form a continuous strand of an α-helix corresponding to residues 5–32, while that of 1Bx7 forms several shorter regions of α-helices (Köhler et al., 1997). An intrachain SS bond stabilizes the structure of domain A. Domain B is characterized by a stretched conformation with repeated β-turns. These occur in regions, where the protein backbone abruptly changes direction. Such regions in domain B include four amino acid residues, consisting of proline, glutamine, and glycine (QPGQ), and form repeated β-turns, which create a loose spiral structure (β-spiral) that is thought to confer elastic properties to the proteins (Kasarda et al., 1994; Tatham et al., 1985).

ω-Gliadins almost entirely contain proline-rich repetitive units, which cause a stretched conformation of the molecule characterized by β-turns and random coils in between (Purcell et al., 1988; Tatham & Shewry, 1985). There are no detectable α-helices or β-sheets or SS bonds,

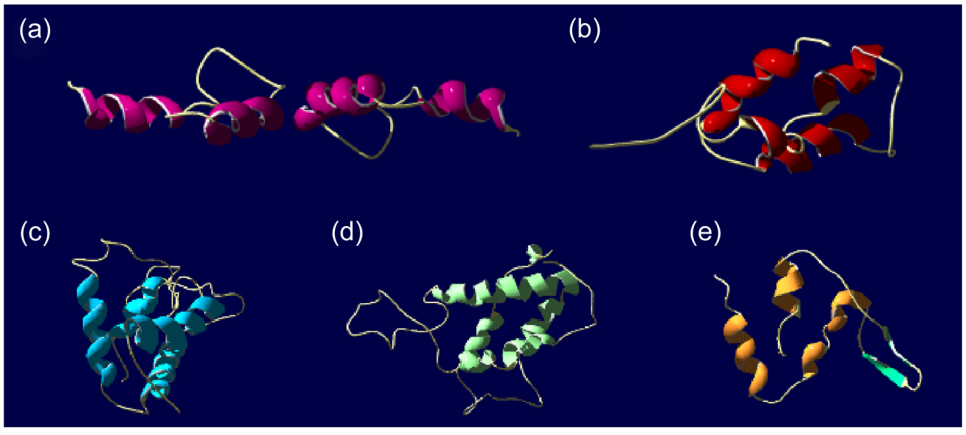

FIGURE 5 Models representing the three-dimensional structure of high-molecular-weight glutenin subunits (HMW-GS) x-type (a) and y-type (b), α-gliadins (c), γ-gliadins (d), and low-molecular-weight glutenin subunits (LMW-GS). Proteins were modeled with SWISS-MODEL (Waterhouse et al., 2018). The minor constituents ω5- and ω1,2-gliadins are missing, because no sufficient quality homology model template was available. [Color figure can be viewed at wileyonlinelibrary.com]

whereas hydrophobic interactions are important. According to partially homologous amino acid sequences, α-gliadins and γ-gliadins have related secondary and tertiary structures. Segment I is characterized by a β-turn conformation similar to that of ω-gliadins (Tatham & Shewry, 1985). Segment II of α-gliadins, that is, the polyglutamine sequence, is characterized by an α-helical conformation. The C-terminal domains (segments III–V) contain considerable proportions of α-helices and β-sheet structures with small regions of β-turn and random coil. The tertiary structure is stabilized by three (α- gliadins) and four (γ-gliadins) intrachain SS bonds, respectively. Information on LMW-GS steric structures is rare. Due to corresponding sequence segments, they are related to those of α- and γ-gliadins. The repetitive segment I is rich in β-turns, while segments III–V have globular structures with elements of α-helix, β-turn, β-sheet, and random coil (Tatham et al., 1987). Three intrachain SS bonds stabilize the steric structure of the C-terminal domain.

5 | MOLECULAR WEIGHT DISTRIBUTION

Gluten proteins are characterized not only by their high number of individual components and their exceptional amino acid sequences but also by their broad MW distribution in the native state (Koehler & Wieser, 2013; Southan & MacRitchie, 1999). Monomeric gliadins (α-, γ-, and ω-gliadins), corresponding to ≈50% of total gluten proteins, have MWs of approximately 28,000 to 55,000. They are alcohol-soluble and are either devoid of SS bonds (ω-gliadins) or have intrachain SS bonds (α-gliadins and γ-gliadins). In contrast, there are apparently no glutenin monomers.

Apart from monomers, the gliadin fraction contains alcohol-soluble oligomers with MWs ranging from 70,000 to about 700,000. Thus, around 2 to 20 protein units are linked by interchain SS bonds. This subfraction has been called HMW-gliadin, aggregated gliadin, or ethanol-soluble glutenin and accounts for ≈15% of gluten proteins (Huebner & Bietz, 1993; Schmid et al., 2016; P. R. Shewry et al., 1983; Southan & MacRitchie, 1999). HMW-gliadin is chemically less well characterized in comparison with total gliadin and glutenin fractions. After reduction of SS bonds, the following proportions of gluten protein types were quantitated in HMW-gliadin isolated from the German common wheat variety Akteur: 48% LMW-GS, 18% γ-gliadins, 13% α-gliadins, 9% ω1,2-gliadins, 8% HMW-GS, and 4% ω5-gliadins (Schmid et al., 2016) It has been shown that modified gliadins with an odd number of cysteine residues act as terminators and stop the polymerization of HMW-GS and LMW-GS resulting in alcohol-soluble oligomers. Free cysteine and glutathione naturally occurring in flour have been identified as further terminators (Schmid et al., 2017).

The alcohol-insoluble glutenin fraction (≈35% of total gluten proteins) contains polymers, mainly consisting of SS-linked LMW-GS and HMW-GS with MWs ranging from around 700,000 to more than 10 million. The largest polymers termed "glutenin macropolymer" (GMP) or "unextractable polymeric protein" have MWs well in the multimillion range and may belong to the largest protein aggregates in nature (Don et al., 2003; Wrigley, 1996). GMP may be defined as the large-size portion of the glutenin fraction that is insoluble in an aqueous SDS solution. GMP can be prepared from flour after extraction of other components with a diluted SDS solution (e.g., 1.5%) followed by centrifugation and decanting of the supernatant (Moonen et al., 1982). The GMP-containing gel layer, formed on the surface of the starch pellet, is scraped off, washed with ethanol and water, and lyophilized. The amount of GMP in common wheat flour is in the range of 20–40 mg/g of flour (≈3% of gluten proteins) and is correlated with dough strength and bread volume (Thanhaeuser et al., 2014; Weegels et al., 1996). Recent quantitative data for two common wheat varieties (Akteur/Winnetou) revealed that GMP consists of 54%/50% LMW-GS, 31%/39% HMW-GS, and around 10% gliadins. The ratios of LMW-GS to HMW-GS (1.7/1.3) were lower than the ratios found in the total glutenin fraction (1.9/1.6), indicating enrichment of HMW-GS in GMP compared to total glutenins (Mueller et al., 2016).

The MW distribution of native glutenins has been recognized as one of the main determinants of dough properties and baking performance (P. R. Shewry et al., 2003). A shift in the distribution curve towards higher MWs, corresponding to higher GMP content, results in a stronger dough with greater resistance to mixing and increased stability. The greater the average length of the polymers, the more they can overlap, interacting to form a continuous matrix surrounding the starch granules in the dough. Three main factors appear to govern the MW distribution of glutenins, (a) the HMW-GS/LMW-GS ratio, (b) the allelic variation at Glu-1 loci (e.g., the presence of HMW-GS 1Dx5 + 1Dy10 vs. 1Dx2 + 1Dy12) and (c) the proportions of chain terminators (Lafiandra et al., 1999; MacRitchie, 1999).

6 | DISULFIDE STRUCTURE

SS bonds play a key role in determining the structure of gluten proteins. They are formed between thiol (SH-) groups of cysteine residues either within one protein

(intrachain) or between more than one protein (interchain). Intrachain bonds stabilize the tertiary structure of the protein, whereas interchain bonds induce the formation of protein aggregates. Thus, cysteine is decisive for the structure of gluten proteins, although it belongs to the minor amino acids (≈2 mol%). SS-formation via SH-oxidation of cysteine residues mediated by protein disulfide isomerase starts shortly after protein synthesis in the lumen of the endoplasmic reticulum of endosperm cells as an integral part of protein folding (Osipova et al., 2012). It is likely that intrachain bonds form more rapidly than interchain bonds. The oxidation process continues until most of the cysteine residues are linked by SS bonds. The importance of SS bonds for dough quality can be demonstrated by adding reducing agents that result in dough weakening and thiol blocking or oxidizing agents that lead to dough strengthening (Lagrain et al., 2007).

ω-Gliadins are predominantly devoid of cysteine and occur as monomers, apart from ωb-gliadins. Most α- and γ-gliadins contain six and eight cysteine residues, respectively, and form three or four homologous intrachain SS bonds either within sequence segment III or between segments III and V (Figure 6). They are responsible for the compact three-dimensional structure of the α- and γ-gliadin molecules. Due to point mutations, some ω-, α-, and γ-gliadins ("modified gliadins") contain an odd number of cysteine residues and this leaves one SH group available for crosslinking to other modified gliadins or to glutenins by interchain SS bonds (Lutz et al., 2012).

LMW-GS contain eight cysteine residues, six of which form three intrachain SS bonds homologous to those of α- and γ-gliadins. Two cysteine residues, located in segments I and IV, are unique to LMW-GS and are not known to build an intrachain SS bond, probably because of steric hindrance. Instead, they are involved in interchain SS bonds with cysteine residues of modified gliadins, LMW-GS, and HMW-GS.

Both x- and y-type HMW-GS clearly differ from LMW-GS in number and position of cysteine residues and thus in SS bond formation (Figure 6). x-Type subunits, except subunit 1Dx5, have three cysteine residues in domain A and one in domain C. Two residues of domain A form an intrachain SS bond and the other residues form interchain SS bonds with other HMW-GS molecules (so-called head-to-tail linkages). Subunit 1Dx5 has an additional cysteine residue in domain A and might form another interchain SS bond. y-Type subunits have five cysteine residues in domain A and one each in domains B and C. Interchain links were identified for adjacent cysteine residues of domain A, which are connected in parallel with corresponding residues of another y-type HMW-GS. An additional cysteine residue is present in domain B, which is linked to the cysteine residue located in segment IV of LMW-GS. In summary, HMW- and LMW-GS fulfill the requirement that allows polymerization with at least two cysteine residues per molecule available for interchain SS bond formation.

The most recent glutenin model suggests a backbone consisting of HMW-GS linked by head-to-tail SS bonds (Figure 7) (Köhler et al., 1993, Wieser et al., 2014). LMW-GS align as linear polymers via cysteine residues of

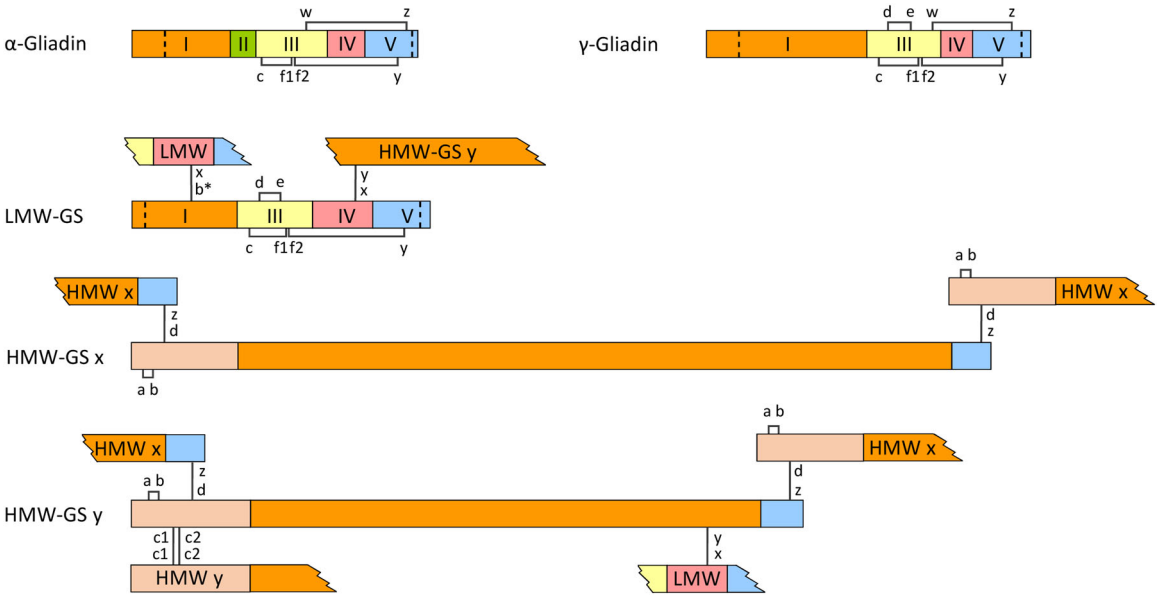

FIGURE 6 Representation of the disulfide structure of wheat gluten proteins. Nomenclature according to Köhler et al. (1993). GS, glutenin subunit; HMW, high molecular weight; LMW, low molecular weight. [Color figure can be viewed at wileyonlinelibrary.com]

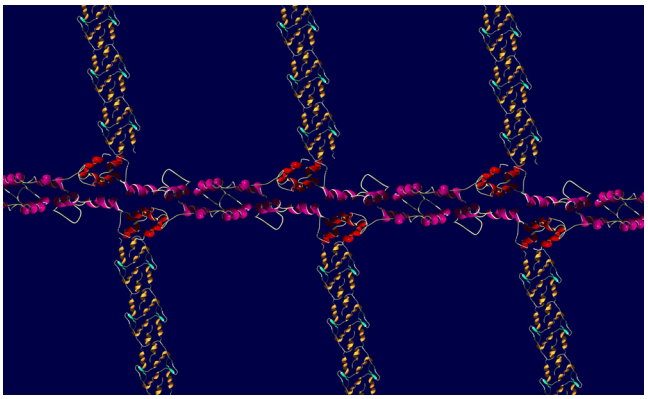

FIGURE 7 Model of the three-dimensional glutenin network made up of x- and y-type HMW-GS and LMW-GS modified from Wieser et al. (2014). Disulfide-linked HMW-GS are displayed horizontally, and LMW-GS are attached to this backbone via intermolecular disulfide bonds. HMW-GS, high-molecular-weight glutenin subunit; LMW-GS, low-molecular-weight glutenin subunit. [Color figure can be viewed at wileyonlinelibrary.com]

segments I and IV and they are linked to domain B of y-type HMW-GS. The SS structure of native gluten proteins, described here, undergoes significant changes during the dough-making and baking process (Lagrain et al., 2007). For example, most monomers containing intrachain SS (α- and γ-gliadins) are bound to the glutenin polymers via interchain SS bonds upon heating. Disulfide interchange reactions have been shown to be involved in the heat-induced effects, next to non-SS crosslinks, for example, isopeptide crosslinks, lanthionine, and lysinoalanine crosslinks (Rombouts et al., 2011). Similarly, treatment of gluten proteins with high hydrostatic pressure provokes a shift of α- and γ-gliadins into the glutenin fraction, indicating the cleavage of intrachain SS bonds and their rearrangement into interchain SS bonds (Kieffer et al., 2007).

Polymerization of HMW- and LMW-GS is stopped by so-called terminators (Kasarda, 1989). These include free thiols such as glutathione or cysteine as well as proteins with an odd number of cysteine residues. To identify possible terminators, HMW-gliadin was partially hydrolyzed with thermolysin and SS bonds were identified in the resulting peptides. Apart from 26 peptides, that contained SS bonds with known gliadin and glutenin crosslinks, 15 peptides with SS bonds unique to HMW-gliadin were detected. They include bonds between modified gliadins and glutenins such as α-gliadin/HMW-GS, γ-gliadin/HMW-GS, and ω-gliadin/LMW-GS as well as bonds between glutathione and HMW-GS (Schmid et al., 2017). In conclusion, glutenin polymerization occurring after synthesis is partially interrupted by terminators. The result is a complex mixture of SS links, involving between 2 and 20 units present in HMW-gliadin and up to more than 300 units present in GMP.

7 | GLUTEN CHEMISTRY AND FUNCTIONALITY

The characteristic molecular structures of gluten proteins are the basis for the unique physical properties of dough and the bread-making quality of wheat. When wheat flour is mixed with water, hydrated gluten proteins enable the development of a dough characterized by a balanced interrelation of cohesivity, viscosity, extensibility, and elasticity. The gluten network mediates the gas-holding properties of dough during fermentation and baking. When the fully developed dough is washed with water to remove starch granules and soluble constituents, a rubber-like, water-insoluble gluten mass remains. This wet gluten mass is a concentrate of all rheological dough features and can be stored as so-called vital gluten after drying. Vital gluten has many applications not only in baking to standardize dough and bread quality but also in other food and nonfood products (Day et al., 2006).

The insolubility of gluten proteins in water or salt solution is an essential prerequisite for the formation of a gluten network during dough mixing. Gluten isolated from wheat dough binds about twice its own weight of water due to the high water-binding capacity of glutamine residues (Schopf et al., 2021), but the proteins still remain insoluble. The nonrepetitive sequences that contain relatively high proportions of hydrophobic amino acid residues (phenylalanine, leucine, isoleucine, and valine) and less glutamine apparently prevent protein solubilization.

The presence of both hydrophilic and hydrophobic sequence segments confers emulsifying properties to gluten proteins, especially, to partially hydrolyzed gluten. Acid treatment of gluten, for example, increases the hydrophilic character of gluten proteins via partial deamidation of glutamine to glutamic acid residues and enhances the foaming and emulsifying properties. Alternatively, enzymatic hydrolysis, using, for example, alcalase or trypsin, produced peptides with emulsifying properties (Joye & McClements, 2014). These so-called hydrolyzed wheat proteins are frequently used as additives for food (e.g., soup and ice cream) and cosmetics (e.g., soap and shampoo) and show considerable differences regarding MW distribution, solubility, and hydrophilicity/hydrophobicity (Gabler & Scherf, 2020).

Apart from insolubility, the cohesivity of gluten proteins is important for gluten network formation and stability. Cohesivity results from strong binding forces between proteins. The gluten structure determined by

covalent SS bonds is superimposed by noncovalent hydrogen bonds, ionic bonds, and hydrophobic bonds, which are important for gluten aggregation. Glutamine residues are not only responsible for water binding, but also for interchain hydrogen bonds. The dough weakening effect of hydrogen-bond breaking agents such as urea provides experimental evidence for the presence of hydrogen bonds between gluten proteins. Vice versa, heavy water (D_2O) has a dough strengthening effect compared to water (H_2O) because deuterium bonds are considerably stronger than hydrogen bonds (Inda & Rha, 2007). Although charged amino acid residues are rare, ionic bonds are also important for interactions between gluten proteins. Increasing the number of ionic bonds by adding salts (e.g., NaCl) is known to strengthen dough due to the promotion of ordered interactions of glutenins with gliadins (Ukai et al., 2008). Dipolar ions such as amino acids or dicarboxylic acids also strengthen gluten isolated from the dough by acting as spacers and the formation of additional ionic bonds within the gluten network. Hydrophobic bonds involving the side chains of phenylalanine, leucine, isoleucine, and valine, can also significantly contribute to the stabilization of gluten protein structures and are particularly important when the dough is heated during baking.

Elasticity, as a particularly important physical property of wheat dough, has been ascribed to the glutenin fraction, and in particular, to domain B of HMW-GS. The regularly repeated sequence unit QPGQ generates β-turns that are linked by GQ and form a loose β-spiral similar to the repetitive sequences of elastin, an elastic protein of the connective tissue. Dough mixing has been shown to increase α-helix, β-turn, and β-sheet secondary structures, indicating that the proteins form a more ordered conformation (Seabourn et al., 2008).

A different study postulated that interchain hydrogen bonds between domains B of HMW-GS contribute to gluten elasticity. Results of infrared spectroscopy suggest that there is an equilibrium between regions forming interchain hydrogen bonds ("trains," β-sheets) and those without interchain bonds ("loops," β-turns). Stretching results in the conversion of β-turn to β-sheet in relation to the force applied. In the case of full elastic recoil, the original loop-train equilibrium should be reinstated, but experimental evidence suggests incomplete elastic recoil and buildup of β-sheets with each extension/relaxation cycle (Wellner et al., 2005).

Gluten proteins are fundamental contributors to the viscosity, extensibility, and elasticity of dough, but the specific functions of gliadins and glutenins are divergent. Hydrated gliadins have little elasticity and are less cohesive than glutenins and mainly determine dough viscosity and extensibility. In contrast, hydrated glutenins are both cohesive and elastic and are thus responsible for dough strength and elasticity. For ease of understanding, the gluten network represents a two-component glue, in which gliadins can be seen as a "plasticizer" or "solvent" for glutenins. An appropriate ratio of both fractions is, therefore, essential to impart the viscoelastic dough properties required to achieve a high-quality end product. A high ratio of gliadins to glutenins (e.g., 2.4 to 3.1) leads to less viscous and more extensible (soft) doughs, whereas a low ratio (e.g., 1.7 to 2.2) generates highly viscous and less extensible (strong) doughs (Kieffer et al., 1998; Wieser & Kieffer, 2001).

8 | FUTURE PERSPECTIVES

As outlined above, significant discoveries have been made by a number of dedicated research groups worldwide to elucidate the qualitative composition of wheat gluten proteins since the pioneering work of T. B. Osborne in the 1900s (Osborne, 1907). Despite this progress, there are still many unresolved questions related to the complex chemistry of wheat gluten proteins and their dynamic interactions. While SS bonds are an important determinant of gluten structure and functionality, not all positions and redox states of SS bonds have been identified on a molecular level. Even less knowledge is available on how different processing techniques from milling to dough-making and baking or extrusion influence SH/SS exchange reactions and other stabilizing forces, particularly hydrogen bonds. Most research activities are dedicated to common wheat, some on durum wheat, and only a few to other wheat species such as spelt, emmer, and einkorn. Renewed interest in utilizing these species for specialty products, will likely spark further investigations into the qualitative composition of gluten proteins from these species. With modern omics workflows being increasingly adopted in cereal chemistry, new approaches are now available to gain insights into wheat protein structure at unprecedented sensitivity, accuracy, and speed. Deep learning algorithms, like AlphaFold (Jumper et al., 2021), provide exciting new opportunities in predicting highly accurate three-dimensional protein structures, even in the absence of similar structural models, as is currently the case for wheat proteins. Taken together, these novel tools will enhance our understanding of gluten protein structure and how it is affected by different genetic and environmental factors as well as processing. This knowledge will be essential to adapt wheat production to elevated CO_2 levels in the atmosphere and to climate change with its associated increasingly extreme weather conditions. Selecting and breeding more resilient wheat varieties

with favorable processing properties will be a key element to help ensure nutrition and food security worldwide.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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