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Biochemical and Functional Properties of Wheat Gliadins: A Review

Sheweta Barak[a], Deepak Mudgil[a] & B. S. Khatkar[b]

[a] Department of Dairy & Food Technology, Mansinhbhai Institute of Dairy & Food Technology,
Mehsana-384002 Gujarat, India

[b] Department of Food Technology, Guru Jambheshwar University of Science and Technology,
Hisar 125001, India
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Biochemical and Functional Properties of Wheat Gliadins: A Review

SHEWETA BARAK,[1] DEEPAK MUDGIL,[1] and B. S. KHATKAR[2]

[1]Department of Dairy & Food Technology, Mansinhbhai Institute of Dairy & Food Technology, Mehsana-384002 Gujarat, India
[2]Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar 125001, India

Gliadins account for 40–50% of the total storage proteins of wheat and are classified into four subcategories, α-, β-, γ-, and ω-gliadins. They have also been classified as ω5-, ω1, 2-, α/β-, and γ -gliadins on the basis of their primary structure and molecular weight. Cysteine residues of gliadins mainly form intramolecular disulfide bonds, although α-gliadins with odd numbers of cysteine residues have also been reported. Gliadins are generally regarded to possess globular protein structure, though recent studies report that the α/β-gliadins have compact globular structures and γ- and ω-gliadins have extended rod-like structures. Newer techniques such as Mass Spectrometry with the development of matrix-assisted laser desorption/ionization (MALDI) in combination with time-of-flight mass spectrometry (TOFMS) have been employed to determine the molecular weight of purified ω- gliadins and to carry out the direct analysis of bread and durum wheat gliadins. Few gliadin alleles and components, such as Gli-B1b, Gli-B2c and Gli-A2b in bread wheat cultivars, γ -45 in pasta, γ -gliadins in cookies, lower gliadin content for chapatti and alteration in Gli 2 loci in tortillas have been reported to improve the product quality, respectively. Further studies are needed in order to elucidate the precise role of gliadin subgroups in dough strength and product quality.

Keywords   Wheat, gluten, gliadins, structure, rheology, product quality

INTRODUCTION

Wheat is undoubtedly the supereminent cereal crop in the world due to its widespread distribution and extensive utilization for baked food products. Hexaploid wheat (*Triticum aestivum*) accounts for 95% and tetraploid wheat (*Triticum durum*) for the remaining 5% of the worldwide production of wheat (Shewry, 2009). Wheat kernels comprise of 13–17% bran, 2–3% germ, and 81–84% endosperm. The major components of the endosperm are starch (60–75%), proteins (6–20%), moisture ($\sim$ 10%), and lipids (1.5–2%). The unusual properties of the wheat are ascribed to the presence of gluten forming storage proteins of the endosperm which are composed of two fractions—the alcohol soluble gliadins and the alcohol insoluble glutenins (Wieser, 2007; Singh et al., 2011). The rheological and functional properties of wheat gluten are found to be dependent upon the ratio of gliadins to glutenins (Khatkar et al., 1995; Pedersen and Jorgensen, 2007), molecular size distribution, structure of the glutenin polypeptides, high/low *M*r glutenin polypeptides ratio,

bond strength between gliadins and glutenins, and reduction or oxidation activity of glutenins. The cohesiveness and extensibility of the gluten is attributed to the monomeric gliadins while glutenins contribute to the elasticity and strength of gluten (Rodrigues et al., 2005). Thus, the ratio of glutenins to gliadins controls the dough strength and extensibility (Khatkar et al., 2002b; Wrigley et al., 2006). Moreover, the property of the wheat flour to form viscoelastic dough is lost upon the removal of gluten proteins from the flour. The influence of the gluten components on the functionality of dough is complex (Khatkar et al., 2002a) and requires extensive studies. Gliadins, one of the major gluten storage proteins of wheat, account for 40–50% of the total storage protein (Anderson et al., 1997) and confer viscous character to wheat gluten (Khatkar et al., 2002a; Wieser, 2007). Significant effects of gliadins on the gluten strength (Weegels et al., 1996; Metakovsky et al., 1997) have been reported. Past researches have suggested that gliadins may play an important role in determining the functional property of wheat flour. However, toxicity studies have also revealed that gliadins trigger the occurrence of celiac disease in genetically susceptible individuals with HLA-DQ2 or -DQ8 haplotypes. In this review paper, efforts have been made to explain the biochemical and

functional properties of gliadins and their relationship with the end product quality.

WHEAT KERNEL PROTEINS

Based on the classical fractionation process by Osborne (1907) wheat proteins have been separated into four groups—albumins (soluble in water), globulins (soluble in dilute salt solution), gliadins (soluble in 70% ethyl alcohol), and glutenins (soluble in dilute acids and bases). The baking performance of a wheat variety does not depend on the composition of the nongluten forming proteins—the albumins and globulins (MacRitchie, 1984) as their composition does not vary among different wheat varieties. The ability of the wheat flour to be processed into numerous baked products primarily depends on the quality and quantity of the gluten proteins (Weegels et al., 1996). Gluten proteins are also known as prolamins because of the presence of proline and glutamine amino acid (AA) residues in their structures. Extensive and intensive research is being carried out on gluten proteins to determine their properties and structure. Based on their solubility in aqueous alcohol solutions, prolamins of wheat have been divided into two classes, the gliadins and glutenins (Francin-Allami et al., 2011). Together, gliadins and glutenins represent 80–85% of the total proteins of wheat flour (Veraverbeke and Delcour, 2002) and impart the unique properties—extensibility and elasticity to the wheat dough. The glutenin polypeptides (30–140 kDa) are further fractionated into high molecular weight (MW) glutenin subunits (HMW-GS: 90–140 kDa) (Klindworth et al., 2005) and low MW glutenin subunits (LMW-GS: 30–75 kDa) by sodium dodecyl polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Wellner et al., 2005; Anjum et al., 2007). Disulphide bonds between the cysteine residues significantly stabilize the glutenin polymers (Lefebvre and Mahmoudi, 2007), functioning as interchain bonds between HMW glutenin subunits. Though regarded as the minor components, HMW-GS are the prime determinants of elasticity of the gluten which in turn have a profound effect on the loaf volume of bread (Tatham et al., 1985; Cornish et al., 2006). HMW-GS are encoded by genes on the long arm of chromosomes 1A, 1B, and 1D at the *Glu-A3, Glu-B3,* and *Glu-D3 loci* (Table 1). It has been found that the HMW-GS *1Dx5+ 1Dy10* encoded by *Glu-d1d* locus improves the bread quality by increasing the strength of dough. On the other hand, the HMW-GS *1Dx2+1Dy12* encoded by *Glu-D1a* gives poor loaf volume in bread.

Gliadins are the monomeric proteins linked by either no disulphide bonds (ω-gliadins) or by intrachain disulphide bonds (α-, β-, and γ-gliadins) (Singh and MacRitchie, 2001). Upon hydration, the gliadins behave as a viscous liquid (Singh and Khatkar, 2005; Song and Zheng, 2008) which imparts extensibility to the dough. AA sequence determination has revealed that α- and γ-gliadins are both related to the LMW-GS (Veraverbeke and Delcour, 2002). The LMW-GS has been subdivided into three groups—B, C, and D based on their electrophoretic mobility and isoelectric point. D'ovidio and Masci (2004) reported that out of these, C and D groups are mainly composed of α-, β-, γ-, and ω-gliadins mutated in the cysteine residues. LMW-GS can act as either chain terminators or chain extenders, depending on their ability to form disulfide bonds. Typical LMW-GS can act as chain extenders by forming two interchain disulfide bonds while gliadin like LMW-GS are expected to act as chain terminators of glutenin polymers by forming one interchain disulfide bond (Muccilli et al., 2010).

The composition of the gliadins in wheat differs from variety to variety. As a result of this extensive polymorphism, gliadins are used for the identification of the cultivar in hexaploid and tetraploid wheat. Differences in the gliadin to glutenin ratio among wheat cultivars are considered an important source of intercultivar variation in physical properties and end product quality. An inverse relationship exists between the gliadin/glutenin ratio and the elasticity of gluten. Doughs that are too elastic and inextensible give poorer bread making performance than doughs that have an appropriate balance of extensibility and elasticity (Khatkar et al., 2002a). Thus, the knowledge of composition of gliadins and glutenins, their properties, and rheological behavior has become increasingly important in the baking industry.

CLASSIFICATION OF GLIADINS

Gliadins have been described as heterogeneous mixtures of single chained polypeptides soluble in 70% aqueous alcohol. They account for about half the gluten proteins and have been divided into four groups- α- (fastest mobility), β-, γ-, and ω-gliadins (slowest mobility) based on their electrophoretic mobility in A-PAGE at low pH (Wieser, 2007; Banc et al., 2009). According to the analysis of primary structure and MWs, a new classification is given as ω5-, ω1, 2-, α/β-, and γ-gliadins (Wieser, 2007). Because of their structural homology as revealed by the AA sequencing, α- and β-gliadins have been grouped under one heading- the α-type gliadins (Zilic et al., 2011).

Table 1 Wheat gluten proteins and their genetic control (Pena et al., 2002)

Proteins	Chromosome arm			Locus		
		Glutenins				
High molecular weight glutenins	1AL	1BL	1DL	*Glu-A1*	*Glu-B1*	*Glu-D1*
Low molecular weight glutenins	1AS	1BS	1DS	*Glu-A3*	*Glu-B3*	*Glu-D3*
		Gliadins				
γ- and ω-gliadins	1AS	1BS	1DS	*Gli-A1*	*Gli-B1*	*Gli-D1*
α- and β-gliadins	6AS	6BS	6DS	*Gli-A2*	*Gli-B2*	*Gli-D2*

The MW range of gliadins is ≈30,000–75,000 Da (Fido et al., 1997). The monomeric gliadins confer their characteristic property—viscosity through noncovalent interactions such as hydrogen bonding, vander Waal's forces, electrostatic, and hydrophobic interactions. Furthermore, gliadins can also interact with the glutenin polymers via noncovalent hydrophobic interactions and the glutamine residues via hydrogen bonds (Wellner et al., 2003).

The genes coding the gliadin proteins (Table 1) are located on the short arms of group 1 and 6 chromosomes (Wrigley and Shepherd, 1973; Brown and Flavell, 1981). They are tightly linked genes located at three homologous loci of the group 1 chromosome—*Gli-A1*, *Gli-B1*, and *Gli-D1* and group six chromosomes—*Gli-A2*, *Gli-B2*, and *Gli-D2* loci. Most γ- and ω-gliadins are encoded by *Gli-1* genes and all the α-/β- and some of the γ-gliadins are encoded by the *Gli-2* genes (Ferranti et al., 2007). There is a tight linkage between the ω- and γ-gliadins encoded at the *Gli* 1 locus and LMW glutenin subunits. Wieser and Kieffer (2001) pointed out that α/β- and γ-gliadins are major components, accounting for 28–33% and 23–31%, respectively, but the ω-gliadins occur in much lower proportions, only 4–7% and 3–6% for the ω1,2- and ω5-gliadins, respectively. Qi and colleagues (2011) recently reported ten novel α-gliadin genes isolated from *Triticum aestivum* L.

Close associations have been found between the gliadin blocks and Zeleny sedimentation value which is regarded as an important criterion for predicting the bread making performance of the wheat variety (Sozinov and Poperelya, 1982). Branlard and Dardevet (1985) reported quality differences between gliadins located on chromosomes 1A, 1B, and 1D and on chromosomes 6A, 6B, and 6D. However, the correlation between the wheat quality parameters and gliadins located on chromosome group 1 has been attributed to the LMW subunits of the glutenins as the genes encoding both these proteins have been found to be tightly linked. Furthermore, deletions in the gliadin locus (*Gli 1*) increase the dough strength and percentage of polymeric proteins.

Due to high structure heterogeneity of gliadins, powerful separation techniques are needed for isolation and characterization of gliadins (Piergiovanni and Volpe, 2003). At present, various modes of electrophoresis and chromatography are widely used methods for gliadin separation and wheat varietal identification. Great numbers of biochemical, genetical, and technological investigations of gliadin proteins have been conducted by electrophoresis (Seva et al., 2005; Ojaghi and Akhundova, 2010; Brezhneva et al., 2010). Electrophoregrams of gliadin proteins provide information about identity of cultivars, protein polymorphisms, technological quality of grain, flour, and dough of wheat (Rashed et al., 2007; Wu et al., 2007). Gliadin markers are easier and more powerful tools for wheat genotype identification than DNA molecular markers, which normally exhibits lower level of intervarietal polymorphism. pH 3.1 is extensively used in all PAGE and starch gel electrophoresis methods.

Various gliadin components have also been separated by ion-exchange and gel-filtration column chromatography. Bietz (1983) first reported the use of High Performance Liquid Chromatography (HPLC) to characterize wheat proteins. Another powerful technique, Capillary Zone Electrophoresis (CZE) has shown much potential for protein analysis and varietal identification (Bean and Lookhart, 2000; Siriamornpun et al., 2001). Capillary electrophoresis is increasingly being recognized as an important separation technique because of its speed, efficiency, reproducibility, ultra-small samples volume, and low consumption of solvents. It overcomes some of the disadvantages of gel electrophoretic methods. CZE gives better resolution and shorter analysis times than either A-PAGE or HPLC (Rodriguez-Nogales et al., 2006). Recently, mass spectrometry (MS), with the development of "soft" desorption/ionization methods such as electrospray ionization and matrix-assisted laser desorption/ionization (MALDI), has become important as an alternative and powerful technique in the genomic and proteomic fields. Particularly, MALDI in combination with time-of-flight MS (TOFMS) has been used to determine the molecular masses of purified wheat α-gliadins. Furthermore, MALDI-TOF-MS has also been used to study the alteration of gliadins during the baking process (Sorensen et al., 2002). This methodological approach has been extended to the direct analysis of bread and durum wheat gliadins (Camafeita et al., 1998). Compared with conventional methods for gluten protein separation (gel electrophoresis and reversed-phase HPLC), MALDI-TOF is much more accurate and much faster, requiring less than 1 pmol of sample and only a few minutes per sample to perform the measurement (Cunsolo et al., 2003).

Alpha and Gamma Gliadins

The average molecular mass of α- and γ-gliadins have been reported to be 31,000 and 35,000 Da, respectively. The composition of AAs of α-gliadins is quite similar to that of γ-gliadins. Both are relatively rich in sulfur containing AAs such as methionine and cysteine but contain few proline, glutamine, and phenylalanine residues. Thus, they have also been classified as the S-rich prolamins by Shewry et al. (1986). Presence of even number of sulfur rich cysteine residues in α- and γ-gliadins leads to the formation of intrachain disulphide bonds responsible for their folded structure which further determines the nature of noncovalent bonding. These noncovalent protein–protein interactions (mainly hydrogen bonds and hydrophobic interactions) are mainly responsible for the viscosity of gliadins and extensibility of gluten (Hamer and van Vliet, 2000).

Omega Gliadins

These belong to the medium-molecular-weight group of gluten protein with their MW ranging from 44,000–80,000 Da. The ω-gliadin differs from the other gliadin subgroups in its AA composition. Shewry et al. (1986) have classified ω-gliadins as the S-poor prolamins as it lacks the sulfur containing AA

(cysteine or methionine) while contains low amounts of AAs of basic nature. Thus, it does not form disulphide bonds and interacts in dough through the hydrogen bonds (Shewry and Tatham, 2000). Omega gliadins are reported to be more polar than α-, β-, and γ-gliadins (Banc et al., 2009). Charbonnier (1974) reported that nearly 80% of the AAs comprising the ω-gliadins are Glx (45–56%), Pro (20–30%), and Phe (9–10%). Alanine, threonine, or seronine formed the N-terminal region. The reported value of amide content revealed that Asx and Glx had a degree of amidation of 99.6%.

STRUCTURE AND AMINO ACID COMPOSITION OF GLIADINS

The overall structure of gliadins consists of a central domain (CD) containing repetitive AA sequences rich in proline (Pro) and glutamine (Gln), and two terminal nonrepetitive domains which are hydrophobic and contains most of the ionizable AAs (histidine, arginine, and lysine), although the latter are present only in low levels (Gianibelli et al., 2001). The knowledge of the complete gliadin AA sequences come from the analysis of cDNA and genomic DNA sequences. Glutamic and aspartic acids exist as amides. The sequence of gliadin proteins is of prime importance as they are the major determinants of the toxicity and functionality in dough (Bietz et al., 1977).

Different types of gliadins have different secondary structure associated with the molecules. Tatham and Shewry (1985) studied the secondary structures of gliadins using circular dichroism spectroscopy and found that ω-gliadins were rich in randomly coiled β-turns without detectable α-helix or β-sheet, but α/β- and γ-gliadins contained 30–35% α-helix and 10–20% β-sheet conformations. They also reported that ω-gliadins were mainly stabilized by strong hydrophobic interactions and α/β- and γ-gliadins were stabilized by covalent disulphide bonds and non-covalent hydrogen bonds in their α-helices and β-sheets. However, there has been no consensus on the structure of gliadins in solutions. Friedli (1996) proposed a doughnut-like structure for gliadin molecules in which there was a large central hole. However, gliadins are still widely considered to adopt a globular protein structure in 70% aqueous ethanol (Foulk and Bunn, 2001). But recent researches reveal that α/β-gliadins have compact globular structures and γ- and ω-gliadins have extended and rod-like structures (Paananen et al., 2006; Ang et al., 2010).

Alpha and Beta Gliadins

These two types of gliadins proteins have similar primary structures consisting of around 250 and 300 AA residues. The sequences are composed of the N-terminal domain with five residues, repetitive CD consisting of about 113–134 AA residues particularly rich in proline and glutamine sequences ((heptapeptide: Pro-Gln-Pro-Gln-Pro-Phe-Pro and pentapeptide: Pro-Gln-Gln-Pro-Tyr) (Ferranti et al., 2007) and the C-terminal domain

of 144–166 residues (nonrepetitive domain). The protein structure is stabilized by the disulphide bonds formed between the cysteine residues (Shewry and Tatham, 1997). About 90% of the AA residues in these gliadins are present as the glutamic and aspartic acid residues in amide form (Ewart, 1983). The repetitive domain is composed of repeat units of PQPQPFP and PQQPY (Shewry and Tatham, 1990). Alpha gliadins contain six cysteine residues which form three intrachain cross-links (Altenbach et al., 2010) and thus there are no free cysteines preventing gliadins from participating in the polymeric structure of glutenin. However, α-gliadins with odd numbers of cysteine residues have also been reported (Anderson et al., 1997). Such gliadins can form one intermolecular S-S bond and act as chain terminators of glutenin and probably decrease the MW of the glutenin in the network.

Gamma Gliadins

The γ-type gliadins starts with a 20 residue signal peptide, followed by a short 12 residue N-terminal nonrepetitive domain, a highly variable repetitive domain of 72–161 residues, a nonrepetitive domain containing most of the cysteine residues and the C-terminal nonrepetitive domain containing the final two conserved cysteine (Figure 1) residues (Cassidy et al., 1998). All the cysteine residues form intramolecular disulfide bonds. About one quarter of γ-gliadins have been reported to contain an uneven number of cysteine residues (Anderson et al., 2001). The free-SH groups left after the formation of intramolecular disulfide bonds form intermolecular disulfide bonds. Thus α-, β-, and γ-gliadins have a possibility of a greater interaction with the gluten because of the higher number of cysteine residues as compared to the ω-gliadins. The repetitive domain in γ-gliadins forms the extended structure and is rich in β reverse turns, while the nonrepetitive domain is rich in helices (Tatham et al., 1990).

Omega Gliadins

Proline, glutamine, and phenylalanine residues account for 80% of the total AAs in ω-gliadins compared to 50–60% for the other gliadins (Hisa and Anderson, 2001). They may also contain few or no methionine and cysteine (sulfur containing AAs). Their methionine level may be less than 0.1%, lack cysteine AA and are not able to produce S-S-type bonding. Thus, a compact structure (Figure 2) cannot be observed in ω-gliadins. These gliadins have few charged AAs such as lysine (Kasarda et al., 1976). Also, they have few basic AAs and comparatively higher level of phenylalanine than other gliadin subgroups (Kasarda et al., 1983). Their surface hydrophobicity is lower than that of α- and γ-gliadins. They are the first peptides to elute out from the RP-HPLC column (Popineau and Pineau, 1987). Moreover, ω-gliadins have also been shown to include modified gliadins having one cysteine residue and, therefore, can act as chain terminators (Gianibelli et al., 2002).
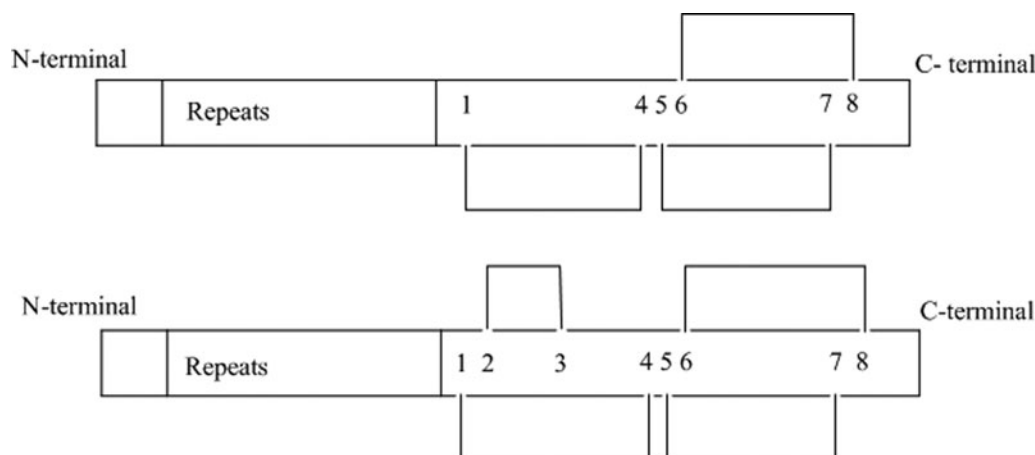
Figure 1 Structure of α and γ-gliadins (Muller and Wieser, 1995; Muller and Wieser, 1997).

On the basis of the N-terminal sequences, three different types of ω-gliadins have been observed in wheat and in related proteins such as C-hordeins and ω-secalins. These sequences have been named ARQ-, KEL-, and SRL-types depending on the first three AAs of their N-terminal sequences (Tatham and Shewry, 1995). According to Kasarda et al. (1983) and Tatham and Shewry (1995), the KEL-type differs from the ARQ-type due to the absence of the first eight residues in its structure. The third type of ω-gliadin – SRL-type is a characteristic of ω-gliadins encoded by chromosome 1B (DuPont et al., 2000).

ROLE OF GLIADINS IN DOUGH RHEOLOGY

Basic rheological instruments provide the fundamental rheological behavior of a material. Viscoelastic properties of gluten affect the rheological properties of wheat dough. When the dough is developed by mixing, the gluten proteins form continuous three-dimensional viscoelastic network throughout the dough with starch granules behaving as filler. The three-dimensional structure of gluten matrix is stabilized by covalent (disulfide), hydrogen, and noncovalent ionic bonds and hydrophobic interactions. The balance between gliadins and glutenins is responsible for important rheological properties such as viscosity and elasticity (Khatkar et al., 1995; Gomez et al., 2011). Understanding of the relationship between the gliadins and the dough rheology still remain the major objectives of cereal biochemistry and rheology. In an attempt to deduce the relationship between seed storage proteins and gluten strength, Damidaux et al. (1978) studied different seed storage proteins of durum wheat cultivars. They found that the cultivars having γ-45 gliadin component exhibited a stronger gluten as compared to cultivars with γ-42 gliadin component. The component was named depending on its mobility in A-PAGE (Bushuk and Zillman, 1978).

The proteins encoded by gliadin alleles vary in their structure, level of expression, and influence on the quality of flour (Payne, 1987). Certain gliadins have been reported to contain long repetitive domains which could have an effect on the flour quality. Kasarda et al. (1983) proposed that gliadins with an odd number of cysteines could form intermolecular disulfide bond and thus participate in the gluten polymer. Significant positive effects of certain gliadin alleles have been reported on gluten strength (Metakovsky et al., 1997). For example, wheats giving flour of the strong type have been found to carry gliadin blocks 1A3 (or 1A4), 1B1, 1D1 (or 1D5), 6A3, 6B1, and 6D1 (or 6D2), which are markers of high gluten quality, frost hardness, and drought resistance. Functional studies of single gliadin by reduction and oxidation of flours containing proteins are useful in assessing the effect of a particular protein on functional properties of dough (Clarke et al., 2003). Redaelli et al. (1997) have shown strong positive effects on dough extensibility by *Gli-D1* (gliadins)/*Glu-D3* (LMW-GS) alleles. Khatkar et al. (1995) reported that alteration in the glutenin to gliadin ratio affect the rheological property of gluten dough. They suggested that glutenin contribute to elasticity and gliadin to the viscous property of hydrated gluten. Uthayakumaran et al. (2001) showed that upon increasing the glutenin content, the rupture viscosity increased, whereas increased level of gliadins lowered the rupture viscosity.
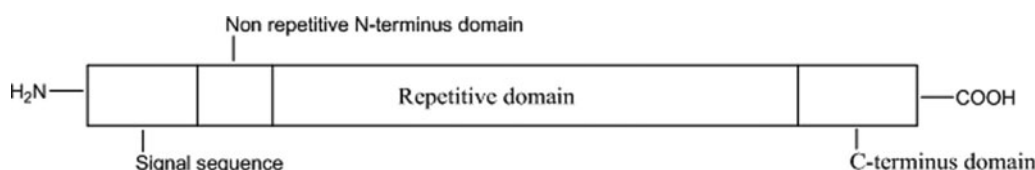

Figure 2 Structure of ω-gliadins based on Hisa and Anderson (2001) and Shewry and Tatham (1990).

During formation of the dough, the gliadins act as a "plasticizer" and promote the viscous flow and extensibility, considered as important rheological characteristics of dough. They might also interact through hydrophobic interactions and hydrogen bonds. Addition of total gliadins to the dough has shown to decrease the overall strength of dough. Gliadin-supplemented doughs generally have a shorter mixing time, greater resistance breakdown (RDB), lower maximum resistance to extension, and decreased loaf volume (Uthayakumaran et al., 1999). Analogous results were observed by MacRitchie (1987) while studying the effect of addition of increased levels of gliadin rich fraction to base flour. He found that the gliadin addition shortened the mixing time and stability of dough. Khatkar and colleagues (2002b) studied the effects of addition of total gliadins on the dynamic rheology of gluten proteins. The addition of total gliadins increased the storage modulus (G′) up to 0.5% total gliadin addition (Figure 3), but any further increments decreased it. However, tan δ values increased with the addition of total gliadins. This was primarily due to differential rate of change in the values of storage modulus (G′) and loss modulus (G″) as a result of total gliadins addition.

Pioneering work on the effect of various gliadin subfractions on the mixograph properties was carried out by Khatkar et al. (2002a). They observed that the RBD values increased with addition of gliadin and its subgroups. Within gliadin subgroups RBD values increased in the sequence ω1- < γ- < α- < β-gliadins (Figure 4). They postulated that the differential interactive behavior of gliadin with the gluten proteins was responsible for the above stated differential increase of RBD values. They also pointed out that addition of gliadin subgroups (α-, β-, and γ-gliadins) increased the PDR (peak dough resistance) of the base flour to a greater extent than gluten and ω1-gliadin addition. Contrary to this, Uthayakumaran et al. (2001) while studying the effect of gliadins and its subgroups on the rheological parameters and functional properties of the dough observed that addition of gliadin subgroups reduced PDR and RBD. They further reported that among all the gliadin fractions,
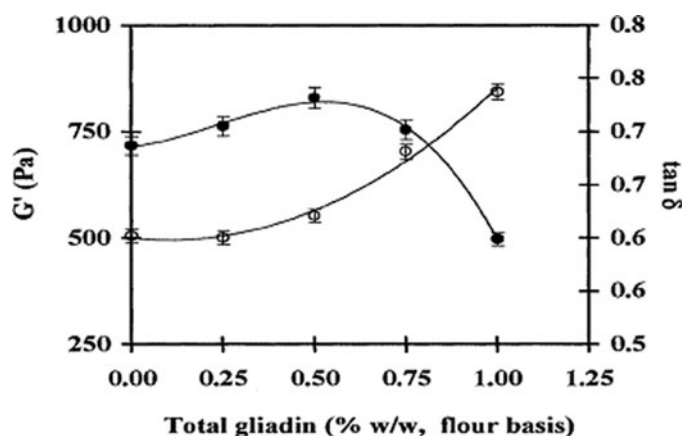
Figure 3   Effect of addition of total gliadin on G′ (•) and tan (○) profiles of cv. Hereward gluten. Stress amplitude, 25 Pa, Frequency 1 Hz (Khatkar et al., 2002b).
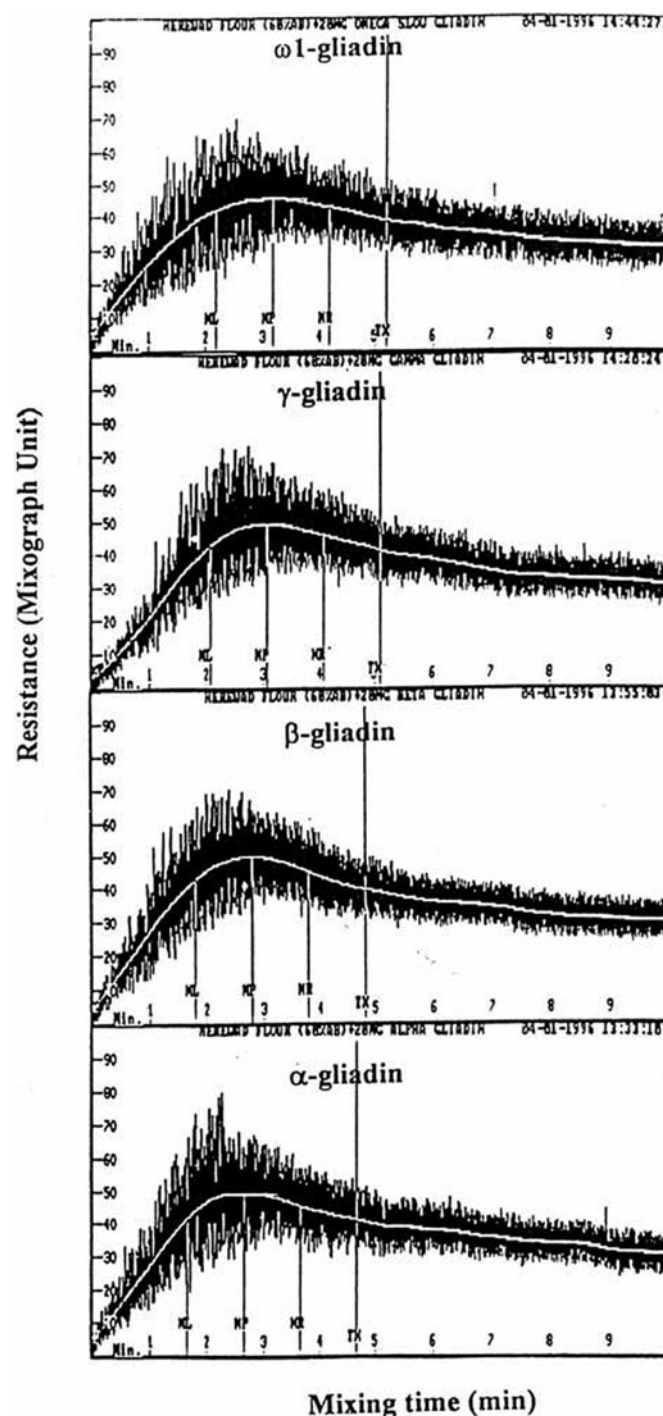
Figure 4   Effects of addition (1%, w/w) of different gliadin subgroups on mixing curves of cv. Hereward flour. Addition of different gliadins is indicated in top middle of each Mixogram (Khatkar et al., 2002a).

γ-gliadin caused maximum reduction in the mixing time and resistance to extension. They postulated that the mixing time and maximum resistance to extension decreased as the hydrophobicity of the gliadin fractions increased in the order ω- < α- and β- < γ-gliadins. Similarly, Fido et al. (1997) reported that among all the gliadin fractions, ω-gliadins showed the largest

weakening effect on the flours, followed by α/β gliadins, while γ-gliadins demonstrated the least effect on the mixing time. However, Khatkar (1996) found that α-gliadins reduce the mixing time to the maximum. He postulated that size/charge of the gliadin is mainly responsible for the reduction of mixing time. It appears more reasonable that the smallest gliadins should have maximum weakening effect on the mixing time of the flour due to their action similar to ball-bearing. Thus, it is evident that there are conflicting thoughts with regard to the effect of specific group of gliadins on the mixing time of the flour. It can be concluded that wheat quality can be improved by acquiring the desired knowledge linked to the role of gliadins and its subfractions on the rheological and end-product properties.

INFLUENCE OF GLIADINS ON PRODUCT QUALITY

The studies on the significance of gliadins and its subgroups on the wheat product quality have been carried out in various parts of the globe. But no single conclusion has been drawn even after numerous studies. Some researchers suggest that gliadin affects the loaf volume of bread whereas others regard glutenin as the sole determinant of the bread quality. A few gliadin alleles and components, such as *Gli-B1b, Gli-B2c,* and *Gli-A2b*, in bread wheat cultivars (Wrigley et al., 1982) and γ-45 in durum wheat (D′Ovidio and Masci, 2004) have been found to contribute significantly to gluten strength.

Bread Quality

The influence of gliadins and its subgroups on the quality of bread has been debatable for many years. The type and quantity of the gluten proteins are important in determining the bread making properties of the wheat flour (Gomez et al., 2011). Certain gliadin bands have shown significant correlation with hardness of grain and dough strength (Wrigley et al., 1981). Huebner and Beitz (1986) identified a correlation between a specific gliadin fraction and a general bread-making score and named it "antibaking-quality fraction" as it had negative effect on the bread quality. Ohm et al. (2010) also observed negative relationship of gliadins on the loaf volume of bread. The effect of genetic variation in the glutenin and gliadin protein alleles of wheat on the mixing characteristics of the dough and quality of bread and noodle were evaluated by Wesley et al. (1999). They concluded that both gliadin and glutenin influence bread and noodle-making properties of wheat flour. They suggested that differences at the *Glu-3/Gli-1* loci, coding for LMW subunits and gliadins or any other biochemical components that affect dough rheology, could be a reason for variation in the bread and noodle-making performance.

Many researchers have observed positive relationship between bread loaf volume and gliadins. Park et al. (2006) measured the protein and protein fractions in wheat flours to investigate their relationship to bread-making properties. The percentage of gliadins based on flour and protein content of the

wheat varieties exhibited a positive correlation with the bread loaf volume ($r = 0.73$, $P < 0.0001$ and $r = 0.46$, $P < 0.001$, respectively). Composition of gliadins and HMW-glutenin subunits were studied by SDS-PAGE and A-PAGE method by Lan et al. (2009) to deduce the relationship of gliadins with the bread making performance of the wheat varieties. A close association was observed between the ω-gliadin fraction and bread making quality. Certain gliadin bands correlated positively with baking quality of bread. Thus, they suggested that gliadins could be used as an important parameter when breeding for bread-making quality.

Different subfractions of gliadins have found to be differently associated with the bread quality. Van Lonkhuijsen et al. (1992) carried out research on 32 wheat samples having similar composition of HMWGS (null, 7 and 2+12) and different gliadin composition. The bread loaf volumes ranged from 445 to 616 ml per 100g of flour. The gliadin composition of the wheat samples accounted for 82% of the observed variation. The ω-gliadin fraction was negatively associated with the bread quality whereas γ-gliadin was found to be present in the good bread quality wheat line. They concluded that both gliadins and HMW glutenin subunits control the bread making properties of wheat flour. Weegels et al. (1994) also observed similar effects of γ-gliadin on the loaf volume of bread. Khatkar et al. (2002a) reported that addition of total gliadins and its subgroups (α-, β-, γ-, and ω-gliadins) to the dough significantly improved its bread-making performance. The addition of total gliadins at $\geq 0.5\%$ level increased the loaf volume significantly by about 10.6% while among the different subgroups α increased the loaf volume of bread by 13.9%, followed by γ- and β-gliadins while ω1-gliadin subfraction caused 11% increase. Omega gliadin proved less effective than α-, β-, and γ-gliadins in improving the loaf volume of the bread as they lack the sulfur containing AA cysteine (Tatham and Shewry, 1995), therefore, ω-gliadins interact with other proteins only noncovalently and thus are probably less effective in influencing viscoelastic properties and bread-making quality. Dynamic rheological measurements determined by Khatkar (1996) for different gliadin subgroups revealed that the dynamic moduli, G′ and G″, had significant ($r = 0.74$ and 0.77) positive relationship with loaf volume for gliadin subgroups added (Figure 5). Contrary to this, Uthayakumaran et al. (2001) reported that addition of gliadins decreased the loaf volume of bread with ω-gliadin reducing the loaf height to the maximum while α- + β-gliadins causing least reduction in loaf height. Furthermore, some investigators have reported a correlation between gliadin surface hydrophobicity and loaf volume; the order of hydrophobicity being ω- < α- < β- < γ-, with the more hydrophobic gliadins being beneficial (Van Lonkhuijsen et al., 1992; Weegels et al., 1994). However, Lookhart and Albers (1988) found that particular ω-gliadin is related to good bread quality whereas β-gliadin is responsible for poor bread making.

As evidenced from the above literature, different researchers have reached to different conclusions regarding the effect of gliadins subfractions on the bread quality. α-, β-, and γ-gliadins might have a greater tendency to interact with the gluten as they
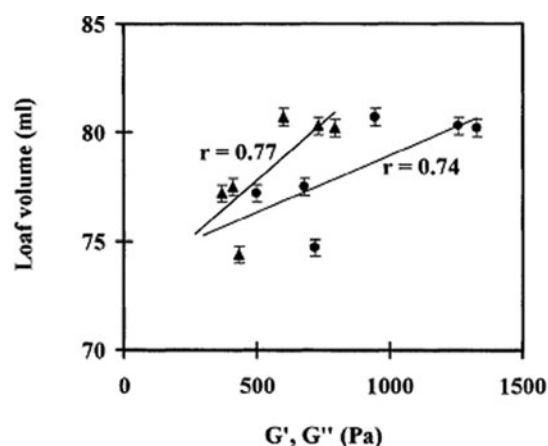
Figure 5 Relationships of G′ (circles) and G″ (triangles) of cv. Hereward gluten with loaf volume for adding different gliadin subgroups. (Khatkar, 1996).

form a compact structure due to the presence of cysteine residues in their structure as compared to ω-gliadins. Thus, these gliadin subfractions may improve the loaf volume of the bread.

Cookie Quality

Although little literature is available on the contribution of gliadins to the cookie quality, there is some evidence of a positive relationship of gliadins with cookie making quality. Cookie making requires highly extensible soft wheats. Kuragano et al. (1991) reported that cookies prepared by addition of gliadins to the base flour resulted in cookies with better spread and soft texture. Ornebro et al. (2001) suggested that interfacial behavior of the gliadins and glutenins might influence the quality of baked products. Uthayakumaran et al. (2001) reported that the γ-fraction of gliadins increases the extensibility of the wheat dough to the most and thus wheat flours with higher content of this gliadin subfraction improve the cookie making quality of the wheat cultivar. The contribution of gliadins to the cookie quality is least explored, thus further studies need to be carried out in this area.

Pasta Quality

Semolina and water are the essential ingredients for pasta (Fuad and Prabhasankar, 2010). For pasta making, dough properties are an important aspect of quality and it is the storage proteins of the wheat endosperm that are the main determinants of dough properties, such as dough extensibility, dough stability, and dough strength. Durum wheats are used primarily around the world for the manufacture of pasta. Semolina flour obtained from hard durum winter wheat form strong and elastic dough due to its high gluten content (Fuad and Prabhasankar, 2010). The best pasta-making characteristics are associated with the presence of a specific allelic form of typical LMW-GS, named LMW-2 (Payne and Lawrence, 1983). The commonly grown

durum wheat cultivars have either LMW-2/ γ-45 associated with superior pasta quality or LMW-1/γ-42 associated with poor pasta quality (Khan et al., 2010). This is probably due to the fact that gluten extracted from durum varieties having γ-45 band is highly firm and viscoelastic in nature. Autran and Galterio (1989) observed negative relationship of ω-gliadins with pasta quality. Du Cros et al. (1982) observed close association between dough strength and a group of gliadin proteins (including band 45), and between dough weakness and a different group of gliadins (including band 42). All breeding lines with weak dough had band 42 and its associated bands, while 90% of the strong-dough lines had gliadin 45 band. Most of the durum wheat breeding programs have fixed the LMW-2/γ-45/ω-gliadin 35 loci because of their positive effect on pasta performance.

Tortilla Quality

The influence of the gliadin alleles on the extensibility of the dough makes them of prime importance for studying the effect of gliadins on the tortilla quality. Uthayakumaran and colleagues (2005) observed that upon increasing the glutenin to gliadin ratio the cooked tortilla puncture force increased. Gliadin functionality in tortilla quality was also studied by Mondal et al. (2009) using near-isogenic wheat lines having deletions in either *Gli A1*, *Gli D1*, *Gli A2*, or *Gli D2* gliadin loci. They found that deletions in the *Gli 2* loci, reduced the proportion of α- and β-gliadins thus lowering the amount of cross-linking in the gluten. The resulting gluten was highly extensible and improved diameter and overall quality of the tortillas. The study proposed that deletions in the *Gli 2* loci could be useful in developing wheat cultivars producing better quality tortillas and thus could cater to the needs of the growing tortilla market.

Indian Flat Bread (Chapatti) Quality

Chapatti, an unleavened flat baked whole wheat flour product, is the staple food of more than 60% of the Indian population. Wheat (*Triticum aestivum*) is extensively used for the production of flat breads. Protein quality and composition, wet and dry gluten content, starch properties, mineral content and composition, type of enzymes present in wheat flour, and water absorption capacity of whole wheat flour play a role in the overall acceptability of chapatti (Sharma et al., 2004). Good chapatti must be soft, pliable, and tear easily, without being extensively brittle or leathery, but a slight chewiness is desirable (Rao, 1993).

Prabhasankar (2002) predicted the suitability of wheat for chapatti making by assessing the biochemical and immunochemical characteristics of 10 commercial Indian wheat varieties. He concluded that wheat varieties having higher amounts of gliadin protein result in poor quality chapatti. Ram and Nigam (1981) suggested that wheat varieties having about equal

quantities of gliadins and glutenins give fully puffed and pliable chapattis, while wheat varieties with more gliadins give relatively stiffer chapatti. Navnidhi (2010) also found that wheat varieties with gliadin/glutenin ratio near to 1 produced softer, pliable and superior chapattis. Numerous scientists all over the world have found correlations between presence of particular HMW subunit of glutenin and chapatti making quality of wheat flour. Many researchers have associated good chapatti quality to the presence of 5+10 (HMW-GS) (Srivastava et al., 2003), HMW-GS 1B20 together with 1 A null (Sreevamulu et al., 2003) and null along with HMW-GS 20 or 7+8 (Navnidhi, 2010) while HMW-GS 2+12 (Srivastava et al., 2003) has been associated with poor chapatti quality. To conclude, the influence of HMW-GS on the chapatti quality has been well established but the role of gliadins and its different subgroups on the chapatti quality still remains an uncharted territory.

GLIADIN AND CELIAC DISEASE

While studying the biochemical properties of gliadins it is important to note that gliadin proteins have certain potential toxic fraction which can cause celiac disease defined as an immune mediated enteropathy triggered by the ingestion of gliadin proteins in wheat and other similar type of protein from barley and rye. The disease is highly heritable and the genes which code human leukocyte antigen HLA- DQ2 or HLA-DQ8 molecules are the prime genetic risk factor. The major symptoms of celiac disease are gastrointestinal disorders such as flatulence, diarrhea, and weight loss due to malabsorption. The small intestine is the main target site of the disease. The gliadin toxicity is confined to α-gliadins and to some extent γ-gliadins and glutenins. In celiac disease patients, peptides that originate from incomplete digestion of gliadins, either in their native state or deamidated state by tissue enzyme transglutaminase, bind to HLA-DQ2 or -DQ8 receptors of antigen presenting cells and thus activate the lamina propria infiltrating CD4+ T cells. In response the CD4+ T cells release proinflammatory cytokines, chiefly g-interferon. This leads to the remodeling of the profound tissue resulting in the atrophy of the small intestinal villi and hyperplasia of crypts (Darewicz et al., 2008; Sollid 2000; Molberg et al., 2001). In short, the native gliadin acts as an antigen against which the genetically susceptible individuals produce an inappropriate T cell mediate immune response. Till date, there is no cure for this disease except for feeding on a non-gluten diet for life.

CONCLUSION

Gliadins have received a great deal of attention from scientists in the past. There have been several investigations on the role of glutenin and gliadin in dough rheology. Some reports suggest that the overall function of wheat proteins derives mainly from glutenin, and that gliadin is only a diluent. Others, however, suggest that gliadin is an important contributor to gluten's properties. Introduction of new protein separation and characterization techniques such as RP-HPLC, SE-HPLC, CZ electrophoresis, and MALDI TOFMS have made it possible to separate gliadins into many individual polypeptides and determine the MW of gliadins. The role of gliadin subgroups on dough rheology requires further study. The role and functionality of different subfractions of gliadins on bread quality have been studied but conflicting rather than conclusive results have been obtained. Moreover, the effect of individual subgroups of gliadins on the quality of cookies, pasta, chapatti, and tortillas require more investigation. Quality ranking of gliadin alleles including those having relatively small but inherited effects can provide a useful tool in predicting the dough quality of progeny in breeding programs. Thus, more studies are needed to elucidate the precise role of gliadin subgroups in dough strength and product quality.

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