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Chapter IV

Wheat Gluten: Composition and Health Effects

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Abstract

Grains of cereals of the *Gramineae* family have been a required source of food for millennia. Wheat, rye, barley are unique among the edible grains because their flours have the protein complex called „gluten“ that can be formed into a dough with the rheological properties required for the production of leavened bread. The gluten proteins consist of monomeric gliadins and polymeric glutenins. Glutenins and gliadins are recognised as the major wheat storage proteins, constituting about 60-85% of the total grain proteins and they tend to be rich in asparagine, glutamine, arginine or proline but very low in nutritionally important amino acids lysine, tryptophan and methionine. The gliadins are a polymorphic mixture of proteins soluble in 70% alcohol, and can be separated into α-, β-, γ-, and ω-gliadins with a molecular weight range of 30 to 80 kDa. Glutenins can be broadly classified into two groups, the high molecular weight (HMW) and the low molecular weight (LMW) subunits, with the molecular weight range of 75 to 120 kDa and 30 to 74 kDa, respectively, according to mobility on SDS-PAGE. They link together and form heterogeneous mixtures of polymers by disulfide bonded linkages of polypeptides. Generally, it is believed that gliadin controls the viscosity of dough and glutenin controls the elastic or strength properties. The precise balance between viscosity (extensibility) and elasticity (dough strength), or the glutenin to gliadin ratio, is important for bread making.

In addition to their role in dough quality, gluten proteins can affect health in genetically susceptible individuals. Among the different gluten subunits, the α-gliadins are considered the most immunogenic, while γ-gliadins and glutenins are much less

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responsible for gluten intolerance. About 1% of the general population suffers from CD and numbers are increasing not only because of better diagnosis but also because of increased intake and usage of wheat constituents as food additives.

1. Wheat Proteins

Wheat is one of the most important cereal crops worldwide, in terms of production and utilisation. It is a major source of energy, protein, and dietary fibre in human nutrition and animal feeding. It provides approximately one-fifth of the total calorific input of the world's population (FAO, 2009).

The ability of wheat flour to be processed into different foods is largely determined by the proteins. *According to previously obtained results, t*he content of total protein in bread and durum wheat genotypes ranged from 10.87 to 13.04 and 11.46 to 16.53%, respectively (Žilić et al., 2010). However, initial screening of the USDA World Wheat Collection showed that the protein content of 12,600 wheat lines varied even more, i.e. from about 7% to 22% (Vogel et al., 1978), with the genetic component accounting for about a third of this (i.e. about 5%). Hence, the greater part of the variation was due to non-genetic factors the more difficult breeding for high protein was due to this strong environmental impact. The total protein content of the grain can be manipulated by adding fertiliser nitrogen but genes conferring high grain protein have been identified in wild tetraploid wheats (Brevis et al., 2010). High-density genetic maps of wheat (Boeuf et al., 2002) helped breeders to locate precisely the position of these genes on an individual chromosome. However, these genes have either not yet been exploited to increase the protein content of cultivated wheat or have not been successful when incorporated into cultivated lines. Wheat proteins show high complexity and different interactions with each other, thus making them difficult to characterise. The molecular weight (MW) of proteins generally ranges from thousands to millions, with those of wheat proteins being from 30,000 to more than 10 million Daltons (Da) (Wieser, 2007).

Usually, they are classified according to their solubility. Following the sequential Osborne extraction procedure, highly heterogeneous group of nongluten proteins (albumins and globulins) and gluten proteins (gliadins and glutenins) are isolated.

An alternative classification to that described above has been proposed based on the composition and the structure rather than solubility (Shewry et al., 1986). Wheat proteins naturally occur as oligomers of different polypeptides containing more than 35% hydrophobic amino acid residues (isoleucine, leucine, tryptophan, tyrosine, valine, phenylalanine, and proline) (Žilić et al., 2011a). There is 6% to 12% proline in wheat proteins (Sivam et al., 2010).

1.1. Albumins and Globulins

The nongluten proteins (albumins and globulins) of wheat endosperm represent 20 to 25% of the total grain protein (Belderok et al., 2000; Merlino et al., 2009) and the majority of them are monomeric. However, screening the Serbian wheat collection showed that the average value of bread and durum wheat genotypes for the contribution of albumin and

globulin fractions was 38.50% of the total protein (Žilić et al., 2011b). According to Stehno et al. (2008) and Abdelrahman et al. (2004), albumins-globulins constitute from 22.29 to 30.81% and 14.25 to 33.46% of the total grain proteins in cultivars grown in the Czech Republic and Sudan, respectively. The nongluten proteins MWs are mostly lower than 25,000 Da, although a significant proportion has a MW between 60,000 and 70,000 Da (Singh et al., 1990).

In a previous study (Žilić et al., 2011b), wheat albumins-globulins were characterised by a rich protein pattern – the number of bands varied from 19 to 23 and they were defined by the molecular weight 76.4–12.4 kDa. Furthermore, both albumins and globulins contain proteins that occur as polymers stabilised by inter-chain disulfide bonds (Gupta et al., 1991). Nutritionally, the albumins and globulins have a very good amino acid balance. They are relatively high in lysine, tryptophan and methionine (Pomeranz, 1968) and contribute about 50% of the total lysine content in the grain proteins (Fra-Mon et al., 1984).

Many of these proteins are enzymes involved in the metabolic activity. In addition, polymeric globulins (triticins) are strongly related to 11S legume-type globulins. Such globulins form a minor group (approximately 5% of total proteins) of wheat storage proteins (Singh et al., 1991). However, several other proteins have unknown functions and are not well characterised. Some nongluten proteins, particularly those belonging to a family of trypsin and α-amylase inhibitor, are also implicated in plant defence (Carbonero et al., 1993), but the role of α-amylase and trypsin inhibitors as wheat allergens in baker's asthma has been demonstrated (Posch et al., 1995). Although the albumin and globulin fractions are not known to play a direct role in bread making, as gluten proteins, they may be necessary for normal baking properties (Peruffo et al., 1996). The same authors indicated the possibility of forming a covalent bond between β-amylase and the LMW (low molecular weight) glutenin subunit that would mean that the role of albumins and globulins in defining the functional properties of the flour is not so irrelevant. Most of the physiologically active nongluten proteins influence the processing and rheological properties of wheat flour. In recent years, the benefits of the use of amylases, xylanases, lipoxygenase, pentosanase, glucoseoxidase, peroxidase has stimulated further interest in the bread-making industry (Jiménez and Martínez-Anaya, 2001; Toyosaki, 2007). From the food quality point of view, the potential effects of the products formed during enzymatic reactions are much more important than the reaction itself. The reaction of lipoxygenase on its substrate generates highly reactive compounds that are initiators of a cascade reaction in which components may be affected secondary, resulting in direct losses of a nutritive value, alterations of organoleptic properties and colour (Žilić et al., 2009). The loss of colour observed during pasta processing is due to the lipoxygenase-linoleic system, which is responsible for carotenoid oxidation (Trono et al., 1999; Serpen and Gökmen, 2007). Although other enzymes such as peroxidases and polyphenoloxidases can contribute to semolina bleaching a major role appears to be played by lipoxygenase (Taha and Sagi, 1987). The lipoxygenase action is probably the causal agent of the increase of the amount of free lipids in dough and of the increase of mixing tolerance and relaxation times of dough, which results in enhanced loaf volume (Permyakova and Trufanov, 2011). On the other hand, the positive effect of peroxidase in bread-making has been attributed to crosslinking of feruloylated arabinoxylans into larger aggregates that have a better water holding capacity and cause a redistribution of water in the dough (Hilhorst et al., 2002). According to the study carried out by Žilić et al. (2012a), the lipoxygenase activity is

concentrated in the endosperm and embryo, while the peroxidase activity is mostly concentrated in the bran fraction of wheat.

1.2. Gluten

Wheat is unique among the edible grains because wheat flour has the protein complex called „gluten“ that can be formed into a dough with the rheological properties required for the production of leavened bread (Uthayakumaran et al., 2002). The rheological properties of gluten are needed not only for bread production, but also within a wider scope of foods that can only be made from wheat, viz., noodles, pasta, pocket breads, pastries, cookies, and other products (MacRitchie, 1992). In practice, the term „gluten“ refers to the proteins, because they play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity and elasticity on dough. In addition to wheat, grain sources of gluten are barley, rye, triticale, spelt, einkorn, emmer and kumut. The common sources of gluten include many home staples such as pasta, bread, cereals, soups and deserts. Ingredients where gluten is also a main component can be found in the following: soy sauce, hydrolysed wheat proteins, wheat bran hydrolysate, wheat protein isolate, wheat starch, glucose syrups, wheat maltodextrin, sorbitol, lactitol, maltitol, caramel, β-glucan, alcohol/ ethanol, vinegar, wheat germ oil, medications, etc. It should be noted that wheat gluten, an important by-product of the wheat starch industry, is produced worldwide in enormous quantities. Research has been performed to develop techniques for converting wheat gluten into more useful products. One approach for the effective application of wheat gluten is enzymatic hydrolysis, which is broadly used in the food industry to improve its functional properties (e.g. solubility, emulsification and film-forming properties) or to prepare extensively hydrolysed proteins for hypoallergenic infant diets and nutritional therapy (Day et al., 2006). Nevertheless, a few researchers have reported the occurrence of antioxidative peptides in wheat gluten hydrolysates (Suetsuna and Chen, 2002; Wang et al., 2007). The native gluten protein had a high antioxidant capacity of 74.39 mmol Trolox/kg (Žilić et al., 2012b). Gluten can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water-soluble constituents. In average, durum wheat contained significantly higher amounts of wet gluten than bread wheat. Wet gluten ranged from 17.35 to 29.65% and 20.00 to 32.20% in bread and durum genotypes, respectively (Žilić et al., 2011c). Depending on the thoroughness of washing, the dry solid contain 75–85% protein and 5–10% lipids; most of the remainder is starch and nonstarch carbohydrates (Wieser, 2007). Gluten contains hundreds of protein components, which either are present as monomers or, linked by inter-chain disulphide bonds, as oligo- and polymers. Traditionally, gluten proteins have been divided into roughly equal fractions: the monomeric gliadins and polymeric glutenins. Gluten proteins classification is shown in Figure 1.

According to the alternative classification, wheat gluten can be separated into three large groups: sulphur-rich (Mw of ~50 kDa; α-, β-, γ-gliadins and B- and C-LMW glutenins), sulphur-poor (Mw ~50 kDa; ω-gliadins and D-LMW glutenins) and high molecular weight (Mw ~100kD; HMW glutenins) proteins. Glutenins and gliadins are recognised as the major wheat storage proteins, constituting about 60-90% of the total grain proteins (Abdel-Aal et al., 1996) and they tend to be rich in asparagine, glutamine, arginine or proline but very low in nutritionally important amino acids lysine, tryptophan and methionine (Shewry, 2007).

Though cysteine belongs to the minor amino acids of gluten proteins (≈ 2%), it is extremely important for the structure and functionality of gluten (Wieser, 2003). Most cysteines form either intra-chain disulphide bonds within a protein or inter-chain disulphide bonds between proteins. During dough development, these disulphide bonds can be mobilised through disulphide inter-change reactions (Zaidel et al., 2008). Wheat gluten, as the most complex protein, had a high concentration of disulfide bonds (45.37 nmol/mg) (Žilić et al., 2012b). Additional covalent bonds formed during break making are tyrosine–tyrosine crosslinks between gluten proteins and tyrosine–dehydroferulic acid crosslinks between gluten proteins and arabinoxylans (Tilley et al., 2001; Piber and Koehler, 2005). The covalent structure of the gluten network is superimposed by non-covalent bonds (hydrogen bonds, ionic bonds, hydrophobic bonds).

Although hydrogen bonds are individually weak, they create stability to the dough when large numbers of bonds are established during the dough development.

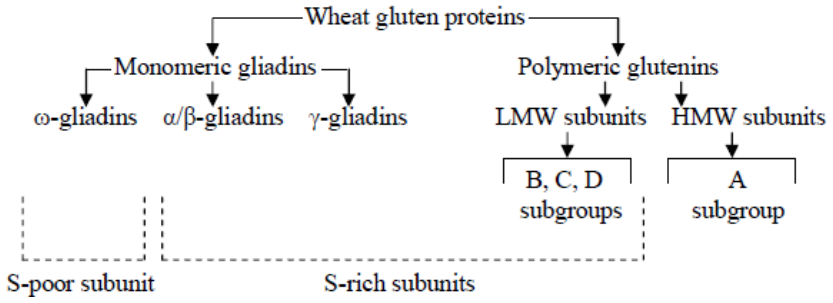

Figure 1. Classification of gluten proteins adapted from Shewry et al. (1986).

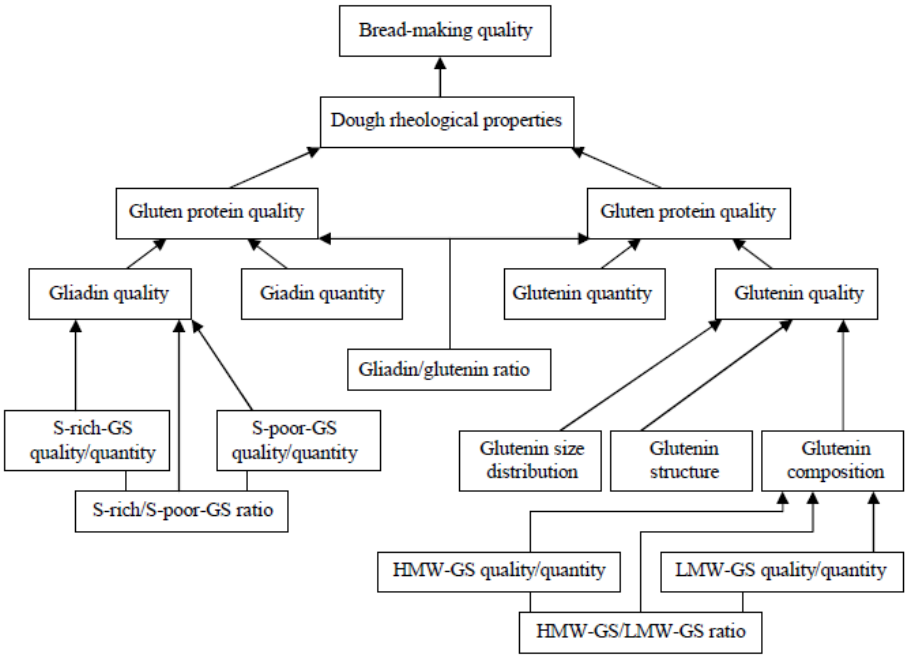

Figure 2. Factors affecting wheat dough rheological properties adapted from Veraverbeke and Delcour (2002).

Hydrophobic and ionic bonds, although present in very small amounts, play significant roles in the interactions among the biopolymers within bread dough that consequently promote dough stability (Sivam et al., 2010).

The glutenin to gliadin ratio clearly affects the mechanical properties of gluten dough. The greater gliadin content was the resistance to extension was lower, while the extensibility was higher. Based on measurements on glutens reconstituted at various glutenin/gliadin ratios, Janssen et al. (1996) found that at the constant protein content the main factor determining the rheological behaviour of hydrated gluten is the glutenin to gliadin ratio. The gliadin/glutenin ratio range (0.49 to 1.01) was obtained for bread wheat genotypes grown in Serbia in 2009-2010 growing season (Žilić et al., 2011c). The effect of gluten proteins on wheat dough rheological properties and bread-making quality is shown in Figure 2.

1.2.1. Gliadins

The gliadins constitute from 30 to 40% of total flour proteins and represent a polymorphic mixture of proteins soluble in 70% alcohol. This protein fraction can be separated into α-, β-, γ-, and ω-gliadins with a molecular weight ranging from 30 to 74 kDa as determined by SDS-PAGE. The molecular weights of ω5-, ω1,2-gliadins are between 52 and 74 kDa, while α-, β- and γ-gliadins have lower MWs, ranging from 30 to 51 kDa (Abdel-Aal et al., 1996; Kasarda et al., 1983). The latter approach has shown that the α- and β-gliadins are closely related and thereby they are often referred to as α-type gliadins. According to the study carried out by Žilić et al. (2011c), there was strong-staining band or polypeptide chain with molecular weight of about 42 to 44 kDa that appeared in all durum wheat genotypes. This polypeptide chain was in the γ-gliadin region and was absent in bread wheat genotypes. This was in agreement with Abdel-Aal et al. (1996) who detected that this band was absent in common wheat. Therefore, this γ-gliadin band might be used to differentiate the durum from bread wheat. Electrophoretic separation of gliadin components present in the bread wheat material used is reported in Figure 3A. The amino acid compositions of the α/β-, γ- and ω-gliadins are similar to each other although, the ω-gliadins contain little or no cysteine or methionine and only small amounts of basic amino acids.

All gliadins are monomers with either no disulphide bonds (ω-gliadins) or intra-chain disulphide bonds (α/β-, and γ-gliadins). With a few exceptions, α-gliadins contain six and γ-gliadins eight cysteine residues located in the C-terminal domains and forming three and four homologous intra-chain disulfide bonds (Müller and Wieser, 1997). The ω-gliadins are characterised by the highest contents of glutamine, proline and phenylalanine which together account for around 80% of the total composition (Wieser, 2007). Studies on the secondary structure have indicated that the N-terminal domains of α/β-, and γ- gliadins are characterised by β-turn conformation, similar to ω-gliadins (Tatham and Shewry, 1985). The non-repetitive C-terminal domain contains considerable proportions of α-helix and β-sheet structures. The α/β- and γ- gliadins are the major components, whereas the ω-gliadins occur in much lower proportions of wheat varieties (Wieser and Kieffer, 2001). According to the SDS-PAGE analysis, bread wheat genotypes had high concentrations of α/β + γ-subunits of gliadin (on the average 61.54% of extractable proteins) and low concentrations of ω-subunit (0.50 to 2.53% of extractable proteins) (Žilić et al., 2011c). The ratio of α/β- and γ-gliadins to ω-gliadin influence the sulphur amino acid content, quality of wheat grain proteins and the structure and functionality of gluten. To simplify matters, gluten is "two-component glue", in which gliadins can be understood as a "plasticizer" or "solvent" for glutenins.

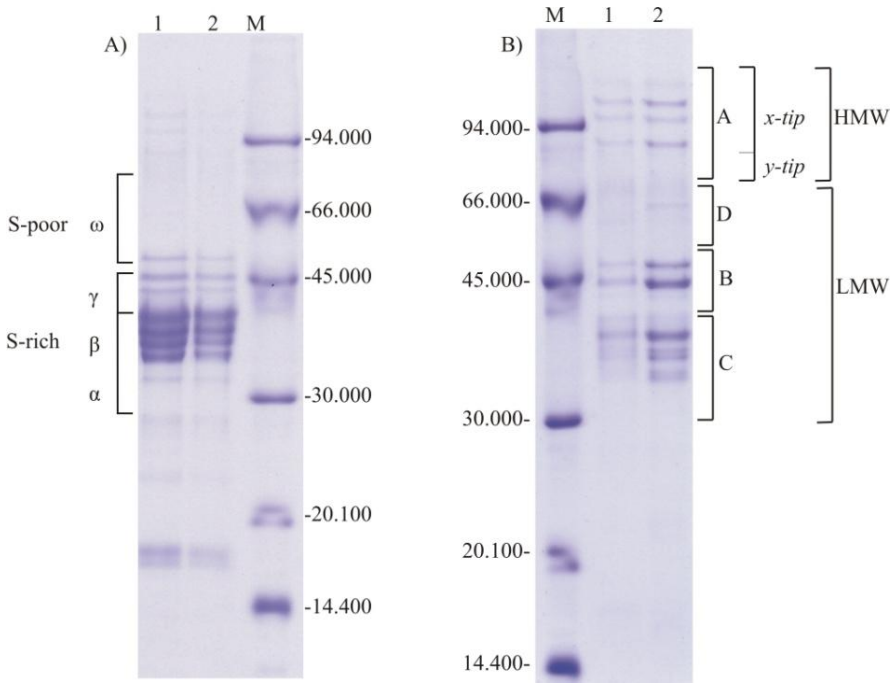

Figure 3. SDS-PAGE patterns of gliadins (A) and glutenins (B) of bread wheat genotypes 1-ZP 87/I, 2-ZP 7/I. M-standard. ω, γ, β and α indicate subunits of gliadins; B-, C-, D-LMW and A-HMW indicate low and high molecular weight subunits of glutenins.

The gliadins as "plasticizers", promote a viscous flow and extensibility, which are important rheological characteristics of dough. In durum wheat a highly significant correlation has been detected between specific durum wheat γ-gliadin and gluten strength. γ-Gliadins 45 and 42 are useful markers for good and poor pasta quality, respectively and this is due to the genetic linkage with low molecular weight glutenin subunits (Sapirstein et al., 2007). On the other hand, β-gliadin subunits may be associated with elevated loaf volumes, and could be the target for indirect selection for breeding programs improving durum wheat for bread-making quality. Gliadins may also interact with the glutenin polymers by non-covalent forces, although these interactions are traditionally considered to contribute to gluten viscosity rather than elasticity (Shewry et al., 2001).

1.2.2. Glutenins

Glutenin polymers are made up of single polypeptides linked through intermolecular disulfide bonds that account for about 45% of the total proteins in the grain endosperm. The content of soluble glutenins ranged from 9.49 to 14.91% of total proteins and from 7.24 to 11.69%, while in average, content of insoluble glutenin was 26.76 and 24.59% of total proteins in wholemeal of bread and durum wheat genotypes, (Žilić et al., 2011c). In addition to these inter-chain cystine bonds, glutenins, like gliadins, also contain intra-chain disulphide bridges. The amino acid compositions of glutenins are very similar to those of gliadins, with high levels of glutamine and proline and low levels of charged amino acids. Glutenins can be broadly classified into two groups, the high molecular weight (HMW) and the low molecular weight (LMW) subunits, with molecular weight (MW) range of 75 to 120 kDa and 30 to 74 kDa, respectively (Goesaert et al., 2005). The LMW subunits most closely resemble γ-

gliadins in sequence (Müller et al., 1998) and comprise about 20 to 30% of the total proteins, while the HMW subunits account for about 5 to 10% of the total proteins (Payne, 1986). The glutenin subunits released has also been further classified into four subgroups (A, B, C and D) based on electrophoretic mobility on SDS-PAGE. The subgroup A is determined to be HMW-GS and subgroups B, C and D are referred to as LMW-GS. B and C subgroups contain about 60% of total LMW-GS. The subgroups B and C of LMW-GS have molecular weights of 42–51 kDa and 30–41 kDa, respectively. The amino acid sequences of LMW-GS in C subgroup are similar to the amino acid sequence of γ- and α-gliadins. Highly acidic LMW-GS, having the molecular weight of 52-74 kDa, are present in the subgroup D (Gianibelli et al., 2001). SDS-PAGE of polymeric protein subunits is shown in Figure 3B. Each common wheat cultivar possesses 3 to 5 HMW subunits. These are encoded at the *Glu-1* loci on the long arms of the group 1 chromosomes (1A, 1B, and 1D) (Payne et al., 1987). Each locus includes two genes linked together encoding two different types of HMW-GS, x- and y-type subunits (Shewry et al., 2006). The x-type subunits generally have a slower electrophoretic mobility in SDS-PAGE and higher molecular weights than the y-type subunits.

The types and characteristics of wheat grain gluten proteins is shown in Table 1. HMW-GS have high contents of proline and glycine and low contents of lysine with the unusually high content of glutamic acid. They consist of nonrepetitive N- and C-terminal domains flanking a central repetitive domain that confers elasticity to protein molecules (Gianibelli et al., 2001). The amino acid composition of HMW-GS indicated the hydrophilic nature of the central repetitive domain and the hydrophobic characteristics of the N- and C-terminal domains.

Table 1. Summary of the types and characteristics of wheat grain gluten proteins (adapted from van Herpen, 2008)

Gluten class	Size (kDa)	% of total	S- residues	Intra-chain bonds	Inter-chain bonds	Chromosome location
HMW glutenins x-type	75-120	5-10	2-5	0-1	2-3	Long arm of 1 (ABD)
HMW glutenins y-type	75-120		6-7	0-2	3>	Long arm of 1 (ABD)
α-gliadins	30-41	70-80	6	3	0	Short arm of 6 (ABD)
C-type LMW glutenins	30-41		7-8	3-4	1	Short arm of 1+6 (ABD)
γ-gliadins	42-51		8	4	0	Short arm of 1+6 (ABD)
B-type LMW glutenins	42-51		7-8	3	1-2	Short arm of 1 (ABD)
D-type LMW glutenins	52-74	10-20	1	0	1	Short arm of 1 (ABD)
ω-gliadins	52-74		0	0	0	Short arm of 1 (ABD)

The N- and C-termini are richer in charged residues than the repeat domain and contain most, or all, of the cysteine residues present in the subunits (Anjum et al., 2007). The glutenin subunits link together and form heterogeneous mixtures of polymers by cysteine residues and disulfide bonded linkages of polypeptides.The glutenin proteins, therefore, are among the largest protein molecules in nature, with molecular weights of up tens of millions (Wrigley, 1996): the actual sizes of the largest glutenin polymers have not yet been accurately determined because of their enormous sizes. Another reason for the difficulties in the size determination of glutenins is their poor solubility in conventional buffers. The glutenin subunits are released from glutenin by disulphide reducing agents such as β-mercaptoethanol or dithiothreitol. Differences in glutenin subunits size, polarity, and number of cysteine residues influence the ability to form disulfide bonds necessary for building up the glutenin polymer structure. This variation in glutenin subunits is a critical factor in determining bread dough end-product quality, particularly through its influence on polymer size distribution (Kasarda, 1999). Glutenins apparently impart to dough its property of resistance to extension. The HMW-GS make doughs elastic and allow them to trap the gas bubbles produced by yeast and to rise (Cornish et al., 2006).

2. Health Effects of Gluten Proteins

Gluten is one of the most commonly used proteins in the food industry. Its characteristic properties make it an essential ingredient for the preparation of high quality dough, hence its popularity in the food (baking) industry. However, some wheat gliadin proteins are strong allergens that may cause various symptoms of food allergies and baker's asthma. The most immunoreactive ω-5 gliadin fractions are the main allergens in wheat dependent exercise induced anaphylaxis (WDEIA). While the allergenicity of ω-5 is quite well understood, knowledge about α, β, γ and ω-1.2 gliadins is much more scanty. Results of Waga et al. (2011) show ω-1.2 gliadins to be almost as immunorective as ω-5. The α-, β- and γ-gliadins also recognise specific IgE antibodies, but their binding capacity is only about half that of ω-fractions.

Apart from their role in dough quality, gluten proteins can affect health in genetically susceptible individuals. Many gluten proteins contain T-cell stimulatory epitopes that can cause celiac disease (CD; gluten intolerance) (Sollid, 2002). Genetic predisposition based on specific alleles of the human leucocitary antigen (HLA-DQ2 and HLA-DQ8) is a prerequisite for the mechanism of CD pathogenesis (Briani et al., 2008). After consumption of gluten proteins from wheat, rye or barley, the epitopes trigger an immune response that causes damage to the small intestine. CD-patients are therefore restricted to a lifelong gluten-free diet.

In many circles, a zero tolerance approach to gluten in GF foods is considered impractical. With derivatives of wheat, and to a lesser extent barley, used widely in mainstream food channels, GF foods are susceptible to contamination, even when produced in dedicated facilities. CD is an inflammatory disorder of the small intestine resulting in a wide variety of chronically symptoms (diarrhoea, bowel pain, headache, growth retardation, osteoporosis, infertility, lymphoma, etc.) in about 1% of the wheat consuming world population.

Among the different gluten epitopes that have been identified, the α-gliadin epitopes are considered the most immunogenic (Camarca et al., 2009). In HLA-DQ2.5-positive adults, responses to the α- and ω-gliadin-derived peptides are dominant, while responses to the γ-gliadins and LMW glutenins are (much) less frequently observed (Vader et al., 2002). In this respect, it is significant that the immunodominant α- and ω-gliadin peptides contain four proline residues, while the γ-gliadin peptides have two or three and the LMW glutenin peptides only one or two. It has been shown that the proline-rich nature protects gluten peptides from degradation in the gastrointestinal tract so that they will persist (Shan et al., 2002), increasing the chance that they will bind to HLA-DQ and trigger T-cell responses.

It is therefore tempting to speculate that this favours the survival of the particularly proline-rich α- and ω-gliadin peptides while the less proline-rich γ-gliadin and LMW glutenin peptides are degraded more rapidly, thus explaining the immunodominance of the α- and ω-gliadin peptides. Gliadin peptides, with their high proline and glutamine contents, are perfect substrates for human tissue transglutaminase reaction of TG2, which is critical for the creation of active T-cell epitopes involved in CD (CD epitopes) (Molberg et al., 1998). An unresolved issue is the immunogenic nature of the HMW glutenins. To date, only one immunogenic peptide has been found in these proteins and this peptide is only recognised by T-cells from HLA-DQ8-positive patients (Wal et al., 2000).

Therefore, more work are needed to establish if the HMW glutenins pose a problem to patients, an issue that is relevant as the HMW glutenins in particular are essential for the generation of high quality dough. Many CD patients may tolerate wheat varieties with very low amounts of T-cell stimulatory epitopes (Vader et al. 2003).

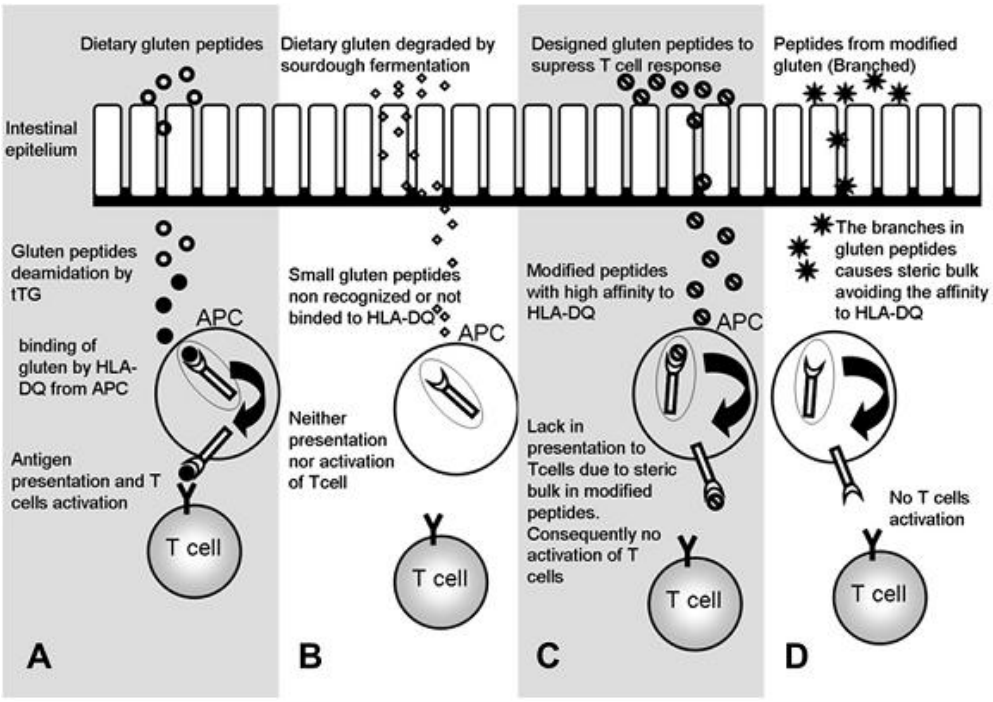

Figure 4. Adaptive immune pathogenesis mechanism in CD (part A) and different ways to avoid the T cell activation by gluten peptides modifications (parts B, C and D) (from Cabrera-Chávez and Calderón de la Barca, 2010). Abbreviations: tTG: tissue transglutaminase; APC: antigen presenting cell.

The reduction of the amount of the major T-cell stimulatory epitopes in food will especially benefit children in whom the onset of CD may be delayed or even prevented, and in non-diagnosed CD patients (the vast majority of all CD patients) to strongly reduce their symptoms. This means that breeding for wheat with considerably reduced T-cell stimulatory epitopes is to be considered as a serious option. Currently, the only effective treatment for CD is the strict lifelong renunciation of gluten-containing foods, although alternative treatments, such as oral doses of microbial endopeptidases to degrade wheat peptides, are under trial (Ehren et al., 2008).

Beyond therapeutic treatments, attempts are being made to modify the immunogenic sequences (epitopes) of gluten proteins to prepare foods for CD patients. These include long-time fermentation by sourdough, as well as enzymic modification to ensure that the epitopes are no longer recognised by the immune system of CD patients (Cabrera-Chávez and Calderón de la Barca, 2010).

Adaptive immune pathogenesis mechanism in CD and different ways to avoid the T cell activation by gluten peptides modifications is shown in Figure 4. In view of the fact that gluten is the major structure-forming protein present in wheat bread, it is a challenge to produce high-quality gluten-free bread.

Therefore, ingredients that have the ability to mimic the properties of gluten are generaly used. According to Guarda et al. (2003), the utilization of polymeric substances such as xanthan gum or hydroxypropylmethylcellulase (HPMC) is requiredfor the production of gluten-free bread.

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