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Structure and functionality of wheat gluten proteins

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ABSTRACT

Wheat gluten proteins consist of gliadins and glutenins. An appropriate balance in the amount of these two major protein components of gluten is required for achieving the desired end product quality. Variation in the composition and physical properties of the glutenin polypeptides appear to be largely responsible for the differences in the gluten viscoelasticity of wheat varieties. Using improved protein separation and purification techniques, physical methods and genetic engineering, it has now become possible to understand the structure-functional relationship of wheat gluten proteins, but much remains to be explored in the years to come.

Introduction

Among plant crops, only wheat flour, and to limited extent rye flour, has the ability to form dough that retains gases and produces a good quality baked product, particularly leavened bread, with the desired eating quality. A number of research workers have made considerable efforts over many years to elucidate which constituents of wheat is responsible for the end product quality. There is currently an increased need to understand the relationships between gluten protein composition and grain/flour functionality. This arises first from more sophisticated processing brought about by automation, with the resulting demands for compliance with strict-limits for properties of ingredients. Second there is an increasing range of food products, all with special requirements. Research into the basis of functionality can be applied directly to optimize processing in the commercial situation or more indirectly to wheat varieties having properties that match specific end-use requirements.

Composition of wheat gluten proteins

The gluten forming proteins, which represent 80-90% of the total proteins of wheat flour, have been classified into two major groups, viz., gliadins and glutenins, based on their extractability and unextractability, respectively, in aqueous alcohol. The gliadin and glutenin fractions can be divided into subclasses. The gliadins are usually separated by lactate polyacrylamide gel electrophoresis (PAGE) into four main sub-categories, α-, β-, γ- and ω-gliadins in decreasing order of electrophoretic mobility under acidic conditions and increasing order of relative molecular mass. Amino acid sequencing has revealed that the α- and β-gliadins are structurally closely related polypeptides and, therefore, both of these gliadin polypeptides have been classified into one group, i.e. α-type gliadins. The relative molecular masses of α-type and γ-gliadins are between 30000-45000, whereas ω-gliadins generally have relative molecular mass of 44000-80000. The γ-gliadins, in general, have somewhat higher relative molecular masses than α-type gliadins.

According to sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions, the glutenin fraction can be divided into two sub-categories, i.e. low and high molecular weight subunits. The high molecular weight (HMW-GS) glutenin subunits, also known as A subunits are the largest polypeptides and contain the highest level of glycine residues among gluten proteins. Their molecular weights measured by SDS-PAGE range from 80000-160000. The HMW subunits are further divided into x-type and y-type. The x-type subunits are somewhat large polypeptides, while the y-type subunits are smaller. The molecular weights of LMW subunits measured by SDS-PAGE range from 30000-51000. The HMW-GS accounts for about 5-10% of the total protein. The LMW-GS most closely resembles γ-gliadins in sequence and comprises about 20-30% of the total protein. Three to six HMW-GS and 15-20 different LMW-GS proteins are recognized in 1 and 2D gels of hexaploid wheat.

Structural properties

Comparison of amino acid sequences of purified individual gluten proteins sequences reveals that all types of gluten protein polypeptieds, in general, have at least two or three distinct structural domains, i.e., a central repetitive domain, flanked by non-repetitive C-terminal and N-terminal domains [1]**.** The S-rich prolamins are characterised by

a shorter repetitive domain and a longer C-terminal domain compared with HMW prolamins and S-poor prolamins. S-rich prolamins have their cysteine residues in the C-terminal domain, the only exception being the LMW subunits of glutenin, which also contain one cysteine residue in the N-terminal domain. S-poor prolamins have no cysteine residues, as indicated from ω-secalin of rye, a homologue of wheat ω-gliadins. On the contrary, HMW prolamins have the majority of their cysteine residues (3 and 5 in x-type and y-type subunits, respectively, in the N-terminal domains and only one in the C-terminal domain [2]. Several y-type subunits and x-type subunit 5 contain an additional cysteine residue in the central repetitive domain.

Total numbers and relative positions of cysteine residues are important for polymer size and the different polymerisation behaviour of gliadins and glutenin subunits. Inter-molecular disulphide linkages are considered responsible for giant size of glutenin polymer. According to Ewart [3] the subunits of glutenin are cross-linked in a head to tail fashion to form a linear macropolymer of glutenin known as 'concatenations'. Based on Ewart's findings and understanding of the structure of glutenin subunits, in particular HMW glutenin subunits, many hypothetical models for glutenin polymer have been proposed. All models propose a central linear polymer of either HMW glutenin subunits only (e.g. model of Graveland et al., [4] or a mixture of HMW and LMW subunits of glutenin [5]**.** Some studies have shown that gluten protein polymers have a wide range of size distribution, ranging from dimers to polymers with molecular weights up to millions. Wrigley [6] proposed a model that shows the interaction of glutenins with gliadins and non-gluten components, such as lipids and starch, which appears to explain the complete model of the gluten network.

Molecular basis of gluten viscoelasticity

Wheat flour dough is largely composed of starch, proteins, lipids, water and air cells. If the gluten proteins are removed from the flour, then the property of forming a viscoelastic dough is lost. It is generally agreed, therefore, that the gluten proteins form the framework of the dough structure. When flour is wetted, the formation of gluten is mainly caused by a complex interaction between the endosperm storage proteins, viz. gliadins and glutenins. Usually the elastic properties of gluten are ascribed to the glutenin fraction, whereas the viscous properties come from the gliadin fraction. It is understood that the gluten of wheat owes its unique viscoelastic behaviour to an appropriate balance in the amounts of gliadin and glutenin proteins, but variation in the composition and elastic properties of the glutenin proteins appear to be largely responsible for the differences in the gluten viscoelasticity among wheat varieties. For this reason, it is widely believed that the viscoelasticity of gluten is controlled by the glutenin proteins.

The ‘linear glutenin hypothesis’ proposed by Ewart [3] has received wide acceptance in explaining the viscoelasticity of gluten and flour doughs. This hypothesis has passed through different stages of sophistication. According to Ewart, the glutenin polypeptide chains form long linear ‘concatenations’ with two S-S bonds connecting each chain in a head-to-tail fashion to the next chain. The ‘concatenations’ of glutenin molecules are assumed to participate in the formation of a three-dimensional entangled network structure. The entanglement may be purely physical and/or at the point of entanglement, known as nodes, non-covalent interactions might occur to form cross-links (branching). The branching of glutenin polymers by S-S bonds is also not ruled out. The effectiveness of entanglement and cross-linking will depend on the length of the glutenin ‘concatenations’, which in turn depends on the number and distribution of covalent bonds and non-covalent interactions.

Under stress conditions, the entangled and non-covalently cross-linked structure in glutenin offers resistance to deformation, which is manifested in increased elasticity (elastic modulus, G′) during controlled stress rheometry. It also enables glutenin polymers to recoil after stress conditions are withdrawn. Viscous flow depends predominantly on molecular slippage at nodes and the labile nature of weak secondary forces acting between the glutenin polymers. Sulphydryl-disulphide bonds interchange and mechanical scission of S-S bonds may also contribute to viscous flow. Mechanical scission of S-S bonds probably occurs when the rate of deformation exceeds the rate at which molecular slippage or SH/SS interchange can occur resulting in glutenin polymers being subjected to stress values more than their elastic limit. The viscous nature of gliadins may be attributable to the absence of effective entanglements owing to their low M_rs. Gliadin polypeptides help molecular slip acting analogously to ball bearings.

Spiral structure hypothesis has also been suggested to explain the elasticity of glutenin [7]. This hypothesis is based on the structural analogy of high M_r subunits of glutenin to the mammalian connective tissue protein, elastin, in which the elasticity is attributed to the presence of β-Spiral structure. Circular dichroism spectroscopy and structural prediction studies have indicated that the central repetitive domain of high M_r glutenin subunits are rich in β-turns.

The β-turns are hypothesised to occur so frequently and regularly that they have been suggested to form a β-spiral structure, as in elastin. The β-spiral structure is assumed to be intrinsically elastic. It has been suggested that under stress conditions, the β-spirals undergo deformation and on release of stress, the β-spiral structures resume their original energetically favourable conformation. Experimental evidence for the β-spiral conformation of the high M_r glutenin subunits has been obtained using scanning tunnelling microscopy of a purified high M_r glutenin subunit (1Bx 20) from durum wheat. There is no direct evidence as yet for the elastic nature of β-spiral, however. Some experiments have shown that the high M_r subunits of glutenin are not elastin-like in their interaction with water, however. It has also been argued that the spiral structure of high M_r of glutenin is based on γ-turns rather than β-turns. Thus, there is still a need for a more complete structural model to explain the viscoelastic behaviour of gluten and flour doughs.

Importance of gluten viscoelasticity in gas retention and bread making

Understanding the structure of the gluten proteins, and how they interact with themselves and other flour constituents to convert a wheat flour into a viscoelastic system, still largely remains a challenging problem. Also, much remains to be learned about how a viscoelastic dough retains gas and how the expanded dough is transformed into bread. However, some progress has been made in this area of research, and in the discussion that follows the role of gluten proteins in gas retention is explained. The discussion below, although based on available evidence, may be somewhat speculative. From a colloidal point of view, in a mixed flour-water dough the hydrated gluten proteins form the continuous phase with starch and air cells as the discontinuous phase embedded in it and the yeast cells are dispersed throughout the aqueous dough phase. The 'free' water in the dough represents the aqueous dough phase, which dissolves the water-solubles and, acts as the medium for chemical reactions and for dissolving CO_2 up to its saturation point.

Yeast ferments sugars and continuously produces CO_2 in the aqueous dough phase. When the aqueous dough phase is saturated with CO_2, most of the CO_2 diffuses into the air cells that are formed in the dough during mixing. This is attributed to the fact that the rate of diffusion of CO_2 in the dough and hence its ultimate evaporation into the surrounding atmosphere is slow. This is due to the presence of continuous gluten protein film, pentosans and lipids. The diffusion of CO_2 into gas cells increases the pressure within gas cells that provides the driving forces for dough expansion. The viscous flow properties of dough owing to its monomeric proteins (mainly gliadins) allow the gas cells to expand. The expansion of gas cells releases the pressure within gas cells, however, a little pressure does exist in the dough system during fermentation. The pressure in gas cells has been reported to be slightly greater than the atmospheric pressure, i.e. about 1.01 atmosphere. This small over-pressure results from the surface tension at the gas dough interface and the resistance of dough to deformation, i.e. expansion.

During baking, the temperature increases, water evaporates from the liquid dough phase into the gas cells, CO_2 and ethanol produced by the yeast and dissolved in the liquid dough phase also diffuse into the gas cells, which together result in an overall increase in the pressure within the gas cells. The viscous components, i.e. monomeric proteins (mainly gliadins) allow gas cells to expand to equalise the internal gas cell pressure, whereas the elastic components, i.e. polymeric proteins (mainly glutenins) provide strength to prevent the gas cells from over expanding, and thus preventing the rupture of the gluten film network enveloping the gas cells. In a flour-water dough containing insufficient amounts of good quality polymeric proteins, the gluten network would be too extensible, allowing uncontrolled expansion of gas cells, which would ultimately rupture the continuous gluten film network and, thus continuity between gas cells would be established. The continuity between gas cells or the discontinuity in the gluten film network is seen as pinholes at the dough surface. This results in the rapid loss of CO_2 even at very low temperature during baking and very early end of the oven spring. Consequently, bread of low loaf volume and dense open crumb is produced. On the other hand, good quality flour-water dough with enough strength retains CO_2 until more advanced stages of baking, i.e. ~70°C. This produces a good bread loaf volume and even crumbs texture on completion of baking.

Differences in the loss of CO_2 between poor and good quality flour doughs are not related to changes in the starch or to changes in the gluten proteins but appear to reside in the inherent differences in the gluten proteins. The ratio of monomeric to polymeric proteins and composition as well as properties of glutenin subunits in flours appears crucial to the gas retention and dough expansion. Dynamic rheological studies showed that gluten-water doughs from poor quality flours had lower elasticity and greater viscosity than those from good quality flours [8]. The rapid loss of CO_2 at ~70°C is assumed to be the result of the increased elastic character of the dough due to starch gelatinisation and

polymerisation of the glutenin proteins via disulphide cross-linking. This leads to a sharp increase in the tensile stress in the dough system, thus causing rupture of the gluten film network at the gluten-starch interface and subsequently loss of gas retention. Rupture of the liquid film surrounding the gas cells has also been considered to contribute to the rapid loss of gas during baking. The liquid film may have an additive effect with the gluten film network in gas retention.

Microstructural analysis of wheat gluten

Scanning Electron Microscopy (SEM) was used to visualize the microstructure of gluten samples. Micrograph of good bread quality wheat variety revealed that the gluten proteins from this variety showed a foam like matrix (Fig. 1 AI), whereas gluten of poor bread quality wheat variety (Fig. 1 AII) demonstrated extensively open gluten matrix. Open, non uniform structure of gluten proteins of poor bread quality wheat may be held responsible for poor gas retention capacity and hence lesser bread loaf volume.

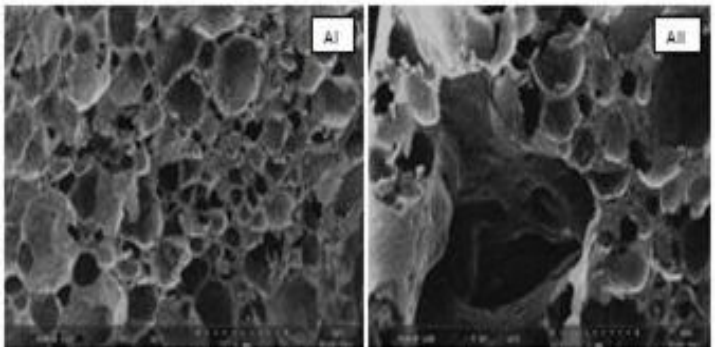

Figure 1: Scanning electron micrographs of glutens good (A1) and poor (AII) bread quality wheat varieties

IR spectroscopy characterisation of gluten proteins

Proteins exhibit three characteristic absorption bands in the mid infra-red spectrum designated as the amide I (1580-1720 cm^{-1}), amide II (1480-1580 cm^{-1}) and amide III (1430-1480 cm^{-1}) bands and the positions of these bands are sensitive to protein secondary structure. The most sensitive spectral region of protein is the amide I band, which mainly arise due to amide carbonyl stretching of peptide linkages. The frequencies of amide I band components are found to be closely related to secondary structural element of proteins. A peak centred between 1658-1650 cm^{-1} is associated with α-helical and random structure, the shoulder at ~1668 cm^{-1} is associated with β-turns and may also be related to glutamine side chains. Bands at 1612 cm^{-1} and 1633 cm^{-1} are assigned to intermolecular and intramolecular β-sheet structure, respectively. It was observed that relative intensities of β-sheet at 1633 cm^{-1} and 1612 cm^{-1} were higher for good bread quality wheat variety HI 977 as compared to poor bread quality variety C 306 (Fig. 2A). It was also noticed that β-turn intensity at ~1668 cm^{-1} for gluten was also greater in good bread quality wheat variety HI 977. Hence, it can be presumed that the gluten of good bread making wheat variety HI 977 was more elastic and stable as β-turn and β-sheet structure of gluten is considered to be related to elasticity and stability in gluten, which is an essential property of gluten for good bread making.

The conformations of glutenin fractions of good and poor bread quality wheat varieties HI 977 and C 306 respectively are shown in Fig. 2B. It was perceived from the results that good bread quality wheat variety HI 977 had more pronounced β-turn intensity at 1665 cm^{-1} indicating that glutenin fraction of this variety is very rich in β-turns, which has been related in past to the high molecular weight glutenin subunits present in glutenin [7]. β-turn structure of HMW-GS organizes to give a regular β-spiral structure. Such behaviour is important to bread quality as the ability of glutenin to form viscoelastic gluten and a gluten film network is essential for gas retention. Furthermore, glutenin is rich in highly polar amino acids, in particular glutamine. Glutamine, acts as an H-bond donor and acceptor. Therefore, it participates in intra-molecular H-bond formation with other donor and acceptor amino acids as well as itself. It has been observed that a large amount of glutamine facilitates formation of β-structures in synthetic polypeptides of glutamine. In this study, it was observed that the glutenin fraction of poor bread quality wheat variety C 306 had weaker intensity for β-turns (band shift from 1665 to 1660 cm^{-1}) suggesting shorter β-turn region, leading to less elastic region of glutenins and hence poor bread making potential. These conformational and network structure

differences in glutens and glutenins of good (HI 977) and poor (C 306) bread quality wheat varieties may be held responsible for the variations in the bread making performances between the flours of these two varieties.

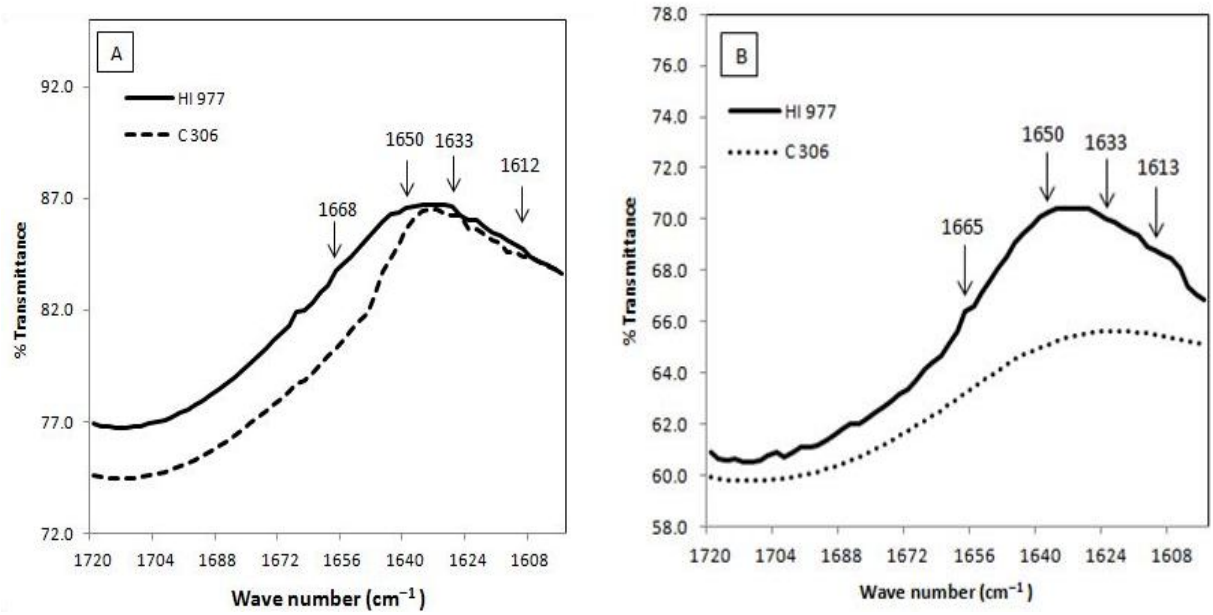

Figure 2: Amide I region for FTIR spectra of glutens (A) and glutenins (B) of good (HI 977) and poor (C 306) bread quality wheat varieties

Associations of wheat gluten proteins with functional properties

It has long been recognized that the mixing properties and bread quality of wheat flours are governed by the quantity and quality of their proteins. In a study conducted by Khatkar and co-workers [9] indicates that the mixing characteristics of flours from wheat varieties in the medium-strong range are significantly influenced by their gluten protein contents. On the other hand, the mixing properties of 'extra strong' or weak flours are relatively less affected by their gluten protein contents, but gluten protein quality primarily controls their behaviour during mixing. Weak gluten develops quickly, where as strong and 'extra strong' glutens need a longer time to mix to peak dough development (PDF). This clearly suggests that the mixing requirement of flour is related to its gluten protein composition. It has been demonstrated that gliadin fraction generally decreases mixing time, whereas certain glutenin fraction increases the mixing requirements of a wheat flour. Addition of different gliadin subgroups to a base flour decreases mixing time in the order ω- > γ - > β- > α-gliadins in accordance with the decrease in their molecular size and \ or the increase in their charge [10].

The type and composition of HMW glutenin subunits have also been considered important for determining the mixing characteristics and bread making performance. It is generally considered that subunits 5+10, 17+8, 1 and 2* are related to longer mixing times, dough strength and superior bread quality, whereas subunits 6+8, 2+12, 3+12 and 20 are related to shorter mixing times and dough weakness [2,9]. Both quantitative and qualitative effects may contribute to quality difference associated with specific HMW glutenin subunit alleles. According to Gupta and MacRitichie [11], the alleles at the *Glu-B1* and *Glu-D1* loci produce HMW subunits (17+18 and 20 or 5+10 and 2+12) in similar amounts. Their results indicate that the qualitative differences in HMW subunits may be responsible for the differences in the mixing characteristics and bread making quality among wheat varieties. This is consistent with the previous observations that the superior subunit 5 has one additional cysteine residue and has a greater molecular mass than the inferior subunit 2. The superiority of the subunits 17+18 over subunit 20 in the case of genome B has been attributed to the number and position of the cysteine residues. Furthermore, on the basis of amino acid sequence differences in the regions towards the C-terminal ends of the repetitive domains of HMW glutenin subunits 10 and 12, it has been postulated that subunit 10 may form a more regular beta-spiral structure than subunit 12, and that this in turn may lead to improved elastic properties [2]. Beta-spiral structure has been proposed to be intrinsically elastic.

It has been argued that it is the quantity of glutenin subunits and not the composition that is important. Studies have been reported in which the HMW subunit pairs 5+10 and 2+12 failed to show the expected differences in dough strength and bread making performance in many genotypes. Bread wheat, such as Egret, contains superior subunit pair 5+10 yet produces weak dough, whereas inferior subunits 2+12 are found present in many Australian good bread making quality wheats, for example, Cook, Gabo, Oxely and Timgalen and also in the European wheats, such as Courtot, Hardi and Capitole [9].

Conclusion

Wheat gluten consists mainly of the storage protein of wheat endosperm, i.e. gliadins and glutenins. Research work has confirmed that the elastic properties of gluten are due to the glutenin fraction, whilst the viscous properties come from the gliadin fraction. In the past genetic lines have proved to be powerful tools in investigating the effect of specific high M_r subunits on wheat dough and gluten functionality. Although it is now evident that the high M_r subunits of glutenin play a significant role in gluten viscoelasticity and breadmaking performance, the basis of the differential effect of various glutenin polypeptides on functionality of a wheat flour remains to be defined. Also, little is known concerning the role of low M_r glutenin subunits and gliadin subgroups in gluten viscoelasticity and breadmaking potential. Therefore, further research is needed in this area as well.

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