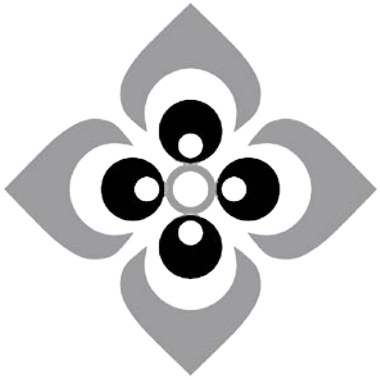

Consortium for Educational Communication

Module
on
Gluten In Bread Making

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TEXT

Introduction

Gluten is defined as “a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, which is insoluble in water. Gluten proteins are storage proteins and occur exclusively in the starchy endosperm of the grains and make up around 70-80% of total grain proteins. The remaining grain proteins are water- and salt-soluble albumins and globulins, mainly enzymes and enzyme inhibitors, and insoluble structural proteins such as membrane and lipoproteins. Traditionally, gluten proteins have been divided into two fractions based on the differences in solubility, prolamins and glutelins. The prolamin fraction contains mainly monomeric proteins which are insoluble in water and salt solutions but soluble in aqueous alcohol (e.g., 60% ethanol or 50% propanol). Whereas glutelins are polymerised by interchain disulphide bonds and are insoluble in water, salt and aqueous alcohol solutions. They are only partly soluble in diluted acids and bases or solvents containing detergents or disaggregating agents. Wheat prolamins are called gliadins and glutenins, barley prolamins are hordeins, rye prolamins are secalins, oat prolamins are avenins, maize prolamins are zein, rice prolamins are oryzins and kafirins are the prolamins of millet and sorghum. Gliadin and glutenin are known to impart entirely different physical properties to the gluten network in dough. Gliadin behaves mainly as a viscous liquid when hydrated and imparts extensibility, allowing the dough to rise during fermentation, whereas glutenin provides elasticity and strength, preventing the dough from being over-extended and collapsing either during fermentation or in baking.

Classification and structural features of gluten protein

According to Osborne fractionation (1907), grain proteins on the basis of their solubility

can be divided into albumins (water soluble), globulins (salt soluble), prolamins (alcohol soluble), and glutelins (alkali soluble). Albumins and globulins contain higher quantity of tryptophan & arginine whereas glaidins and glutelins are rich in glutamine, proline & phenyl-alanine. Amino acid sequencing indicates that the storage proteins of wheat, rye, barley, and oats (only prolamins) are closely related and differ from those of other cereals. Based on homologous sequences and similar molecular weights, they can be divided into three groups: (1) the high-molecular-weight (HMW) group; (2) the medium-molecular weight (MMW) group; and (3) the low-molecular-weight (LMW) group. Shewry and colleagues have proposed an alternative classification in 1986 that reflect biological, chemical and genetic relationships among component polypeptides of the gluten complex. They divided gluten proteins into three main categories namely: sulphur-poor prolamins, sulphur-rich prolamins and high molecular weight (high M_w) prolamins. All these gluten protein groups are considered typical prolamins because they are rich in proline and glutamine amino acid residues and are extractable, at least partially, in aqueous alcohol, particularly after addition of reducing agent.

Gluten proteins

Monomeric gliadins | Polymeric glutenins

ω-gliadins | α-type gliadins | γ-gliadins | Low M_w | High M_w

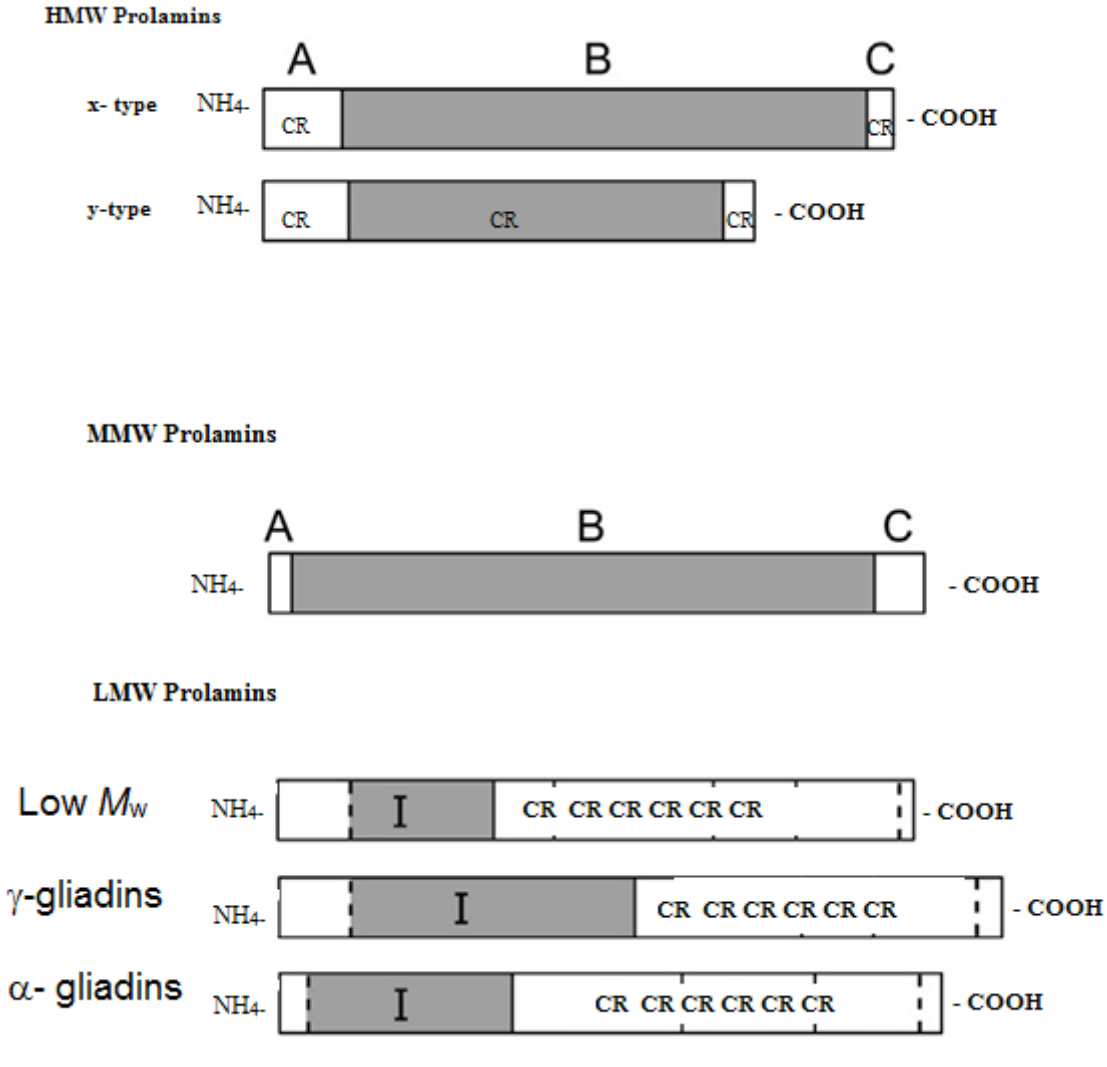
S-poor prolamins (MMW) | S-rich prolamins (LMW) | HMW prolamins

Fig.1. Classification of gluten proteins by Shewry & colleagues

The high-molecular-weight (HMW) prolamins contain three protein types: HMW glutenin subunits of wheat, HMW-secalins of rye, and D-hordeins of barley. Oat proteins are missing from this group. Proteins of the HMW group consist of around 600- 800 amino acid residues corresponding to molecular weights of around 63,000 - 88,000. The amino acid compositions are characterised by high contents of glutamine (26-36%), glycine (16-20%), and proline (10-15%), which account for around 60% of the total amino acid content. The high molecular weight (HMW) subunits are further divided into x-type and y-type. The x-type is somewhat larger polypeptides (M_w 83,000-88,000) while the y-type is smaller (M_w 67,000-74,000). DNA sequencing technique revealed that all types of gluten protein polypeptides have at least two or three distinct structural domains: a central repetitive domain, flanked by non-repetitive C- terminal and N-terminal domains. HMW prolamins have the majority of their cysteine residues in the N-terminal domains and only one in the C-terminal domain. Several y-type subunits also have one additional residue in the central repetitive domain towards the C-terminal end, and x-type subunit has one residue close to the N-terminal end of its central domain. The medium-molecular weight (MMW) group which is sulphur poor prolamines consists of the homologous ω1,2-gliadins of wheat, ω-secalins of rye, and C-hordeins of barley. These proteins consist of 320-380 amino acid residues, which correspond to molecular weights of around 40,000. Beside ω1,2-gliadins, wheat contains unique ω 5-gliadins with around 420 residues and molecular weights of approximately 50,000. These type of proteins are also absent in oats. MMW group contain high contents of glutamine (37-53%), proline (20-29%), and phenylalanine (9-10%), which together account for about 80% of total amino acid residues. S-poor prolamins have no cysteine residues. The LMW group (sulphur rich prolamines) contains monomeric proteins including α/β and γ-gliadins, γ-40k-secalins,

γ-hordeins, and avenins. Their sequences consist of approximately 300 amino acid residues corresponding to molecular weights of around 28,000-35,000. The amino acid composition is dominated by glutamine (28-36%) and proline (11-22%) and high contents of hydrophobic amino acids such as leucine (5-9%) and valine (5-8%). The S-rich prolamins are characterised by a shorter repetitive domain and a longer C-terminal domain compared with high M_w prolamins and S-poor prolamins. S-rich prolamins have their cysteine residues in the C-terminal domain, the only exception being the low M_w subunits, which also contain one cysteine residue in the N-terminal domain.

Total numbers and relative positions of cysteine residues are important for polymer size and the different polymerisation behavior of gliadins and glutenin subunits. The presence of cysteine residues in the form of intramolecular disulphide bonds in gliadins and inter- and intramolecular disulphide bonds in glutenin subunits is due to the fact that gliadins have even numbers of cysteine residues whereas glutenin subunits have odd number of residues within a single structural domain. It is the intermolecular disulphide linkages which are considered responsible for the gaint size of glutenin polymer. It has been suggested that intermolecular sulphur –sulphur bonds are limited i.e., only two per polypeptide chain connecting each chain in a head –to-tail fashion to the next chain, thus forming a linear macropolymer of glutenin known as “concatenations”.



Schematic representation of gluten protein

Bonds involved in the gliadin-glutenin interaction

During the early stage of dough mixing, glutenin interacts with gliadin to form gluten, the viscoelastic matrix of the dough. Several chemical bonds are involved in the development of the optimum dough structure. Disulphide bonds are the principal covalent bonds within and between gluten polypeptides. Non-covalent interactions include ionic, hydrogen and van der Waals interactions. Gliadins have either intramolecular (as

in α-type and γ-gliadins) or no (ω-gliadins) disulphide linkages, whereas in glutenin there are both inter- and intramolecular disulphide linkages. Disulfide bonds play a key role in the formation and development of dough. They form strong cross-links within and between polypeptide chains, thereby stabilizing hydrogen bonds and hydrophobic interactions. During dough formation and development, disulfide bonds can be mobilized through disulfide-interchange reactions. The interchange reaction requires a mobile (soluble or low-molecular-weight) sulfhydryl-containing substance to initiate the series of disulfide interchanges resulting in extensive polymerization of gluten proteins. The total number of S-S bonds do not change, only their location in the glutenin molecule is altered. Hydrogen bonds are formed as a result of the affinity of hydrogen atoms in hydroxyl, amide or carboxyl groups for oxygen in carboxyl or carbonyl groups. Gluten proteins are rich in highly polar amino acids, in particular glutamine. Glutamine constitutes over 33% of the amino acids present in gluten; the amide group in glutamine actively participates in hydrogen bonding and, in fact it forms two hydrogen bonds per glutamine residue. Hydrogen bonds are much weaker than covalent bonds but, because of their large numbers that act cooperatively and contribute significantly to the structure of the dough. Another unique feature of hydrogen bonds is their ability to interchange under stress and thereby facilitate re-orientation of gluten proteins. Gluten proteins also contain large proportions (~30% of total amino acid residues) of apolar amino acids, such as phenylalanine and proline, which are considered potential source of hydrophobic interactions. Their functionality is similar to that of hydrogen bonds but the overall effect is much smaller. Hydrophobic interactions differ from other chemical bonds because their energy increases with increasing temperature, which could result in increased stability during baking. Another important feature of gluten proteins is that they have a very low charge density. This

is due to their low level of basic amino acids, such as lysine, histidine, arginine and tryptophan, and also due to the fact that glutamic and aspartic acids occur mainly as amides. As a consequence of this low charge density, the wheat gluten proteins are not repelled and associate strongly by non-covalent interactions. Such behaviour is important to baking technology in that it results in the ability of gluten proteins to form viscoelastic gluten network that is essential for gas retention.

The “linear glutenin hypothesis” proposed by Ewart has received wide acceptance in explaining the viscoelasticity of gluten. 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 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). Under stress conditions, the entangled and non-covalently cross linked structure in glutenin offers resistance to deformation, which is manifested in increased elasticity. It also enables glutenin polymers to recoil after the stress conditions are withdrawn. Viscous flow depends predominantly on molecular slippage at nodes and liable nature of weak secondary forces acting between 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 occurs when the rate of deformation exceeds the rate at which molecular spillage or SH/SS interchange can occur resulting in glutenin polymers being subjected to stress values more than their elastic limits.

Gluten in bread making

Bread making process is accomplished through three basic operations i.e., mixing, fermentation, and baking. Mixing transforms the flour and water into cohesive

viscoelastic dough. Another key function of the mixing operation is the incorporation of air. Air is essential for introduction of gas cells into which the carbon-dioxide produced by the yeasts during fermentation diffuses as yeasts cannot produce new gas cells. If there is no air cell, the crumb of the final bread would be coarse with few large cells.

Several physical and chemical transformations occur during mixing and kneading of a mixture of flour and water. Under the applied shear and tensile forces, gluten proteins absorb water and partially unfold. The partial unfolding of protein molecules facilitates hydrophobic interactions and sulfhydryl–disulfide interchange reactions, which result in formation of thread-like polymers. These linear polymers in turn are believed to interact with each other, via hydrogen bonding, hydrophobic associations, and disulfide cross-linking, to form a sheet-like film capable of entrapping gas. Because of these transformations in gluten, the resistance of the dough increases with time until a maximum is reached and this is followed by a decrease in resistance indicative of a breakdown in the network structure. The breakdown involves alignment of polymers in the direction of shear and some scission of disulfide cross-links, which reduces the polymer size. The time it takes to reach maximum dough strength (R_{max}) during kneading is used as a measure of wheat quality for bread making—a longer time indicating better quality. The visco-elasticity of dough is related to the extent of sulfhydryl–disulfide interchange reactions. This is supported by the fact that when reducing agents, such as cysteine, or sulfhydryl blocking agents, such as N-ethylmaleimide, are added to dough, visco-elasticity decreases greatly. On the other hand, addition of oxidizing agents, such as iodates and bromates, increase the elasticity of the dough. This implies that gluten rich in SH and S−−S groups might possess superior bread making qualities.

Yeasts ferment sugars and continuously produce carbon-dioxide in the aqueous

dough phase. When the aqueous dough phase is saturated with carbon-dioxide, most of the carbon dioxide diffuses into the air cells that are formed in the dough during mixing. The diffusion of carbon-dioxide into gas cells increases the pressure within gas cells that provides the driving force for dough expansion. The viscous flow properties of dough owing to its monomeric proteins (mainly glaidins) allow the gas cells to expand and release the pressure. During baking, the temperature increases, water evaporates from the liquid dough phase into the gas cells, carbon-dioxide and ethanol produced by yeasts also diffuses into the gas cells, resulting in overall increase in the pressure within gas cells. The viscous component i.e., monomeric proteins – mainly glaidins allow gas cells to expand to equalize the internal gas cell pressure, whereas the elastic components i.e., polymeric proteins (mainly glutenins) provide strength to prevent gas cells from over expanding and thus preventing the rupture of gluten network, enveloping the gas cell.

Factors affecting bread making quality

Protein quantity and quality

It has long been established that bread making performance of wheat flours are related to the quantity and quality of their proteins. When the total protein content of a wheat grain increases, the total amount of gluten proteins also increases, but, the amount of the non-gluten forming proteins (i.e. albumins and globulins) changes very little. Thus, wheat of high-protein content usually has a higher proportion of gluten proteins compared with those with lower protein contents. Fractionation and reconstitution studies (i.e. experiments in which the different flour constituents are first separated and then systematically exchanged between reconstituted flours of good and poor quality) have confirmed that the gluten proteins are primarily responsible for quality

differences among wheat varieties.

Gliadin / glutenin ratio

Differences in the gliadin to glutenin ratio among wheat cultivars have long been considered an important source of inter-cultivar variation in physical properties and bread making quality. The technological significance of gliadin and glutenin in bread making has been attributed to their contribution to dough extensibility and elasticity, respectively. It has been reported that addition of complete gliadin and its subgroups (ω-, γ-, α- and β- gliadins) substantially improved bread making quality, as measured by loaf volume.

Molecular size distribution

Molecular size distribution has been considered another important factor in relation to functional properties of flour. Gel filtration chromatography studies have indicated that the glutenin fractions of flours with longer mixing times have a higher average molecular weight than the glutenin fractions of short mixing flours. Flours with short mixing times give doughs that break down rapidly during mixing and are easily extensible. Such flours are considered unsuitable for bread making. Conversely, flours with longer mixing times are noted for doughs of high mixing stability and greater resistance to extension and which perform well in bread making.

Glutenin polypeptides

Upto 20 different high Mw subunits have been identified in different bread wheats. Each bread wheat cultivar contains 3-5 high Mw subunits, which together account for about 1% of the dry weight of the mature endosperm of a wheat grain. This indicates that, although high Mw subunits are quantitatively minor, but they are functionally important

polypeptides of gluten proteins. The high Mw subunits of glutenin are encoded at the Glu-1 loci on the long arms of the chromosomes 1A, 1B and 1D. These loci are designated as Glu-A1, Glu-B1 and Glu-D1, respectively. Glu-1 quality scores are assigned to the individual subunit or subunit pair based on their values determined by the SDS-sedimentation test, an indirect measure of bread making quality. The Glu-1 scores of bread wheats usually range from 3 (for poor bread making quality) to 10 (for good bread making quality).