Impact of Biochemical Characterization of Gliadins of Indian Wheat Varieties

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ABSTRACT

Due to their gluten components which regulate their dough properties, eight varieties of durum wheat-PDW 233, MACS-9, HI-8498, A-9-30-1, Raj 911, Raj 1555 with good quality characteristics and Bijaga yellow, NIDW-15 with poor quality characteristics have been identified. The content of protein in these varieties ranged from 11.1(Yo to 13.8 percent and the content of ~-carotene from 4.1 to 5.85 ppm with maximum in PDW-233 and minimum in A-9-30-1. Only in the good quality durum varieties was a 45 kD polypeptide present. However, PCR amplification of the genomic DNA from the selected varieties using unique primers of the LMW glutenin gene showed no polymorphism between the go

Keywords – *Gliadins, Glutenin, LMW Glutenin Gene, Storage Proteins, T. Durum.*

INTRODUCTION

Wheat is one of the world's three main developed cereal crops, the other two being rice and maize. India's wheat production is forecast at 90.23 million tonnes in the 2011-12 crop year (July-June), compared to 86.87 million tonnes in 2010-11. In India, wheat is eaten in various forms as a staple food, primarily as chapati, bread, pasta, macaroni, spaghetti, cookies, pizzas, doughnuts, etc. Locally known as rava or sooji, it is also consumed as semolina to prepare various food items such as kheer, upma, sooji, halwa etc. It has been estimated that about 65 percent of wheat grain is directly used as human food, suggesting its acceptance as the main staple food and 21 percent as live stock feed, 8 percent as seed material, and 6 percent as industrial raw material for other uses. Commonly used as straw or fresh forage are the raw parts of wheat plants, the stem and leaves. Triticum aestivum (bread wheat), Triticum durum (durum wheat) and Tritium dicoccum (dicoccum wheat) are three varieties of wheat that are grown in India (Gupta, 2004). At present, hexaploid bread wheat is around 95% of the wheat grown worldwide, with much of the remaining 5% being tetraploid durum wheat (Shewry, 2009). Wheat flour consists primarily of starch (70-80%), protein (8-18%), lipids (1-2%), pentose (2%), enzymes, inhibitors of enzymes, and other minor components. The majority (78-85%) of endosperm protein is gluten, a very large complex consisting primarily of proteins known as glutenins and gliadins, respectively, polymeric (multiple polypeptide chains connected by

disulfide bonds) and monomer (single-chain polypeptides) proteins (Mac Ritchie, 1984). The significance of wheat is due to the gluten storage proteins that give dough specific viscoelastic properties.

In addition, the property of forming viscoelastic dough is lost with the removal of gluten proteins from the flour the gluten proteins have therefore been the focus of extensive studies for a time exceeding 250 years. This has shown that gluten proteins have peculiar structures and properties, making them of particular interest for studies The properties of wheat gluten that make it special are; ability to shape a viscoelastic mass, ability to form films, ability to thermoset and ability to absorb water The functions of the individual gluten components in the functionality of dough are complicated Studies have shown that stora Within glutei, the subunits of high molecular weight glutenin (HMW-GS) contribute most to variance in the consistency of baking.

In terms of quantity, the HMW-GS are minor components (5-10% of total protein; Payne, 1987), but they are key factors in the bread-making process because they are major determinants of gluten elasticity that allow efficient gas trapping for dough to rise. These proteins are genetically determined, although the relative amount and size distribution of the proteins varies as a result of the enviroleum distribution The functional and rheological properties of wheat gluten depend on the ratio of gliadins to gluten, the distribution of molecular size, the structure of glutenin polypeptides, the high/low ratio of glutenin polypeptides (Khatkar,1996), the strength of bonds between gliadins and glutenins and the reduction or oxidation activity of glutenins.

When hydrated, Gliadin functions primarily as a viscous liquid and gives extensibility, enabling the dough to rise during fermentation, while glutenin provides elasticity and strength, preventing the dough from over-extending and collapsing during fermentation. Gliadins have been commonly used for cultivar recognition in hexaploid and tetraploid wheat due to widespread polymorphism. The composition of gliadin is typical of the variety of wheat. Differences between wheat cultivars in the gliadin/glutenin ratio are considered a significant source of intercultivar variation in physical properties and consistency of bread making. There is an inverse relationship between the ratio of gliadin/gluten in and gluten elasticity. Doughs that are too elastic and inextensible offer lower performance for bread making than doughs that have an acceptable balance of elasticity and extensibility. In the banking industry, therefore, the understanding of rheological behavior and dough properties has become increasingly relevant.

OBJECTIVES

1. To study the compositional variation of gluten proteins in wheat varieties.

2. To carry out biochemical characterization of gliadins of diverse wheat varieties.

3. To investigate the relative importance of gliadins to end use qualities of wheat varieties.

Wheat Kernel Proteins

Wheat is among the world's most widely grown cereals. The wheat kernel consists of bran, germ and endosperm (Fig 1.1) Based on the classical fractionation method by Osborne (1907), wheat proteins were divided into four groups: albumins (water soluble), globulins (dilute salt soluble), gliadins (70% ethyl alcohol soluble) and glutenins (70% ethyl alcohol soluble) (soluble in dilute

acids and bases). The baking performance of a wheat variety does not depend on the composition of albumins and globulins, the non-gluten-forming proteins (MacRitchie, 1984), as their composition does not vary between different varieties of wheat.

Owing to the presence of proline and glutamine amino acid residues in their structures, the ability of wheat flour to be processed into various baked goods mainly depends on the quality and quantity of gluten proteins. Gluten proteins are also known as proclams. To determine their properties and structure, comprehensive and intensive research is being performed on gluten proteins. The wheat prolamins have been classified into two groups, gliadins and glutenins, on the basis of their solubility in aqueous alcohol solutions. Together, gliadins and glutenins constitute 80-85 percent of the total wheat flour proteins and give the wheat dough its unique properties-extensibility and elasticity. High molecular weight glutenin subunits (HMW-GS: 90 to 140 kDa) and low molecular weight glutenin subunits (LMW-GS: 30 to 75 kDa) are further fractionated into high molecular weight glutenin subunits (HMW-GS: 90 to 140 kDa) and low molecular weight glutenin subunits (LMW-GS: 30 to 75 kDa) by sodium dodecyl polyacrylamide gel electrophoresis under decreasing conditions. Although considered to be the minor components, HMW-GS are the primary determinants of gluten elasticity, which in turn have a profound impact on the bread loaf volume HMW-GS is encoded at the Glu-A3, Glu-B3 and Glu-D3 loci by genes on the long arm of chromosomes 1A, 1B and 1D. (Table 1.1).

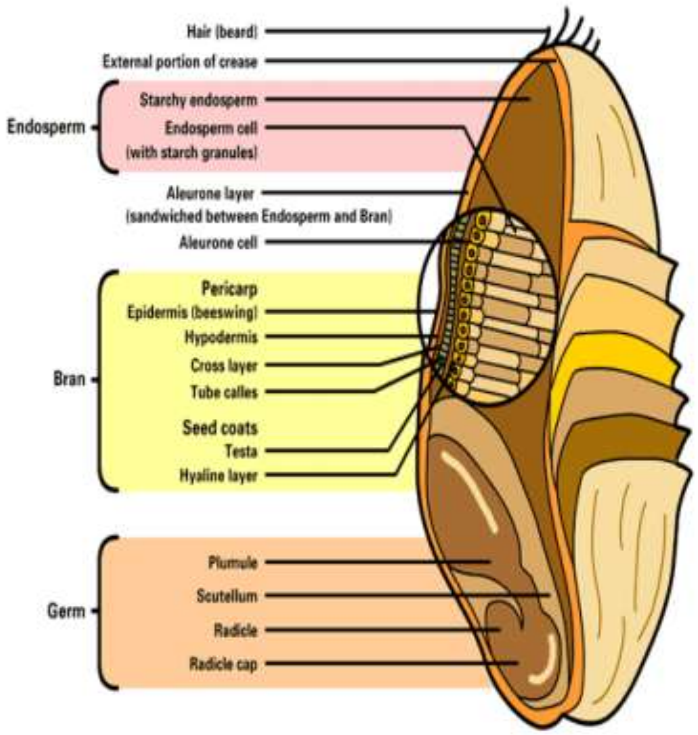

Figure 1.1 Schematic diagram of a wheat kernel

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.

Table 1.1 Wheat gluten proteins and their genetic control (Pena et al., 2002)

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

Gliadins are monomeric proteins associated with either no disulphide bonds (alpha-gliadins) or intrachain disulphide bonds (alpha-, beta- and gamma-gliadins) (Singh and MacRitchie, 2001). Gladdens serve as a viscous liquid during hydration, which gives the dough extensibility. The determination of the amino acid sequence revealed that alpha- and gamma-gliadins are both related to the LMW-GS The LMW-GS are subdivided into three classes, B, C and D, based on their mobility to electrophoresis and is an electric point. It was stated that the C and D groups consist mainly of alpha-, β—, γ- and ⁇ -gliadins mutated in the cysteine residues from these groups. Depending on their ability to form disulfide bonds, LMWGS can serve as either chain terminators or chain extenders. By forming two interchange disulfide bonds, traditional LMW-GS may act as chain extenders, while gliadin such as LMW-GS is supposed to act as chain gluten terminators in polymers by forming one interchain disulfide bond. The composition of gliadins in wheat varies from variety to variety.

As a result of this widespread polymorphism, gliadins are used for the identification of the cultivar in hexapod and tetraploid wheat. A significant source of inter-cultivar variation in physical properties and end product quality is considered to be the differences in the gliadin/glutenin ratio among wheat cultivars. There is an inverse relationship between the ratio of gliadin/glutenin and gluten elasticity. Doughs that are too elastic and inextensible offer lower bread production efficiency than doughs that have an acceptable balance of elasticity and elasticity (Khatkar et al., 2002a). Awareness of the structure, properties and rheological actions of gliadins and glutenins has thus become increasingly important in the banking industry.

Classification of Gliadins

Gliadins have been identified as heterogeneous mixtures of 70 percent aqueous alcohol soluble single chained polypeptides. They account for about half the gluten proteins and have been divided into 4 classes- α- (fastest mobility), β-, γ-, and ω-gliadins (slowest mobility) based on their electrophoretic mobility in A-PAGE at low pH According to the analysis of primary structure and molecular weights (MWs), a new classification is given as ω5-, ω1, 2-, α/β- and γ-gliadins (Wieser, 2007). Because of their structural homology, as shown by the sequencing of amino acids, alpha- and beta-gliadins were grouped under one heading: alpha-type gliadins. The

molecular weight range of gliadins is between 30,000 and 75,000. Through non-covalent interactions such as hydrogen bonding, vander Waal forces, electrostatic and hydrophobic interactions, the monomeric gliadins confer their characteristic property-viscosity. In addition, via no covalent hydrophobic interactions, gliadins can also interact with the glutenin polymers and via hydrogen bonds with the glutamine residues.

Gliadin protein-coding genes (Table 2.1) are located on the short arms of chromosome groups 1 and 6. Gli-A1, Gli-B1, and Gli-D1 and chromosome group's 6-Gli-A2, Gli-B2, and Gli-D2 loci are closely related genes located at three homologous loci of chromosome group 1. Gli-1 genes encode most γ- and ⁇ -gliadins and all the alpha-/β- and some of the γ-gliadins are encoded by Gli-2 genes (Ferranti et al., 2007). The ⁇ - and γ-gliadins encoded in the Gli 1 locus and LMW glutenin subunits are closely related. Ten novel alpha- gliadin genes isolated from Triticum aestivum L have recently been reported by Qi and colleagues (2011). Near correlations between the gliadin blocks and the sedimentation value of Zeleny have been established, which is considered to be an important criterion for predicting the bread-making efficiency of the recorded quality differences between gliadins located on chromosomes 1A, 1B and 1D and on chromosomes 6A, 6B and 6D. The association between the parameters of wheat quality and gliadins located on chromosome group 1 was, however, attributed to the LMW glutenin subunits as the genes encoding both these proteins were found to be closely related. In addition, deletions in the locus of gliadine (Gli 1) increase the strength of the dough and the percentage of polymeric proteins.

Effective separation techniques are required for the isolation and characterization of gliadins due to high structural heterogeneity of gliadins. Currently, different electrophoresis and chromatography modes are commonly used to isolate gliadins and classify wheat varieties. Electrophoresis has carried out a large number of biochemical, genetic and technological studies of gliadin proteins Electrophoregrams of gliadin proteins provide information on the identity of cultivars, protein polymorphisms, technological grain consistency, wheat flour and dough Gliadin markers are simpler and more effective tools for the identification of wheat genotypes than DNA molecules. For all polyacrylamide gel electrophoresis (PAGE) and starch gel electrophoresis techniques, pH 3.1 is commonly used. Various components of gliadin were also isolated by column chromatography for ion-exchange and gel-filtration. The use of High Performance Liquid Chromatography (HPLC) to classify wheat proteins was first reported by Bietz (1983). Capillary Zone Electrophoresis (CZE) has shown great promise for protein analysis and varietal recognition, another important technique (Bean and Lookhart, 2000; Siriamornpun et al., 2001). Because of its speed, performance, reproducibility, ultra-small sample volume and low solvent consumption, capillary electrophoresis is increasingly recognised as a significant separation technique. It overcomes some of the drawbacks of methods of gel electrophoresis. CZE has higher resolution and shorter time for review than either A-PAGE or HPLCC (Rodriguez-Nogales et al., 2006). Mass spectrometry (MS) has recently become relevant in the genomic and proteomic fields as an alternative and effective technique with the advent of 'soft' desorption/ionization methods such as electro spray ionisation (ESI) and matrix-assisted laser desorption/ionization (MALDI). In particular, the molecular masses of purified wheat alpha-gliadins have been calculated using MALDI in conjunction with time-of-flight mass spectrometry (TOFMS). In addition, MALDITOF-MS was also used during the baking process to research gliadin alteration (Sorensen et al., 2002). The direct study of bread and durum wheat

gliadins was applied to this methodological approach (Camafeita et al., 1998). MALDI-TOF is much more precise and much quicker compared to traditional methods for gluten protein separation (gel electrophoresis and reverse phase HPLC), requiring less than 1 pmol sample and just a few minutes per sample to perform the measurement (Cunsolo et al., 2003).

Alpha and Gamma Gliadins

It was stated that the average molecular mass of alpha- and γ-gliadins was 31,000 and 35,000 Da, respectively. The amino acid composition of γ-gliadins is somewhat similar to that of γ-gliadins. Both are relatively abundant in amino acid-containing sulphur, such as methionine and cysteine, but have few residues of proline, glutamine and phenylalanine. Thus, by Shewry et al., they were also labelled as S-rich prolamins (1986). The presence in the alpha- and gamma-gliadins of an even number of sulphur-rich cysteine residues contributes to the creation of intrachain disulphide bonds responsible for their folded structure, further determining the existence of non-covalent bonding. These non-covalent protein-protein (mainly hydrogen bonds and hydrophobic interactions) interactions are primarily responsible for gliadin viscosity and gluten extensibility.

Omega Gliadins

These belong to the medium-molecular-weight (MMW) group of gluten protein with their molecular weight ranging from 44,000-80,000 Da. The ω- gliadin differs from the other gliadin subgroups in its amino acid composition. have classified ω- gliadins as the S-poor prolamins as it lacks the sulphur containing amino acid (cysteine or methionine) while contains low amounts of amino acids of basic nature. Thus, it does not form disulphide bonds and interacts in dough through the hydrogen bonds Omega gliadins are reported to be more polar than α-, β- and γ-gliadins reported that nearly 80% of the amino acids 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 proline (Pro) and glutamine (Gln) rich repetitive amino acid (AA) sequences and two hydrophobic terminal non-repetitive domains containing most of the ionizable amino acids (histidine, arginine and lysine), although the latter are present only in low concentrations (Gianibelli et al., 2001). Awareness of the complete amino acid sequences of gliadin comes from cDNA and genomic DNA sequence analysis. Amides exist as glutamic and aspartic acids. The gliadin protein sequence is of primary importance since they are the key determinants of dough toxicity and functionality. Different forms of gliadins have distinct molecular-related secondary structures. Using circular dichroism spectroscopy, Tatham and Shewry (1985) analysed the secondary structures of gliadins and found that al-gliadins were rich in randomly coiled β-turns without detectable alphelix or β-sheet, but alpha/β- and γ-gliadins contained 30-35 percent alpha-helix and 10-20 percent β-sheet conformations. They also stated that al-gliadins were mainly stabilised by strong hydrophobic interactions, and alpha/β- and γ-gliadins were stabilised in their alpha-helices and β-sheets by covalent disulphide bonds and noncovalent hydrogen bonds. However, no consensus on the structure of gliadins in solutions has been reached. For gliadin molecules, in which there was a

large central void, Friedli (1996) suggested a doughnut-like structure. Gliadins, however, are still commonly regarded as following a globular protein structure in 70% of aqueous ethanol (Foulk and Bunn, 2001). But recent research shows that alpha/β-gliadins have compact globular structures, and expanded and rod-like structures have γ- and alpha-gliadins.

CONCLUSION

The study paper revealed that gliadins affect the properties of dough. Upon the addition of gliadins at various concentrations, the flour exhibited different mixing properties. Gliadins shortened the processing time of the dough and improved the dough's softening. After gliadins were introduced, the peak dough consistency was drastically reduced. Upon the addition of gliadins, the values of pasting properties have decreased. Greater gluten was retrieved from the flours added with gliadin and glutenin. The presence of gliadin proteins was negatively affected by the gluten index. The study results clearly indicated the effect of gliadins on the properties of flour. The study's results could help to improve the properties of the flour in order to improve the quality of the final product.

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