USE OF NEAR-ISOGENIC WHEAT LINES TO DETERMINE GLUTENIN AND GLIADIN COMPOSITION AND FUNCTIONALITY IN FLOUR TORTILLAS

A Thesis

by

SUCHISMITA MONDAL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Plant Breeding

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ABSTRACT

Use of Near-Isogenic Wheat Lines to Determine Glutenin and Gliadin Composition and Functionality in Flour Tortillas. (August 2006)

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The synthesis of high molecular weight (HMW) glutenin, low molecular weight glutenin and gliadin proteins are controlled by nine major loci present in wheat chromosomes. The loci Glu A1, Glu B1, Glu D1 and Gli A1, Gli B1, Gli D1 and Gli 2 and their allelic variants play important roles in determining the functional properties of wheat flour. This study focused on understanding the functionality of these protein subunits with respect to tortilla quality for use in developing varieties with ideal tortilla baking quality. Near-isogenic wheat lines in which one or more of these loci were absent or deleted were used in the study. These lines were analyzed using SSR primers to verify the chromosome deletions. A standard SDS PAGE gel and a Lab on Chip Capillary Electrophoresis method were used to confirm the protein composition of the deletion lines. Tortillas were prepared from each deletion line and the parent lines used to derive the deletion lines, and tortilla quality evaluations were analyzed. The analysis has revealed that elimination of certain HMW glutenins results in gain of function both for tortilla diameters and overall tortilla quality. The deletion line possessing 17+18 at Glu B1 and deletions in Glu A1 and Glu D1 had a gain of function in tortilla diameter, yet tortilla stability was compromised. The deletion line possessing Glu A1, Glu D1 (1,

5+10) and a deletion in Glu B1 improved both the diameters and stability of the tortillas. Presence of subunits 5+10 is important for maintaining tortilla stability. Deletions in gliadin monomeric proteins also affected the tortilla diameters and stability.

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ABBREVIATIONS

AACC – American Association of Cereal Chemists

DNA- Deoxyribonucleic acid

Gli- Gliadins

Glu- Glutenins

HMW- High Molecular Weight Glutenins

HPEC – High Performance Capillary electrophoresis

HRRW- Hard Red Winter Wheat

IPP- Insoluble Polymeric Proteins

LMW- Low Molecular Weight Glutenins

min- Minutes

mA- Milliamperes

MU- Mixograph Units

NIR- Near Infrared Reflectance Spectrophotometer

PAGE- Polyacrylamide Gel Electrophoresis

PCR- Polymerase Chain Reaction

PPP- Polymeric Protein Percent

Sarat- Saratovskaja cultivar

SDS Page- Sodium Diocyl Sulphate Polyacrylamide Gel Electrophoresis

SE-HPLC – Size-exclusion High Performance Liquid Chromatography

SKHT- Single Kernel Hardness Test

SSR- Single Sequence Repeat Primers

μL- Microliters

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CHAPTER I

INTRODUCTION

Wheat is one of the most important crops of Texas. Most of the wheat produced is sold in bulk and part of it supplied to the bread and tortilla industry. Tortilla is flat, circular, light colored bread. Wheat flour tortillas contribute significantly to the wheat market. According to the Texas Industry Association it represents a $6.1 billion dollar industry in USA in 2004 (www.tortilla-info.com). The ethnic flat bread that has been considered a Mexican specialty has now moved mainstream into the American diets and is the second most popular bread after white bread (Lovgren 2006). Tortillas are offered on 2/3rd of the menus nationwide (www.washingtonpost.com). Most of the U.S. tortilla industry is based in Texas. The wheat grown in Texas and across the Southern Great Plains is hard red winter wheat (HRWW). It has high protein levels and high gluten strength that is most suitable to bread making. While wheat protein functionality is also important for tortilla quality, most hard wheats produce poor quality tortillas. Consumers usually prefer tortillas that have acceptable appearance, taste and texture. However as the tortillas are not consumed on the day they are baked, shelf stability is an important issue in tortilla quality. As such, it has become a challenge for the tortilla producers to make tortillas with good quality attributes and shelf stability.

Most research in the last 50 yr has focused on improving wheat quality for bread production. Wheat, having the ability to form dough and exhibiting rheological properties is unique among cereal grains.

This thesis follows the style of Cereal Chemistry.

Much of cereal science research for optimal bread quality has focused on the study of wheat storage protein functionality. However, little research has focused on the storage functionality requirements for optimal tortilla quality. The critical tortilla parameters are diameter, opacity and rollability. According to George L. Lookhart, a cereal chemist at USDA, ARS, tortillas that are "about 2 mm thick evenly opaque with ample diameter and at least 3 weeks shelf life" approaches ideal. Wheat flour, the major ingredient in tortillas, contributes to this quality and shelf stability (Wang and Flores 1999a, Waniska 1999, Waniska et al. 2002). Wheat flour quality affects the dough extensibility and tortilla shelf stability that determine the tortilla processing parameters. Tortillas must resist breaking and crumbing while processing and consumption (Dally and Navarro 1999; Waniska 1999). Thus, shelf stability of tortillas is an important characteristic. The gluten network plays a major role in the shelf stability of the tortillas. Rapidly extensible dough and tortillas with a resilient gluten network are needed to retain flexibility in the baked product (Waniska 1999). The shelf life and the extensibility of the tortillas thus depend on the glutenin and gliadin proteins (Pascut et al 2004). The shelf life of tortillas are greater than the bread as tortillas retain their protein functionality and have decreased starch dispersion and firming compared to bread (Bejosano and Waniska 2004). The diameter of the tortilla depends on the extensibility of the dough (Bache and Donald 1998). The extensibility of the dough depends of the gluten protein and their interactions to form the gluten network. Thus rapid dough extensibility during hot pressing and flexibility of protein is required after tortilla baking, counter to the strong viscoelastic gluten functionality needed for bread.

The tortilla industry uses bread wheat flours and depends on the use of various chemicals and additives to increase the diameter, extensibility and the shelf stability of the tortillas. L-Cysteine is used widely to increase the diameter of the tortilla. It is a common perception in the tortilla industry that increasing the amount of leavening agents gives rise to good tortillas, however recent studies observed small or insignificant improvements by using more chemicals (Cepeda et al 2000). The additions of these compounds also have adverse effects on the taste and quality of the tortillas. The lack of research and knowledge in the gluten functionality requirements for tortilla quality is the basic problem in tortilla processing. The contribution of the wheat storage proteins to the dough and tortilla properties is unknown.

The viscoelastic nature of the gluten proteins in wheat make them unique. The gluten network is developed during the flour mixing. The gluten molecules interact during the mixing and form polymers that give rise to dough strength. Highly extensible and elastic dough properties are ideal for bread; tortilla requires the dough to be highly extensible with a much less elastic quality. The gluten molecules are grouped into the high molecular weight (HMW), low molecular weight (LMW) glutenins and gliadins and their allelic variants. The glutenins are the main component responsible for the end – use quality differences among genotypes (Weegels et al 1994). The total protein content and the glutenin and gliadin ratio also affect dough and baking properties (Uthayakumaran et al 2004). The HMW glutenins are polymeric proteins. They are minor components in terms of quantity, yet they play key factors in bread making as they are the primary determinants of gluten elasticity (Tatham et al 1985a, b). The HMW subunits of wheat gluten have been studied extensively and it has been demonstrated that allelic differences

among genotypes affects the amount and the properties of HMW polymers and the bread making properties of individual genotypes (Payne et al 1987; Shwery et al 2003). The HMW glutenin subunits influence the bread baking quality. This has resulted in the selection of particular HMW glutenin subunits to improve bread making quality. The dough mixing strength and the bread making quality are reduced when HMW glutenins levels are low. Varieties have been developed with higher gluten strength and improved bread baking qualities.

The HMW glutenins are located in long arm of chromosome 1A, 1B, and 1D. The HMW glutenins represents 5-10% of the total seed protein. The HMW glutenins are further subdivided into allelic pairs on 1B and 1D and a single subunit on 1A and each of these subunits influence wheat flour and dough quality. It has been demonstrated that the high molecular weight glutenins encoded at the Glu D1 locus (5+10, 2+12), followed by those encoded at the Glu A1 (1, 2*, nil) and those encoded at the Glu B1 locus (7+9, 17+18) are the principal components contributing to quality, based on statistical analysis of Payne et al (1987). It has also been hypothesized that the differences in the allelic pair on 1D locus (5+10 vs. 2+12) are important determinants of end-use quality. The allelic pair 5+10 has been associated with strength. In bread making strong flour is required and it has been suggested that the introduction of the HMW glutenin allelic pair 5+10 can improve the strength. Similarly the Glu B1 subunit 17+18 is strong while subunit 20 is associated with weak dough strength. The high amounts of glutamine residues present in HMW glutenins have a high capacity to form intra and inter-molecular hydrogen bonds that influence the elasticity of the dough (Gianibelli et al 2001).

While the HMW glutenins are the major determinants of bread quality, LMW glutenins and gliadins are also important. Genes encoding the LMW glutenins are present on the short arm of chromosome 1A, 1B, and 1D.The LMW glutenins are one-third of the total seed protein and 60% of the total glutenins. The HMW and the LMW glutenins form extensive disulphide linked polymers that influence the dough quality. The LMW glutenins form aggregates that may be important for dough strength. The cysteine residues in the LMW structure helps to separate two different HMW polymer-building subunits. The chain extenders (having two or more cysteine residue) allow the formation of stronger dough's, while chain terminators have the opposite effect (Greenfield et al 1998; Masci et al 1998). The chain extender proteins have increased strength and stability due to the longer repetitive domains. The polypeptides with single cysteine residue have decreased dough strength and stability as they act as chain terminators in the glutenin polymers. The reduction in proportion of LMW glutenins, results in dough properties shifting towards greater strength due to an increase in the HMW/LMW glutenin ratio (MacRitchie and Lafiandra 2001; Lawerence et al 1998). The increase in the polymeric proteins results in a stronger dough strength that is good for bread quality. In contrast the dough mixing strength is reduced in deletion lines missing the HMW glutenins. An increase in the amount of polymeric protein and better flour performance has also been demonstrated (Ciaffi et al 1995; Rogers et al 1997; Lafiandra et al 1998).

Gliadins are monomeric proteins. They are grouped into α, β, ω, γ gliadins. The genes encoding gliadin proteins are located on short arm of chromosome 1 and 6 (Wrigley and Shepard 1973). The Gli 1 loci has tightly linked genes located at the three homeologous loci on the short arm of chromosome 1, Gli-A1, Gli-B1, and Gli-D1 and in

short arm of chromosome 6, Gli-A2, Gli-B2, Gli-D2 for Gli 2 loci. The ω, γ gliadins encoded at the Gli 1 loci are tightly linked to the LMW glutenins. The α, β gliadins are encoded by the Gli 2 loci (Beitz et al 1984). The proportion of the glutenins and the gliadins in wheat are known to influence the viscoelastic properties of the wheat. Deletions in the gliadin locus (Gli1) exhibit increased dough strength and percentage of polymeric proteins (MacRitchie 1985). The reduction in the gliadin monomeric proteins increases the proportion of polymeric proteins.

The quality of end-use products of wheat is determined by the molecular structure of the storage proteins (Bhusuk 1998; Shwery et al 1999). The glutenins, high molecular weight (HMW) and low molecular weight (LMW) and the gliadin loci and the allelic variants on the A, B, and D genomes of wheat are known to determine the flour protein functionality. In an effort to understand the relationships between the wheat protein composition and functional quality near-isogenic lines have been used. Near-isogenic lines have been developed to transfer particular genes through backcrossing into a common genetic background. The lines have different useful characters with unique value and have been used in studies aspect in understanding the functional contribution of particular genes. The isogenic nature of the lines allow individual affects of the glutenins, its allelic subunits and gliadins to be studied without the confounding effects of the different genetic backgrounds. A series of near-isogenic lines developed from the cultivar Chinese Spring has been used for studying of wheat proteins (Sears 1954). A set of similar lines was developed from the cultivar Sicco by Payne et al (1987) and used for the study of wheat glutenins. The testing of the near-isogenic lines in different agro-climatic conditions can also help to understand the functionality of the individual allele's

combinations. Lawerence et al (1988) developed a set of lines in which the number of HMW glutenins varied from a full complement of five to zero. These lines in which the genes for the glutenin and the gliadin loci are absent have been used to deduce the effects on bread dough functionality of deleting or substituting specific proteins or subunits while maintaining the same genetic background (MacRitchie and Lafiandra 2001). Two important conclusions were generated from studies using these deletion lines: first, the dough mixing strength and the bread making quality are dramatically reduced as HMW glutenins are deleted (MacRitchie and Lafiandra 2001); second the allelic variation at the Glu D1 locus is associated with the dough strength and bread making quality. Near-isogenic deletion lines in the gliadin loci are also available and have been used in studies made by Gianibelli et al (1998).

The proportion of glutenins to gliadins also influence the dough properties and thus the bread making quality, however the gluten functionality needed for tortilla making differs from that needed for bread making. While bread firms and stales in a week, tortilla flexibility and rollability are exhibited for more than 3 weeks depending on flour properties, formulation and processing (Seetharaman et al 2002, Waniska et al 2004). The less heat received by the tortillas during processing help to retain the gluten functionality that is exhibited by the rollability and the flexibility of tortillas. While in bread the functionality of the proteins is masked by the retrograded starch. As such, the glutenin and gliadin functionality required to improve the quality of tortilla is different from that of bread. The specific protein composition and functionality required for tortillas however, is not yet known. Research is thus needed to define the glutenin and gliadin functionality requirements needed for optimal tortilla making. In this study near –

isogenic deletion lines were used to determine the HMW, LMW glutenins and the gliadins affecting the tortilla quality. The contributions of the individual glutenin and gliadin loci for dough processing and tortilla quality were defined. Once the glutenin and gliadins loci that contribute to the tortilla functionality have been identified, the near-isogenic deletion lines themselves can be used to develop new wheat cultivars having good tortilla quality. This can be particularly helpful to meet the needs of the growing tortilla industry. This may lower the cost of tortilla production by reducing the use of chemicals and other additives. It may also represent a premium to the farmers for the production of identity preserved wheat. The tortillas with better diameter and shelf life characteristics would also enhance the marketing and quality of tortilla.

The specific objectives of this project are:

1. Determine the functional role of the wheat storage protein glutenins in the manufacture and storage of tortillas
2. Determine the functional role of the wheat storage protein gliadins in the manufacture and storage of tortillas

CHAPTER II

FUNCTIONALITY OF GLUTENINS IN WHEAT FLOUR TORTILLAS

1. Introduction

The wheat kernel or grain is divided into three constituents, the bran, germ and the endosperm. The germ is separated from the endosperm and bran during milling. The milled endosperm fraction produces flour. The functional properties of wheat grains are dependent on the chemical composition of the grain. The chemical composition of the mature wheat grain consists of 72% starch of the total grain weight (Lasztity 1984) and 6-16% protein content. While starch is present only in the endosperm, protein is distributed in all parts of the grain. The highest amount of protein is present in the germ followed by the aleurone layer, then by the endosperm. The endosperm proteins are typically storage proteins. The wheat storage proteins are present as a continuous matrix with starch granules embedded in the matrix (Lasztity 1984). Wheat endosperm proteins were among the first proteins to be studied. The classical fractionation procedure of Thomas Osborne (1907) has been used for years to divide cereal proteins into four major groups (Wrigley and Beitz 1988). The albumins are water soluble, globulins are soluble in dilute salt solutions, prolamins are soluble in aqueous alcohols, and glutelins are soluble in acid or alkali solutions. Through many decades of study it has been demonstrated that the unique properties of wheat is due to the presence of the glutenin proteins. Beccari in 1745 reported the isolation of glutenins. The wheat storage protein genes exhibit a co-dominant Mendelian inheritance (Payne et al 1981, Gupta and Shepherd 1990). The wheat storage protein is grouped into glutenins and gliadins. Gluten is described as a bimodal distribution of the gliadin and glutenin proteins (Wrigley and Bekes 1999). In

this chapter the study will focus on the glutenins and their functionality with respect to tortilla quality.

Glutenins are among the largest proteins found in nature (Wrigley 1996). They are polymeric proteins with molecular weights more than twenty million daltons. These proteins form large groups of polymers linked by disulphide bonds. They can be grouped into two groups based on their electrophoretic mobility. The high molecular weight glutenins (HMW) have molecular weight of 80, 000- 120, 000 daltons and the low molecular weight glutenins (LMW) have molecular weight 30, 000-40, 000 daltons. The genetic loci controlling the synthesis of these gluten proteins lie on the chromosome 1 of wheat. The HMW glutenins are encoded by the Glu 1 loci on the long arm of chromosome 1. Three genetically unlinked loci Glu A1, Glu B1, and Glu D1 present on homeologous chromosomes 1A, 1B, 1D control the synthesis of the HMW glutenins (Gálová et al 2002, MacRitchie and Lafiandra 2001) (Figure1). The LMW glutenins are encoded by the Glu A3, Glu B3 and Glu D3 loci on the short arm of chromosome 1A, 1B, 1D respectively. In this chapter only the HMW glutenins of the Glu 1 loci will be examined with respect to tortilla quality.

This amino sequence of the protein forms the primary structure of the protein. While the R group is not involved in peptide bond formation, the R group influences the interaction of the protein with the other proteins or other constituent in the system. The glutenins and the gliadins have similar amino acid composition. The glutenins have a content of basic amino acids and a relatively lower glutamine and proline content than the gliadins. Proline is present as 10-12% of the glutenin amino acid composition. Proline has a cyclic R group structure that allows a bend in the chain of the amino acids.

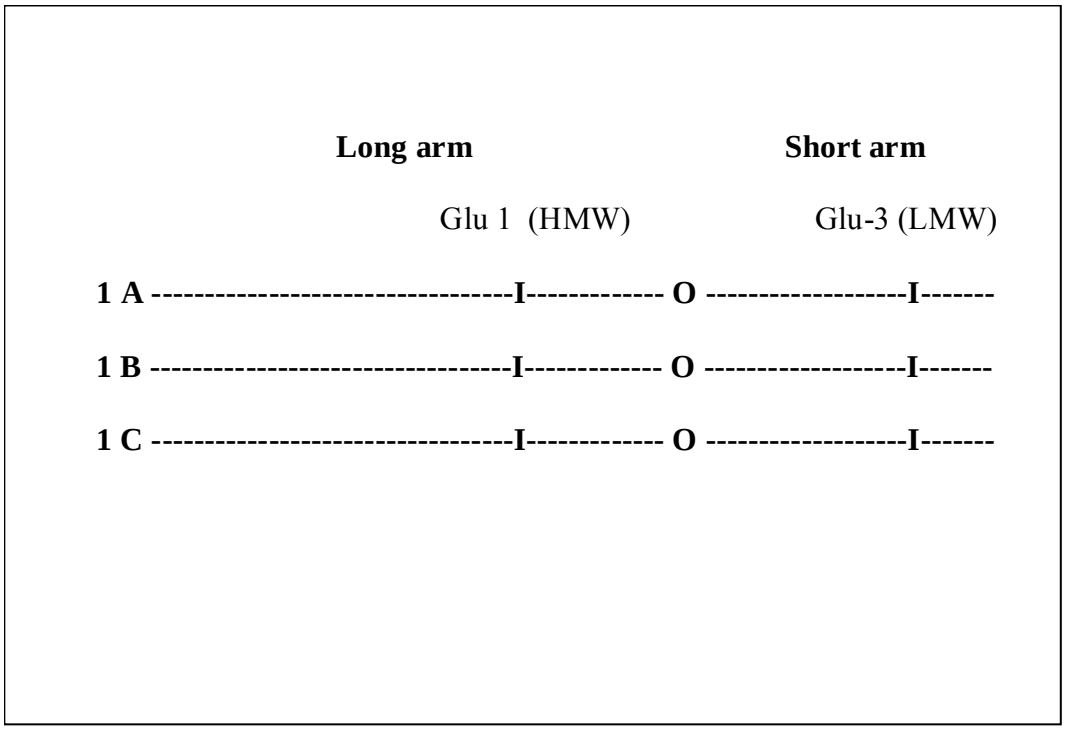


Figure 1: The chromosomes 1A, 1B, 1D of wheat identifying the location of Glu 1 (HMW) and Glu-3 (LMW) glutenin in the long and short arm of the chromosomes.

The protein chain is often coiled to form helices that are considered as the secondary structure. Proline inhibits the formation of these types of structures in wheat. Another important amino acid present is cysteine. Cysteine has a unique ability to form bonds connecting protein chains. The structure of the HMW glutenins as commonly believed to be a mixture of polypeptide subunits crosslinked by intermolecule disulphide bonds in a way that yields a wide spectrum of molecular weights ranging up to molecular weights in millions. The amino acids: glutamine, proline, and cysteine play a major role in explaining the characteristics of the glutenin proteins.

The LMW glutenin structure consists of cysteine residues that help in forming glutenin polymers. The number of cysteine residues present in LMW glutenins differentiates two different LMW type polymer building subunits. Chain extenders have two or more cysteine residues that form the intermolecular disulphide bonds between the HMW glutenins; while the chain terminators have single cysteine residues that terminates the growth of the glutenin polymers (Masci et al 1998). Studies have revealed that allelic variation at the LMW glutenin loci can also affect the wheat end use quality (Gupta et al 1989). However it has been suggested that the effect of these alleles can be studied more accurately in conjunction with the HMW alleles. The characters such as formation of large polymers and dough strength are influenced more by the HMW glutenins than the LMW glutenin subunits and it has been estimated that the effect of one HMW glutenin subunit is equivalent to four times that of one LMW subunit molecule (Gupta 1994). The close association of the LMW glutenins and the gliadins on the short arms of homologous chromosome 1 has led to the belief that the quality characteristics may be associated with the gliadins.

Based on the current theories it appears that the effect of the glutenins on the dough rheological properties depends first on the quantity and quality of the proteins present in the gluten complex, and second on the interactions (disulphide bonds, hydrogen bonds) between the proteins fractions present in the gluten complex. The mathematical-statistical evaluations of the overall amino acid compositions of the glutenins and the rheological characteristics do not exhibit a significant correlation, yet the total number of disulphide bonds has a positive correlation with the rheological properties (Laszitity 1984). The correlation varies from 0.3 to 0.6 which may indicate that not only the absolute number of disulphide bonds but their distribution is important for dough rheological characteristics. Similarly a strong positive correlation between the cysteine content and the wheat flour baking value has also been demonstrated (Laszitity 1984). Investigations have also demonstrated the importance of the hydrogen bonding in determining the rheological properties. A study reported that freshly washed gluten becomes stronger and elastic after dipping in D_2O (Laszitity 1984). Many other studies have also illustrated the importance of the hydrogen bonds in the rheological properties of wheat flour. The subunit composition of the HMW and the LMW glutenins are also important. The subunit composition of wheat has been shown to be both random and ordered with multiple-allelism at Glu 1 loci (Gianibelli et al 2001).

The HMW glutenins are classified into two subgroups: x-type and y-type subunits (Payne et al 1981, Shwery et al 1992). The x-type subunits have lower electrophoretic mobility in SDS page and thus higher molecular weight than the y-subunits. The Glu A1 locus codes for only one x-type subunit, Glu B1 locus codes for either one x-type and y-type or only x-type while Glu D1 codes for both x-type and y-type subunits. SDS page

separation of HMW glutenins from several wheat cultivars have demonstrated a number of alleles at each loci (Payne & Lawerence 1983) The Glu 1 loci has two allelic forms in Glu A1, four allelic pairs and one single allele pair at Glu B1 and two allelic pair at Glu D1 (Table I). Each cultivar of wheat expresses three to five different subunits. The number of expressed subunits is less due to the effects of gene silencing that has occurred through wheat evolutionary history (Lafiandra et al 2000). The numbering system of the subunits was developed by Payne and Lawerence (1983) based on the electrophoretic mobility of the HMW glutenins and this system is still in use. The HMW glutenins have three distinct domains (Shwery et al 1989); a central domain, composed of repetitive sequences (Harberd et al 1986), flanked by the non-repetitive N and the C terminal ends (Halford et al 1987). The amino acid sequences of the HMW glutenins have four to seven cysteine residues located in the N-terminal and C-terminal domains (Shwery et al 1992). Subunit 1Dx5 has an additional cysteine residue in its N-terminal domain that is not present in 1Dx2. Cysteine is important for the disulphide bond formation and also controls most of the functional properties of wheat flour.

The HMW glutenin subunits encoded by Glu A1 (1, 2*), Glu B1 (17+18) and Glu D1 (5+10, 2+12) are important in wheat quality (Payne et al 1987, Weegels et al 1994). Based on these studies we have chosen a set of near-isogenic lines that lines vary in one or more glutenin loci. The series of near-isogenic deletion lines with specific deletions in HMW glutenin loci was used in this study to determine the functional contribution of specific HMW subunits to tortilla quality. The same near-isogenic lines were previously used to study of the functionality of the HMW glutenin subunits in bread making quality.

Table I: The various HMW glutenin subunits present in wheat.

HMW glutenin subunit in each loci		
Glu A1	Glu B1	Glu D1
1	17+18	5+10
2*	13+16	2+12
	7+9	
	7+8	
	20	

Based on Payne et al (1981).

The HMW glutenin allelic subunits have been associated with various aspects of quality. The HMW glutenins subunits have been linked to both dough mixing strength and bread making quality in wheat. Bread making quality depends on the elasticity and the extensibility of the dough which dependent on the HMW glutenins present (Payne et al 1987). The molecular weight distribution of the glutenin polymers plays an important role in determining the viscoelastic properties of the dough (Wrigley & Beitz 1988). The subunits in the Glu D1 loci, 5+10 and 2+12 have been associated with dough mixing strength. The subunit 5+10 is associated with higher dough mixing strength, possibly due to the presence of one extra cysteine residue not found in 2+12 subunit (Kasarda 1999). That said varieties such as an old Hungarian variety Bankuti 1201 that possesses a 2+12 subunit in its Glu D1 locus and yet has high technological quality properties. The subunit 1 at Glu A1 locus is associated with gluten strength while the subunits 2* of Glu A1 locus and 17+ 18 of Glu B1locus are associated with extensibility of the dough (Barnlard and Dardevet 1985). The glutenin subunit 1 has been suggested to have a positive effect due to its unique structure that enables larger and more stable protein aggregates. The use of near-isogenic glutenin deletion lines have confirmed that both the dough mixing strength and bread making quality are reduced as the HMW glutenins are deleted (MacRitchie and Lafiandra 2001, Lawrence et al 1988).

Tortilla quality is also dependent upon dough quality. Reduced dough mixing resistance and greater dough extensibility are related to good quality tortillas (Waniska et al 2004). The glutenin proteins that affect bread making quality also influence tortilla quality. Differences in processing parameters also influence the functionality of these proteins. Thus in this study the tortillas were processed by a standard formulation of

Bello et al (1991) and standard processing parameters. The individual influence of the glutenin protein subunits will be defined via comparisons of the tortillas prepared from the unique near-isogenic deletion lines.

2. Methodology

2.1. Plant Material and Growth Conditions

The near-isogenic deletion lines were developed from a mutant line of cultivar Olympic, null at Glu B1 locus, and an isogenic line of cultivar Gabo, null at Glu A1 and Glu D1 loci. The lines were backcrossed into Gabo (Lawerence et al 1988). The Fm series were developed from this set of deletion lines. The lines are near-isogenic, varying in one or more glutenin loci. A set from this series of deletion lines was obtained from Dr. Finlay MacRitchie (Kansas State University, Kansas) to study the protein composition-functionality in tortillas (Table II). The parent cultivars Gabo and Olympic were also grown along with the set of deletion lines.

The wheat lines were grown in a greenhouse in College Station, and in field in the Texas Agricultural Experiment Station at College Station and at McGregor, Texas in year 2005. The lines were also grown in South Dakota by Dr. Karl Glover, South Dakota State University, Brookings, South Dakota. Performances of these lines in both the locations are evaluated for their protein composition and tortilla making ability. The DNA analysis was performed on the lines grown in greenhouse in College Station, Texas.

Table II: Protein composition of parents and the HMW glutenin deletion lines.

Wheat Lines	HMW glutenin alleles		
	Glu A1	Glu B1	Glu D1
Olympic	1	17+18	5+10
Gabo	2*	17+18	2+12
Fm 2B	-“	17+18	-
Fm 3	-	17+18	-
Fm 4	1	17+18	-
Fm 6	1	-	5+10
Fm 7	1	17+18	10
Fm 9	-	17+18	2+12
Fm 13	2*	17+ 18	2+12

“Deletions in a line are indicated by (-) in the chart.

2.2. DNA Analysis

The DNA composition of the deletion lines PCR with a set of single sequence repeat (SSR) primers were used (Rőder et al 1998). The SSR primers were chosen based on their location in or around the glutenin loci. The primers selected were used to identify the presence of deletion in there long arm of chromosme 1 at Glu A1, Glu B1, and Glu D1 locus were Xgwm497 and Xgwm99 for Glu A1 locus, Xgwm124 for Glu B1 locus, Xgwm642 for Glu D1 locus

DNA Extraction Procedure

The DNA was extracted using a rapid extraction protocol (Brown-Guedira 2002). The leaf tissues was collected from the leaves in a 96-well flat bottom tissue culture plate and frozen. A 40 µL 0.25 NaOH solution was added to the wells and heated in a water bath for 1min at 95°C. The tissue was then ground for 7-10 min using a 96-pin steel rod made to fit the 96 well plates. After grinding 130 µL of 0.1 Tris-HCl was added to each well, centrifuged (Hermile 2383K, Labnet International Incorporation, Woodbridge, NJ) at 3500 rpm for 10 min and a 150 µL supernatant aliquot was transferred to a 200 µL 96-well conical bottom PCR plate (Mid West Scientific, St. Louis, MO). A solution of 15 µL 3 M NaOAc and 120 µL 100% isopropanol was added to the supernatant transferred to -20°C overnight, and centrifuged at 3500 rpm for 30 min. The isopropanol was removed and was centrifuged upside down 600rpm for 1min. A solution of 200 µL 70% EtOH was added and centrifuged again at 3500 rpm for 20 min. The EtOH was removed and centrifuged upside down at 600rpm for 1 min. The pellet obtained was resuspended in 20 µL TE buffer. PCR reactions were performed with the extracted DNA.

Polymerase Chain Reactions and DNA Analysis

The PCR reactions were performed in a volume of 25 µL in DNA Engine-Tetrad 2 (Model-PTC220, MJ Research Incorporation, St. Weltham, MA) thermocyclers. 2 µL of the resuspended DNA was (Beta-Pette, Continental Lab Products, San Diego, CA) mixed with a reaction mixture containing 1 µL of primer (10µm), 2.5 µL of 2.5mM of each deoxynucleotide, 25mM $MgCl_2$, 2.5 µL of 10X PCR buffer (Biolase), 0.25 µL of Biolase (Bioline, UK) and 14.5 µL of distilled water. The PCR cycle was set at 3 min at 94°C, 45 cycles were performed with 1 min at 94°C, 1 min at 55°C, 2 min at 72°C. It was then incubated for 10 min at 72°C and then maintained at 4°C until analysis.The DNA analysis was performed on 2% agrose SFR gels stained with 0.1% ethidium bromide and 0.5X TBE buffer. The gel was run for 20 min at 80 V.

2.3. Protein Analysis

The HMW glutenin proteins present in the deletion lines were tested using the SDS (sodium dodecyl sulphate) PAGE (polyacrylamide gel electrophoresis) and Lab on Chip Capillary Electrophoresis method.

SDS PAGE Test

The SDS method used was rapid 1-D SDS PAGE procedure (Gupta and MacRitchie 1991). The monomeric proteins were removed in the first step. A 5mg sample of flour was taken and mixed with 1.4 ml of 100% DMSO (Dimethyl sulphoxide) and agitated for 10 min in a Vortexer (VX100, Labnet Vortex Mixer, Labnet International Incorporation, Woodbridge, NJ) at 20, 000 rpm, centrifuge for 5 min (15900 rpm), and the pellet obtained was resuspended in 1.4 ml 70% ethanol stirred for 10min and centrifuged for a minute to obtain the final pellet. In the second step the glutenin

subunits were extracted. The residue obtained in the first step was dispersed in 160 µL of 70% ethanol and sonicated (Vibracell, Sonics & Material Incorporation, Danbury, CT) for 5s and mixed with 5 ul 2-mercapthanol (ME) and incubated for 5 min in a water bath (Aquabath, Labline Instruments Incorporation, Meirose Park, IL) at 65°C. Samples were centrifuged for 2 min and then the supernatant containing the glutenin subunits was mixed with 160 µL of SDS buffer and alkylated with 5 µL of 4-vinylpyridine and incubated for 30 min at 65°C. In the third step electrophoretic separation of glutenins was performed in 1D SDS PAGE gels. The electrophoresis was carried out in a 1 mm think 10% acrylamide (1% w/v cross-linker) separating gel (18.5 cm wide, 14.5 cm long) at a constant current of 30mA/gel for 4 hr. The gel was stained with Comassie Blue G-250 for 12-16 hr following Neuhoff procedure. The gels were rinsed with 0.003% w/v Brij solution for 1hr. Gels were stored in 20% (w/v) ammonium sulphate at 4°C for further analysis. The gels were then observed in a white light transilluminator instrument under white light and the images were taken.

Lab on Chip Capillary Electrophoresis

Lab on chip Capillary Electrophoresis is a comparatively new method that was used to identify the protein composition of the deletion lines (Uthayakumaran et al 2004). This work was conducted by Dr. Mike Tilley, USDA-ARS, Kansas, Manhattan. A 10mg sample of flour samples was extracted with 0.5 ml 1% SDS solution containing 1% dithiothreitol (DTT) by vortex –mixing (5 sec) and shaking for 3 min at 65°C. After centrifugation extracts were ready for loading. Ten extract s4 µL each were applied with Agilent sample buffer for Analysis in the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).The software present in the system provided results both as

quantitative profiles (like conventional liquid chromatography) and as simulated gel patterns.

Polymeric Protein Analysis

The analysis of % insoluble polymeric proteins was conducted by Dr. Scott Bean, ARS, Kansas, Manhattan. Flour (0.01 g) was suspended in 0.5% (w/v) SDS buffer (1.0ml). It was then stirred for 5 min at 20, 000 rpm and centrifuged for 20 min at 15900 rpm to obtain a supernatant (extractable protein). The residue was sonicated for 30 sec in 0.5% (w/v) SDS buffer (1ml) to solubilize the remaining protein (unextractable protein). Both the extracts were filtered through 0.45 μm filters. The percentages of extractable and unextractable polymeric protein in the total polymeric protein were calculated as [peak 1 area (extractable)/peak 1 area (total)] x 100 and [peak 1 area (unextractable)/peak 1 area (total)] x 100 respectively. Peak 1 (total) refers to the sum of peak 1(extractable) and peak 1 (unextractable).

2.4. Evaluation of Wheat Grain and Flour

The field grown wheat deletion lines used in this study were combine harvested

Single Kernel Hardness Test

The kernel hardness test was performed in a single kernel hardness tester (SKHT4100). A 300 kernel sample was used for determining kernel hardness, diameter, weight and moisture content.

Tempering of the Wheat Grains

Cleaned grain was tempered to 14% to improve the flour yield, based on the moisture content of kernel determined single kernel hardness test.

It was determined by the formula:

$$\frac{100 - \text{Moisture content}}{100 - 14\ \%} - 1 * \text{weight of grain} = \text{g of water required for tempering}$$

The grains were maintained in plastic bottles with water added and shaken overnight \for the proper distribution and tempering of water.

Milling of the Wheat

The tempered grains were allowed to rest before milling them. The grain was then milled (Barbender Instruments, Incorporation, South Hackensack, NJ) to obtain the flour. The amount of flour obtained was weighed and recorded.

Near-infrared Reflectance Spectrophotometer (NIR)

Near-infrared reflectance spectrophotometer was used to estimate the flour protein content and moisture content from wheat flour samples from each of the deletion lines and parent lines. A control flour (ADM tortilla flour, ADM Milling Company, Overland Park, Kansas) was used to calibrate the instrument. The flour from each sample was filled in a cup and placed in the NIR instrument (Perten PDA 7000 Dual Array with Grams Software). Three replicates of each sample were done and the values were recorded (AACC, Method 44-16, 2000).

Mixograph

Mixographs were used to determine the dough mixing time and the dough strength of the flour. The wheat flour protein and the moisture values obtained from NIR were used to determine the flour water absorption values from the mixograph handbook. The values obtained from the manual were subtracted by 10 and then used to calculate

the water to be added for the mixograph. A 35g sample of flour from each line was used for each Mixograph obtained (Lincoln Manufacturing Company, Lincoln, NE). The graphs were interpreted using standard procedure (AACC, Methods 55-40A, 2000). The dough mixing resistance and the dough mixing time were recorded from the mixographs.

2.5. Tortilla Processing

The flour from each line grown in fields in Texas and South Dakota were processed into tortillas. The tortillas were prepared according to the standard formulation (Bello et al 1991). Commercially available tortilla flour (ADM Tortilla Flour, ADM Milling Company, Overland Park, Kansas) was also included in the testing. This was used to compare the tortilla quality obtained from the commercial flour and the selected experimental lines. The tortillas from each of the experimental lines were made in two batches. The first batch was made with smaller amount of flour to standardize the formulation and the water requirement. The second batch was made from 500g of flour and the tortillas obtained were used for evaluation.

Tortilla Formulation

The formulation used for making the tortillas followed that previously described by Bello et al (1991), except that Cysteine was not added. The formulation was standardized as 500g of flour, 7.5g of salt, 2.5g of sodium stearoyl lactylate, 2g of potassium sorbate, 2.3g of encapsulated fumaric acid, and 30g of shortening. The amount of water added was based on the mixograph water absorption.

Dough Making Process

The dry ingredients were mixed with the flour in a mixing bowl. The bowl was placed over copper tubes through which heated water at 70°C was pumped in by a water

bath at 70°C using a water bath to modify the bowl temperature (Model A-200, Hobart Corporation, Troy, OH). The dry ingredients were mixed with a paddle at a low speed for 1 min. Shortening was then added and mixed for 2 min at a low speed with a paddle. Water was then added and mixed for 1min at low speed and then mixed at a medium speed for 6 min with a hook.

The dough was placed in a plastic tray and the dough quality measurements were obtained. The dough was then placed in a proof chamber (model 57638, National Manufacturing Company, Lincoln, NE) at 35°C and 70% relative humidity for 5 min. The dough was then pressed by hand on a stainless steel round plate. It was divided and rounded in a press for 30 sec (Duchess Divider/Rounder, Bakery Equipment and Service Company, San Antonio, TX) into 36 dough balls of 43 g each. The dough balls were transferred to the plastic tray and again rested in the proof chamber for 10 min at 35°C and 65% relative humidity. The dough balls were then used for making tortillas.

Tortilla Making Process

The dough balls were taken out of the proof chamber and were pressed and baked into tortillas. A three tier oven (Micro-Combo Tortilla Oven, Model 0P01004-02, Lawerence Equipment, South El Monte, CA) was used. The oven was set at a temperature of 350-360°F. The dough balls were placed on a hot press (Micro-Combo model 0P01004-02, Lawerence Equipment Company Incorporation, South El Monte, CA) and pressed at 1100 psi. The tortillas were then baked in a three tier oven. The dwell time was adjusted to 30 sec. The tortillas were cooled on a three tier cooling chain (model 3106 INF, Food Machinery Incorporation Pivo Machinery Incorporation, Pico Rivera, CA). The cooled tortillas were removed from the cooling conveyer and placed on a table.

The tortillas were set aside for 1 min and then flipped on the other side for cooling. The tortillas were then collected and packed in low density polyethylene bags and stored at 23°C for tortilla quality evaluation.

2.6. Dough Evaluation

The dough quality properties were evaluated subjectively. The dough was placed on a plastic tray and the temperature was measured using a thermometer and the values recorded. The other dough properties such as softness, smoothness, extensibility and force to extend were evaluated subjectively (Waniska 2005).

Smoothness refers to the appearance and texture of the dough. It was rated from 1 to 5, 1 being very smooth and 5 being rough. The ideal smoothness rating is 2.0

Softness refers to the firmness of the dough when compressed by hand. It was rated from 1 to 5, 1 being very soft and 5 being very firm. The ideal softness rating is 2.0

Extensibility refers to the length to which the dough extends when pulled apart. It was rated from 1 to 5, 1 implying that it breaks immediately and 5 implying that it extends readily. The ideal extensibility is 3.0

Force to extend measures the elasticity of the dough. It is rated from 1 to 5, 1 is less force required and 5 is extreme force required.

2.7. Tortilla Evaluation

The tortillas were evaluated for their weight, diameter, height, opacity and rollability. 10 tortillas were chosen from each of the lines and evaluations were performed (Friend et al 2000, Waniska 2005).The weights of 10 tortillas were measured using an analytical balance and the values recorded and averaged to obtain the weight of one tortilla. Diameters were measured using a ruler at two points across the tortillas, with

values averaged to obtain the average diameter of the tortilla prepared from each line. The height of the tortilla were measured using a digital caliper and was averaged for 10 tortillas. The pH was determined by mixing 10g of ground tortillas with 40ml of distilled water and measuring the pH in the first minute of the mixing. The moisture was determined using a two-stage procedure (AACC, Methods 44-15A, 1995). The initial weight of one tortilla in a weigh pan was recorded, followed by a second weight record after maintaining the same tortilla for 24 hr in open air. This gives the weight gained. Half of the tortilla was then ground and 2 g of the ground sample transferred to an aluminium pan. The sample was maintained in a drier oven (Model 16, Precision Scientific Company, Winchester, VA) for 1.5-2 hr. The sample was then maintained in a dessicator (Nalgene 5317-0180, Rochester, NY). The dry weight of the sample was measured and the moisture calculations were estimated based on wet method measurements.

$$M = w - d / w$$

Where,

M= % moisture content,

w= initial weight of sample (g)

d= weight of sample after drying (g).

The opacity of 10 tortillas was subjectively evaluated using a continuous scale of 1-100% (1% being fully translucent and 100% being high opacity). The values recorded averaged. The color parameters, L*(lightness), $\pm$ a*(red-green), and $\pm$ b*(yellow-blue) were measured for each tortilla using a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan). Three measurements were taken on each side of the tortillas.

Tortilla shelf stability was evaluated by the rollability test (Bello et al 1991). Rollability scores were determined by wrapping a tortilla in a round dowel (1.0 cm in diameter). Ratings on a scale of 1 to 5 were recorded with 1 being immediate breakage and 5 being no cracks or breakage. The rollabilities were evaluated on the 4^{th}, 10^{th}, 14^{th} days of tortilla processing for each of the lines. Three tortillas from each of the lines were used for the measurements. The specific volume was then calculated for each of the lines (mm.cm.mm/g). The specific volume indicates the fluffiness of the tortillas. It ranges from 1.5 – 3.5 cm3/g. The specific volume was calculated by the formula-

π * (Diameter/2)2 * height * 1000/weight

The quality index was then calculated based on the opacity, rollability and specific volume by using the formula-

Opacity * Specific volume * Rollability score (14^{th} day of rollability)

2.8. Method of Analysis

The measurements were recorded and were arranged in Microsoft Excel File (MS Office 2003) and analyzed. The data was also analyzed using SPSS statistical software (SPSS 13.0 for Windows) to estimate the correlations, compare the means, test for significance by Tukey HSD and ANOVA analysis. The main effects and the interaction effects of the genotypes and the environments were estimated by a all fixed model. The ANOVA analysis of the locations separately for the main parameters IPP, protein, diameter, rollability and opacity indicated significant genotypic effects (Table VI, Table VII). A combined analysis (fixed model) for both the locations had significant genotypic, location and genotype x location interaction effects (Table VIII). Due the significance of the genotype x location effect for the major parameters IPP, protein, diameter, rollability

and opacity, the quality parameters will be discussed separately for two locations in the results section.

3. Results

3.1. PCR Analysis

The PCR analysis verified the glutenin deletions in the wheat chromosomes. The primers used were located closely to the HMW loci. The use of SSR primer Xgwm497 located near GluA1, produced bands in Fm 4, Fm 6, Fm 7 and Fm 13 that have Glu A1 present, yet no amplification of the 200bp product was found Fm2B, Fm 3 and Fm 9 that lacks Glu A1 (Figure 2). Using the primer Xgwm124, located near the Glu B1 locus, in chromosome B1 verifies the deletion in Glu B1 locus of Fm6 (Figure 3). The absence of bands in Fm 2B and Fm 3 when the PCR reactions are performed with primer Xgwm642 verifies the absence of Glu D1 locus (Figure4) All other cultivars were similarly analyzed by the use of the specific primers to confirm the genetic composition of the deletion line.

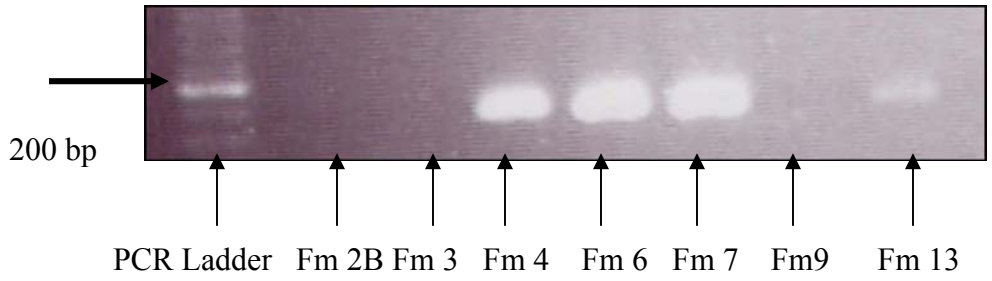


Figure 2: The PCR reaction performed with primer Xgwm497 located near Glu A1 loci.

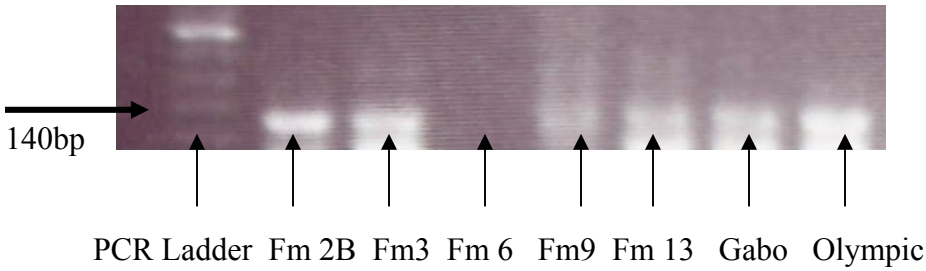


Figure 3: The PCR reaction performed with primer Xgwm124 located near Glu B1 loci.

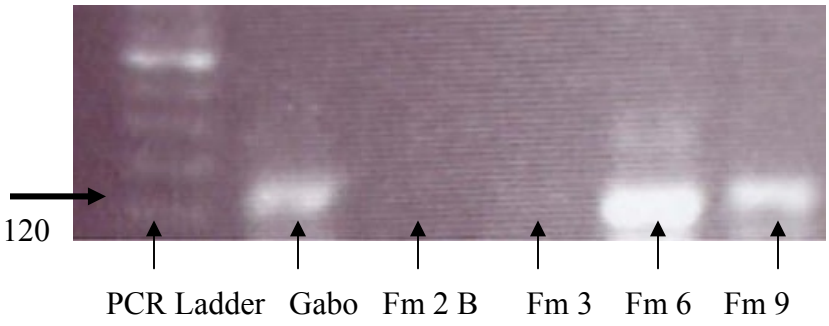


Figure 4: The PCR performed with primer Xgwm642 located near the Glu D1 locus.

3.2. Glutenin Protein Analysis

The SDS PAGE was used to further verify the HMW glutenin protein allelic patterns of the deletion lines and the parent cultivars. The cultivar Gabo has subunits 2*, 17+18 and 2+12 from Glu A1, Glu B1 and Glu D1 loci respectively (Figure 5). The SDS page results of the other deletion lines demonstrated their respective HMW glutenins present and the absence of the subunits indicated the deletions in the chromosomes.

The Lab on Chip Capillary electrophoresis also supports the SDS page gel results. In Gabo and Fm2B, a peak shows the position of the subunit 2* of Glu A1 that is present in Gabo while absent in Fm2B (Figure 6). Similarly a peak in the position of the subunit 17+18 of Glu B1 locus is present in both Fm 2B and Gabo (Figure 6). The subunits 2+12 of Glu D1 are present in Gabo and absent in Fm3. Thus the presence of deletions in Glu A1 and Glu D1 locus in Fm2B and Fm 3 were confirmed. The HMW glutenin subunit composition of Olympic, Gabo, Fm2B and Fm3 is shown in Figure 7. The Glu A1 subunit 1, Glu B1 subunit 17+18 and the Glu D1 subunit5+10 were present in Olympic while Gabo has subunits 2*, 17+18 and 2+12 of Glu A1, Glu B1 and Glu D1 respectively. Fm 2B and Fm 3 have only HMW glutenin Glu B1 subunit 17+18. Thus deletions in Glu A1 and Glu D1 in Fm2B and Fm3 are confirmed. Similar protein analysis was carried out for the lines Fm6, Fm9 and Fm13 to confirm their glutenin subunit composition and deletions. Line Fm13 had a HMW glutenin allelic pattern similar to parent line Gabo, subunits 2* at Glu A1, 17+18 at Glu B1 and 2+12 at Glu D1 locus were present. The protein analysis results were supported by the PCR analysis. The protein profile of the South Dakota lines were also verified to insure no mislabeling of plots or harvested seed occurred.

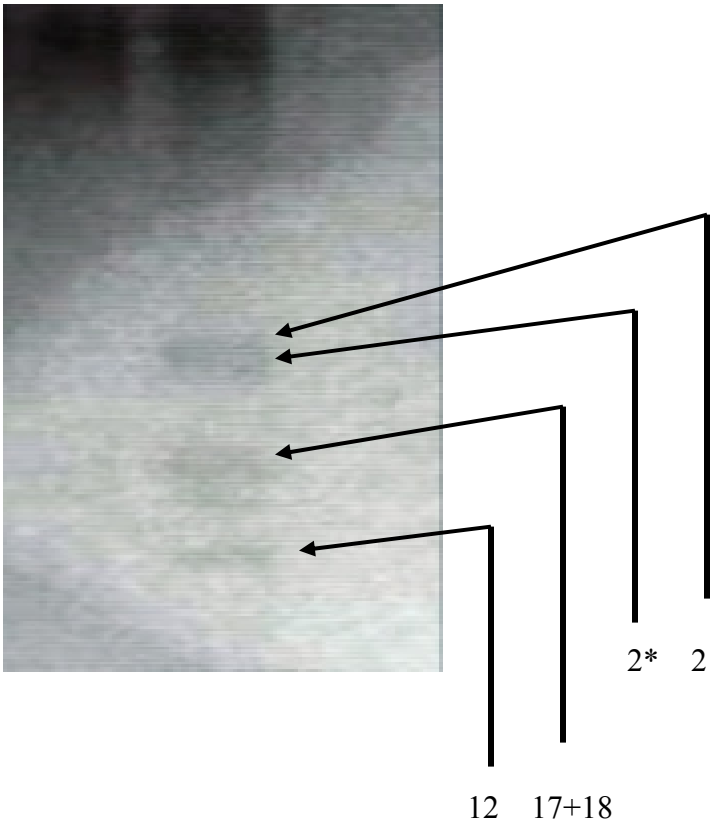


Figure 5: The SDS gel picture of wheat line ‘Gabo’. The presence of the bands 2*, 17+18 and 2+12 are marked.

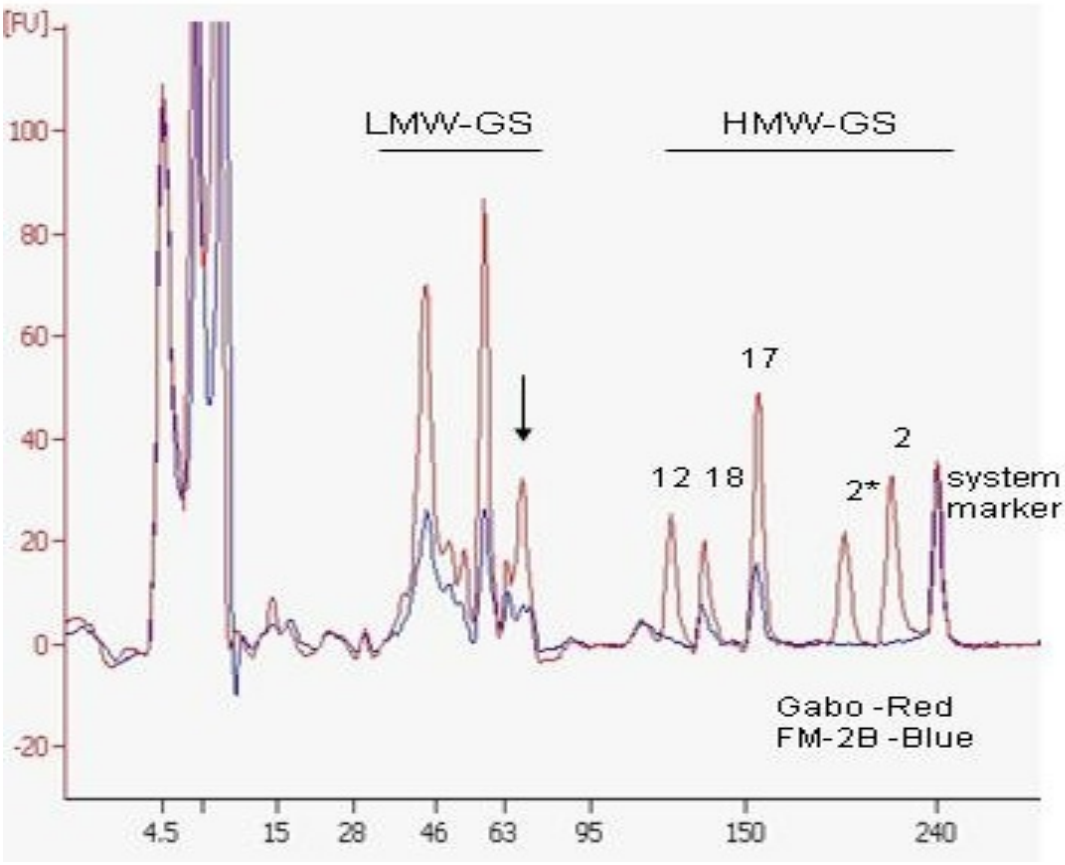


Figure 6: Lab on Chip Electrophoresis performed to observe the HMW glutenins. The electrophoretic pattern of 'Gabo' and Fm 2B are shown here. Absence of 2, 2* and 12 can be seen in Fm 2B.

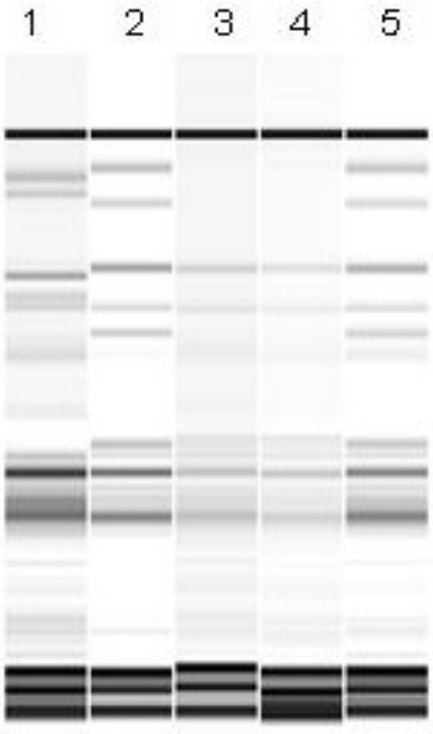


Figure 7: A simulated gel picture obtained from the Lab on Chip Electrophoresis. Line 1 –Olympic, Line 2 – Gabo, Line 3- Fm 2B, Line 4- Fm 3, Line 5- Gabo.

The deletions in the glutenin loci resulted in significantly (α=0.05) reduced insoluble polymeric protein content (Table III) in Texas (Tukey HSD= 0.06) and South Dakota (Tukey HSD=0.11). Deletions in 1A and 1D reduced the IPP (Insoluble Protein Percent) in the lines Fm 2B and Fm3 in Texas. Similar results were obtained in South Dakota. A deletion in the Glu 1A and Glu 1D loci of HMW glutenins reduced the % IPP in the flours. Fm6, which has a deletion in Glu B1 loci, had an intermediate range of IPP content in Texas and South Dakota. A significant correlation ($p<0.05$) was observed between the % IPP and the dough mixing time (Table IV and Table V). Significant genotypic and location effects present in both the locations (Table VI, Table VII and Table VIII).

3.3. Single Kernel Hardness Test

The SKHT was used to determine the kernel hardness of the lines grown in Texas and South Dakota. Grain hardness index of 60 and above are hard grains, while below 40 are soft grains. Olympic, Fm 3 and Australith are soft grains with a grain hardness index less than 40. The other lines Fm2B, Fm 6, Gabo, and Fm 9 have higher grain hardness index (Figure 8). The hardness gene is located in the Chromosome 5B in wheat (Singh and MacRitchie 2000). In the absence of replicated data a combined analysis performed for the kernel hardness in two locations. Significant genotypic and environmental effects were estimated from the analysis of the hardness index. Fishers LSD (LSD=4.31) revealed significant differences between the genotypes for hardness. Fm 2B and Fm 3 have similar deletions, yet Fm 2B was much harder than Fm3. While deletions in glutenin loci do not affect the kernel hardness, Fm 3 may have inherited the hardness gene from parent Olympic.

Table III: The % insoluble polymeric protein and dough development time in the glutenin deletion lines and the parent cultivars 'Gabo' and 'Olympic'.

Wheat lines	Glutenin Composition			% IPP		MDDT	
	A	B	D	Texas	South Dakota	Texas	South Dakota
Fm 2B	-	17+18	-	3.6	2.5	1.5	1.0
Fm 3	-	17+18	-	4.6	3.3	2.0	2.0
Fm 6	1	-	5+10	5.5	3.2	2.9	2.3
Fm 9	-	17+18	2+12	6.3	4.2	3.5	2.5
Fm13	2*	17+18	2+12	6.7	5.3	3.6	2.8
Gabo	2*	17+18	2+12	6.1	4.1	3.3	2.5
Olympic	1	17+18	2+12	-	4.8	5.4	2.6

'-'indicates absence of the specific glutenin loci.
MDDT- Mixograph dough development time.
Tukey HSD analysis identifies % IPP to be significantly different between the genotypes grown separately in Texas (HSD=0.06) and South Dakota (HSD=0.11).

Table IV: Pearson correlations of % insoluble polymeric protein and % polymeric protein with the dough and tortilla quality parameter in Texas.

	Extensibility	Elasticity	Diameter	Rollability	Protein%	MDDT	Mixograph resistance
% IPP	-0.781	-0.474	-0.811	0.749	-0.459	0.950*	0.695
% PPP	-0.562	-0.263	-0.697	0.600	0.004	0.765	0.757

*at 0.05 level of significance.

Table V: Pearson correlations of % insoluble polymeric protein and % polymeric protein with the dough and tortilla parameters in South Dakota.

	Extensibility	Elasticity	Diameter	Rollability	Protein%	MDDT	Mixograph resistance
% IPP	-0.592	0.241	-0.538	-0.201	-0.514	0.821*	0.528
% PPP	-0.133	0.641	0.226	-0.386	-0.288	0.460	0.337

*at 0.05 level of significance.

Table VI: Mean squares from ANOVA analysis of the genotypes grown in Texas.

	IPP	Protein	Diameter	Rollability	Opacity
Genotypes	3.044*	1.027*	389.394*	2.238*	278.381*
Error	0.001	0.002	9.937	0.310	13.907

*at 0.05 level of significance.

Table VII: Mean squares from ANOVA analysis of the lines grown in South Dakota.

	IPP	Protein	Diameter	Rollability	Opacity
Genotypes	1.671*	0.641*	632.275*	1.864*	221.727*
Error	0.003	0.033	10.239	0.589	13.510

*at 0.05 level of significance.

Table VIII: Mean squares from combined analysis (Fixed model) of the lines grown in Texas and South Dakota.

	IPP	Protein	Diameter	Rollability	Opacity
Genotypes	4.481*	0.412*	323.259*	1.529*	80.655*
Locations	34.476*	10.707*	305.021*	7.922*	300.00*
G x E	0.267*	1.424*	117.878*	1.577*	11.905*
Error	0.002	0.015	6.641	0.26	10.938

*at 0.05 level of significance.

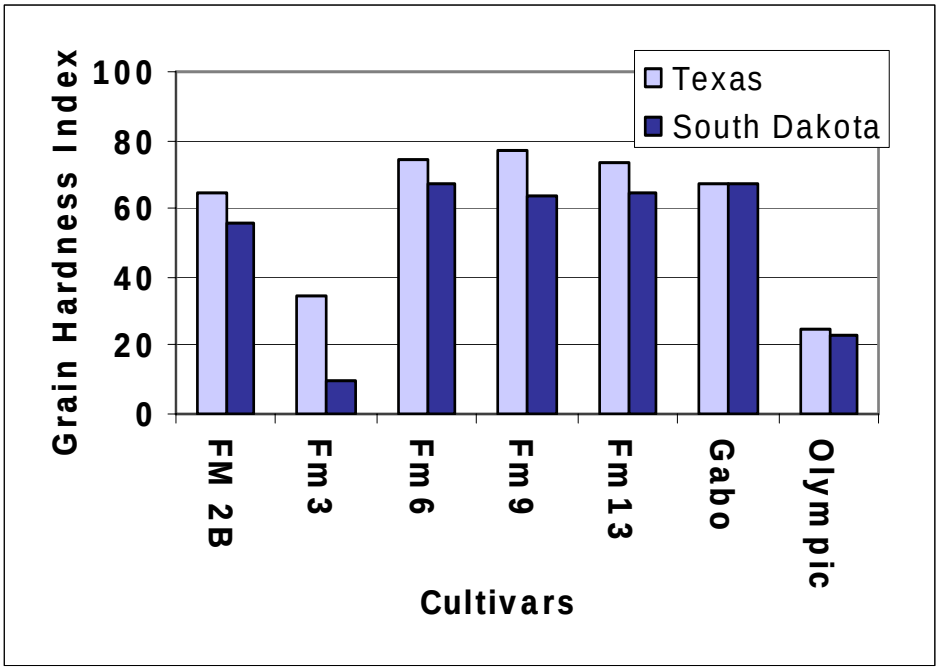


Figure 8: The grain hardness index (300 wheat kernels) of the glutenin deletion lines and the parent cultivars 'Gabo' and 'Olympic' in Texas and South Dakota (LSD= 4.31).

3.4. NIR Results

The Near infrared reflectance spectrophotometer was used to estimate the protein and moisture content of the wheat flour. When grown in South Dakota the deletion lines and the parent cultivars and other varieties had 1-2% reduction in flour protein content compared to same lines grown in Texas. The ANOVA analysis of the flour protein content demonstrates a significant genotypic and location effects (Table VI, Table VII and Table VIII). The contrasts between the deletion lines and the parents indicate that the deletions did not cause any significant effect in the flour protein content (Table IX). The flour protein content of the lines Fm 2B, Fm 9 and Olympic was significantly higher than other lines grown in Texas (Figure 9). The flour protein content of the line Fm 6 was significantly higher in South Dakota (Figure 10). The flour protein content of Fm 6 and Gabo was similar in both the locations while other showed a reduction. Even though there was a significant genotype x location effect, Fm 6 and Gabo were stable in their flour protein content. Dough extensibilities had a significant correlations with the flour protein content in Texas and in South Dakota (Table X and Table XI).The flour protein content had a positive correlation of 0.533 ($p<0.05$) with dough elasticity in Texas (Table X and Table XI). The correlation indicates that an increase in protein content increases the elasticity of the dough. The NIR was also used to measure the flour moisture content of the grains. The flour moisture content in Texas was less than that in South Dakota. The cultivars showed moisture content from 13-14% in both the locations. The flour moisture content is important to predict the flour water absorption rates.

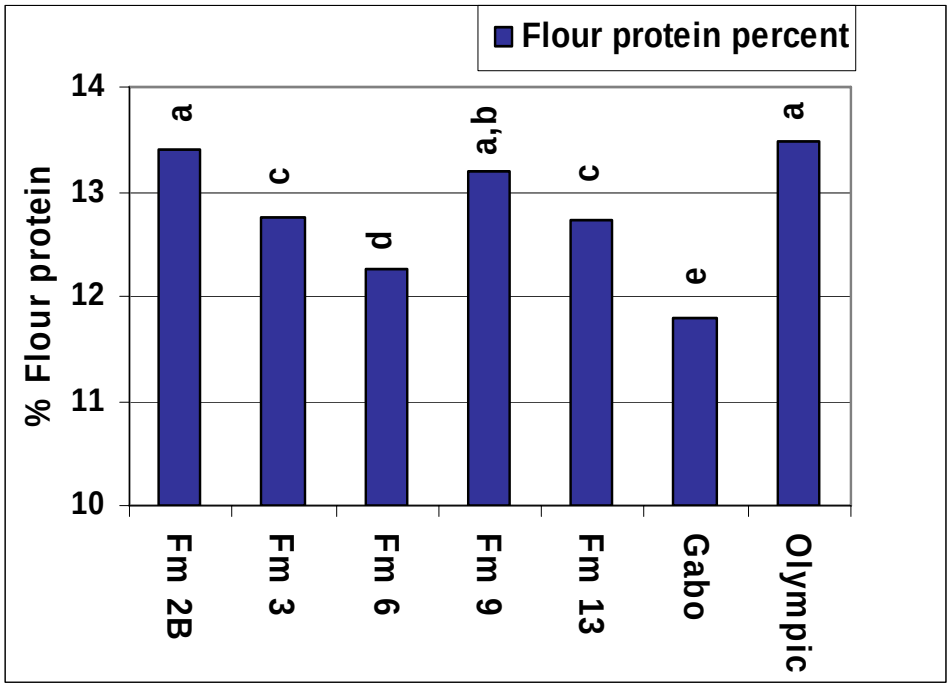


Figure 9: The % flour protein of the parent cultivars and deletion lines in Texas. The symbols a, b, c, d, e, indicate the significant (HSD=0.389, α=0.05) subgroups based on Tukey HSD Test.

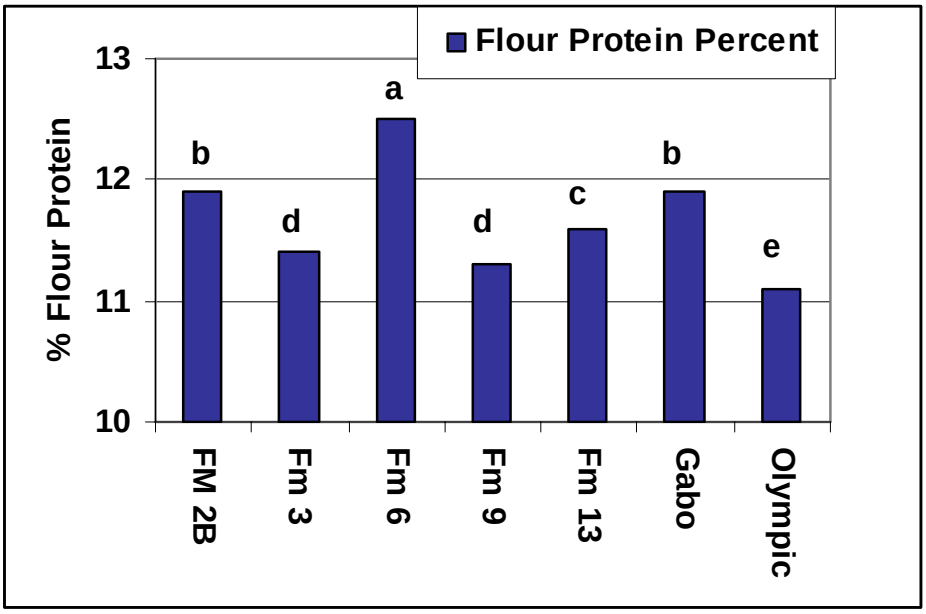


Figure 10: The % flour protein of the lines grown in South Dakota.
The symbols a, b, c, and d indicate the significant (HSD=0.197, α=0.05) subgroups based on Tukey HSD test.

Table IX: Contrasts between the glutenin alleles absent in the glutenin deletion lines and parent cultivars

Wheat lines	**Glu A1**	**Glu B1**	**Glu D1**
Fm 2B	-	+	-
Fm 3	-	+	-
Fm 6	+	-	+
Fm 9	-	+	+
Fm 13	+	+	+
Gabo	+	+	+
Olympic	+	+	+
Contrast			
Protein	0.46	0.58	0.45
Diameter	0.02*	0.01*	0.004*
Rollability	0.00*	0.73	0.00*

*at 0.05 level of significance

Table X: Correlation of the dough and tortilla quality parameters of the wheat lines grown in Texas

	Elasticity	Extensibility	Height	Diameter	Opacity	Sp. vol.	Rollability	Protein	MDDT	Mixograph resistance
Elasticity	1	0.380	-0.366	0.301	-0.126	-0.127	0.198	0.689*	-0.448	-0.234
Extensibility		1	0.036	-0.48	0.149	0.029	0.203	0.533*	-0.052	0.068
Height			1	-0.553*	0.296	0.326	-0.133	-0.200	-0.742*	-0.124
Diameter				1	0.216	0.553*	-0.396	0.146	-0.211	-0.396
Opacity					1	-0.566*	-0.822*	0.067	0.021	-0.424
Sp. Vol.						1	-0.563*	0.003	-0.111	-0.584*
Rollability							1	0.272	0.034	0.298
Protein								1	-0.211	-0.366
MDDT									1	0.368
Mixograph resistance										1

*at 0.05 level of significance

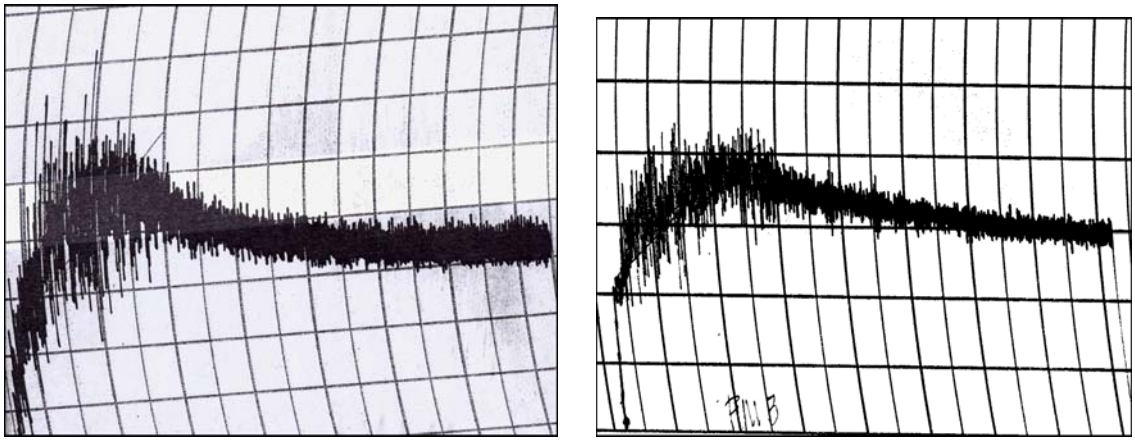
Table XI: Correlation of the dough and tortilla quality parameters of the wheat lines grown in South Dakota

	Extensibility	Elasticity	Height	Diameter	Opacity	Sp. Vol.	Rollability	Protein%	MDDT	Mixograph resistance
Extensibility	1	0.084	0.079	0.154	-0.242	0.225	0.230	0.74*	-0.266	0.278
Elasticity		1	-0.244	0.497	-0.311	0.135	0.302	0.235	-0.81	0.234
Height			1	-0.679*	-0.222	-0.238	0.012	0.106	0.145	0.521
Diameter				1	-0.379	0.741	-0.045	0.072	-0.498	-0.572
Opacity					1	0.717*	-0.480	-0.351	-0.377	-0.715*
Sp. Vol.						1	-0.333	-0.080	-0.415	-0.484
Rollability							1	0.633*	0.173	0.275
Protein%								1	-0.332	0.340
MDDT									1	0.393
Mixograph resistance										1

*at 0.05 level of significance

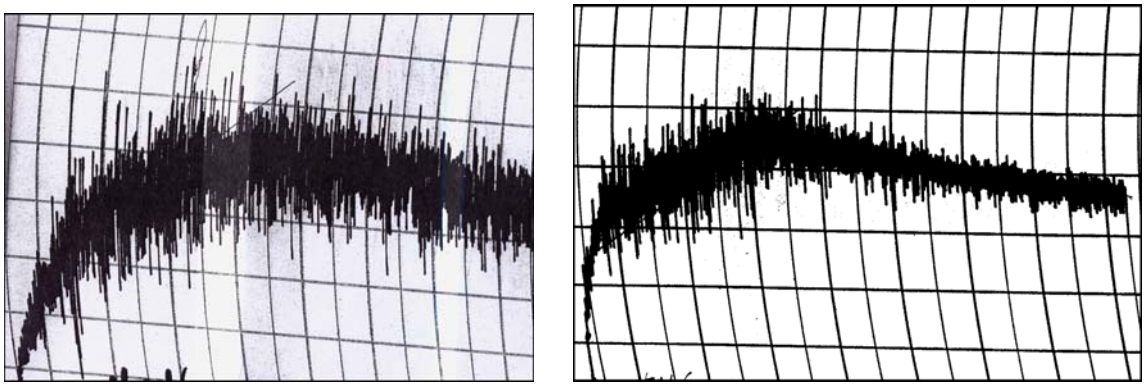
3.5. Mixograph

The mixographs observed in Texas and South Dakota showed similar trends yet the mixographs from South Dakota flours were lower in intensity, presumably due to the lower flour protein content in South Dakota. The dough mixing time (MDDT) and resistance were used to describe the strength of the flours. The deletion lines FM2 B and Fm3 had weak dough mixing strength in both Texas and South Dakota as indicated by the quick rise in peak and immediate fall indicating lack of resistance in the flour (Figure 11). The dough prepared from Fm 6 had intermediate strength in both the locations (Figure 12). Doughs prepared from Fm 9 and Fm 13 exhibited strong dough mixing strength in both the locations. The parent Gabo was strong in Texas yet had an intermediate dough mixing strength in South Dakota (Figure 13). Olympic, the other parent had intermediate flour strength in Texas but was weak in South Dakota (Figure 14). The changes in location and environment had affected protein content and thus the dough mixing strengths. Table III contains the dough mixing time and the % IPP in Texas and South Dakota. The other varieties such as Fang 60 and Diebre had strong dough mixing strength in Texas but an intermediate strength in South Dakota. No significant correlations were observed between the dough resistance and the % IPP and % PPP though they seemed to vary with the variations in % IPP (Table IV, Table V). The dough mixing time had a significant correlation with the % IPP ($p<0.05$) in both the locations. FM 2B and Fm 3 had a lower % IPP values and a lower dough mixing time than Fm 9, Fm 13 and Gabo which had a higher percentage IPP and stronger dough mixing time (Table III) and the mixographs reflected the changes. Lowering the % IPP resulted in weaker doughs.



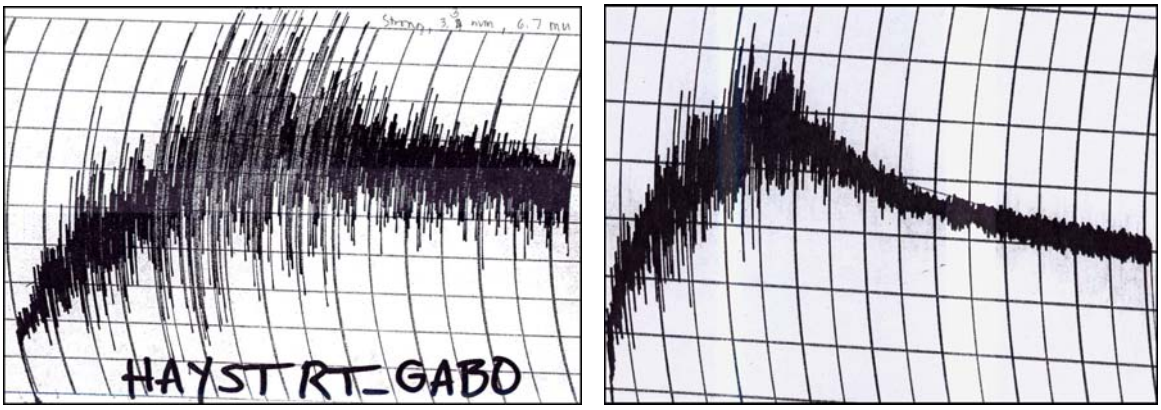
Texas South Dakota

Figure 11: The mixograph of the glutenin deletion line Fm 3 in Texas and in South Dakota.



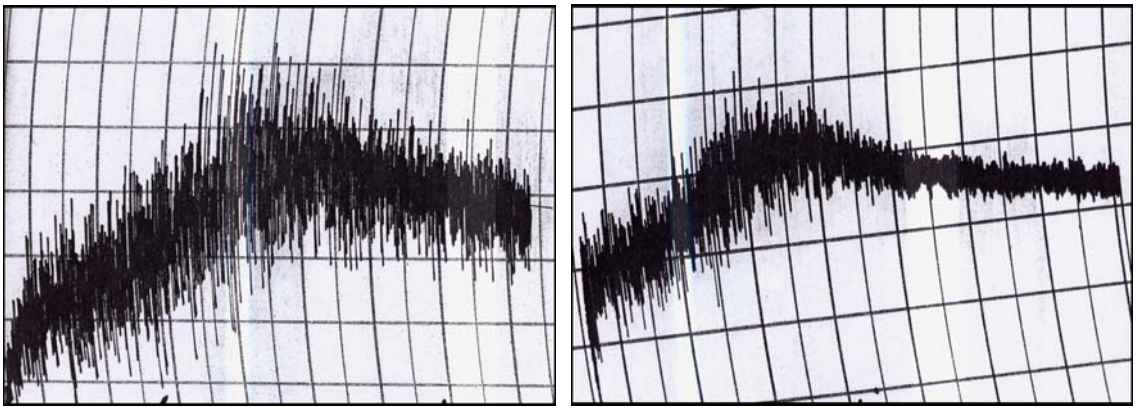
Texas South Dakota

Figure 12: The mixograph of the glutenin deletion line Fm 6 in Texas and in South Dakota.



Texas South Dakota

Figure 13: The mixograph of the parent line ‘Gabo’ in Texas and in South Dakota.



Texas South Dakota

Figure 14: The mixograph of the parent line ‘Olympic’ in Texas and in South Dakota.

3.6. Dough Quality Test Results

Subjective dough quality evaluations were also performed. The extensibility of dough is an important parameter that influences the tortilla quality. The ideal score of dough extensibility is 3.0 on a scale of 1 to 5 where 5 is very extensible and 1 has low extensibility. The doughs prepared from glutenin deletion line Fm 2B and Fm 3 had dough extensibility scores of 4.5 and 3.5 in Texas and South Dakota respectively. Fm 2B and Fm 3 lines have extensible doughs. The lines Fang 60 and Olympic had low dough extensibility score in South Dakota. The other lines have extensibility scores of 3.0 that are near ideal. The elasticity scores indicate the force needed to extend. The ideal score of elasticity is 2.0 on a scale of 1 to 5 where 5 is highly elastic and 1 is less elastic. Doughs prepared from lines Fm 2B and Fm 3 had ideal elasticity scores of 2.0. Dough extensibility score of line Fm 6 was 2.3. The other lines had higher elasticity scores of 3.0. The higher the elasticity score the greater the force required to extend the dough's. The low elasticity scores with high extensibility scores indicated that these lines had doughs that had good extensibilities and required less force to extend. The control flour that is commercially available tortilla flour also had a good extensibility score but higher elasticity scores of 3.5 and 3.0 respectively. Good extensibility with good elasticity indicates the nature of the dough to extend and then subsequent shrink back due to elasticity.

The dough moisture content was around 37-39 % for most of the lines and also the control flour. The deletion lines FM 2B, Fm 3 and Fm 7 had dough moisture content of 35%. The ideal dough smoothness score is 2.0 on a scale of 1 to 5 where 5 is highly rough and 1 is very smooth. The lines Fm 2B and Fm 3 had an ideal smoothness score of

2.0 in both the locations. The doughs prepared from other lines grown in both the locations had a smoothness score above the ideal score. The ideal softness score is 3.0. The dough prepared from the lines Fm 2b and Fm 3 had softness scores of 3.5 and 3 respectively in both locations indicating these lines produce softer doughs. The doughs prepared from the other lines and the control flour had dough softness scores of 2-2.3; the doughs were firmer.

Significant correlations were observed between the force to extend and the protein content (Table X, Table XI). A significant correlation 0.533 ($p<0.05$) was observed in Texas. The increased protein content caused the force to extend the dough to increase. The dough extensibilities also are significantly correlated ($p<0.05$) with the flour protein content.

3.7. Tortilla Quality Evaluation Results

The tortilla quality parameters were affected by the deletions in the HMW glutenin loci. The diameters of the wheat deletion lines had significant genotypic and location differences (Table VI, Table VII and Table VIII). In Texas the tortillas prepared from the control flour had a diameter of 163 mm. The tortillas prepared from the parent cultivars Olympic and Gabo had tortilla diameters of 155 mm respectively and were significantly smaller than the control tortilla flour. Tukey HSD analysis of the tortillas prepared from the lines grown in Texas had some of the deletion lines with significantly larger diameter (Figure 15). The tortillas prepared from deletion lines Fm 2B and Fm 3 had diameters of 176 mm and 171 mm respectively, which was significantly ($p<0.05$) larger (by 1-2 cm) than the diameter of the tortillas prepared from the control tortilla flour, the parent cultivars and other varieties grown in Texas. The tortilla prepared from

Fm 6 and Fm 13 had diameters 167 mm and 165 mm respectively, which was also significantly ($p<0.05$) larger than the control flour and the parent cultivars. The tortillas prepared from line Fm9 had smaller diameter of 155 mm (Table XII).

The South Dakota tortilla diameter evaluations supported the Texas results. Significant differences were observed for tortilla diameters by Tukey HSD analysis of tortillas prepared from the lines grown in South Dakota. The tortillas prepared from the lines Fm 2B, Fm 3, Fm7 had diameters of 181 mm, 181 mm and 191 mm respectively (Figure 16). The tortillas prepared from the control flour had a diameter of 170 mm. The tortilla prepared from lines Fm 6 and Fm13 had diameters of 174 mm and 175 mm respectively and were significantly larger than the control flour. The tortillas prepared from the parent lines Gabo and Olympic had diameters of 156 mm and 162 mm respectively (Table XIII). The tortillas prepared from the other lines grown in South Dakota had diameters of 170 mm on an average. Some of the glutenin deletions lines had significantly larger tortilla diameters than control flour tortilla and the other wheat varieties. The analysis of the tortilla diameters by the Tukey–HSD test demonstrates that Fm 2B, Fm 3 and Fm 7 had significant larger diameters ($\alpha=0.05$) than the other lines in South Dakota.

The opacity scores of the tortilla prepared from the glutenin deletion lines were higher than the control flour and the other varieties. The ideal opacity scores are above 85. The tortillas prepared from the glutenin deletion lines Fm2B and Fm 3 had better opacity scores of 86. While the tortillas prepared from other deletion lines and the other varieties had opacity scores similar to the control flour (Table XII).

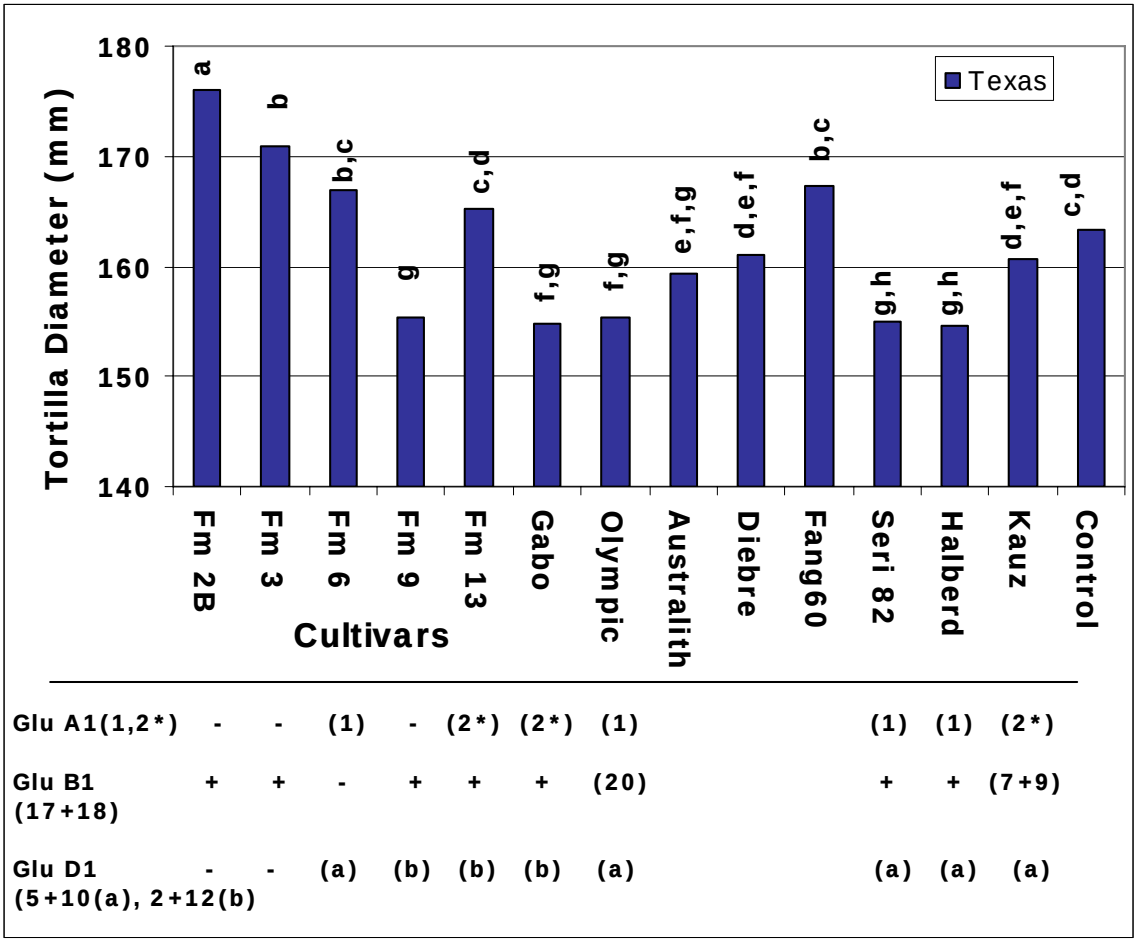


Figure 15: The average diameters of tortillas (10 tortillas) prepared from flours from the glutenin deletion lines, the parent cultivars, adapted cultivars grown in Texas and the commercial control tortilla flour.

The glutenin subunit composition of the deletion lines, the parent cultivars and other well adapted varieties are also included. The (-) sign indicates the absence of the glutenin loci, the (+) sign indicates the presence of the loci. The subunits present in each of the loci are in the brackets. The protein subunit compositions of 'Australith', 'Diebre', and 'Fang 60' are not known. The symbols a, b, c, d, e, f, g, h indicate significance ranks of the cultivars based on the significant differences (HSD=6.178, α=0.05) obtained from Tukey-HSD test.

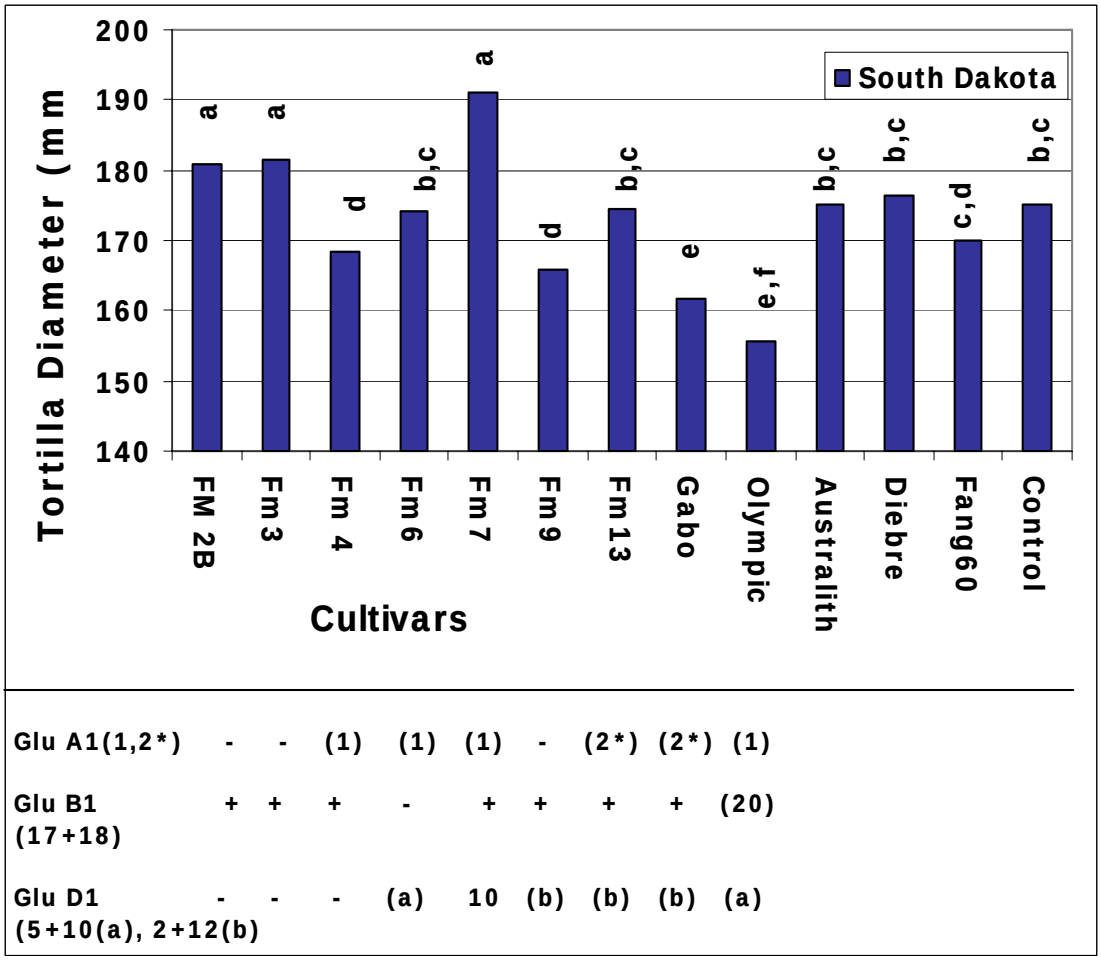


Figure 16: The average diameter of tortillas (10 tortillas) prepared from the flours from glutenin deletion lines, the parent cultivars, adapted cultivars grown in South Dakota and the commercial control tortilla flour.
The glutenin subunit composition of the deletion lines, the parent cultivars and other well adapted varieties are also included. The (-) sign indicates the absence of the glutenin loci, the (+) sign indicates the presence of the loci. The subunits present in each of the loci are in the brackets. The symbols a, b, c, d, e, f, indicate significance ranks of the cultivars based on the significant differences (HSD=5.32, α=0.05) obtained from Tukey-HSD test.

Table XII: Dough and tortilla quality results of the wheat lines grown in Texas

Genotype	A	B	D	Protein (%)	Q. Index (14d)	Rollability (14d)	Diameter (mm)	Opacity (%)	Sp. Vol. (cm^3/g)	Mixtime (min)	Resistance (MU)
Control	?	?	?	12	284	2.5	163	80	1.42	4.2	5.4
Fm2B	-	17+18	-	13.39	212	1.5	176	86	1.65	1.7	5.5
Fm 3	-	17+18	-	12.75	300	2.3	171	86	1.55	1.7	5.0
Fm 6	1	-	5+10	12.26	383	3.0	167	75	1.70	2.9	5.0
Fm 9	-	17+18	2+12	13.19	365	3.3	155	78	1.44	3.1	6.4
Fm 13	2*	17+18	2+12	12.72	300	2.5	165	78	1.54	3.6	6.5
Gabo	2*	17+18	2+12	11.78	272	2.5	155	80	1.36	3.3	6.7
Olympic	1	20	5+10	13.47	335	2.5	155	86	1.57	4.2	5.4
Australith	?	?	?	13.07	148	1.0	159.3	91.9	1.6	4.0	5.2
Diebre	?	?	?	11.05	127	1.0	161.3	83.5	1.5	3.3	6.0
Fang 60	?	?	?	11.97	208	1.0	167.3	84	1.7	3.2	5.8
Seri 82	1	17+18	5+10	11.71	301	2.3	154.0	84.4	1.6	4.3	6.5
Halberd	1	20	5+10	12.85	329	3.8	154.6	70.5	1.2	3.5	6.3
KAUZ	2*	7+9	5+10	11.74	244	1.8	160.6	84.5	1.6	3.8	6.0

?-composition unknown, '-'indicate the absence of the glutenin loci

Table XIII: Dough and tortilla quality results of the wheat lines grown in South Dakota.

Genotype	A	B	D	Protein (%)	Q. Index (14d)	Rollability (14d)	Diameter (mm)	Opacity (%)	Sp. Vol. (cm^3/g)	Mixtime (min)	Resistance (MU)
Control	?	?	?	12.0	304	2.75	175	85	1.30	-	-
Fm 2B	-	17+18	-	11.9	136	1.0	181	91	1.50	1.0	5.2
Fm 3	-	17+18	-	11.4	147	1.0	181	95	1.56	2.0	4.6
Fm 4	1	17+18	-	11.7	110	1.0	168.3	82	1.34	2.0	6.0
Fm 6	1	-	5+10	12.5	330	3.0	174	84	1.28	2.3	5.5
Fm 7	1	17+18	10	11.2	127	1.0	191	90	1.42	1.1	3.5
Fm 9	-	17+18	2+12	11.3	115	1.25	166	80	1.16	2.5	6.0
Fm 13	2*	17+18	2+12	11.6	115	1.0	175	84	1.37	2.8	6.4
Gabo	2*	17+18	2+12	11.9	246	2.5	162	80	1.24	2.5	6.6
Olympic	1	20	5+10	11.1	109	1.0	156	89	1.23	2.6	5.2
Australith	?	?	?	11.2	192	1.75	175	84	1.31	3.0	4.7
Diebre	?	?	?	10.1	134	1.0	176	90.5	1.4	3.0	4.8
Fang 60	?	?	?	10.2	110	1.0	170	80.5	1.27	2.5	5.4

? - Unknown composition, '-'absence of specific glutenin loci.

In South Dakota the tortillas prepared from the glutenin deletion lines Fm 2B and Fm 3 had higher opacity scores of 91 and 95 respectively while the control flour and the other varieties had opacity scores of 80-85. The tortillas prepared from the glutenin deletion lines with greater diameters had better opacities (Table XIII). Significant tortillas opacities ($p<0.05$) differences between the wheat lines were observed.

The specific volume of the tortillas indicates the fluffiness of the tortillas. The specific volume of the tortillas above 1.4 g/cm^3 is ideal for tortillas. The control flour tortilla had specific volume of 1.42 g/cm^3, which is similar to the industry. The tortillas prepared from the glutenin deletion lines grown in Texas had a higher tortilla specific volume (1.50-1.60 $g/cm^{3)}$ than the control flour tortilla. The other varieties also had higher tortilla specific volume than the control flour tortilla (Table XII). They had tortilla specific volume ranging from 1.40 g/cm3-1.60 g/cm^3. The specific volume of the tortillas prepared from the lines grown in South Dakota had similar results supporting the Texas results (Table XIII). The control flour tortilla had lower specific volume of 1.30 g/cm^3. The tortillas prepared from the glutenin deletion lines Fm 2B and Fm 3 had specific volume of 1.50 g/cm3 and 1.56 g/cm3 respectively. The tortilla specific volumes Fm 2B, Fm 3 were better in both the locations and better than the control flour and the parent cultivars.

The rollability scores of the tortillas were recorded on the 4th, 10th and 14th day following the day they were processed from the flours from the parent deletion lines, and cultivars grown in Texas and South Dakota. The rollability scores had significant genotypic effects in both locations (Table VI, Table VII). In Texas the control flour tortilla had a shelf life of 2.5 on the 14th day (Figure 17). Rollability scores of 3.0 and

above on the 14th day indicate a good shelf life. The parent cultivars Gabo and Olympic had a rollability scores of 2.5 on the 14th day. A rollability score of the tortillas prepared from the glutenin deletion lines Fm 6 and Fm 9 were 3.0 on the 14th day. Tortillas prepared from lines Fm 2B and Fm 3 had poor rollability scores of 1.5 and 2.3 on the 14th day respectively. The other varieties Halberd, TX 8444 and Glenson 81 had rollability scores above 3.0 (Table XII). The tortillas prepared from other lines had poor rollability scores of 1.5-2. Tukey HSD analysis of the rollability scores on the 14^{th} day indicates that Fm 6 had significantly higher shelf stability.

In South Dakota the control tortilla flour showed a rollability of 2.75 on the 14th day. In general all the lines in South Dakota had poor rollability scores. The tortillas prepared from the lines Fm 9, Fm 13 had a rollability score of 1 on the 14th day (Figure 18). Poor rollability scores were observed in the tortillas prepared from the lines Fm 2B, Fm 3 and Olympic in South Dakota on the 4^{th} day. The only exceptions were tortillas prepared from the line Fm 6 and parent the cultivar Gabo that had rollability scores of 3.5 on the 14th day. Tukey HSD analysis of the rollability scores of the tortillas prepared from the lines grown in South Dakota indicates that Fm 6 and Gabo had significantly higher shelf stability.

The quality index calculations were based on the rollability scores on the 14th day. Based on an average quality score we found that some of the lines had very good quality index in Texas (Table XII). The control flour tortilla had a good quality index of 284. The parent wheat cultivar Olympic had a quality score of 335 which was greater than the control flour. Gabo had a lower quality index. The other deletion lines had quality scores above 300 with Fm 6 being the best with a quality score of 383.

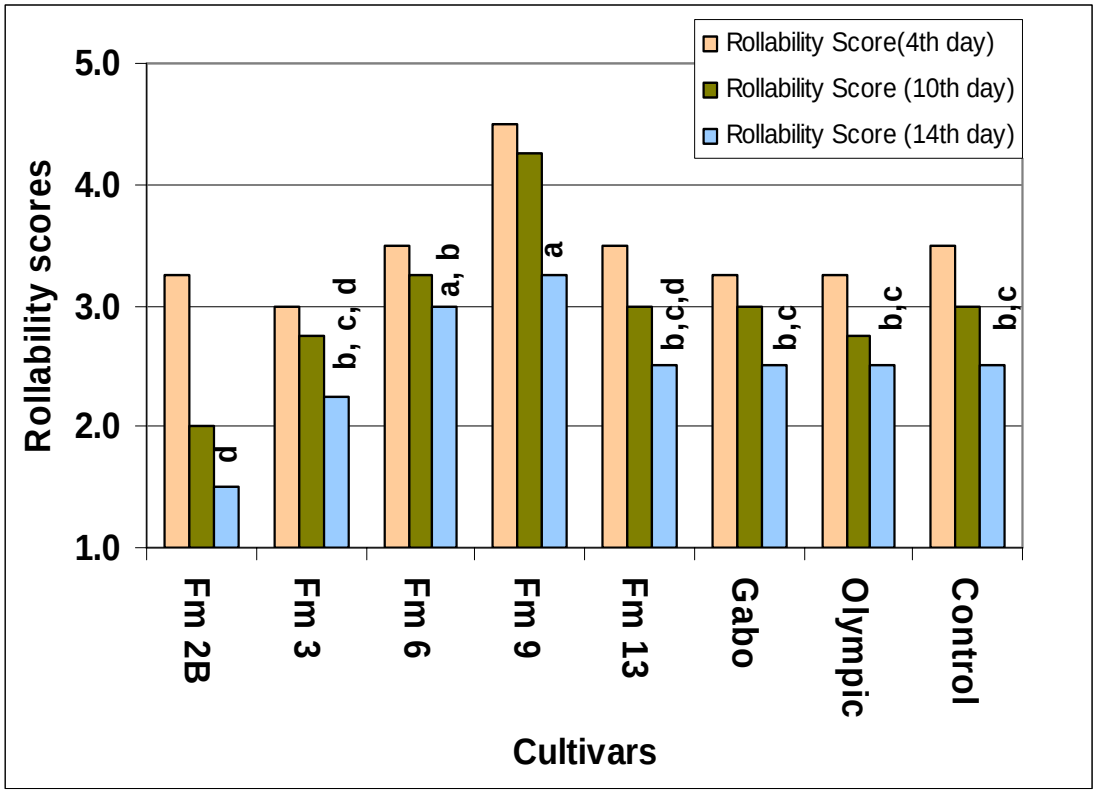


Figure 17: The rollability scores of tortillas prepared from the lines grown in Texas on the 4th, 10th and 14th day after processing.
The symbols a, b, c, d indicate significance ranks of the cultivars based on the significant differences (HSD=1.13, α=0.05) between the rollability scores on the 14th day obtained from Tukey HSD tests.

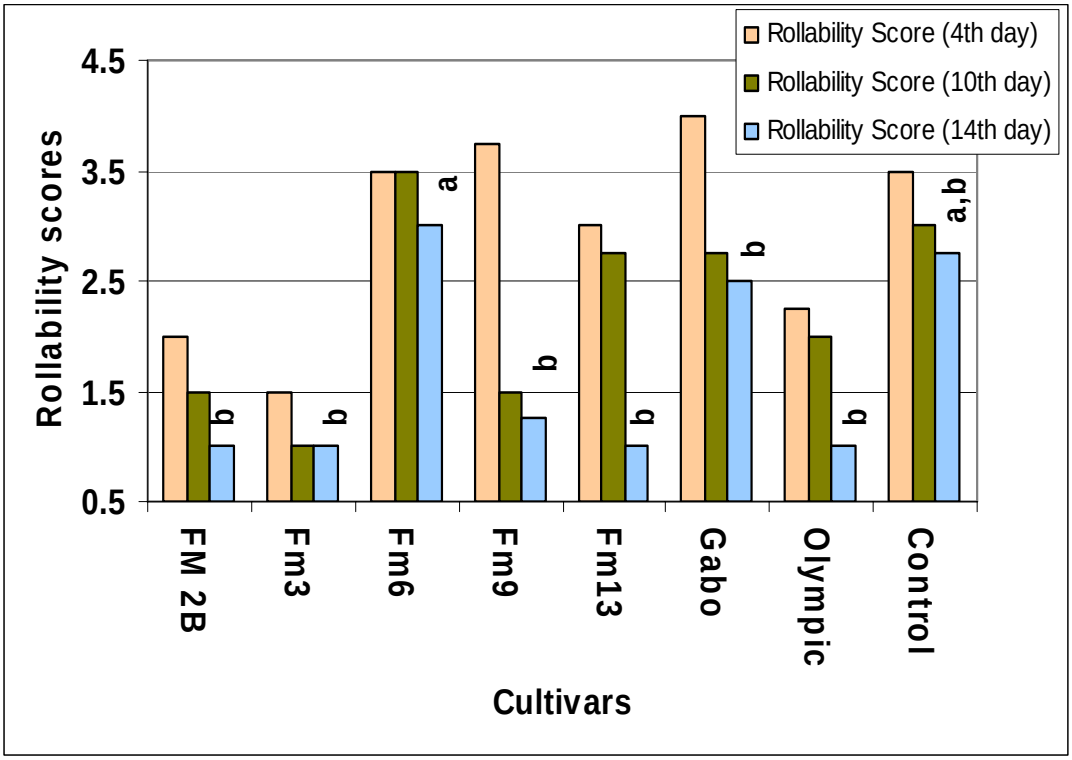


Figure 18: The rollability scores of tortillas prepared from the lines grown in South Dakota at 4th, 10th and 14th day after processing.
The symbols a, b, c, d indicate significance ranks of the lines based on the significant differences (HSD=1.59, α=0.05) between the rollability scores on the 14th day obtained from Tukey HSD tests.

The only exception is Fm 2B which had a lower quality index due to the low rollability scores on the 14th day (Table XII). Lines with better rollabilities have shown better quality index. The lines Halberd, TX 8444 and Glenson 81 had quality index of above 300. In South Dakota a similar quality index calculations was found for the rollability scores at the 14th day. The rollability scores in South Dakota were lower on the 14th day thus the quality index were also low (Table XIII).

In South Dakota the control tortilla flour had a quality index of 304. The glutenin deletion lines as well as the other lines had poor quality index of less than 200 though Fm6 and Gabo were exceptions having higher quality scores (Table XII). As both of these lines had better rollability scores on the 14th day, their quality index scores were also higher. The quality index of Fm 6 was better than the control flour in both Texas and South Dakota.

Correlations were calculated between the tortilla quality parameters separately in both the locations using SPSS software (Table X, Table XI). In Texas a significant correlation of 0.533 ($p<0.05$) was found between the height and the diameter of the tortillas. Diameters had significant correlations ($p<0.05$) with the specific volume of the tortillas. In South Dakota the rollability and the protein content had a significant correlation ($p<0.05$). Tortilla diameters had a strong negative correlation ($p<0.05$) with the dough mixing resistance and tortilla height in South Dakota. Correlations between the tortilla quality parameters were not similar in Texas and South Dakota. The % IPP and the % PPP had no significant correlation with any of the tortilla quality parameters.

4. Discussion

The effect of the HMW glutenins is evident from the results that were obtained. The flour protein content in Texas was higher by almost 2% from South Dakota. The higher temperatures in Texas may have increased the protein accumulation by suppression of starch accumulation that resulted in higher flour protein content. The deletion lines had decreased protein content in South Dakota except the lines Fm6 and Gabo that had an increase in protein content in South Dakota. While the protein content increased, the % IPP in Fm 6 and Gabo had a decrease similar to the other lines in South Dakota (Table III). The mixographs had evident differences in their protein contents in both the locations. The reduced protein contents lowered strength in the mixographs of the lines from South Dakota. The effects on the dough mixing strength due to the specific subunit composition of the HMW glutenin in the deletions were significant. The parent line Gabo which has subunits 2*, 17+18 and 2+12 at Glu A1, Glu B1 and Glu D1 and had higher % IPP values and strong dough mixing strength (Table III) even though the presence of 2+12 is associated with weak dough strength (MacRitchie 1985). The line Fm 9 that has subunits 17+18 and 2+12 at Glu B1 and Glu D1 respectively and a deletion in Glu A1, had % IPP almost similar to Gabo and a strong mixing strength (Table III). Thus 17+18 and 2+12 together can give rise to stronger dough mixing strengths. The line Fm 6 which has subunits 1 and 5+10 and a deletion in Glu B1 had significantly a lower % IPP than Fm 9 and Gabo and had an intermediate dough strength. Olympic having 1, 20 and 5+10 at Glu A1, Glu B1 and Glu D1 had higher % IPP than Fm6. The subunit 5+10 is associated with the dough strength but the presence of subunit 20 instead of 17+18 may have reduced the % IPP. The lines Fm 2B and Fm3 have subunit 17+18 only at Glu B1

and deletions at other locus. These two lines showed lower % IPP than Fm6, Fm9 and Gabo. Thus the subunits at Glu B1 and Glu D1 are important in contributing to greater dough mixing strength. The strong correlations with of the % IPP and the dough mixing time support the above statement (Table IV & Table V).

The HMW glutenin functionality also altered the tortilla properties. Since cysteine was not used in any of these experiments the tortilla properties where due to the functionality of the glutenins present in the flour. The major tortilla parameters are the diameter, rollability, opacity. The deletion lines with deletions in specific HMW glutenin loci affected the tortilla qualities. The deletions resulted in the changes in the % IPP, which also influenced tortilla quality. The lines Fm 2B and Fm 3 have larger diameters in both Texas and South Dakota (Figure 15, Figure 16). The diameters are nearly 1-2 cm more than the control tortilla flour and the parent cultivars. The diameters of these two lines were better than the other deletion lines. Fm 2B has subunits 17+18 at Glu B1 and poor rollability scores in both the locations. Fm 3 had a better rollability score in Texas, though the rollabilities were poor in South Dakota. The line Fm 6 with subunits 1 and 5+10 and a deletion in Glu B1 had better rollabilities on the 14th day and also significantly ($p<0.05$) larger diameter to the control flour and the parent cultivars, though less than the diameters of Fm 2B and FM 3. Line Fm 4 with 1 and 17+18 had a smaller diameters as well as a poor rollability (Figure 16 & Table XIII). In this line the interactions of subunit 1 from Glu A1 with 17+18 on Glu B1 have altered the diameter versus Fm 3 and Fm 2B with only 17+18. Thus the absence of the Glu D1 loci has a negative effect on the rollability of the tortillas. The line Fm 7 which has 1, 17+18 and 10 had a larger diameter than most of the lines yet the rollabilities were poor with breakage

by at the 4th day. Thus while the absence of subunit 5 of Glu D1 yielded larger diameter tortillas the rollabilities were none the less lowered. The subunit 5+10 thus appears important for tortilla rollability. The line Fm 9 which has 17+18, 2+12 and a deletion in Glu A1 had small diameters. While it had good rollabilities in Texas the rollabilities were significantly reduced in South Dakota. The lines Fm 13 and Gabo had a similar composition of 2*, 17+18 and 2+12 and produced tortillas with small diameters and poor rollabilities. The interactions between the subunits 2*, 17+18 and 2+12 did not seem to contribute to good tortilla diameter. Though similar in composition Fm 13 and Gabo had differences between their diameters and rollabilities. The HMW glutenin compositions are not able to account for all the discrepancies in the lines and the changes may have been due to other reasons. The presence of subunits in Glu D1 is related to a gain in function in tortilla rollability. The subunits at Glu B1 alone do not confer good rollabilities, yet when combined with the Glu D1 it does contribute to better rollability. The subunits 5+10 had better rollability scores than subunits 2+12. This could have been better explained if a glutenin deletion line having 17+18 and 5+10 were also available for comparison. The rollability of Fm 6 was stable by the 14th day; Fm 9, Fm 13 and Gabo had reduced rollability by the 14th day. The 5+10 subunit appears to be more important than 2+12 to maintain good rollabilities.

The comparisons can be further made with the cultivars such as Seri 82 and Kauz that were processed into tortillas (Table IX). The cultivar Kauz has 2*, 7+9 and 5+10 subunit composition. The Glu B1 locus has the presence of 7+9 subunit. The cultivar has poor diameter and rollability scores. The other line Seri 82 has 1, 17+18 and 5+10 subunit composition. Though the tortilla diameters were small the rollabilities were better

than Kauz. The rollability scores were not as good as Fm6. The functionality of the subunit 5+10 in contributing to the rollability of the tortillas is thus observed. The diameter of Seri 82 was significantly ($p<0.05$) lower than Fm 6. This result can indicate that the interaction of 17+18 with 5+10 results in loss of functionality. This interaction has possibly resulted in poor diameters in Olympic though it has a comparable rollability score.

A contrast between the glutenin subunits supports the results obtained for the effects of the subunit composition on the tortilla quality parameters (Table XI). Deletions in Glu A1 significantly affected the diameter and rollability of the tortillas. The deletions in Glu A1 loci did not affect the flour protein content in the lines. The variation in the flour protein content is due to the genotype and environment effects. The deletions in Glu B1 loci also improved the diameter significantly with no significant effect on rollability and flour protein content. Deletions in Glu D1 loci significantly affected the diameter and the rollability of the tortillas. The deletions in the Glu D1 did not significantly affect the flour protein content. Thus the deletions in the glutenin loci did not affect the flour protein content. As also observed in the ANOVA analysis the genotypic and location effects are significant that influence the flour protein content. Tortillas prepared from Fm 2B and Fm 3 with deletions in Glu A1 and Glu D1 had significantly larger diameters and lower shelf stability. The tortillas prepared from Fm 6 with deletion in Glu B1 had slightly smaller diameters than Fm 2B and Fm 3 but had good shelf stability. Interaction of the Glu A1 and Glu D1 is important for shelf stability.

The % IPP also supports the effects of the glutenin subunit composition in tortilla properties. The deletion of Glu A1 and Glu D1 have reduced the dough mixing strength

significantly (Table III). The % IPP in Fm 2b and Fm 3 were low. The reduction in the HMW glutenins in these two lines also resulted in increased extensibility of the dough. The HMW /LMW ratio reduced and thus the polymer network formed was weaker as is evident from the dough mixing strength. The lower amount of altered glutenins would form a weak network that would be able to extend itself yet the weak structure reduces its stability and the network ruptures quickly. A lack of polymer forming glutenin also reduced the elasticity of the network. The deletion lines Fm 2B and Fm 3 were thus able to extend but could not maintain their stability. In Texas the % IPP were higher hence the stability of the network was better yet the highly reduced % IPP in South Dakota resulted in the rupture of the network by the 4th day after processing. The lines Fm 13, Gabo and Olympic had higher % IPP. The proportions of HMW glutenins were higher because of the presence of all the glutenin loci Glu A1, Glu B1 and Glu D1. Olympic has an intermediate dough mixing strength due to subunit 20 present in Glu B1, which contributes less to the strength than the subunit 17+18. The HMW glutenin network is stronger due to higher proportion of HMW glutenins; the extensibility of the network was reduced. This resulted in smaller diameters of these lines. This complex network resulted in increased elasticity of the network. The stability of the network was good initially but it seemed to drop by 14th day. The lines missing Glu B1 have shown as intermediate dough strength and % IPP were also moderate. The HMW glutenin network formed was mellow with good extensibility. The stability of the network was also good. The better quality tortillas with bigger diameters and the longer shelf stability can be obtained with moderate % IPP that forms a mellow gluten network with intermediate dough strength. Intermediate dough strength is ideal for better dough and tortilla quality.

The line Fm 6 had the best overall tortilla quality attributes than the other deletion line. The absence of the subunit 17+18 at Glu B1 locus resulted in gain of functions in this line. The other lines such as Fm 2B and Fm 3 have also shown good dough extensibility and tortilla diameters. However the shelf life of these tortillas was poor though not different from the control flour and parent lines, thus breeding for better adaptation may be useful for these lines. Similar results for these lines were observed in both Texas and South Dakota. The introgression of these deletion compositions into a more adapted background may however help compensate for the poor shelf stability. The lines Fm 9 and Fm 13 have a better shelf life, but the tortilla diameters are very low. They yielded tortillas with diameters less than the commercially available flour. Fm 6 among all the deletion lines, parent lines and the other lines included in the comparison had a better combined compromise of greater diameter with a longer shelf life. It was better in quality than the commercial tortilla flour possessing L-cysteine for improved extensibility. Fm 6 has performed better in both the locations in Texas and South Dakota. This line has a subunit composition of 1 and 5+10 at Glu A1 and Glu D1 has a potential as a line with better tortilla quality attributes. Fm 6 thus can be identified as a line that can be further introgressed into a cultivar suitable for tortilla production. It also has acceptable loaf volume and may be compatible in a hard red winter wheat distribution system that targets bread quality (MacRitchie and Lafiandra 2001). The lines Fm 2B and Fm 3 having better dough properties and diameter can also be developed to use them in tortilla mixes. These tortilla mixes are usually used to make tortillas that are eaten fresh. These lines can be used to produce tortillas with good diameters that can be consumed fresh. The tortillas from these lines were fluffier and whiter in color and would be liked

by the consumers for its appearance and light texture. As well household or small business would appreciate the ease of mixing and dough processing attributes that these lines possess.

CHAPTER III

FUNCTIONALITIES OF GLIADINS IN WHEAT FLOUR TORTILLAS

1. Introduction

The wheat gluten proteins provide unique attributes to wheat flour. The gluten proteins are classified based on their electrophoretic mobilities into glutenins and gliadins. The function of the glutenins in wheat tortilla quality has been discussed in Chapter I and Chapter II. Here I will focus my study on the function of gliadins in wheat flour tortillas. Gliadins are monomeric proteins. Their molecular weight ranges from 30, 000 – 75, 000 Daltons. Gliadins are known to have extensive genotypic polymorphism. Based on the electrophoretic mobilities, the gliadins are classified into four different groups α, β, γ, and ω-gliadins. The genes coding for these proteins are present on the short arm of chromosome 1 and chromosome 6 (Figure 19). They are tightly linked genes present in the three homologous loci of chromosome 1 as Gli A1, Gli B1 and Gli D1. In chromosome 6 they are present as Gli A2, Gli B2 and Gli D2 (Wrigley and Shepard 1973, Brown and Flavell 1981). The Gli 1 genes code for the ω and γ-gliadins and the Gli 2 genes code for α and β-gliadins. The Gli 1 locus is tightly linked to the LMW glutenin loci Glu 3 in chromosome1 (Figure 19). The gliadin loci are known to be inherited in simple Mendelian inheritance and multiple-allelism has been reported for both the Gli 1 and Gli 2 loci (Metakovsky et al 1984, Metakovsky 1991).

Gliadin components have high glutamine amino acid content. For instance ω-gliadins have a higher than 50% glutamine content (Lasztity 1984). The proline content is also high in gliadins exceeding that found in HMW glutenins. The high proline content plays an important role in the secondary structure of gliadins.

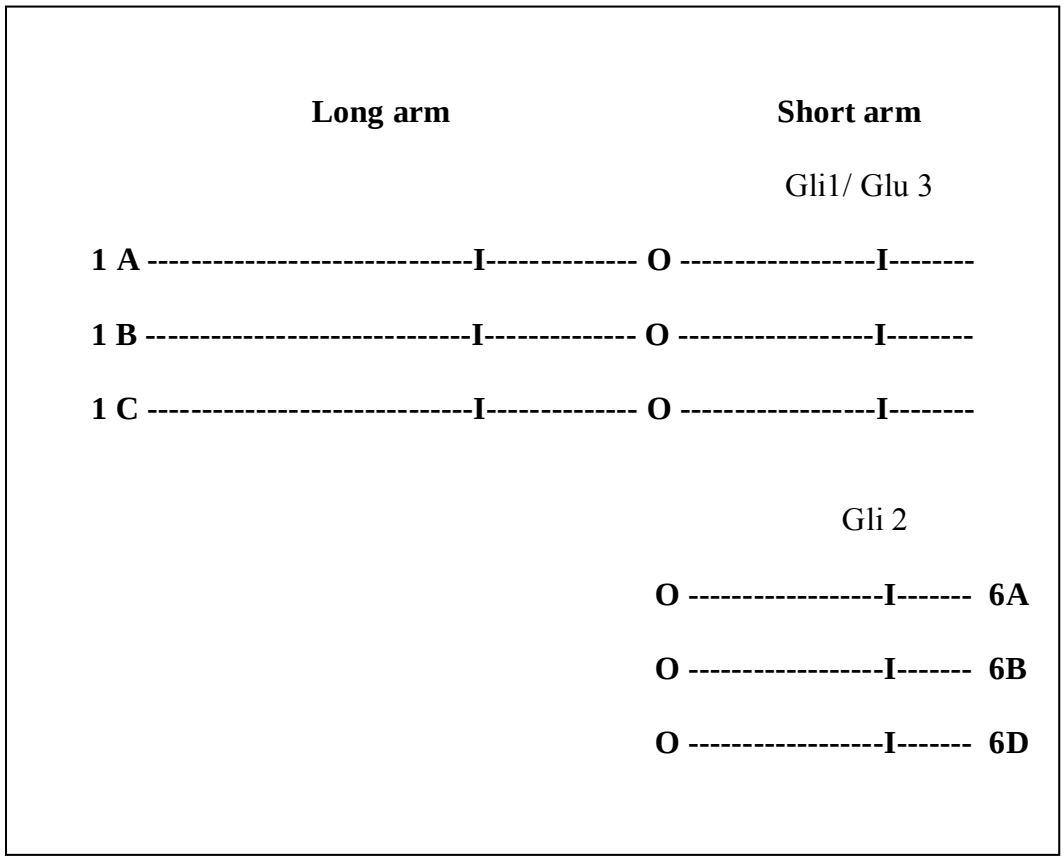


Figure 19: The location of the gliadin loci in short arm of chromosome 1 and 6.
The 'I' indicates the position of the Gli 1/Gli 3 loci and Gli 2 loci. The 'O' indicates the position of the centromere.

The gliadins are however poor in other basic amino acids like lysine, arginine and histidine (Kasarda et al 1974). The determination of the N-terminal sequence of the gliadins has supported the theory that a large number of gliadins is due to a genetic mutation of a common precursor during natural evolution of bread wheat. Most of α, β-gliadins and some of the γ gliadins have similar N-terminal sequence. The α, β, γ-gliadins have six to eight cysteine residues, as a result three to four intramolecular disulphide bonds occur (Kasarda et al 1984). Although the HMW and LMW glutenins form the disulphide cross-linked gluten matrix a small proportion (5-10%) of α and γ-gliadins occupy the matrix cross-link function. The ω gliadins also may take part in the polymer formation (Kukataite et al 2004).The gliadins like the LMW glutenin can also function as the chain terminators. The gliadins are generally considered to contribute to the viscosity and extensibility of the dough. The glutenin to gliadin ratio is thought to be an important determinant of quality. The higher glutenin to gliadin ratio is shown to contribute to greater dough strength (MacRitchie 1985). While study by Edwards et al (2001) suggests that an increase in the relative gliadin content is associated with increase extensibility and loss of dough strength. Glutenin enrichment can circumvent this and increase dough strength. Although gliadins have been associated to certain parameters of bread making quality, they are considered unimportant to dough strength (Gianibelli et al 2001).

The contribution of the gliadin alleles to the extensibility of the dough makes them an interesting object of study for tortilla quality. Good quality tortillas require extensible and mellow gluten network. High or low gliadin content depending on the allele may influence positively or negatively the tortilla quality. In order to test these hypotheses near-isogenic lines are available with deletions in Gli 1 and Gli 2 loci will

again be employed (Gianibelli 1998). In the previous studies deletions in the Gli 1 loci were found to exhibit greater dough strength (MacRitchie & Lafiandra 2001). Gianibelli (1998) compared the protein composition and the dough mixing strength of the near-isogenic lines missing Glu B3/ Gli B1 with its recurrent parent. A reduction in the polymeric proteins was observed in association with a reduction in dough strength. However these reductions were minor with regards to the reductions seen from the deletion of Glu 1 loci that reduce the glutenin to the gliadin ratio. This was thought to be due to the greater expression of LMW glutenins versus glutenins at Gli 1/Glu3 locus of the parent lines (MacRitchie and Lafiandra 2001).

Changes in the glutenin to gliadin ratios modify the bread making quality. In this study we will examine the functionality of the gliadin loci in relation to tortilla manufacture and quality. Significant contributions of the gliadin loci to tortilla quality may be observed. The near-isogenic wheat lines with deletions in the Gli 1 and Gli 2 loci provide an excellent opportunity to determine these contributions. Dough quality and tortilla quality measurements from these lines will enable us to relate the functionality of the gliadins to flour tortillas.

2. Methodology

2.1. Plant Material and Growth Conditions

Near-isogenic lines with deletions in the gliadin loci occur in nature. In the Russian cultivar Saratovskaja (Sarat) mutant deletion lines exists and have been obtained and increased (MacRitchie and Lafiandra 2001). This cultivar was selected as the plant material for the study of the effect of gliadins functionality in tortilla quality. The mutant lines of Sarat with deletions in their Gli 1 and Gli 2 loci, respectively (Table XIV).

Table XIV: Protein composition of the gliadin deletion and cultivar 'Saratovskaja'.

Wheat Lines	Gliadin alleles			
	GliA1 (1A)	GliA2 (6A)	GliD1 (1D)	GliD2 (6D)
Saratovskaja	ω γ	α β	ω γ	α β
GliA1	-	α β	ω γ	α β
GliA2	ω γ	-	ω γ	α β
GliD1	ω γ	α β	-	α β
GliD2	ω γ	α β	ω γ	-

The '– 'signs indicate the absence of the locus. 'Saratovskaja' has all the gliadin loci present. The other lines have deletions in either of the gliadin loci. The chromosome numbers are indicated in the brackets.

These selected gliadin deletion lines and their parent cultivar Sarat were grown in South Dakota under supervision of Dr. Carl Glover in 2005. They were grown along with the glutenin deletion lines and other selected lines in South Dakota under similar agronomic and weather conditions.

2.2. DNA Analysis

The deletion in the chromosome 1 and 6 were identified by the use to SSR based polymerase chain reaction. A set of SSR primers was selected based on their position near the gliadin loci in chromosome 1 and 6. The SSR primers that were used are Xgwm136, Xgwm147 for Gli A1 locus and Xgwm459 for Gli A2 locus, Xgwm106 for Gli D1 locus and Xgwm469 for Gli D2 locus.

The DNA extraction procedure, PCR and the DNA analysis were performed in a similar way as mentioned in Chapter II, Section 2.2.

2.3. Protein Analysis

The gliadin protein analysis was conducted using of high pressure capillary electrophoresis (HPEC) (Lookhart and Bean 1995). The analysis was conducted by Dr. Scott Bean, Agricultural Research Service, Kansas, Manhattan. The procedure followed for the analysis included in the extraction of the gliadins from each of the selected gliadin deletion line and also the parent cultivar Sarat and the separation of the gliadin subunits by the HPEC. The wheat kernels were ground in a mortar and pestle to produce flour. The gliadins were extracted from the flour using 70% ethanol and water (Lookhart and Bean 1995a). The samples and reagents were filtered through 0.45µm filters, and separated on a Beckman P/ACE 2100 system for HPEC analysis. The separations were performed at 22 kV and 45°C in 20µm i.d., uncoated fused silica capillaries that were 27

cm in length (Lookhart and Bean 1995a). Capillary cleaning was performed according to known procedures (Lookhart and Bean 1995b). A 0.1mM phosphate buffer was used in HPEC. The proteins were detected at an UV absorbance of 200nm. Gold's software collected the sampling data and the analysis was performed with Origin software (Microcal Software, North Hampton, MA). The gliadin profiles obtained were compared to confirm the absence of gliadin subunit in the deletion lines.

2.4. Evaluation of Wheat Grain and Flour

The grain quality evaluation of Sarat and the gliadin deletion lines were identical to that of the glutenin deletion lines. The single kernel hardness test was done for determining the grain hardness and moisture. The grain was milled into flour and the flour quality evaluations such as NIR and mixographs were also performed. The procedures followed for each of the tests for the evaluation of the gliadin deletion lines is discussed in Chapter II, Section 2.4.

2.5. Tortilla Processing

The flour from each of the lines was processed into tortillas. The standard formulation was followed to make the tortillas (Bello et al 1991). The formulation and the procedure used to prepare the tortillas are similar to that mentioned in Chapter II, Section 2.5.

2.6. Dough Quality Evaluation

The dough properties were measured on the following traits: dough temperature, dough softness, elasticity and extensibility were done. The procedures that were used for the measurements are same as mentioned in Chapter II, Section 2.6

2.7. Tortilla Quality Evaluation

The tortilla prepared from the gliadin deletion lines and cultivar Sarat were evaluated for diameter, weight, height, opacity, pH, moisture, color and rollability. The tortilla measurements were conducted according to the procedures mentioned in Chapter II, Section 2.7. The tortilla calculations of specific volume and quality index also followed the method as in Chapter II, Section 2.7.

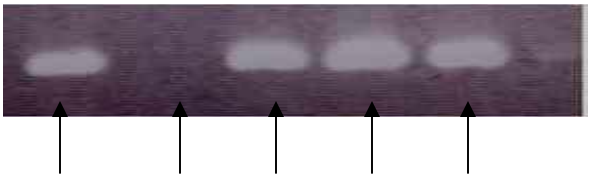
2.8. Data Analysis

The data obtained from the evaluations of gliadin deletion lines and the cultivar Sarat were analyzed using SPSS and Microsoft Excel. The Tukey HSD test for significance was performed.

3. Results

3.1. PCR Analysis

Polymerase chain reactions were performed with the SSR primer pair Xgwm147 located near the Gli 1 locus in short arm of chromosome 1A. A deletion in the short arm of chromosome 1 containing the Gli A1 locus in the gliadin deletion line Gli A1 locus resulted in no amplification with the primer Xgwm147. The other lines Gli A 2, Gli D1, Gli D2 and parent cultivar Sarat had bands in the agrose gels, verifying the presence of the Gli A1 locus (Figure20). The PCR was performed similarly to verify the deletions in the Gli 1 and Gli 2 loci present in the gliadin deletion lines Gli A2, Gli D1 and Gli D2 with the SSR primers pairs Xgwm459, Xgwm106 and Xgwm469 respectively. The PCR results were further verified using the HEPC analysis.



Saratovskaja Gli A1 Gli A2 Gli D1 Gli D2

Figure 20: The PCR analysis of the wheat lines with primer Xgwm147.
The Gli A1 shows no bands. The deletion in Gli A1 locus in short arm of chromosome 1 in the wheat line Gli A1 is confirmed.

3.2. Protein Analysis

The HEPC analyses performed by Dr. Scott Bean supported the results from the PCR analysis. The HEPC analysis verified the presence of gliadin deletions in the line Gli A1, Gli A2, Gli D1 and Gli D2 loci. In Figure 21 the deletions in the gliadin deletion lines are demonstrated with respect to the parent line Sarat. Absences of some peaks indicate the deletions. Line Gli A2 had some of α and β- gliadin peaks absent. In the line Gli D2 ω-gliadin peaks are absent indicating the deletions in Gli 2 locus.

The extraction of the flour proteins reveals that the polymeric protein percent was increased in the lines with gliadin deletions. This was expected as the reduction in the monomeric proteins corresponded with an increase in the polymeric proteins and an increase in the glutenin to gliadin ratio. The Tukey HSD analysis confirmed the significant increase in % IPP in the gliadin deletions lines compared to the parent cultivar. The only exception was the line Gli A2 that has similar % IPP as the parent cultivar. Table XV contains the deletions present and the changes in the % IPP and dough mixing time. The deletions in gliadin alleles have significantly affected the % IPP as obtained from the contrasts.

3.3. Single Kernel Hardness Test

The single kernel hardness test of the gliadins lines and the parent cultivar Sarat indicated that all the lines had hard kernels. The hardness index was above 60 for all the lines. The moisture content of the lines was also determined to estimate the water required for tempering. The seeds of all the lines were tempered overnight (12 hrs) and milled to obtain the flour.

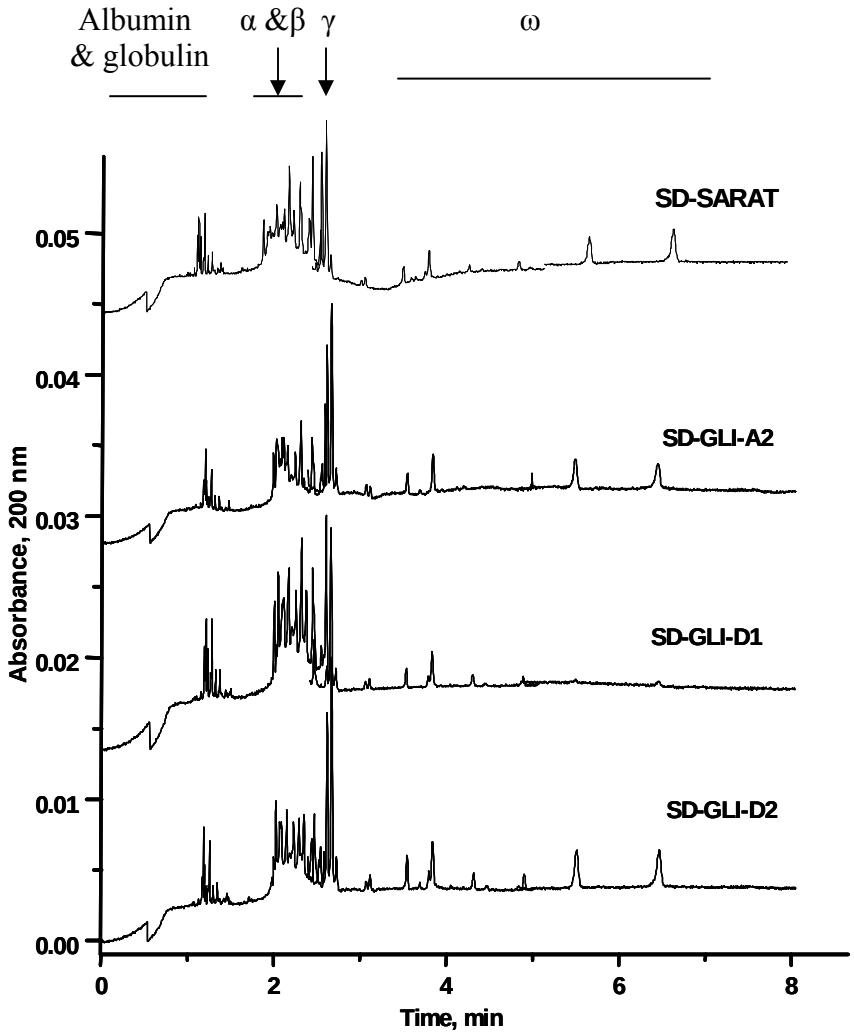


Figure 21: The capillary electrophoresis of the gliadins deletion lines and parent cultivar. The absence of ω-gliadin peaks in Gli D1 indicates the deletions in Gli 1 locus. The absence of some of the peaks in α- and β subunits indicates deletions in Gli A2 and Gli D2.

Table XV: The % insoluble polymeric protein and MDDT of 'Saratovskaja' and the gliadin deletion lines in South Dakota.

Wheat Lines	Gliadin Loci	% IPP	MDDT
Sarat	All gliadin loci present	4.0*	4.2
Gli A1	Gli 1 on short arm of chromosome 1A deleted	5.7*	3.2
Gli A2	Gli 2 on short arm of chromosome 6A deleted	5.2*	3.1
Gli D1	Gli 1 on short arm of chromosome 1D deleted	5.3*	3.4
Gli D2	Gli 2 on short arm of chromosome 6D deleted	3.9	3.4

The % IPP were significantly different by the Tukey HSD analysis (HSD=0.11)
* at 0.05 level of significance

3.4. NIR Results

The flour protein content from NIR of the parent cultivar was 10.4%. The gliadin deletion lines Gli A1, Gli A2, Gli D1 and Gli D2 had flour protein content similar to the parent line of around 10.5% (Figure 22). These lines were not grown in Texas, so the changes in the protein content due to change in location could not be assessed. Though the protein content was lower than the other glutenin lines grown in South Dakota, the % IPP of the flour was similar. No significant genotypic effects were observed using an ANOVA analysis (Table XVI). The flour protein contents were not affected by the deletions in the gliadin alleles (Table XVII).

3.5. Mixographs

The mixographs of the gliadin deletion lines and the parents were different from that the glutenin lines in South Dakota. The parent cultivar had a mixograph that was strong and with no defined peaks. The lines had a constant resistance for 8 min. The gliadin deletion lines Gli A1, Gli A2, Gli D1 and Gli D2 also had similar mixographs to the parent Sarat (Figure 22). No definite peaks were observed, though higher dough resistance was observed. The increase in the % IPP resulted in stronger mixographs, yet they lacked a peak mixing time. The glutenins and the gliadins behaved in a different manner to form the polymer network. The % IPP and the % PPP showed no significant correlations ($p<0.05$) with the dough and tortilla parameters (Table XVIII).

Table XVI: Mean squares of the ANOVA analysis of the gliadin deletion lines and the parent cultivar grown in South Dakota.

	IPP	Protein	Diameter	Rollability
Genotype	0.997*	0.48	1061.20*	0.15
Error	0.04	0.42	4.663	0.617

* at 0.05 level of significance

Table XVII: Contrast of the % IPP, protein, diameter, rollability between the gliadin lines and parent cultivar Saratovskaja.

Wheat Line	Gli A1	Gli A6	Gli D1	Gli D6
Sarat	+	+	+	+
Gli A1	-	+	+	+
Gli A2	+	-	+	+
Gli D1	+	+	-	+
Gli D2	+	+	+	-
Contrast				
IPP	0.00*	0.00*	0.00*	0.00*
Protein	0.210	0.80	0.941	0.941
Diameter	0.00*	0.00*	0.00*	0.00*
Rollability	0.873	0.810	0.873	0.968

*at 0.05 level of significance

Table XVIII: Pearson correlations of the dough and tortilla properties with the % insoluble polymeric protein and % polymeric protein.

	Extensibility	Elasticity	Diameter	Opacity	Rollability	Protein%	MDDT	Mixograph resistance
% IPP	-0.227	0.335	-0.493	-0.076	0.557	-0.042	-0.675	0.549
% PPP	-0.077	0.402	-0.477	-0.154	0.439	-0.038	-0.573	0.413

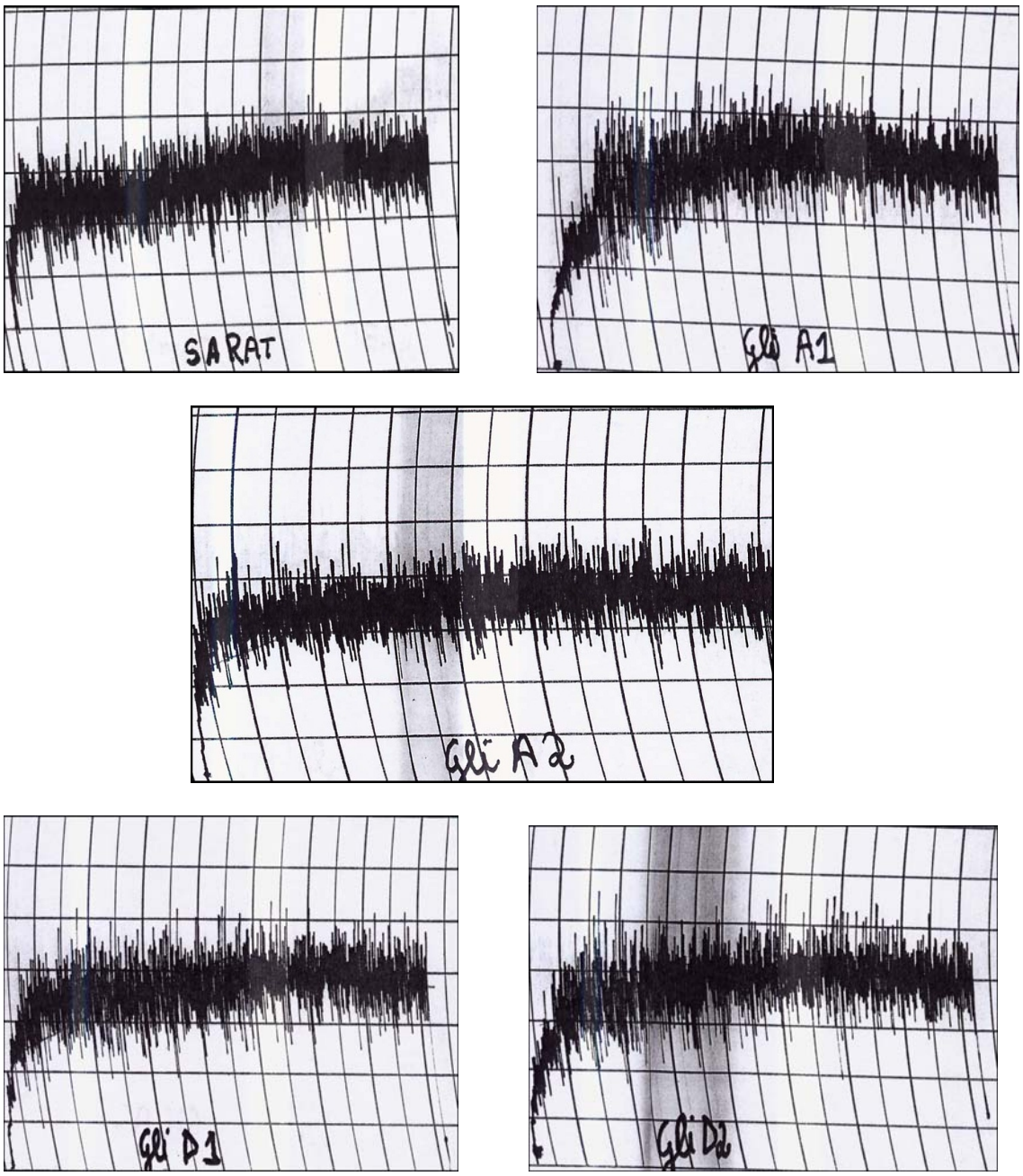


Figure 22: The mixographs of the gliadin deletion lines Gli A1, Gli A2, Gli D1, Gli D2 and the parent cultivar ‘Saratovskaja’.

3.6. Dough Quality Evaluation

The dough quality evaluation demonstrates that the extensibility of the dough is increased even when there is reduction in the monomeric proteins. It is quite contrary to the earlier studies. In all the gliadin deletion lines an extensibility score of 3.0 was found that similar to the score for the parent Sarat and the control tortilla flour. The elasticity scores are above the ideal score of 2.0. The doughs were extensible even though there was an increase in the % IPP. Except for the lines Gli A2 and Gli D2 all the lines had an ideal dough softness rating of 2.0. The gliadin deletion lines Gli A2 and Gli D2 had very soft doughs. The dough formation in the gliadin deletion lines required more water than was calculated by the water absorption rate from the mixographs. This excess water may have been responsible for some of the abnormal quality parameters.

3.7. Tortilla Quality Evaluation

The main tortilla quality parameters are diameter and rollability. The gliadin lines behaved opposite way to our expectations. The deletions in gliadin loci increased the polymeric proteins. The increase in the polymeric proteins should decrease the tortilla diameter. However, the tortillas prepared from the gliadin deletion line Gli A2 and Gli D2 that had significantly large diameters ($p<0.05$). The diameters were 180 mm and 175.2 mm respectively. These tortilla diameters were similar to the diameters obtained from the deletions of the HMW glutenins and larger than the tortilla diameters from the parent cultivar Sarat and the control flour. The tortillas prepared from the gliadin lines Gli A1 and Gli D1 had smaller diameters as expected due to the increase in their % IPP. The parent line Sarat had a diameter of 172 mm (Figure 23).

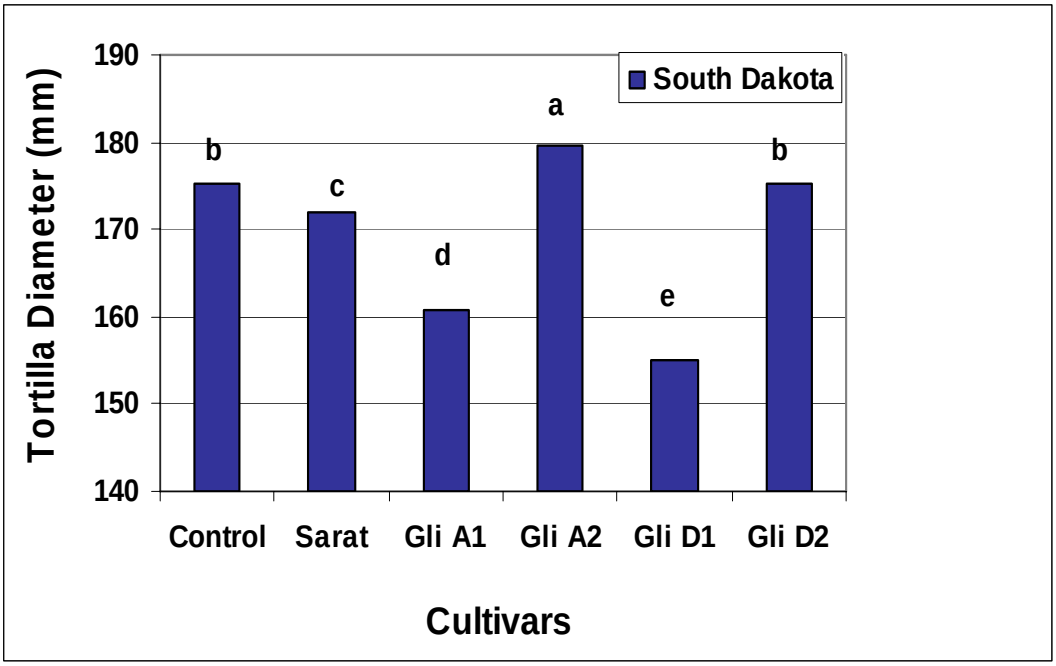


Figure 23: The average tortilla diameters (10 tortillas) of the gliadin deletion lines Gli A1, Gli A2, Gli D1 and Gli D2, parent cultivar 'Saratovskaja' and control tortilla flour. The symbols a, b, c, d indicate significance ranks of the cultivars based on the significant differences (HSD=2.02, α=0.05) obtained from Tukey-HSD analysis.

The ANOVA analysis of the tortillas diameters indicated significant genotypic effects The Tukey-HSD test analysis of the tortilla diameters indicates that Gli A2 had significantly (HSD=2.02, α=0.05) larger tortilla diameters than the other lines and the control flour. Significant contrasts were present between the gliadin deletions affecting the tortilla diameter.

The tortillas prepared from the gliadin deletion lines Gli A1, Gli D1, Gli A2 and Gli D2 had similar poor rollability scores on the 14th day (Figure 24). No significant differences in the tortilla rollability scores observed between the gliadin deletion lines and the parent and the control flour by the Tukey-HSD test on the 14th day. The rollabilities of all the deletion lines were within the range of that found for the control flour (2.3 versus 2.7 respectively).

The specific volume of the tortillas from the gliadin deletion lines were less than the control flour (Table XIX). The opacity scores of the tortillas were above 80 for all the lines (Table XIX). The calculated quality indexes of the gliadin deletion lines were lower than the control tortilla flour (Table XIX).

Some significant correlations were observed. The tortilla diameters were negatively correlated to the tortilla height ($p<0.05$). An increase in tortilla diameters reduces the height of the tortillas. The dough elasticity was positively correlated ($p<0.05$) to the tortilla height. The tortilla opacities showed a positive correlation with the tortilla diameter and the specific volume (Table XX).

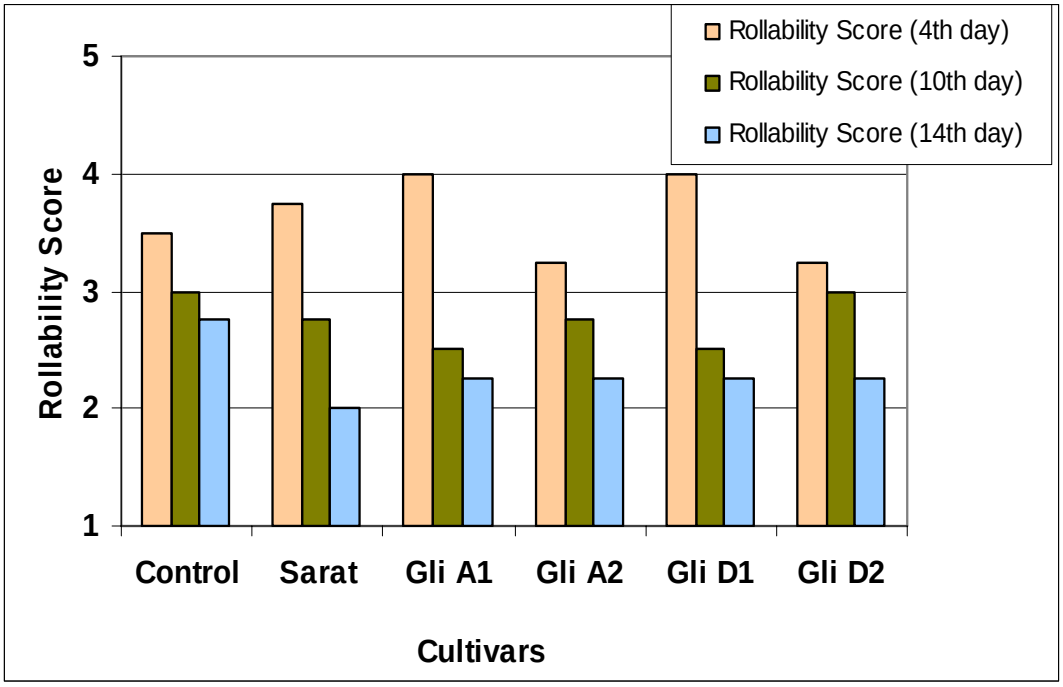


Figure 24: The rollability scores of the tortillas from the gliadin deletion lines Gli A1, Gli A2, Gli D1 and Gli D2 and the parent cultivar 'Saratovskaja' on the 4th, 10th and 14th day after processing of the tortillas.

Table XIX: The dough and tortilla quality evaluation results of the gliadin deletion lines grown in South Dakota.

Genotype	1A	1B	6A	6D	Protein (%)	Q. Index (14d)	Rollability (14d)	Diameter (mm)	Opacity (%)	Sp. Vol. (cm^3/g)	Mixtime (min)	Resistance (MU)
Control	?	?	?	?	12.0	304	2.75	175	85	1.30	-	-
Sarat	+	+	+	+	10.4	201	2.0	172	80.5	1.25	4.2	4.2
Gli A1	-	+	+	+	10.3	220	2.25	161	82	1.19	3.2	5.4
Gli A2	+	+	-	+	10.7	253	2.25	180	85	1.33	3.1	4.8
Gli D1	+	-	+	+	10.5	212	2.25	155	79	1.19	3.4	5.4
Gli D2	+	+	+	-	10.5	248	2.25	175.2	84	1.32	3.4	5.2

Table XX: Pearson correlations of the dough and tortilla properties of the gliadin deletion lines, parent cultivar 'Saratavoskaja' in South Dakota.

	Extensibility	Elasticity	Height	Diameter	Opacity	Sp. vol.	Rollability	Protein%	MDDT	Mixograph resistance
Extensibility	1	0.816*	0.427	-0.270	-0.475	-0.289	0.186	0.403	0.060	-0.019
Elasticity		1	0.837*	-0.587	-0.448	-0.567	0.455	0.458	-0.292	0.403
Height			1	-0.815*	-0.465	-0.707	0.397	0.197	-0.372	0.709
Diameter				1	0.820*	0.949*	0.176	0.378	-0.182	-0.396
Opacity					1	0.841*	0.563	0.559	-0.645	0.162
Sp. Vol.						1	0.279	0.421	-0.292	-0.201
Rollability							1	0.919*	-0.953*	0.699
Protein%								1	-0.805	0.374
MDDT									1	-0.748
Mixograph resistance										1

*at 0.05 significance level.

4. Discussion

The gliadin deletion lines were grown in South Dakota. The gliadin deletion lines had lower protein content similar to the parent cultivar Sarat. The lower protein content may have resulted from environment conditions in South Dakota and or poor adaptability. The % IPP varied in the gliadin deletion lines as expected due to deletions in the monomeric proteins. The % IPP increased in the gliadin deletion lines Gli A1, Gli A2, Gli D1 (Table XV). The % IPP in the gliadin deletion line Gli D2 did not increase but was similar to the parent cultivar Sarat. The deletion in the Gli D2 loci may not have affected the glutenin to gliadin ratios. While the other deletions were effective – the reduction in the gliadins monomeric proteins increased the ratio of polymeric proteins. The behaviors of the polymeric proteins in the gliadin lines were unique. The mixographs of the gliadin deletion lines were intermediate in dough strength to the parent Sarat yet strong. The mixographs lacked a peak and the dough mixing resistances were similar through the entire 8 min. This was unique for the lines. The increase in the polymeric proteins should have produced a strong mixograph with a good peak and strong dough mixing resistance. The polymeric proteins in these lines did not behave this way though, over expression of Glu D1 glutenins have similar mixographs with ultra strong mixographs that don't peak (Popineau et al 2001).

The dough and the tortilla properties also are quite unique. Though there were deletions in the gliadins and an increase in the % IPP, the extensibility and the elasticity of the lines were increased. Earlier studies indicate that increase in the gliadins results in higher extensibilities (Singh & MacRitchie 2000) counter to what was observed with the glutenin deletion lines discussed in Chapter II, the reduction in % IPP increased the

extensibilities and vice versa. The gliadin lines required more water to form the dough than estimated from the water absorption rates. This may indicate that the polymer network was poorly developed or a loss in the gliadin content has unexpected consequences for the network.

The tortilla quality properties of the lines Gli A1 and Gli D1 were as expected. Though lines had ideal extensibility scores the tortilla diameters were smaller. The tortilla diameters of these lines were significantly ($p<0.05$) smaller than that of the parent cultivar Sarat and the control flour. The rollabilities of the lines were good on the 4th day but showed a gradual reduction by the 14th day similar to the control flour. The lines Gli A2 and Gli D2 had excellent tortilla properties. Gli A2 had an increase in % IPP, yet the tortilla diameters were very large. The tortilla diameter was 181mm similar to that obtained from the glutenin deletion lines grown in South Dakota. Line Gli D2 and the parent cultivar Sarat had similar % IPP and similar diameters. The rollability scores of the lines were low on the 14th day after processing, yet in a range similar to the control flour.

The behaviors of some of the gliadin lines were not as expected. The tortillas prepared from the gliadin deletion lines are expected to have smaller diameters and better rollabilities. The possible explanation for the behavior of the gliadin lines may be due the differences in the formation of the glutenin polymer network in the dough. The HMW glutenins are interlinked to form a cohesive mass of dough. The gliadins facilitate the extensibility of the network. The results obtained from the study of the gliadin deletion lines suggest that possibly the polymer network did not form properly. The increase in the ratio of HMW glutenins due to the deletion in the gliadin loci should have resulted in

strong polymer network. The increase in the HMW glutenins and the subsequent absence of the gliadin locus resulted in increased polymeric proteins and high gluten strength. The high gluten strength resulted in abnormal mixing and thus the mixographs obtained showed a very strong gluten network. The mixographs indicate that the proteins had failed to form a homogenous protein network. The glutenins may have formed aggregates rather than being a part of the network. When water was added to form the dough and processed into tortillas these aggregated glutenin subunits provided the extensibility that resulted in good tortilla diameters in the line Gli A2. This line required more water than estimated by the flour protein content, the excess water may have been another reason for the increased tortilla diameter in this line. The hydrogen bonds affect the aggregation of the proteins (Laszitity 1984). The glutenins in absence of gliadins caused a restructuring of the glutenin polymer network that may have affected the behavior of these lines. The deletion in the Gli D2 locus did not cause any significant changes in the dough and tortilla properties different from the parent cultivar Sarat. The other lines Gli A1 and Gli D1, while having abnormal mixograph behavior, reduced tortilla diameters indicate that on processing the polymeric proteins behaved as expected. The increase in the polymeric proteins in absence of gliadins that provide slippage to the network may result in abnormal behavior of the network. (Popineau et al 200).

The oxidizing and reducing agents when added to strong gluten strength causes a reduction in the resistance and increase the extensibilities. The amino acid cysteine is a commonly used reducing agent that competes with the disulphide bridges and increases the extensibilities of the gluten network. The oxidizing or reducing agents were not added during tortilla processing of gliadin deletion lines. The addition of the reducing agent

cysteine may have facilitated the disassociation of the disulphide linkages in the protein aggregates and assisted in the polymer network formation. The addition of cysteine to gliadin deletions lines may have given us a better understanding of the affects of the gliadins in tortilla processing.

While some of the results were quite unique, the study revealed the importance of the monomeric proteins in the gluten network formation. The gliadins provide the slippage and extensibility to the protein polymeric network. The increase in the polymeric proteins in the absence of the gliadins leads to an abnormal network formation that affects tortilla quality parameters. The study of the contrasts between the lines indicates that the deletions in the gliadin locus significantly affect the % IPP and the tortilla diameters. Thus the elimination of gliadin loci Gli 2 may be important for achieving larger tortilla diameter while maintaining shelf stability.

CHAPTER IV

CONCLUSION

Specific glutenins and the gliadins alleles and their specific functionality affect tortilla quality. The formation of the polymeric protein network by the specific glutenin and gliadin subunits in the dough influences the tortilla quality. The quantity of the proteins, the subunit compositions and the interaction of the proteins all influence the polymer network. Lines with deletions in the Glu A1 and Glu D1 subunits had reduced polymeric proteins. A weak mixing resistance and increased extensibility observed. Increased extensibilities resulted in larger tortilla diameters. The reduction in polymeric proteins reduced the resistance points in the polymer network and increased the chain slippage resulting in higher extensibilities (Singh & MacRitchie 2000). The gliadins supposedly provided the slippage properties to the network, increasing extensibilities. The formation of weak gluten network affected the stability of the tortillas. The lines with the deletions in Glu B1 had optimum tortilla properties. The presence of the Glu A1 and Glu D1 formed a gluten network of intermediate strength. The strength increased due to the increase in the resistance points, yet the tortilla diameters obtained were still larger. The diameters were not significantly affected as the chain slippage action provided the extensibilities. The stability of the tortillas was good. The lines with deletion in Glu A1 and those with all the loci present had strong resistance and increased polymeric proteins. The increase in the polymeric proteins increased the disulphide linkages and resulted in strong network. The resistance in the network was greater than the rate chain slippage. This affected the extensibilities and resulted in smaller tortilla diameters. The tortilla stability of these lines was poor. The interaction of the Glu B1 and Glu D1 loci in Fm 9

increased the dough resistance reduced the dough extensibilities resulting in smaller diameters.

The shelf stability of the tortillas reduced was in South Dakota. The deletion line Fm 6 was an exception with better tortilla diameters and stability in both the locations. The interactions of the subunit 1 of Glu A1 and 5+10 of Glu D1 may have resulted in gain in functionality in line Fm 6. Fm 6 had ideal tortilla properties. Fm 2B and Fm 3 with subunit 17+18 at Glu B1 locus had larger diameters, but lower tortilla stability in South Dakota only, in Texas they were statistically similar to the parent lines or control flour tortillas

The behaviors of the gliadin deletion lines were unique. The deletion in monomeric proteins is expected to increase the polymeric proteins and reduce the diameters of the tortilla. The mixographs of the gliadin lines indicate that the increase in polymeric proteins resulted in the abnormal behavior of the polymer network. The mixographs lacked a peak resistance indicating that a cohesive mass was not formed. Uniform resistance was observed in the gliadin deletion lines. While the Gli A1, Gli D1 lines behaved as expected, the mixing behavior was abnormal, the lines had smaller tortilla diameters. The line Gli A2 was exceptional with larger tortilla diameters. This line also showed bubbles in the tortillas that indicate that more water had been added. The glutenins formed aggregates rather then forming a homogenous network. The interaction of the hydrogen bonds may be important. The increased water content may have resulted in more hydrogen bonding between the protein aggregates and water, preventing them from entering into the polymer network. The interactions other than the disulphide linkages of the polymer network thus may be important. A similar exception was

observed in the glutenin deletion lines. Line Fm 13 and parent Gabo had similar protein subunit composition (1, 17+18, and 2+12), yet the lines behaved differently. Fm 13 had a higher % IPP than Gabo, yet had larger tortilla diameter than Gabo. The tortillas of Fm 13 had bubbles on their surface, indicating higher water content. The behavior of the glutenin polymer network is influenced by the amount of water present.

The study of the glutenin and the gliadin proteins exhibit their importance in the functionality for tortilla quality. The increases in the polymeric proteins affect the tortilla diameters. The reduction of the polymeric proteins reduced the stability of the tortilla. Thus an intermediate polymeric protein is important for larger tortilla diameters and good stability. The subunit compositions of the glutenins influence the polymeric network forming ability and the tortilla quality. The monomeric proteins gliadins influence the tortilla quality. Deletions in the gliadin loci resulted in loss of functionalities for diameters while Gli A2 behaved exceptional. Monomeric proteins are required to provide the dough extensibilities that influence the tortilla diameters. Line Gli A2 had good tortilla properties, but is unpredictable. The gain in functionality in the Gli A2 lines due to abnormal mixing behavior may not be repeatable.

The line Fm 6 (1, 5+10) with a deletion in Glu B1 had better tortilla quality attributes. The line was stable in both locations. This line is a promising line that can be selected and tested further for improved adaptability and release as a tortilla cultivar. Lines Fm 2B and Fm 3 can be selected as wheat lines providing better tortillas quality for the fresh market.

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