EFFECTS OF DELETIONS OF HIGH MOLECULAR WEIGHT GLUTENIN SUBUNIT ALLELES ON DOUGH PROPERTIES AND WHEAT FLOUR TORTILLA QUALITY

A Thesis

by

YUNUS EMRE TUNCIL

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 2012

Major Subject: Food Science and Technology

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Approved by:

Chair of Committee,	Joseph M. Awika
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ABSTRACT

Effects of Deletions of High Molecular Weight Glutenin Subunit Alleles on Dough Properties and Wheat Flour Tortilla Quality. (August 2012)

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In wheat (Triticum aestivum L), high molecular weight glutenin subunits (HMW -GS) are synthesized by the loci *Glu-A1*, *Glu-B1*, and *Glu-D1* on the long arm of group 1 chromosome, and their variants play a significant role in the functional properties of flour; hence dough properties and tortilla quality. This study was conducted to understand the effects of HMW-GS on dough properties and tortilla quality using 40 different wheat lines from two different locations; Texas Agrilife Experiment Station at McGregor, and at Castroville, Texas, in 2010.

Wheat lines in which one or more of these loci were absent (deletion lines) and non-deletion lines were used. Flours were evaluated for insoluble polymeric protein (IPP) content and mixograph properties. Dough properties; compression force, stress relaxation test, and dough extensibility, were determined using a texture analyzer. Tortillas were produced by hot-pressed method and evaluated for physical properties and textural change during 16 days of storage.

Flour from deletion lines had lower average IPP content (38.4%) than non-deletion lines (41.9%). Dough from deletion lines were more extensible (44.8 mm) and required lower equilibrium force from stress relaxation test (4.91 N) compared to non-deletion lines (34.2 mm, and 6.56 N, respectively). Deletion lines produced larger diameter tortillas (177 mm) than non-deletion lines (165 mm) and had lighter color (L* = 82.3) than tortillas from non-deletion lines (L* = 81.0). Most of the deletion lines interestingly produced tortillas with acceptable flexibility scores on day 16 of storage (≥ 3.0). Flour IPP content (r = -0.57) and equilibrium force (r = -0.80) were negatively correlated with tortilla diameter, but positively correlated with 16 day flexibility scores (r = 0.72, and r = 0.68, respectively). In general, deletion at *Glu-A1* or *Glu-D1* or presence of 2+12 instead of 5+10 allelic pair at *Glu-D1* locus produced large diameter tortillas, but with poor day 16 flexibility. However, combination of 7+9 at *Glu-B1* locus with deletions at *Glu-A1* or *Glu-D1* or 2+12 at *Glu-D1* consistently produced tortillas that had large diameter and retained good flexibility scores during 16 days of storage. The results indicate the presence of 7+9 at *Glu-B1* may play a crucial role in selection of wheat varieties for tortilla making.

DEDICATION

I dedicate this thesis to my family. More specifically, to my grandmother Ayse Tuncil and grandfather Nuri Tuncil.

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NOMENCLATURE

HMW	High Molecular Weight
GS	Glutenin Subunits
Glu	Glutenin
IPP	Insoluble Polymeric Proteins
min	Minute
sec	Second
mm	Millimeter

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CHAPTER I

INTRODUCTION

Tortillas are the second most consumed bread type in the United States after white bread and are offered on two-thirds of restaurants menus nationwide (Lovgren 2006). The consumption of tortillas is growing throughout the world. While tortilla sales in the United States were \$ 1.37 billion in 2002, they were \$ 1.91 billion in 2005 (U.S. Census Bureau) and the estimated volume of tortilla industry is \$11 billion by the end of 2011 (Hartman 2011). In the market, corn and wheat flour tortillas are currently available.

Gluten proteins are the storage proteins of wheat which give the viscoelastic properties of dough. High molecular weight glutenin subunits (HMW-GS) are a group of wheat gluten proteins which have important effect on dough viscoelastic properties and hence final baked product quality (Anjum et al 2007).

Wheat varieties which have the right HMW-GS alleles have been developed for bread making (Weegels et al 1996) and these varieties are currently blended to produce tortilla flour in the industry. However, tortillas made from bread flour often give smaller diameter due to the strong gluten network, thus require use of additives to weaker gluten structure.

This thesis follows the style of Cereal Chemistry.

Good quality tortillas must be soft without sticking together, flexible without cracking and tearing when folded, and puffed (Bello et al 1991). Good quality wheat flour tortillas have large diameters (17- 18 cm) and good shelf stability (Pascut et al 2004), because most of them are not consumed on the day of production. To provide these desirable properties of tortilla, gluten extensibility in dough must be increased. For this reason, tortilla producers use ingredients such as reducing agents, fats, and enzymes. However, use of these ingredients in high amounts can cause undesirable taste and flavors, besides adding to production cost.

The right HMW-GS combination in wheat for tortilla production is still unknown. Jondiko (2010) reported that wheat lines with HMW-GS 2*, 17- 7, 5 on *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively gives larger diameter tortillas but with inferior flexibility. Mondal et al (2008) also reported that wheat lines with HMW-GS 17+18 on *Glu-B1* and deletions on *Glu-A1* and *Glu-D1* produce tortilla with large diameter but inferior shelf stability. This can be attributed to deletions in HMW-GS causing formation of fewer cysteine linkages and a more extensible gluten matrix in the dough system.

There is need to increase understanding of the effect of variations and deletions in HMW-GS on dough properties and tortilla quality. This will lead to developing of wheat cultivars with optimum gluten functionality for tortillas.

RESEARCH OBJECTIVES

1) Determine the effect of deletions at different HMW -GS alleles at homologous loci on A, B, and D genomes, on dough properties

2) Evaluate the tortilla making quality of wheat lines possessing deletions at different HMW –GS alleles at homologous loci on A, B, and D genomes.

CHAPTER II

EFFECTS OF DELETIONS OF HIGH MOLECULAR WEIGHT GLUTENIN SUBUNIT ALLELES ON DOUGH PROPERTIES AND WHEAT FLOUR TORTILLA QUALITY

INTRODUCTION

Tortilla Production Trends and Uses

Tortillas are flat bread produced from either corn or wheat. Corn tortilla is made from lime-cooked, stone-ground corn, while a major ingredient for wheat tortilla is wheat flour. The tortilla industry had a strong growth in the last five years. Tortilla Industry Association (TIA) reported that the tortilla was $6 billion industry a few years ago. However, estimated volume of the tortilla industry was $11 billion by the end of 2011.

In the U.S., tortillas are now served as substitutes for traditional breads in such popular food as hot dogs, lasagna, sandwiches, and pitas. Good quality tortillas must be large in diameter (larger than 170 mm), flexible over storage (flexibility score is equal 3 or above), light in color (Pascut et al 2004), and thin (1- 5 mm) (Waniska 1999). Wheat flour proteins play a crucial role on tortilla quality, because they are responsible for dough viscoelastic properties and strength. Therefore, functionality of wheat flour proteins must be understood.

Wheat Flour Proteins and Their Synthesis

Proteins are the most important constituent of wheat flour which are responsible for visco-elastic properties of dough and hence final product quality. Flour proteins can be classified into four groups based on their solubility: water soluble proteins are albumins; salt solution soluble proteins are globulins; alcohol soluble proteins (prolamins) are gliadins; dilute acid or base soluble proteins (prolamins) are glutenins (Shewry et al 1986). Prolamins are the major storage proteins (Shewry et al 2002). Wheat flour contains 8- 20% (dry weight) protein, and 80- 85% of the wheat proteins are gluten proteins (Shewry et al 1995). The storage proteins that form a network called gluten, determine viscoelastic properties of dough (Schofield 1994; Shewry et al 1995). Among these groups, gliadins and glutenins constitute the most important part of proteins because they interact with each other through chemical bonds such as disulfide, hydrogen, and hydrophobic interactions (Tilley et al 2001) to form a protein network in the system. Glutenins and gliadins are responsible for elasticity, and viscosity of dough, respectively (MacRitchie 1987).

Gliadins

Gliadins are monomeric proteins that are soluble in 70% alcohol solution. Gliadins are separated into four groups based on their electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): α- gliadins have fastest mobility; β-, γ- and ω- gliadins have slowest mobility (Gianibelli et al 2001). Wrigley and Shepherd (1973) reported that gliadins are encoded by the genes located on the short arms of group 1 and 6 chromosomes. These genes are tightly linked and located at three homologous loci of each group of chromosomes. *Gli-A1*, *Gli-B1*, and *Gli-D1* loci are in group 1 chromosome. *Gli-A2*, Gli-B2, and *Gli-D2* are in group 2 chromosome. ω- Gliadins and most of the γ- gliadins are encoded on *Gli-1* genes, whereas all of the α- and most of the β- Gliadins are encoded on *Gli-2* genes (Mondal et al 2009).

Gliadins are responsible for viscosity and extensibility of gluten (Gianibelli et al 2001). An increase in relative gliadin content results in loss of dough strength, increase in extensibility of dough, and reduction in bread making quality (Edwards et al 2001). An increase in dough extensibility contributes to tortilla quality by providing an increase in diameter. Moreover, Mondal et al (2009) reported that absence of *Gli-2* gliadins contribute to the extensibility of dough even while increasing the polymeric protein content.

Glutenins

Glutenins are polymeric proteins which are among the largest proteins in nature (Wrigley 1996). Based on gel filtration method, their molecular weights reach over twenty million daltons (Bietz and Simpson 1992). According to their molecular weight, glutenins are divided into two groups: low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). Each group of glutenins plays important roles in determining dough properties. Gupta et al (1991) reported that glutenin content of protein in flour is as important as protein content of flour to determine extensibility and resistance of dough.

LMW- GS constitute approximately 60 % of glutenins and one- third of the total seed protein (Bietz and Wall 1972). They are encoded by the genes at the Glu-A3, Glu-B3, and Glu-D3 loci on the short arms of group 1 chromosomes (Galova et al 2002).

Although HMW- GS are quantitatively low (constitute the 5- 10% of total protein) (Payne 1987), they constitute the most important part of wheat storage proteins. They are responsible for gluten elasticity (Flavell 1989). HMW- GS are encoded by the genes at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci on the long arms of group 1 chromosomes (Payne et al 1979). There are two genes, x- and y- type, on each locus which are called subunits (Payne et al 1981; Shewry et al 2001). Two subunits that are encoded by the same allele are tightly linked to each other; therefore, alleles, rather than individual subunits are related to quality (Weegels et al 1996). The most common method used to determine allelic composition is SDS- PAGE and each gene is named based on their mobility in SDS-PAGE. These differences arise from amino acid content and sequences in their structure (Shewry et al 1992, 2001; Gianibelli et al 2001). While there are 3 different alleles at the *Glu-A1*, 11 and 6 different alleles are reported at the *Glu-B1* and *Glu-D1*, respectively (Payne and Lawrence 1983). The common HMW-GS coded by *Glu-1* loci are 1 and 2* at *Glu-A1*; 17+18, 13+16, 7+9, 7+8, 6+8 and 20 at *Glu-B1*; and 5+10, 2+12 and 3+12 at *Glu-D1* (Pierucci 2008).

Effects of HMW-GS on Tortilla Quality

Variations in the HMW-GS composition affect the tortilla properties (Mondal 2006). Mondal et al (2008) reported that HMW- GS 1 and 5+10 at *Glu-A1* and *Glu-D1*, respectively are important for tortilla quality in terms of diameter and shelf stability because these alleles improve dough extensibility and provide sufficient gluten strength. Jondiko (2010) also reported that flours with allelic compositions 2*, 17+18, 5+10 and 1, 2*, 7+9, 5+10 at *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively produced tortillas with good shelf stability, but with smaller diameter. This can be attributed to the fact that 5+10 at *Glu-D1* locus causes the formation of strong gluten network in dough. Strong gluten matrix in the dough increases the flexibility of tortilla and hence increases shelf stability. However, the strong gluten network limits dough extensibility and the dough shrinks back after pressing; causing production of tortilla with smaller diameter.

Deletions at different HMW glutenin alleles at homologous loci on A, B, and D genomes also have potential effect on tortilla quality. Tortillas with larger diameter were produced using wheat flour with deletions at different HMW glutenin alleles at homologous loci on A, B, and D genomes, but their flexibility scores were inferior (Uthayakumaran et al 2003; Mondal 2006). This can be attributed to the fact that sufficient disulphide bonds cannot be formed due to decreased cysteine residues which cause a formation of weaker gluten network. Dough with weaker gluten network spread more during hot-pressing and hence produces larger diameter tortillas, but it does not provide sufficient strength to maintain flexibility over storage. On the other hand, flours possessing 1 and 5+10 at *Glu-A1* and *Glu-D1* loci, respectively and with deletions at *Glu-B1* locus give tortilla with large diameter and higher flexibility (Mondal et al 2008). Apparently, the locus has a big impact on dough properties, and combination of null alleles with the right allelic pairs may produce optimum protein quality for tortilla.

Growing popularity of wheat flour tortillas increases the needs of good quality wheat that can produce optimum tortilla quality. Due to the important role of HMW-GS on dough functionality, they are a logical target for optimizing wheat flour for tortillas. This study aims to provide better understanding of the effect of variations and deletions in HMW-GS, present at the homologous loci of *Glu-1* on the A, B, and D genomes on dough properties and tortilla quality.

MATERIALS AND METHODS

Wheat Lines

40 wheat lines with variations and deletions on HMW glutenin allelic composition were collected from two different fields (Table 1); Texas Agrilife Experiment Station at McGregor, and at Castroville, Texas, in 2010. The wheat was harvested and dry milled into flour and processed into tortillas. Flours which have same allelic compositions and deletions were grouped together for evaluation (Table I). Commercial tortilla flour, which is untreated, bleached, and enriched (ADM milling company, Overland Park, KS) was used as a control.

Evaluations of Kernel and Milling

Single Kernel Hardness Test

To evaluate hardness, diameter, moisture content and weight of single kernel, the single kernel hardness tester (Perten Single Kernel Characterization System SKCS 4100, Perten Instruments, Sprongfield, IL) was used.

Table I

Wheat lines with different high molecular weight glutenin allele composition

Group	Entry	Wheat Lines	Pedigree	*Glu-A1*	*Glu-B1*	*Glu-D1*
1	1	Ogallala		-	-	-
2	21[a]	T76-11	FM3/TX5009/TX9628	-	7+8	-
2	26	T78-34	FM3/Ogallala/Halberd	-	7+8	-
4	33[a]	T80-16	FM3xTX5009/OgallalaxFM3	-	20a+20b/7+9	-
5	8	T68-21	FM3xTX5009/JaggerxFM6	-	7+9	2+12
6	10	T69-32	FM3xTX5009/OgallalaxFM6	-	7+9	5
7	40	T82-37	FM6/TX5009/Ogallala	-	7	10
8	37	T82-1	FM6/TX5009/Ogallala	2*	17+18	-
9	3	TAM 304		1	7+9	5+10
9	5	T66-23X	Fm2a/Seri/Ogallala/	1	7+9	5+10
9	6[a]	T66-25	Fm2a/Seri/Ogallala/	1	7+9	5+10
9	27	T78-38	FM3/Ogallala/Halberd	1	7+9	5+10
9	28	T78-64	FM3/Ogallala/Halberd	1	7+9	5+10
9	29[a]	T78-65	FM3/Ogallala/Halberd	1	7+9	5+10
9	31	T78-74	FM3/Ogallala/Halberd	1	7+9	5+10
9	38	T83-5	FM6xOgallala/JaggerxFM6	1	7+9	5+10
17	2	TAM112		1	7+8	5+10
18	11	T74-4A	FM3/Jagger/TX8618	2*	7+8	5+10
18	12[a]	T74-4X	FM3/Jagger/TX8618	2*	7+8	5+10
18	14[a]	T74-23	FM3/Jagger/TX8618	2*	7+8	5+10
18	15	T74-25	FM3/Jagger/TX8618	2*	7+8	5+10
18	16	T74-25Y	FM3/Jagger/TX8618	2*	7+8	5+10
18	17	T74-26X	FM3/Jagger/TX8618	2*	7+8	5+10
18	22	T76-21X	FM3/TX5009/TX9628	2*	7+8	5+10
18	24	T76-36X	FM3/TX5009/TX9628	2*	7+8	5+10
18	32	T79-62	FM3/TX5009/Austalith	2*	7+8	5+10
18	7	T66-26X	Fm2a/Seri/Ogallala/	2*	7+8	5+10
27	19	T75-1	FM3/Ogallala/SeriM82	1	20a+20b	5+10
27	25[b]	T77-32	FM3/TX5009/Halberd	1	20a+20b	5+10
29	4	T60-1	GLID1/TREGO	1	20a+20b/7+8	5+10
31	9	T69-23	FM3xTX5009/OgallalaxFM6	2*	17+18	2+12
32	13	T74-13X	FM3/Jagger/TX8618	2*/1	7+9	5+10
33	18	T74-42	FM3/Jagger/TX8618	2*/1	17+18	5+10
34	20	T76-9	FM3/TX5009/TX9628	2*	17+18	5+10
35	23	T76-23	FM3/TX5009/TX9628	2*	17+18/7+8	5+10
36	30	T78-66	FM3/Ogallala/Halberd	1	20a+20b/7+9	5+10
37	34	T80-20	FM3xTX5009/OgallalaxFM3	2*	20a+20b	5+10
37	35	T80-27	FM3xTX5009/OgallalaxFM3	2*	20a+20b	5+10
39	36	T80-30	FM3xTX5009/OgallalaxFM3	2*	7+9	2+12
40	39	T82-13	FM6/TX5009/Ogallala	1	17+18	2+12
50	41	Control	Commercial Tortilla Flour (ADM Inc)		Unknown	

[a] wheat lines were collected from McGregor, Texas [b] wheat line collected from Castroville, TX. - refers to null alleles.

Milling Process

Before milling, grains were tempered for 24 hours to moisture content of 14% to improve flour yield during milling. Grains were milled using a Quadrumat Senior mill (C. W. Barbernder Instruments, Inc., South Hackensack, NJ), in accordance with the manufacturer recommendations. Flour yield for samples was ranged from 63.6 to 74.4%, and average flour yield was 69.3%.

Evaluations of Flour

Protein and Moisture Content of Flour

Protein and moisture content of flour were determined using near-infrared reflectance (NIR) spectrophotometer (Perten PDA 7000 Dual Array with Grams Software, Reno, NV). Three replicates of each sample were analyzed. Moisture and protein content were recorded as percentage of total weight. Results were averaged for each sample.

Protein Analysis

Uthayakumara et al (2003) reported that protein electrophoresis on a microfluidic chip (Lab-on-a-chip electrophoresis) can be used to identify the protein composition of the deletion lines. Therefore, Lab-on-a-chip capillary electrophoresis was used to determine HMW-GS composition for each sample. Protein was extracted form flour samples (10 mg) with 1% SDS solution containing 1% dithiothreitol (D-TT) by vortex-mixing for 5 seconds and shaking for 3 minutes at 65 ^{O}C. Samples were centrifuged for 5 min to obtain extracts. Each of 4 µL extracts was mixed with Agilent sample buffer and loaded on the capillary chip (Agilent 2100 Bioanalyzer, Agilent Technologies, Palo

Alto, CA). Results were obtained from the software as both simulated gel patterns and quantitative profiles.

Polymeric Protein Analysis

For this test, 100 mg of flour were mixed with 1.0 ml of 50 % (w/v) 1- propanol. The suspension was mixed in a vortex stirrer (Vortex Genie2, Scientific Industries, Bohemia, NY) for 5 min and then centrifuged (at 12,000 rpm) for 5 min to obtain extractable proteins. This procedure was repeated two more times and the supernatants were discarded which contain the monomeric and soluble polymeric proteins. The pellet, which contains the insoluble polymeric proteins, was then lyophilized. Nitrogen content of dry pellets was determined as total nitrogen (LECO FP-428, St. Joseph, MI). Insoluble polymeric protein percentage (IPP) was calculated by multiplying nitrogen values by a conversion factor of 5.7 and divided by total flour protein (Bean et al 1998).

Mixing properties

Mixing properties of dough, such as mixing time, and tolerance, were determined using a mixograph (National Manufacturing Co., Lincoln, NE) according to approved method 54-40.02 (AACC 2000).

Formulation and Dough Preparation

For tortilla production, dough was prepared from 500 g flour of each wheat line. 30 g of shortening (Sysco Corporation, Houston, TX), 3 g of sodium bicarbonate (Arm and Hammer, Church and Dwight Company, Inc., Princeton, NJ), 2.5 g sodium propionate (Niacet Corp., Niagara Falls, NY), 7.5 g of salt (Sodium Chlorur) (Morton International, Inc., Chicago, IL), 2.9 g of sodium aluminum sulfate (Budenheim USA

Inc., Plainview, NY), 2 g of potassium sorbate (B. C. Williams, Dallas, TX), 2.5 g sodium steroyl lactylate (Caravan Ingredients, Lenexa, KS), and 1.65 g of encapsulated fumaric acid (Balchem Corp., New Hempton, NY) were added to each 500 g batch. Firstly, dry ingredients were mixed in a mixer for 2 minutes (model A-200, Hobart Corp, Troy, OH) with a paddle at slow speed (speed 1). Shortening was then added to the dry ingredients and mixed at slow speed for 3 minutes. After mixing all of the dry ingredients, amount of water, which was based on adjusted value from the mixograph. The water was added to dry ingredients and mixed using a hook at low speed (speed 1) for 1 min and then mixed at medium speed (speed 2) for the time determined for each sample by its mixograph peak time.

After mixing, the dough was rested at 32 ºC and 70 – 75% relative humidity (RH) for 5 minutes in a proofing chamber (Model 57638, National Manufacturing Co., Lincoln, NE)   The dough was then pressed into a stainless steel rounding plate, and divided and rounded into 18 dough balls (Duschess Divider/Rounder, Bakery Equipment and Service Co., San Antonio, TX). The dough balls were then rested at 32 ºC and 70 – 75% RH for 10 minutes in a proofing chamber. After resting, the dough balls were evaluated objectively using the texture analyzer (model TA.XT2i, Texture Technology Corp., Scarsdale, NY).

Evaluation of Dough Properties

Dough Compression Force

Dough compression test was used to measure dough texture, especially hardness in Newtons (N) (Bejosano et al 2005). Maximum dough compression force was observed using a probe with 10 centimeters diameter on a texture analyzer (Model TA-XT2, Micro Systems, Scarsdale, NY). In this test, two dough balls of approximately equal size and weight were subjected to 70% compression after 10 minutes resting time, and maximum force was recorded and averaged for each treatments.

Stress Relaxation

Wheat flour dough is a viscoelastic material which relaxes gradually with equilibrium stress depending on its molecular structure (Steffe 1996). These changes in doughs were measured using a stress relaxation test (Rodriguez-Sandoval et al 2008). Stress relaxation was measured using texture analyzer (TA.XT2i Texture Analyzer, Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). Two dough balls from each sample were placed on the texture analyzer platform after 10 minutes resting and pressed by a cylindrical probe with a diameter of 10 cm. The holding time for dough balls were 100 seconds. Force at 25 seconds, 50 seconds, 75 seconds, maximum force and relaxation time were recorded and averaged for each treatments.

Dough Extensibility Test

In this test, three different variables are measured: Distance to extend (dough extensibility) (mm) is a measure of how far the dough extends in millimeter before it ruptures; force to extend or resistance to extension (N) refers to force required to stretch a sample until it ruptures; work to extend is calculated using the area under the extensibility curve (N.mm) (Smewing 1995; Srinivasan 1996).

For extensibility tests, doughs were prepared using 100 grams of flour from each wheat line. Two grams of salt were added to the flour and mixed in the mixer (Model N-50, Hobart Manufacturing Company Corp, Troy, OH) at speed 1 (slow speed) for 1 min. Water at 35 ºC was added to the dry ingredients and mixed with a paddle at speed 1 for 2 min to hydrate the flour. The amount of water was determined based on an adjusted value from the mixograph water absorption values. It was then mixed at speed 2 (medium speed) for a time equal to mixographs peak time. The doughs were rested at 32 ºC and 68- 79% RH for 25 min in a proofing chamber (Model 57638, National Manufacturing Co., Lincoln, NE).

Dough extensibility test was performed using Kieffer dough extensibility rig (Smewing, 1995). After proofing the dough, 20 grams of dough were weighed and rolled into a cylindirical shape with minimal manipulation. The dough were pressed with a grooved base and a top form to prepare samples. Paraffin oil was spread to simplify the removal of dough strips and prevent sample adhesion. The dough sample was placed on the grooved base with its length perpendicular to the groove direction. The top form was then placed on the grooved base. The dough press was placed in the clamp and screwed down. The dough clamp was placed into a plastic bag and left to relax at room temperature for 40 minutes. After the relaxation, the dough press was removed. A thin spatula was used to remove dough strips. Dough strips were then placed across the grooved region of the sample plate of texture analyzer, and the test was conducted. Extensibility of dough (distance), resistance to extension (force), and work to extend were recorded and averaged for each treatment.

Tortilla Production

Tortillas were produced according to the standard hot-press method (Bello et al 1991; Akdogan et al 2006). Dough balls were pressed at 400 ºF, 1150 psi for 1.35 seconds and baked at 380- 400 ºF for 30 seconds on a three-tier gas fired oven (Model 0P01004-02, Lawrence Equipment, El Monte, CA), then cooled for 1.5 minutes on a three-tier conveyor (Superior Food Machinery Inc., Pico Rivera, CA). After that, tortillas were packed in polyethylene bags and stored at 22 ºC for subjective and objective tests.

Evaluation of Tortilla Properties

Moisture Content

Tortilla moisture content was measured using a two-stage procedure in a hot-air oven according to AACC Approved Method 44-15.02 (AACC 2000). On the day of production, two tortillas were weighed and dried for 60 hours at room temperature. Tortillas were then ground and weighed (2- 2.5 g) into a pre-weighted pan and dried at 130 ºC for one hour in an oven (Model 16, Precision Scientific Co. PS, Chicago, IL). Samples were cooled to room temperature in the desiccator, and moisture content was calculated as a percentage of weight loss from the drying process.

Diameter

Ten tortillas were selected randomly, and diameter of ten selected tortillas was determined at two points across the tortilla. Diameters were averaged to get the diameter of one tortilla in millimeter (mm) (Alviola et al 2008).

Weight

Weight measurements were conducted with an analytic scale (Ohaus, Houston, TX). Ten tortillas were selected randomly and weighed. The values were averaged to report the weight of one tortilla in gram (g) (Bello et al 1991).

Thickness

Tortilla thickness was measured using as automatic caliper (Chicago Brand 12" Electronic Digital Caliper, Chicago, IL).Thickness of ten randomly selected tortillas were recorded and averaged to obtain the thickness of one tortilla in mm (Bello et al 1991).

Specific Volume

After determining the weight, height, and diameter of tortilla, specific volume was calculated with following formula: (Height* πr^2)/ weight (cm^3/g); where r = average radius of a tortilla, centimeter (cm).

Color

Color properties of tortillas were measured using a Color Meter (Chroma Meter CR- 310, Munilta, Tokyo, Japan). This equipment gives color values as L (0- 100, whiteness- grayness), ±a (redness- greenness), ±b (yellowness- blueness). These parameters were measured from four different spot of two randomly selected tortillas, and the values were averaged.

Shelf Stability (Rollability)

Rollability test was conducted to evaluate the shelf stability of tortilla. Rollability is a subjective test which is a 5 point measure of the cracking and breakage of a tortilla.

Two randomly selected tortillas from each wheat line were evaluated using a wooden dowel with 1.0 cm diameter. Each tortilla was wrapped around this dowel and allocated a rollability score from 1 to 5. (Cepeda et al 2000; Alviola and Waniska 2008). In the rate, 1 refers to unrollable tortilla which breaks easily; 2 refers to cracking and breaking imminent on both sides; 3 refers to cracking and breaking beginning on the surface; 4 refers to signs of cracking, but no breaking; 5 refers to no cracking. Rollability score 3 was used as an indicator. Rollability test was performed on the days 4, 8, 12, and 16 of storage. Tortillas with rollability score below 3.0 after 16 days of storage are considered undesirable (Pascut et al 2004).

Two Dimensional Extensibility Test

Tortilla texture evaluations were determined using two dimensional extensibility tests (Bejosano et al 2005; Barros 2009) with the texture analyzer. For this test, two tortillas were evaluated at days 0, 4, 8, 12, and 16 of storage, and the modulus of deformation, force, distance, work to rupture values were recorded and averaged for each treatments.

Data Analysis

Means and standard deviations of data were determined using Microsoft office excel 2010 (Microsoft Co., Redmond, WA). Statistical analysis was done using JMP version 9 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was performed at α=0.05 significance level to determine differences among the samples and controls. Student’s t test was used to see whether mean differences were statistically significant.

RESULTS AND DISCUSSIONS

Flour Properties

Flour protein content is one of the most important quality parameter that determines dough machinability (Uthayakumaran et al 1999; Zhang et al 2007) and hence final tortilla quality (Wang and Flores 2000; Waniska et al 2004; Mondal et al 2008). Flours with high protein content (> 12.5%) generally produce smaller tortillas with good shelf stability, while flours with low protein content (< 10%) generally produce larger tortilla, but with inferior shelf stability. Therefore, flours with intermediate protein content are desirable in tortilla production to obtain tortilla with large diameter and superior rollability (Waniska et al 2004).

In this study, the protein contents of flours ranged between 12.1 and 12.9%, as is (Table II). This range is relatively narrow, thus protein content was not expected to be a major factor in determining tortilla attributes of the lines tested.

Insoluble polymeric proteins (IPP) contain different poly-peptide chains which are linked with each other via intermolecular disulphide bonds (Macritchie 1992); their molecular weight is over twenty million daltons (Huebner and Wall, 1976). The IPP are not soluble in aqueous alcohol solution such as 1-propanol, ethanol, or methanol (Bean et al 1998). IPP content affects dough properties (Gupta et al 1993); higher IPP content increases dough strength (Ciaffi et al 1996); thus may impact tortilla quality.

Amount of IPP was affected by deletions and variations in HMW–GS ($p < 0.05$) (Table II). IPP values were between 30.8% and 44.8%. Deletion lines generally had lower average of IPP content (38.4%) than lines which do not have null genes in their

HMW-GS (41.9%). Ewart (1977; 1987; 1988) found a linear arrangement of HMW-GS in gluten polymers. Therefore, deletion of one or more of these loci may cause a reduction in gluten polymers, which may decrease IPP content. This also suggests that dough from deletion lines may form a weaker gluten network which is more extensible and spread more during hot pressing and produce tortillas with large diameter.

Early reports about effects of HMW-GS on flour properties have demonstrated that presence of 5+10 at *Glu-D1* locus resulted in an increase in flour IPP content (Beasley et al 2002; Mondal et al 2008; Jondiko 2010). This was confirmed by our data that showed flours from wheat lines possessing (1, 7+8, 5+10) (group 17), (1, 20a+20b/7+8, 5+10) (group 29), and (2*, 17+18, 5+10) (group 34) at (*Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively) had the highest IPP content (44.8%, 44.6%, and 44.7%, respectively) (Table II). Therefore, dough from these lines likely form strong gluten networks, which produce tortillas with small diameter. On the other hand, flour from wheat line possessing 1, 17+18, and 2+12 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively had the lowest IPP content (30.8%). This may be attributed to the fact that presence of 2+12 at *Glu-D1* locus cause a formation of one less disulphide bonds compared to 5+10 at the same locus (Shewry et al 1991), which may cause a decrease in formation of polymeric proteins in wheat. A decrease in flour IPP content decrease dough strength and form extensible dough that are likely to produce tortilla with large diameter (Waniska et al 2004; Mondal et al 2008; Jondiko 2010).

Peak time is time (in minute) required for optimum dough consistency (Suas, 2009). Dough development (mixing) time was affected by deletions and variations in allelic composition ($p < 0.05$). Wheat lines possessing deletions at different loci generally require lower dough development time (average of 2.2 minutes) than those without null genes (average of 3.0 minutes) (Table II). This agrees with the IPP data, and previous findings by Lawrence et al (1988) that dough from wheat lines deficient in one or more loci on the long arm group 1 chromosome required lower mixing time. Ciaffi et al (1996) and Mondal et al (2008) also reported that lower amount of IPP cause a decrease in dough development time. In this study, a strong positive correlation ($r = 0.67$) between IPP content and dough mixing time was observed.

Flour from wheat lines possessing 2*, 17+18/7+8, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively required the longest development time (3.7 min). IPP content of flour from these lines was also higher (41.5%) than average IPP content of flours (40.1%). Flours from wheat lines possessing 5+10 at their *Glu-D1* loci generally require longer development time (3.05 min) compared to flours from lines with other alleles (2.24 min), and their IPP content were higher (41.6%) compared to others (38.4%). This confirms that presence of 5+10 contribute to dough strength (Payne et al 1987; Lawrence et al 1988; Shewry et al 1992). On the other hand, presence of2+12 instead of 5+10 at *Glu-D1* resulted in shorter dough development time (2.6 vs 3.4 min). This can be attributed to formation of one less disulphide bond in presence of 2+12 compared to 5+10 at *Glu-D1*, which makes the gluten network weaker (Shewry et al 1992).

Table II

Effects of deletions and variations in high molecular weight glutenin allelic composition on the flour properties[1]

Group	HMW-GS Allelic Composition			Entries[2]	% Protein	% IPP	Mixing Time (min)
	Glu-A1	*Glu-B1*	*Glu-D1*				
1	-	-	-	1	12.71 abc	39.07 abcd	2.48 b-g
2	-	7+8	-	21,26	12.84 a	37.70 cd	2.58 c-g
4	-	20a+20b/7+9	-	33	12.22 cdeg	40.45 abcd	2.45 b-g
5	-	7+9	2+12	8	12.65 abc	40.34 abcd	2.15 b-g
6	-	7+9	5	10	12.54 abc	**35.28 de**	2.00 efg
7	-	7	10	40	12.23 fgh	**35.45 de**	**1.80 fg**
8	2*	17+18	-	37	12.29 efgh	40.68 abcd	2.00 efg
9	1	7+9	5+10	3,5,6,27, 28,29,31,38	12.60 bcd	41.55 ab	3.00 bcd
17	1	7+8	5+10	2	12.39 cde	**44.83 a**	2.78 b-g
18	2*	7+8	5+10	7,11,12,14,15,16, 17,22,24,32	12.65 abc	40.90 abc	3.19 bc
27	1	20a+20b	5+10	19,25	12.64 abc	41.94 abc	3.15 bcde
29	1	20a+20b/7+8	5+10	4	12.65 abc	**44.61 a**	3.08 b-f
31	2*	17+18	2+12	9	12.29 dfgh	43.25 ab	2.55 b-g
32	2*/1	7+9	5+10	13	12.91 ab	40.41 abcd	3.28 bcde
33	2*/1	17+18	5+10	18	12.35 cdfh	36.62 bcde	2.23 c-g
34	2*	17+18	5+10	20	12.45 cdef	**44.72 a**	3.38 bcd
35	2*	17+18/7+8	5+10	23	12.28 efgh	41.46 abcd	**3.73 ab**
36	1	20a+20b/7+9	5+10	30	12.57 abcd	39.87 abcd	3.08 b-f
37	2*	20a+20b	5+10	34,35	12.10 gh	40.73 abcd	2.69 b-g
39	2*	7+9	2+12	36	12.55 abcd	40.86 abcd	2.65 b-g
40	1	17+18	2+12	39	12.28 defg	**30.76 e**	**1.70 g**
50		Unknown		41	12.00 h	X	5.00 a

[1]Average of two lines collected from two locations. [2]Wheat lines with same HMW-GS.
Levels not connected by same letters are significantly different (α=0.05).
X. not determined. – refers to null alleles

Objective Dough Properties

Dough Compression Force

Dough compression force test is used to measure dough hardness in Newtons (N). In tortilla production, dough compression force is an important indicator of spreadability of dough balls during hot-pressing (Holt et al 1992). Alviola et al (2010) reported that dough requiring low compression force spread more during hot-pressing and produced large diameter tortillas and vice versa. Therefore, in tortilla production, dough which require less force to compress is desired to obtain good quality tortillas.

Dough compression force was significantly affected by deletions and variations in HMW- GS ($p < 0.05$). The force to compress ranged between 77 and 137 N (Table III). Dough made from lines with null alleles at *Glu-A1*, GluB-1, and GluD-1 loci (group 1), and from lines possessing deletions at *Glu-A1* and *Glu-D1* combined with 20a+20b/7+9 at *Glu-B1* (group 4) required lowest force to compress (92.2, and 77.4 N, respectively). These can be attributed to the fact that the deletion caused a reduction in disulphide bonds formed in dough network; thus making the dough softer.

On the other hand, presence of 5+10 at *Glu-D1* locus generally contributed to dough hardness. Dough made from flour in group 32 which has 2*/1, 7+9, and 5+10, and in group 36 which has 1, 20a+20b/7+9, and 5+10 at *Glu-A1*, B-1, and D-1 required highest force to compress (Table III). These results agree with Payne (1987) and Jondiko (2010) findings that presence of 5+10 at *Glu-D1* locus contributes to dough strength, which increases the dough hardness.

Dough Stress Relaxation

Wheat flour dough is a viscoelastic material which relaxes gradually with equilibrium stress depending on its molecular structure (Steffe 1996). Stress relaxation test is used to measure these changes in the dough system and to evaluate textural difference of dough (Rodriguez-Sandoval et al 2008). Dough stress relaxation is an important parameter to predict final tortilla quality because it has lower variability between replicates and provides consistent results (Barros 2009).

In this study, equilibrium force for dough was measured at 75 seconds, and relaxation time was calculated. Both parameters were significantly affected by deletions and variations in allelic composition ($p < 0.05$). Force after 75 seconds of compression ranged from 3.80 to 8.97 N (Table III). Dough from deletion lines generally exhibited lower equilibrium force (with an average of 4.91 N) than dough from wheat lines that do not have null genes in their HMW-GS (6.56 N). In tortilla production, dough with lower equilibrium force is desired to obtain larger diameter tortillas because higher equilibrium force corresponds to stronger and elastic dough, which shrink back after hot-pressing and produces tortilla with smaller diameter (Barros 2009).

Dough from wheat lines possessing 5+10 at *Glu-D1* locus generally required higher force at 75 sec than dough from wheat lines possessing 2+12 at *Glu-D1* locus. These results can be attributed to the fact that 5+10 at *Glu-D1* contains an additional cysteine residue which forms intermolecular crosslinks (Shewry et al 1992) and makes the dough stronger. Flour from lines with null allele at *Glu-A1* locus generally produced dough that required lower equilibrium force with an average of 4.32 N (group 4, 5, 6, and 7) compared to other lines (average of 6.04 N). This means flours from these lines produce dough with weak gluten network, which is likely to expand more during hot-pressing and produce larger diameter tortillas. On the other hand, deletions or variations on *Glu-B1* locus did not significantly ($p<0.05$) affect dough equilibrium force. These results suggest that *Glu-A1* and *Glu-D1* loci produce greater effect than *Glu-B1* locus on viscoelastic properties of dough.

Dough from lines possessing 2*, 17+18, 5+10 (group 34) and 1, 20a+20b/7+8, 5+10 (group 29) at *Glu-A1*, *Glu-B1*, *Glu-D1*, respectively showed the highest equilibrium force (8.17 and 7.54 N, respectively) among the lines (Table III). This result agrees with the dough compression test where allelic pair of 5+10 at *Glu-D1* increased compression force. This confirms the dough strengthening role of 5+10 at *Glu-D1* (Payne et al 1987; Shewry et al 1992).

Relaxation time is the time in seconds required for the maximum dough compression force to decay to 36.8% of its value. Like equilibrium force, relaxation time is important determinant of tortilla quality. Doughs that require shorter time to relax have weak gluten network and vice versa (Barros 2009; Barros et al 2010). In this study, relaxation time ranged from 1.79 to 2.36 sec (Table III) and was significantly affected by deletions and variations in HMW-GS ($p < 0.05$). As expected, dough from deletion lines generally required shorter time to relax (with an average of 1.94 sec) than dough from wheat lines that do not have null genes (2.14 sec). This further confirms that dough from deletion lines are likely to produce large diameter tortilla. In general, the dough relaxation data strongly correlated with IPP content and dough mixing time with

Table III

Effects of deletions and variations in high molecular weight glutenin allelic composition on dough objective properties[1]

Group	HMW-GS Allelic Composition			Entries	Compression (N)	Force (N) at 75 sec	Relaxation time (sec)	Extensibility (mm)	Force to extend[3] (N)	Area[4] (Nxmm)
	Glu-A1	*Glu-B1*	*Glu-D1*							
1	-	-	-	1	**92.18 efg** **(53-139)**	6.02 bcde (5.7-6.9) [2]	2.00 ab (1.7-2.2)	38.38 defg (21.1-65.1)	0.58 cde (0.52-0.69)	5.15 ab (2.7-6.9)
2	-	7+8	-	21,26	108.63 abcde (77-135)	6.06 bcde (2.9-9.4)	2.12 ab (1.9-2.4)	**41.18 defg** **(31.5-51.5)**	0.44 efg (0.24-0.51)	4.36 bcde (3.3-5.5)
4	-	20a+20b/7+9	-	33	**77.38 fg** **(52-94)**	4.44 cde (4.2-5.0)	1.98 ab (1.9-2.1)	**55.33 bc** **(47.5-67.4)**	**0.27 ghi** **(0.20-0.32)**	3.74 defg (3.1-4.4)
5	-	7+9	2+12	8	107.32 abcd (70-125)	5.02 bcde (3.7-5.8)	**1.79 b** **(1.7-2.0)**	**59.71 b** **(40.1-86.2)**	0.32 fgh (0.24-0.36)	4.46 bcde (3.1-5.6)
6	-	7+9	5	10	112.95 abcd (65-160)	**3.89 e** **(2.9-4.9)**	1.81 b (1.5-2.1)	**75.46 a** **(66.3-98.7)**	**0.19 hi** **(0.17-0.23)**	3.46 fg (2.8-4.5)
7	-	7	10	40	109.44 abcd (90-127)	**3.92 de** **(2.4-5.9)**	1.84 b (1.7-2.0)	25.99 ijk (17.9-35.0)	0.66 abcd (0.49-0.79)	3.66 efg (2.6-4.8)
8	2*	17+18	-	37	119.96 ab (102-146)	5.02 bcde (3.8-7.9)	2.07 ab (1.7-2.4)	31.84 fghjk (27.5-36.5)	0.61 bcde (0.50-0.73)	4.35 bcde (4.0-4.5)
9	1	7+9	5+10	3,5,6,27, 28,29,31,38	100.26 cde (82-134)	6.59bc (4.1-9.5)	2.00 ab (1.7-2.2)	37.69 efg (18.6-58.9)	0.56 de (0.35-1.39)	3.98 e (3.3-5.1)
17	1	7+8	5+10	2	101.50 abcde (76-115)	6.86 abcd (6.2-7.7)	2.05 ab (1.8-2.2)	34.47 fghi (22.1-51.6)	0.56 de (0.41-0.82)	4.18 cde (3.2-5.8)
18	2*	7+8	5+10	7,11,12,14,15, 16,17,22,24,32	103.84 bcde (73-128)	6.98 bc (3.3-9.4)	2.09 ab (1.7-2.3)	35.37 fgh (24.9-64.5)	0.64 bc (0.14-1.22)	4.49 c (2.9-6.2)
27	1	20a+20b	5+10	19,25	110.63 abcd (89-146)	6.68 bcd (5.6-7.2)	2.12 ab (1.9-2.2)	35.23 ghi (22.2-47.1)	0.53 de (0.41-0.71)	4.07 cdef (2.1-5.0)
29	1	20a+20b/7+8	5+10	4	106.38 abcde (98-111)	7.54 abc (6.3-8.3)	2.07 ab (1.8-2.2)	x	x	x

Table III (continued)

Group	HMW-GS Allelic Composition			Entries	Compression (N)	Force (N) at 75 sec	Relaxation time (sec)	Extensibility (mm)	Force to extend[3] (N)	Area[4] (Nxmm)
	Glu-A1	*Glu-B1*	*Glu-D1*							
31	2*	17+18	2+12	9	109.99 abcd	5.02 bcde	1.81 b	40.91 def	0.51 de	4.51 bcde
					(103-106)	(3.9-6.2)	(1.6-1.9)	(25.1-65.3)	(0.40-0.71)	(3.5-6.4)
32	2*/1	7+9	5+10	13	120.60 a	7.12 abc	2.11 ab	31.31 ghij	0.71 abc	4.35 cde
					(86-140)	(5.8-8.8)	(1.8-2.3)	(18.2-52.6)	(0.52-1.04)	(3.1-6.6)
33	2*/1	17+18	5+10	18	100.02	4.92 cde	2.05 ab	45.27 cd	0.49 e	**5.15 a**
					(80-115)	(2.9-6.7)	(1.9-2.4)	(31.0-56.3)	(0.37-0.55)	**(3.8-6.1)**
34	2*	17+18	5+10	20	106.70 abcde	**8.17 ab**	**2.36 a**	35.34 efghi	0.55 de	4.03 cdef
					(76-120)	**(6.2-9.6)**	**(1.9-2.5)**	(28.4-64.1)	(0.30-0.71)	(2.3-6.8)
35	2*	17+18/7+8	5+10	23	**115.38 abcd**	6.89 bcde	1.98 ab	**23.43 jk**	**0.80 a**	4.02 cdef
					(94-137)	(6.5-7.4)	(1.9-2.2)	**(18.4-26.7)**	**(0.54-0.97)**	(3.1-5.5)
36	1	20a+20b/7+9	5+10	30	**116.55 abc**	7.18 de	2.12 ab	35.86 efghi	0.64 bcd	3.96 def
					(106-130)	(5.7-8.3)	(2.0-2.2)	(20.1-50.9)	(0.33-1.06)	(3.1-4.4)
37	2*	20a+20b	5+10	34,35	106.71 abcde	5.80 bcde	1.90 b	42.65 de	0.49 e	4.05 def
					(93-121)	(4.3-7.5)	(1.7-2.1)	(31.3-65.1)	(0.25-0.59)	(3.3-4.3)
39	2*	7+9	2+12	36	104.85 abcd	5.90 bcde	1.94 ab	27.05 hijk	0.48 def	2.88 g
					(87-120)	(4.9-7.2)	(1.7-1.4)	(26.1-29.1)	(0.21-0.54)	(2.2-3.5)
40	1	17+18	2+12	39	95.94 cdef	3.80 e	1.94 ab	31.46 hij	0.16 i	**1.80 g**
					(73-111)	(3.2-4.8)	(1.7-2.1)	(11.5-58.8)	(0.08-0.24)	**(0.2-3.4)**
50		Unknown		41	96.06 def	8.97 a	2.01 ab	22.79 k	0.73 ab	3.23 g
					(61-120)	(7.4-9.5)	(1.6-2.4)	(17.7-31.2)	(0.50-0.97)	(2.2-3.9)

[1]Average of two lines collected from two locations. [2]Range for wheat lines with same HMW-GS.
[3]Resistance to extension. [4] Work to extend. x Not determined due to insufficient amount of sample.
Levels not connected by same letters are significantly different (α=0.05). – refers null genes

correlation coefficients of 0.73 and 0.82, respectively. This confirms the dough strengthening effect of higher IPP content.

Dough Extensibility

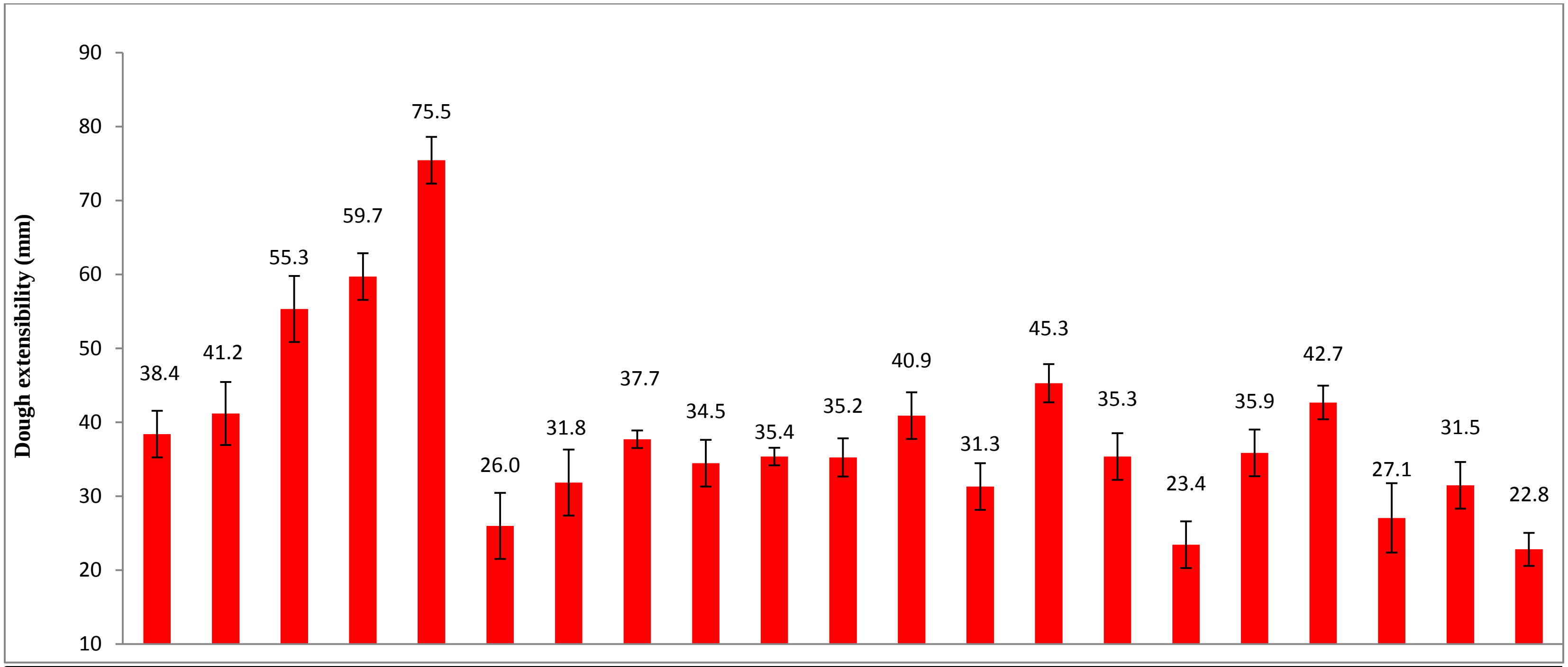
Dough extensibility is one of the most important parameter which determines final tortilla quality. A highly extensible dough is required to produce large diameter tortillas (Waniska et al 2004; Mondal et al 2008; Pierruci et al 2009; Barros 2009). The following variables are measured in dough extensibility test: Distance to extend (dough extensibility) (mm) is a measure of how far the dough extends in millimeter before it ruptures; force to extend or resistance to extension (N) refers to force required to stretch a sample until it ruptures; work to extend is calculated using the area under the extensibility curve (N.mm) (Smewing 1995; Srinivasan 1996).

Distance to extend

Dough extensibility was significantly affected by deletions and variations in HMW glutenin subunits ($p < 0.05$). Dough extensibility values for the lines ranged from 23.4 to 75.5 mm, and all wheat lines tested produced more extensible dough than a commercial control (Table III). Wheat lines in which one or more of *Glu-A1*, *Glu-B1*, and *Glu-D1* alleles were absent produced the most extensible dough (average of 44.8 mm) compared to wheat lines that do not have null genes (average of 34.2 mm) (Figure 1). There was no significant correlation ($p < 0.05$) between flour IPP content and dough extensibility, whereas negative significant correlation ($p < 0.05$) was found between dough extensibility and dough mixing time with a correlation coefficient of -0.50. This means that when dough mixing time decreased, which generally indicates weaker gluten, dough extensibility increased. An extensible dough can spread more during hot-pressing without shrinking back; thus produce large diameter tortillas.

Wheat line possessing null genes at *Glu-A1*, subunit 7 at *Glu-B1*, and subunit 5 at *Glu-D1* loci (group 6) formed the most extensible dough (75.5 mm). IPP content and dough development time of this line were also very low; 35.3%, and 2.0 minutes, respectively. This high extensibility can be attributed to lack of subunit 10 at *Glu-D1* locus which may cause a reduction in disulphide bonds present in the gluten network; thus decrease dough strength and increase extensibility. This hypothesis is strengthened by the observation that lack of 5 (instead of 10) at *Glu-D1* even with deletion at *Glu-A1* (group 7) decreased the dough extensibility (26.0 mm) compared to wheat lines possessing allelic pair of 5+10 at their *Glu-D1* locus (average of 35.7 mm). These results suggest that within allelic pair of 5+10 at *Glu-D1* locus, subunit 10 produces a greater effect on dough strength than subunit 5; thus lack of subunit 10 (but not subunit 5) causes a dramatic decrease in dough strength and increase in dough extensibility, which is desirable for tortilla production to obtain large diameter tortillas.

As previously mentioned, presence of 5+10 at *Glu-D1* increases dough strength (Shewry et al 1992); thus decreased dough extensibility (Mondal et al 2008; Jondiko, 2010). This is confirmed by our findings; wheat lines possessing 5+10 at their *Glu-D1* locus produced less extensible dough (with an average of 35.7 mm) than wheat lines that do not possess 5+10 at their *Glu-D1* locus (average of 42.7 mm) (Figure 1). Additionally, presence of 7+9 instead of 17+18 at *Glu-B1* locus appeared to increase dough strength; hence reduced dough extensibility. For example, in the presence of 2*



Group	1	2	4	5	6	7	8	9	17	18	27	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 1. Effect of deletions and variations in high molecular weight glutenin subunits on dough extensibility (mm).
Glu= Glutenin. Numbers in the x axis refer to group of wheat line with same allelic composition. Error bars represent the ± standard error of means.
– refers to null alleles.

and 2+12 at *Glu-A1* and *Glu-D1* loci, respectively, presence of 7+9 instead of 17+18 at *Glu-B1* locus decreased dough extensibility from 40.9 to 27.1 mm (group 31 vs 39). Similarly, in the presence of 2*/1 and 5+10 at *Glu-A1* and *Glu-D1* loci, respectively, presence of 7+9 instead of 17+18 at *Glu-B1* locus decreased dough extensibility from 45.3 to 31.3 mm (group 32 vs 33). These results suggest that 7+9 at *Glu-B1* locus has improving effect on dough strength; therefore, effect of *Glu-B1* locus on dough functionality needs to be further investigated.

In summary, flours from deletion lines form highly extensible dough with shorter dough development time (average of 2.2 min) than other lines (average of 3.0 min). These results indicate that deletion lines are likely to produce tortillas with large diameter, but their shelf stability cannot be predicted from this information.

Dough Resistance to Extension

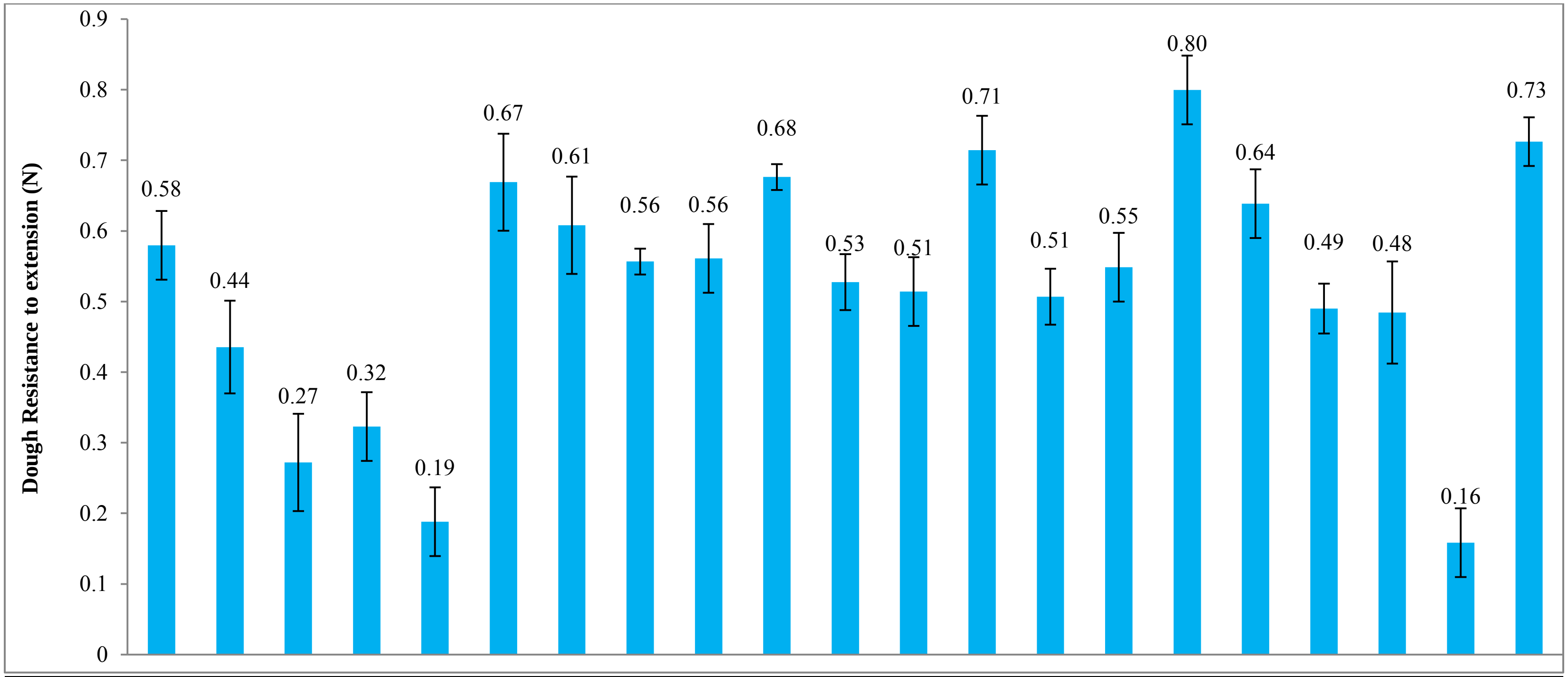
Deletions and variations in HMW- GS significantly affected ($p < 0.05$) dough resistance to extension. The means of dough resistance to extension for lines varied from 0.16 to 0.80 N (Table III). Strong negative correlation between dough extensibility and dough resistance to extension have been reported by Barros et al (2010) and Jondiko 2010. This was confirmed in this study with a correlation coefficient of -0.72. Therefore, dough requiring less force to extend is more extensible and vice versa. Dough resistance to extension was also positively correlated with flour IPP values and dough development time with correlation coefficients of 0.49, and 0.63, respectively. This further highlights the important role of flour IPP content plays in dough properties. As expected, deletion lines generally produced dough which require less force to extent (average of 0.44 N) than other wheat lines (average of 0.56 N), meaning that dough from deletion lines were more extensible than other lines.

It has been reported that presence of 2+12 and 5+10 at *Glu-D1* locus are very important determinants of dough strength. Presence of 2+12 at *Glu-D1* causes formation of weak dough that decrease the dough resistance to extension, whereas presence of 5+10 at *Glu-D1* forms of strong dough with increased dough resistance to extension (Payne 1987). This is confirmed in our study that flour possessing 5+10 at *Glu-D1* generally produced dough which require higher force to extend (average of 0.60 N) than dough from flour possessing 2+12 at *Glu-D1* (average of 0.39 N). Wheat line possessing 2*, 17+18/7+8, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively (group 35) produced dough which had the highest resistance to extension (0.80 N) and dough from wheat line possessing 1, 17+18, and 2+12 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci respectively (group 39) produced the lowest resistance to extension (0.16 N).

The results also indicate that within the allelic pair of 5+10 at *Glu-D1* locus, subunit 10 may play a more effective role on dough strength than subunit 5. For example, when only subunit 10 was present at *Glu-D1* locus (group 7) the dough resistance to extension (0.67 N) was generally similar to the lines possessing 5+10 allelic pair at *Glu-D1* (Figure 2). On the other hand, when only subunit 5 was present at *Glu-D1* locus (group 6), dough required less force to extend (0.19). This further confirms the dough strengthening effect of subunit 10 instead of subunit 5 at *Glu-D1* locus.

Dough Work to Extension

Work to extend is a measurement of energy required to rupture dough and

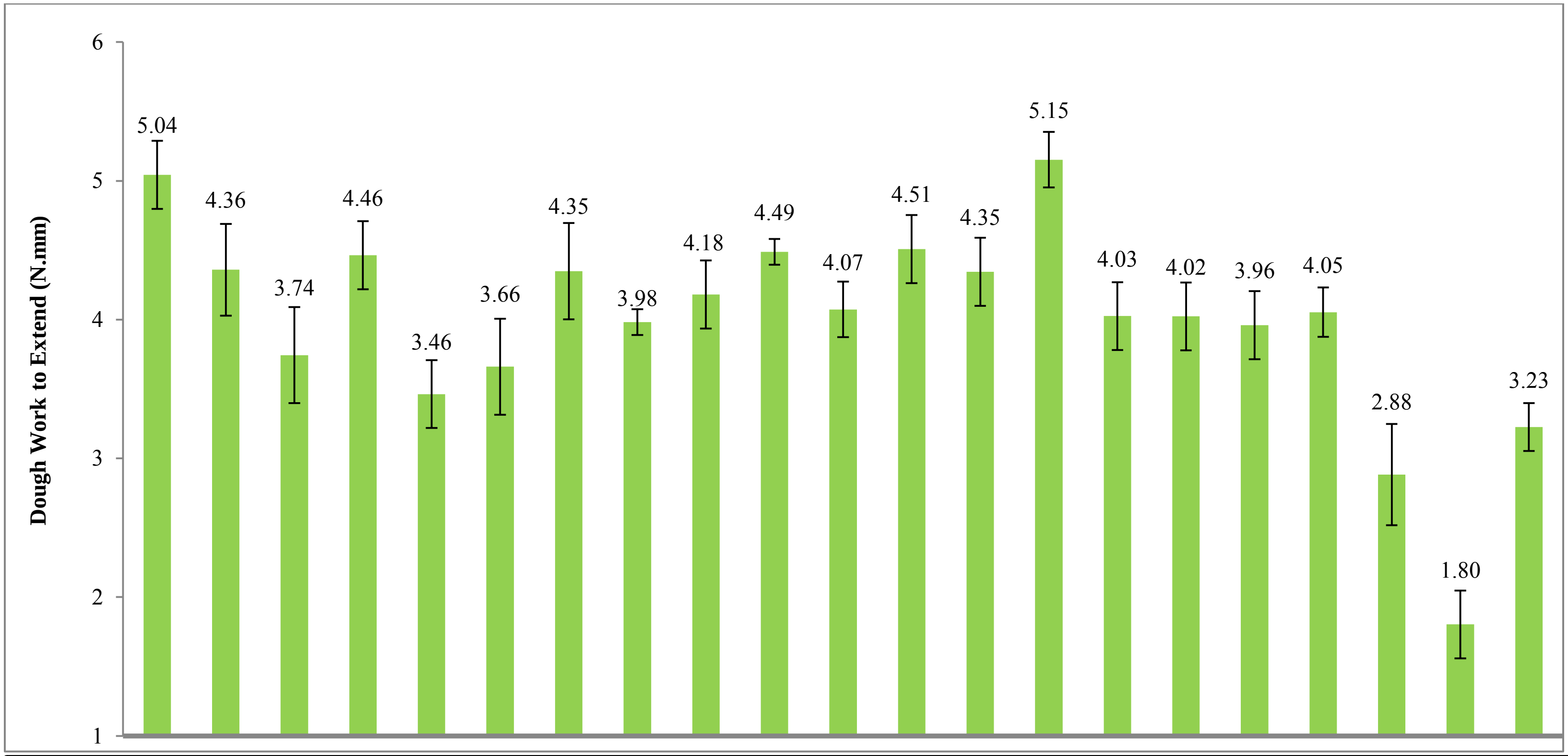


Group	1	2	4	5	6	7	8	9	17	18	27	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 2. Effect of deletions and variations in high molecular weight glutenin subunits on dough resistance to extension (N).
Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic composition. Error bars represent the ± standard error of means.
– refers to null alleles.

calculated using the area under the extensibility curve. Work to extend values is related to dough stiffness, and higher work to extend values refers to stiffer dough and vice versa (Edwards and Dexter, 1987). Work to extend values ranged from 1.80 to 5.15 N.mm and were significantly affected by deletions and variations in HMW glutenin subunit ($p < 0.05$) (Figure 3). Dough from group 39 (2.88 N.mm) and 40 (1.80 N.mm) exhibited lower work to extend value than dough from control (3.23 N.mm). All other lines produced dough with increased work to extend than control, meaning that dough from lines are generally stiffer than a commercial dough. Dough made from wheat line possessing 2*/1, 17+18, 5+10 at *Glu-A1*, *Glu-B1*, *Glu-D1*, respectively required the highest work to extend. This is due to presence of 2* at *Glu-A1* and 5+10 at *Glu-D1* which contributes to dough strength (Payne 1987; Shewry et al 1992), and hence cause a stiffer dough.

In summary, deletions and variations in HMW-GS are very important determinant of dough properties which affect final tortilla quality. Deletion lines generally produced dough with weaker gluten network which were more extensible and require less force to compress. Therefore, tortillas from these lines are expected to be larger in diameter than lines with no deletions. This is a desirable attribute; however, the shelf stability of the tortillas made from these lines cannot be predicted from the data. Presence of 2* at *Glu-A1* and 5+10 at *Glu-D1* generally resulted in dough with strong gluten network which had low extensibility and require high force to compress, thus would produce tortilla with small diameter. Tortillas from these lines are also likely to



Group	1	2	4	5	6	7	8	9	17	18	27	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	Unknown
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig 3. Effect of deletions and variations in high molecular weight glutenin subunits on work to extend (N.mm). Glu= Glutenin. Numbers in the x axis refer to group of wheat line with same allelic composition. Error bars represent the ± standard error of means. – refers to null alleles.

exhibit good shelf stability (rollability scores >3.0 over storage) due to strong gluten network (Mondal et al 2008).

Tortilla Properties

Tortilla physical properties and textural change during storage are important quality parameters, because good quality tortillas must be large in diameter (larger than 170 mm), flexible over storage (flexibility score ≥ 3.0), light in color (Pascut et al 2004), and thin (1- 5 mm) (Waniska 1999).

Tortilla Moisture Content

Tortilla moisture content is generally between 30 and 37% and an important parameter because it influences product yield. Higher moisture content corresponds to higher yield, which is desirable in tortilla industry to increase profit. Tortilla moisture content is also used to monitor tortilla freshness during storage. The higher moisture content corresponds to fresher tortillas and vice versa (Mao and Flores 2001).

In this study, tortilla moisture content ranged from 32.7 to 36.3% (Table IV) and was significantly ($p < 0.05$) affected by deletions and variations in HMW–GS. An increase in dough strength generally resulted in higher moisture content. For example, flour from wheat line possessing 2*/1, 7+9, 5+10 (group 32) at *Glu-A1*, *Glu-B1*, *Glu-D1* loci, formed stronger dough (dough development time = 3.8 min; equilibrium force from stress relaxation test = 7.1 N) compared to other flours tested and produced tortillas with the highest moisture content. On the other hand, flour from wheat line possessing 1, 17+18, 2+12 at *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively, formed weaker dough (dough development time = 1.7 min; equilibrium force from stress relaxation test = 3.8

N). Tortillas from these lines had the lowest level of moisture content. This may be attributed to the presence of 5+10 at *Glu-D1* which resulted in formation one more cysteine residues in the repetitive domain, which provides an additional inter-chain bond between HMW-GS (Shewry et al 1992). This additional bond may resulted in an increase in flour protein water binding capacity and its competition with starch and other components for water (Leon et al 2010); thus an increase in water binding capacity of flour may result in an increase in final tortilla moisture content. Tortilla moisture content was positively correlated with IPP ($r = 0.68$), dough development time ($r = 0.58$), and dough equilibrium force from stress relaxation test ($r = 0.74$). As previously determined, 7+9 at *Glu-B1* is associated with an increase in dough strength; thus, presence of 17+18 instead of 7+9 at *Glu-B1* resulted in a dramatic decrease in tortilla moisture content from 36.3to 32.9% (group 32 vs 33). These results showed that presence of 17+18 at *Glu-B1* decrease the water binding capacity of tortilla and final moisture content.

Tortilla Color (L value)

Lightness is very important quality parameter in terms of consumer acceptance. Good quality tortilla must be highly opaque (Waniska 1999) which is highly correlated with tortilla L value (Alviola and Awika 2010). Retention of air bubbles on tortilla surface produced from leavening agents contributes to tortilla opacity and lightness (Waniska 1999).

Tortilla L value (whiteness) ranged from 79.5 to 84.1 (Table IV), and was significantly affected ($p < 0.05$) by deletions and variations in HMW-GS. Flours tested in this study generally produced lighter tortillas than commercial flours (L value = 80.3) except group 29. Deletion lines produced slightly (non-significant $p < 0.05$) lighter tortillas (average of 82.3) than lines with no deletion (average of 81.7). Tortillas from wheat line possessing 1, 20a+20b/7+8, 5+10 at *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively (group 29) exhibited the lowest L value (79.49), whereas wheat line with 1, 17+18, 2+12 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively (group 40) produced the lightest tortillas (L value = 84.1).

Data from this study show that *Glu-D1* locus has great effect on tortilla lightness rather than *Glu-A1* and *Glu-B1* loci, because alteration of allelic pairs in these loci did not significantly ($p < 0.05$) affect tortilla lightness. For example; wheat lines possessing of 1 at *Glu-A1* locus produced similar tortillas (average L value = 81.6) in terms of lightness, when compared to wheat lines possessing 2* at the same locus (average L value = 81.9). Similarly, wheat lines possessing 7+9 at *Glu-B1* locus produced tortillas with similar L values (average 82.2) compared to wheat lines possessing 17+18 at the same locus (average 82.6). On the other hand, presence of 5+10 at *Glu-D1* locus cause a significant ($p < 0.05$) decrease in tortilla lightness (L value = 81.5) compared to lines without allelic pair of 5+10 at the same locus (average L value = 82.5). Conversely, presence of 2+12 at *Glu-D1* gave lighter tortillas (average L value of 82.8) compared to 5+10 at the same locus. In general, tortilla L values increased, when dough strength decreased. This may mean that weaker dough provides better air bubble expansion which contributes to opacity and lightness. There were negative correlations between tortilla lightness and indicators of dough strength: flour IPP content ($r = -0.66$), dough

Table IV

Effects of deletions and variations in high molecular weight glutenin subunit on tortilla moisture, weight, thickness, and specific volume[1]

Group	HMW-GS Allelic Composition			Entries	Moisture (%)	Weight (g)	Height (mm)	Specific Volume	Lightness L Value
	Glu-A1	*Glu-B1*	*Glu-D1*						
1	-	-	-	1	34.64 abcde	41.33 abcd	3.13 abcde	1.62 bcdef	82.18 bcde
					(33.96-36.17)	(39.41-41.79)	(2.91-3.20)	(1.51-1.86)	(80.68-85.55)
2	-	7+8	-	21,26	34.85 abcde	40.31 abcd	3.21 ab	1.57 cdef	**83.22 ab**
					(32.62-36.48)	(39.77-41.95)	(2.84-3.23)	(1.29-1.85)	**(80.63-86.47)**
4	-	20a+20b/7+9	-	33	34.57 abcde	38.93 abcd	**2.86 efghi**	1.76 abcde	82.65 bcd
					(34.41-34.73)	(37.48-40.37)	**(2.85-2.87)**	(1.66-1.87)	(80.82-85.25)
5	-	7+9	2+12	8	34.83 abcde	39.80 abcd	2.99 cdehi	1.68 abcde	82.45 bcde
					(33.47-36.38)	(39.14-40.52)	(2.80-3.23)	(1.66-1.71)	(79.00-83.42)
6	-	7+9	5	10	34.15 cde	39.62 abcd	2.87 ghi	1.77 abcd	82.29 bcde
					(33.06-35.63)	(37-73-41.24)	(2.71-3.12)	(1.55-1.82)	(80.95-84.20)
7	-	7	10	40	33.50 efg	40.63 abcd	3.16 abcd	**1.89 ab**	81.27 de
					(31.03-34.82)	(39.20-42.28)	(3.00-3.39)	**(1.56-2.14)**	(77.98-85.42)
8	2*	17+18	-	37	34.73 abcde	39.54 abcd	3.23 ab	1.84 abc	82.11 bcdef
					(34.18-35.42)	(37.51-41.38)	(2.97-3.55)	(1.56-2.06)	(79.20-85.05)
9	1	7+9	5+10	3,5,6,27,	34.89 bcde	40.64 abcd	2.97 fgh	1.54 def	81.65 de
				28,29,31,38	(32.22-35.97)	(38.26-42.06)	(2.72-3.13)	(1.43-1.69)	(77.66-83.56)
17	1	7+8	5+10	2	35.43 abcde	41.43 abc	3.13 abcde	1.49 defg	80.71 ghi
					(34.15-35.20)	(40.11-43.03)	(3.00-3.23)	(1.44-1.58)	(75.06-84.69)
18	2*	7+8	5+10	7,11,12,14,15,	35.06 abcde	40.76 ab	3.09 bcde	1.49 efg	81.17 fg
				16,17,22,24,32	(33.78-36.88)	(38.12-43.16)	(2.86-3.18)	(1.38-1.64)	(79.61-83.25)
27	1	20a+20b	5+10	19,25	34.74 abcde	41.15 abc	**3.27 a**	1.53 defg	82.08 cde
					(33.61-35.67)	(39.70-44.78)	**(3.04-3.57)**	(1.42-1.64)	(78.72-83.16)
29	1	20a+20b/7+8	5+10	4	34.59 abcdef	40.4 abcd	2.87 efghi	1.4 efg	**79.49 i**
					(33.65-35.55)	(39.56-42.59)	(2.26-3.00)	(1.36-1.64)	**(75.13-83.66)**

Table IV (continued)

Group	HMW-GS Allelic Composition			Entries	Moisture (%)	Weight (g)	Height (mm)	Specific Volume	Lightness L Value
	Glu-A1	*Glu-B1*	*Glu-D1*						
31	2*	17+18	2+12	9	34.68 abcdef	41.92 abcd	2.94 defgh	1.56 bcdeg	82.7 bcd
					(34.36-34.99)	(36.82-47.01)	(2.87-3.10)	(1.51-1.61)	(80.62-85.22)
32	2*/1	7+9	5+10	13	**36.26 ab**	39.51 abcd	3.08 bcdef	1.45 efg	82.3 bcde
					(34.77-37.67)	(39.05-40.48)	(2.90-3.23)	(1.41-1.61)	(79.28-84.37)
33	2*/1	17+18	5+10	18	32.95 fg	**38.19 d**	3.09 abcdef	1.76 abcd	82.64 bc
					(32.40-34.37)	**(36.59-39.76)**	(2.80-3.39)	(1.47-1.92)	(79.49-84.34)
34	2*	17+18	5+10	20	35.22 abcde	40.1 abcd	3.11 abcdef	1.46 efg	81.23 efgh
					(34.88-35.73)	(38.08-41.77)	(2.99-3.15)	(1.35-1.67)	(78.78-84.08)
35	2*	17+18/7+8	5+10	23	34.40 cdefg	**42.33 a**	3.2 abc	1.51 defg	81.86 cdef
					(32.88-35.17)	**(41.93-43.25)**	(3.06-3.26)	(1.48-1.54)	(79.67-84.37)
36	1	20a+20b/7+9	5+10	30	35.71 abcd	41.21 abcd	3.17 abcd	1.43 efg	81.66 cdefg
					(35.32-35.98)	(38.77-42.89)	(2.97-3.26)	(1.30-1.53)	(80.31-83.04)
37	2*	20a+20b	5+10	34,35	34.07 defg	39.85 abcd	3.05 bcdefg	1.67 abcde	81.75 cdef
					(33.54-34.66)	(38.38-41.60)	(2.88-3.33)	(1.43-1.93)	(79.91-84.11)
39	2*	7+9	2+12	36	35.08 abcde	38.71 bcd	3.06 bcdefg	1.59 bcdeg	82.07 cde
					(34.33-35.48)	(35.07-40.51)	(2.76-3.11)	(1.51-1.68)	(79.97-83.18)
40	1	17+18	2+12	39	**32.67 g**	38.32 cd	2.97 defghi	**1.94 a**	**84.11 a**
					(31.76-34.58)	(37.97-39.26)	(2.48-3.20)	**(1.79-2.05)**	**(82.72-85.82)**
50		Unknown		41	**36.08 a**	40.44 abcd	**2.81 i**	**1.35 g**	**80.28 hi**
					(35.96-36.64)	(39.53-41.82)	**(2.67-2.95)**	**(1.24-1.49)**	**(77.92-83.43)**

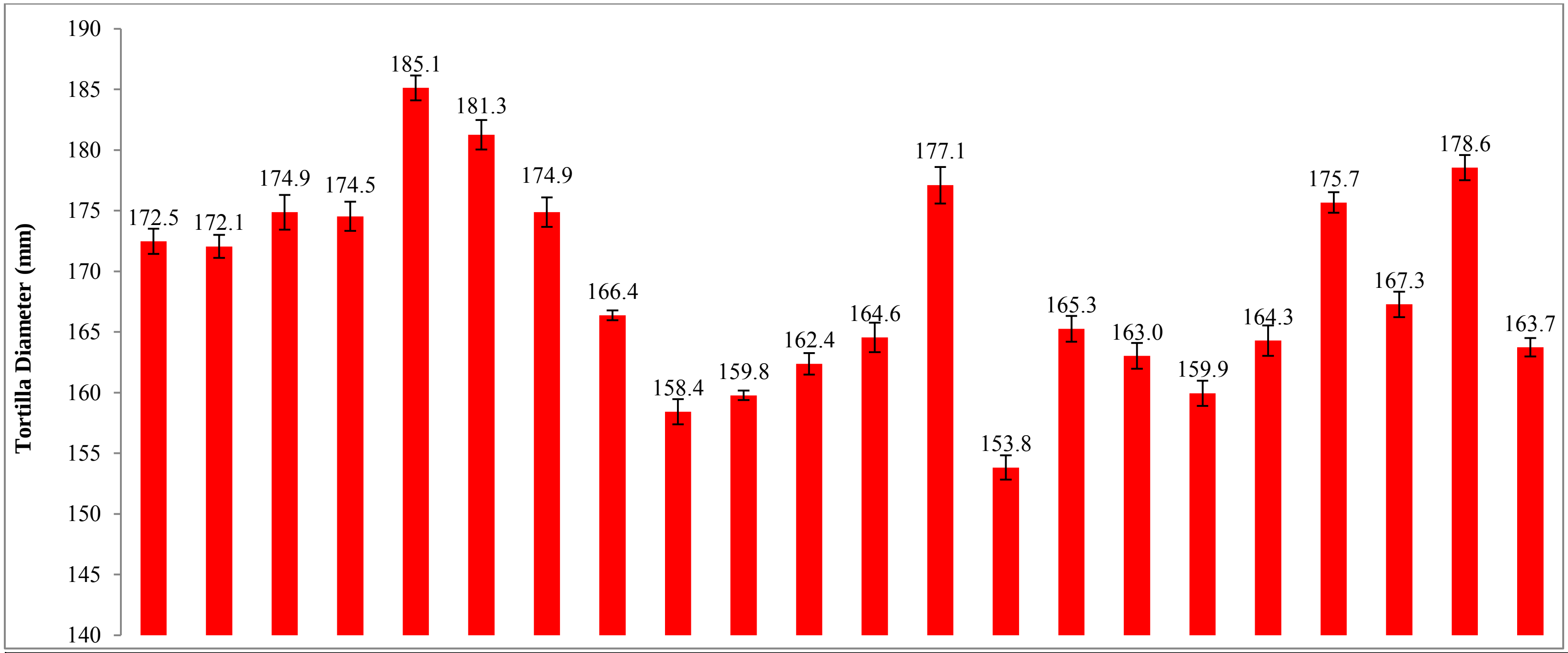
[1]Average of two lines collected from two locations. [2]Range for wheat lines with same HMW-GS.
Levels not connected by same letters are significantly different (α=0.05).

development time (r = -0.58), dough equilibrium force (r = -0.66), and dough resistance to extension (r = -0.66).

Tortilla Diameter

Tortilla diameter is one of the most important parameter that determines tortilla quality. Good quality tortillas must be large in diameter (Pascut et al 2004). Deletions and variations in HMW glutenin allelic compositions significantly affect tortilla diameter ($p < 0.05$) which varied from 153.8 to 185.1 mm (Figure 4). Tortillas from deletion lines generally had larger diameter with an average of 176.5 mm than tortillas from other lines (average of 165.5 mm). These results agree with dough properties and confirm that deletion of one or more loci on the long arm of group 1 chromosome gives more extensible gluten network which spreads without shrinking back during hot-pressing and hence produce larger diameter tortillas. In general the amount of IPP in flour, dough development time, dough equilibrium force from stress relaxation test, and dough resistance to extension values were negatively correlated with tortilla diameter with correlation coefficients of -0.57, -0.55, -0.80, and -0.65, respectively. These data confirms that stronger dough generally result in small diameter tortillas.

Flour from wheat line possessing null allele at *Glu-A1* along with 7+9 at *Glu-B1* and 5 at *Glu-D1* produced tortilla with the largest diameter (185.1 mm) (Figure 4). This agrees with Jondiko (2010) who found that lack of subunit 10 at *Glu-D1* dramatically decreased dough strength and dough elasticity, and hence produced tortilla with large diameter. Tortillas from this group were 13.3% larger in diameter compared to commercial control tortillas (163.7 mm). Similarly, dough from wheat lines possessing



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 4. Effect of deletions and variations in high molecular weight glutenin allelic composition on wheat flour tortilla diameter. Glu= Glutenin. Numbers in the x axis refer to group of wheat line with same allelic composition. Error bars represent the ± standard error of means. – refers to null alleles.

(2*, 17+18, and 2+12) and (1, 17+18, and 2+12) at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively also produced large diameter tortillas (177.1 and 178.6 mm, respectively). This can be attributed to weakening effect of 2+12 at *Glu-D1* on dough strength (Payne 1987; Shewry et al 1992; Jondiko 2010). On the other hand, although wheat lines from group 39 possess 2+12 at their *Glu-D1* locus, tortillas from these lines had smaller diameter (167.3 mm) than wheat lines with 2+12 at *Glu-D1*, but without 7+9 at *Glu-B1* locus. This can be attributed to the fact that interactive effect of 2* at *Glu-A1* with 7+9 at *Glu-B1* counteracted the weakening effect of 2+12 at *Glu-D1*, resulting in dough with strong gluten network.

Flour from wheat line possessing 2*/1, 7+9, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively produced tortillas with the smallest diameter (153.8 mm). This is mainly attributable to dough strengthening effect of 5+10 at *Glu-D1* locus; but as previously mentioned, presence of 7+9 at *Glu-B1* locus also seems to play a significant role on dough strength. Dough from wheat line possessing 2*, 20a+20b, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively, produced large diameter tortillas (175.7 mm). This may imply that presence of 20a+20b at *Glu-B1* decreases dough elasticity and counteract the dough strengthening property of 5+10 at *Glu-D1*.

In the presence of 7+9 and 2+12 at *Glu-B1* and *Glu-D1*, respectively, deletion at *Glu-A1* locus increased the tortilla diameter from 167.3 to 174.5 mm compared to presence of 2* at the same locus (group 5 and 39). This agrees with dough extensibility results that absence of 2* at *Glu-A1* increased the dough extensibility. Similarly, in the presence of 2* at *Glu-A1* and 17+18 at *Glu-B1*, presence of deletion at *Glu-D1* locus significantly ($p < 0.05$) increased tortilla diameter from 163.0 to 174. 9 mm compared to presence of 5+10 at the same locus (group 8 and 34). However, no significant effects were observed, between deletion at *Glu-D1* locus or presence of 2+12 at this locus (group 8 and 31). This shows that presence of 5+10 at *Glu-D1* locus has a major impact on dough strength, whereas presence of 2+12 at *Glu-D1* locus has minimal effect on dough strength. Therefore, at *Glu-D1* locus, genetic alterations affecting 5+10 are more useful when targeting good quality tortilla production.

As previously mentioned, presence of 7+9 instead of 17+18 at *Glu-B1* locus appeared to increase dough strength; hence cause a decrease in tortilla diameter. For example, in the presence of 2* and 2+12 at *Glu-A1* and *Glu-D1* loci, respectively presence of 7+9 instead of 17+18 at *Glu-B1* locus decreased tortillas diameter from 177.1 to 167.3 mm (group 31 vs 39). Similarly, in the presence of 2*/1 and 5+10 at *Glu-A1* and *Glu-D1* loci, respectively presence of 7+9 instead of 17+18 at *Glu-B1* locus decreased tortillas diameter from 165.3 to 153.8 mm (group 32 vs 33). These results suggest that 7+9 at *Glu-B1* locus improves dough strength; therefore, effect of *Glu-B1* locus on tortilla quality needs to be further investigated.

Tortilla Thickness

Dough strength is an important determinant of tortilla thickness. Dough with strong gluten network shrinks back after hot pressing and may produce thicker dough. Moreover, strong gluten network does not allow the air bubble trapping in dough during hot-pressing and baking; hence cause a production of translucent tortillas. On the other hand, dough with weak gluten network may increase tortillas fluffiness due to gas bubble expansion during hot-pressing and baking which is desirable in tortilla production.

Tortillas thickness was between 2.81 and 3.27 mm (Table IV). Although deletion lines formed highly extensible dough (46.8 mm) and produced larger diameter tortillas compared to non- deletion lines (34.2 mm), the deletion lines produced similar tortillas with non-deletion lines in terms of tortilla thickness (3.06, and 3.07 mm, respectively). The similarities can be misleading. Dough from non-deletion lines shrunk back after hot-pressing, but did not produce enough gas bubbles to produce fluffy tortillas. On the other hand, more air bubble expansion occurred in dough from deletion lines which caused an increase in tortillas thickness and contributes to tortilla lightness as confirmed by their higher L values (82.3).

Tortilla Specific Volume

Tortilla specific volume is a better indicator of tortilla quality than thickness since it accounts for both diameter and gas bubble retention (fluffiness). The higher specific volume refers to fluffier tortilla and vice versa (Qarooni 1993; McDonough et al 1996). Tortillas' specific volumes varied from 1.35 to 1.94 (Table IV) and were affected significantly by deletions and variations in HMW glutenin subunits ($p < 0.05$). Tortillas made from control flour had the lowest specific volume. Wheat lines with deletions in HMW allelic compositions generally produced tortilla with higher specific volume than those without deletion. This may be due to the decreased disulphide bonds in the gluten network in deletion lines, which makes the dough weaker and thus allowing gas bubbles in dough to expand more readily during baking. Specific volume was negatively correlated with IPP, dough development time, dough equilibrium force, and dough resistance to extension with correlation coefficients of -0.76, -0.70, -0.93, and -0.59, respectively, meaning that tortilla specific volume increased when dough strength decreased.

Dough from wheat line possessing 1, 17+18 and 2+12 at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (group 40) produced tortillas with the highest specific volume. This is due to negative effect of 2+12 at *Glu-D1* on dough strength which increases the tortilla diameter (178.6 mm) and apparently gas bubble retention. Thickness of these tortillas were 2.97 mm. On the other hand, wheat line possessing 1, 20a+20b/7+8 and 5+10 at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (group 29) produced tortillas with the lowest specific volume among the lines. The dough strength of this line was probably too strong to allow for expansion of air cells during the short baking time (30 seconds).

In summary, all deletion lines and wheat lines possessing following HMW-GS composition (2*, 17+18, 2+12), (2*, 20a+20b, 5+10), (1, 17+18, 2+12) at their (*Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively) produced tortillas which met minimum quality criteria for diameter (> 170 mm). Tortillas from these lines were also lighter in color due to better gas bubble expansion, and hence greater specific volume (fluffier) compared to other lines.

Tortilla Shelf Stability (Rollability)

Tortilla shelf stability was measured subjectively using a 5 point scale of the cracking and breakage of a tortilla on the 4, 8, 12, and 16 days of storage. A score of 3.0 or above is considered acceptable flexibility score after 16 days of storage (Pascut et al

2004). As expected, tortilla rollability scores decreased over storage at room temperature. This is partly explained by the staling theory which can be attributed to starch retrogradation, and loss of moisture over storage (Willhoft 1973). Rollability scores at 4 days of storage ranged from 3.50 to 4.58 and at 16 days of storage varied from 1.63 to 3.92 (Table V). Generally negative correlation has been reported for tortilla diameter and flexibility scores over storage (Mao et al 2002; Pascut et al 2004; Waniska et al 2004; Bejosano et al 2005; Mondal et al 2008). This study agrees with these findings since there was a negative ($p < 0.05$) correlation ($r = -0.50$) between tortilla diameter and rollability scores over storage. Tortilla flexibility score over storage was also positively ($p < 0.05$) correlated with flour IPP ($r = 0.72$), dough development time ($r = 0.45$), and equilibrium force at 75 sec ($r = 0.68$), meaning that as dough strength increased, tortillas shelf stability increased. This highlights the difficulty in finding ideal wheat lines that can produce large diameter tortillas (> 170.0 mm) and still keep good flexibility scores over storage (> 3.0).

Deletions and variations in HMW glutenin allelic composition significantly affected tortilla flexibility ($p < 0.05$). In this study, although wheat lines possessing deletions in their HMW- GS produced tortillas with lower average rollability score over storage (mean = 2.98) compare to those without deletion (mean = 3.30), most samples with deletion in one or more loci on the long arm of group 1 chromosome interestingly produced tortillas with good shelf stability (except group 2 and 7) (Figure 5).

Wheat lines possessing (-, 20a+20b/7+9, -), (-, 7+9, 5), and (2*, 17+18, -) at their *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively produced tortilla with good shelf stability

Table V

Effect of deletions and variations in high molecular weight glutenin subunits on wheat tortillas diameter and rollability scores[1]

Group	HMW-GS Allelic Composition			Entries	Diameter	Rollability (Flexibility)			
	Glu-A1	*Glu-B1*	*Glu-D1*		(mm)	Day 4	Day 8	Day 12	Day 16
1	-	-	-	1	**172.48e**	4.44abc	4.00abc	3.69abcde	**3.25cdefg**
					(165-180) [2]	(4.0-5.0)	(3.5-4.5)	(3.5-4.5)	**(3.0-3.5)**
2	-	7+8	-	21,26	**172.06e**	3.95de	3.55cde	3.15fgh	2.60hi
					(163-184)	(3.0-5.0)	(3.0-4.0)	(2.5-4.0)	(2.0-3.0)
4	-	20a+20b/7+9	-	33	**174.88de**	4.00cdef	3.75abcde	3.38cdefg	**3.00efghi**
					(166-181)	(3.5-4.5)	(3.5-4.5)	(2.5-4.0)	**(2.5-3.5)**
5	-	7+9	2+12	8	**174.54de**	4.08cde	3.33efg	3.33defg	**3.17defgh**
					(169-180)	(3.5-4.5)	(3.0-4.0)	(3.0-3.5)	**(3.0-3.5)**
6	-	7+9	5	10	**185.13a**	4.44abc	3.94abcd	3.50cdefg	**3.31bcdefg**
					(176-192)	(4.0-5.0)	(3.5-4.5)	(3.0-4.0)	**(3.0-3.5)**
7	-	7	10	40	**181.27b**	3.50f	3.00fg	2.67hi	2.42i
					(177-187)	(2.5-4.5)	(2.0-4.0)	(2.5-3.0)	(2.0-3.0)
8	2*	17+18	-	37	**174.88de**	3.92def	3.33efg	3.17efgh	3.08efghi
					(165-180)	(3.5-4.5)	(3.0-4.0)	(3.0-3.5)	(2.5-3.5)
9	1	7+9	5+10	3,5,6,27,	166.38f	4.58a	4.23a	4.03a	**3.85a**
				28,29,31,38	(153-171)	(4.5-5.0)	(4.0-4.5)	(3.5-4.5)	**(3.5-4.0)**
17	1	7+8	5+10	2	158.42k	4.50abc	4.13ab	3.63bcdef	**3.31bcdefg**
					(151-165)	(4.0-5.0)	(4.0-4.5)	(3.0-4.0)	**(3.0-3.5)**
18	2*	7+8	5+10	7,11,12,14,15,	159.78hij	4.34bc	4.06ab	3.79bc	**3.47bcdef**
				16,17,22,24,32	(155-167)	(4.0-5.0)	(4.0-4.5)	(3.5-4.0)	**(3.0-3.5)**
27	1	20a+20b	5+10	19,25	162.38hij	4.42abc	4.29a	4.13a	**3.79abc**
					(157-165)	(4.0-5.0)	(4.0-4.5)	(3.5-4.5)	**(3.5-4.0)**
29	1	20a+20b/7+8	5+10	4	164.55fgh	4.50abc	4.42a	4.08ab	**3.83abcd**
					(157-171)	(4.0-5.0)	(4.0-4.5)	(3.5-4.5)	**(3.5-4.0)**

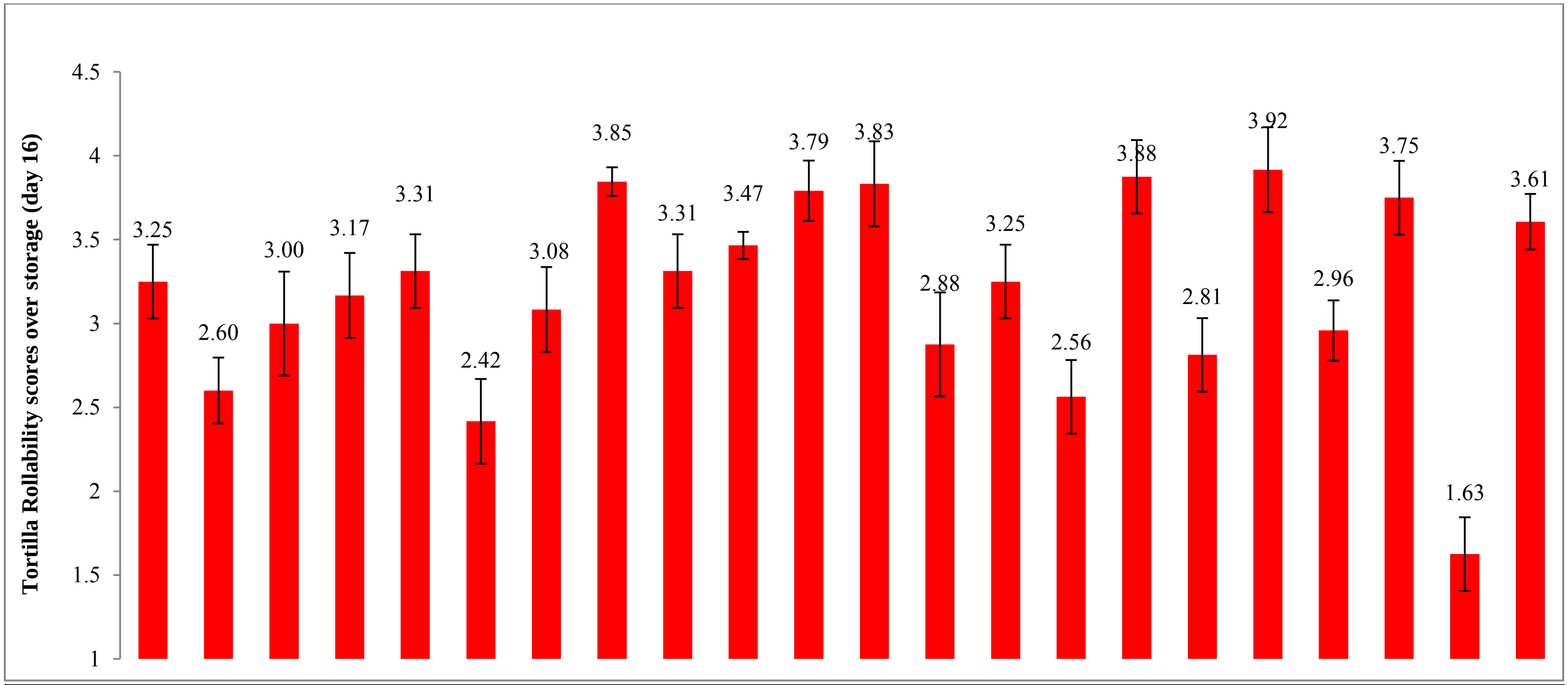
Table V (continued)

Group	HMW-GS Allelic Composition			Entries	Diameter	Rollability (Flexibility)			
	Glu-A1	*Glu-B1*	*Glu-D1*		(mm)	Day 4	Day 8	Day 12	Day 16
31	2*	17+18	2+12	9	**177.11cd**	3.75def	2.75g	2.88gh	2.88fghi
					(172-181)	(3.5-4.5)	(2.5-3.0)	(2.5-3.0)	(2.5-3.0)
32	2*/1	7+9	5+10	13	153.83l	4.50abc	4.19ab	3.63bcdef	**3.25cdefg**
					(146-157)	(4.0-5.0)	(4.0-4.5)	(3.0-4.0)	**(3.0-3.5)**
33	2*/1	17+18	5+10	18	165.25fg	4.25bcd	3.56cdef	3.06gh	2.56hi
					(156-174)	(4.0-5.0)	(3.0-4.0)	(2.5-3.5)	(2.0-3.0)
34	2*	17+18	5+10	20	163.02ghi	4.25bcd	4.13ab	3.94abc	**3.88ab**
					(158-169)	(4.0-5.0)	(4.0-4.5)	(3.5-4.5)	**(3.5-4.0)**
35	2*	17+18/7+8	5+10	23	159.95jk	4.13cd	3.81bcde	3.38defg	2.81ghi
					(155-163)	(4.0-4.5)	(3.5-4.0)	(3.0-4.0)	(2.5-3.0)
36	1	20a+20b/7+9	5+10	30	164.29fgh	4.58ab	4.42a	4.17ab	**3.92ab**
					(155-170)	(4.0-5.0)	(4.0-4.5)	(4.0-4.5)	**(3.5-4.0)**
37	2*	20a+20b	5+10	34,35	**175.68d**	4.04de	3.50def	3.21fg	2.96ghi
					(169-181)	(4.0-4.5)	(3.0-4.0)	(2.5-3.5)	(2.5-3.5)
39	2*	7+9	2+12	36	167.28f	4.56ab	4.13ab	3.81abcd	**3.75abcd**
					(164-171)	(4.0-5.0)	(4.0-4.5)	(3.5-4.0)	**(3.5-4.0)**
40	1	17+18	2+12	39	**178.55bc**	3.69ef	3.00g	2.19i	1.63j
					(172-184)	(3.0-4.5)	(2.5-3.5)	(2.0-2.5)	(1.0-2.0)
50		Unknown		41	163.74gh	4.39abc	3.96abc	3.82abcd	3.61abcde
					(156-173)	(4.0-4.5)	(3.5-4.5)	(3.5-4.0)	(3.0-4.0)

[1]Average of two lines collected from two locations. [2]Range for wheat lines with same HMW-GS.
Levels not connected by same letters are significantly different (α=0.05).

(flexibility scores over storage > 3.0). These tortillas were also large in diameter (174.9, 174.5, and 185.1 mm, respectively. The presence of 7+9 at *Glu-B1* or 2* at *Glu-A1* loci apparently provided adequate gluten strength to maintain good flexibility during storage, while deletion of one or more loci on the long arm of group 1 chromosome reduces dough strength enough to obtain large diameter tortillas. Thus, the presence of null alleles at *Glu-A1* or *Glu-D1* loci along with 2* at *Glu-A1* or 7+9 at *Glu-B1* loci is a promising combination to produce good quality tortillas. On the other hand, wheat lines possessing (-, 7+8, -) and (-, 7, 10) at *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively produced large diameter tortillas, but they had inferior flexibility scores over storage (2.60, and 2.42, respectively). This shows that presence of 7+8 or 7 at *Glu-B1* or subunit 10 at *Glu-D1* do not provide enough strength to compensate for deletions.

Wheat line possessing 1, 17+18, and 2+12 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively produced large diameter tortillas (178.6 mm), but with the worst rollability score on 16 days of storage (1.63). This can be attributed to the fact that presence of 2+12 is associated with formation of weak gluten network (Payne 1987) which cause a decrease in tortilla shelf stability (Jondiko, 2010) and presence of 1 at *Glu-A1* and 17+18 at *Glu-B1* loci could not counteract dough weakening effect of 2+12 at *Glu-D1* locus. On the other hand, wheat line possessing 2*, 7+9 and 2+12 produced smaller diameter (167.3 mm) tortilla, but with superior flexibility on 16 days of storage (3.75). This further indicates that presence of 2* and 7+9 at *Glu-A1* and *Glu-B1* loci, respectively play a major role in dough strength.



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 5. Effect of deletions and variations in high molecular weight glutenin allelic composition on wheat tortilla shelf stability.
Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic composition. Error bars represent the ± standard error of means.
– refers to null alleles.

Presence of 5+10 at *Glu-D1* contributes to dough strength (Payne 1987); hence tortillas from wheat lines possessing this allelic pair generally have smaller diameter, but with superior flexibility scores over storage (Mondal et al 2008; Jondiko 2010). Our data agree with previous findings; tortillas from wheat lines possessing (1, 7+9, 5+10), (1, 20a+20b, 5+10), (1, 20a+20b/7+8, 5+10), (2*, 17+18, 5+10), and (1, 20a+20b/7+9, 5+10) had rollability scores of 3.85, 3.79, 3.83, 3.88, and 3.92, respectively on 16 days of storage.

As expected, deletion at *Glu-D1* compared to presence of 5+10 at this locus decreased the tortillas rollability scores over storage from 3.88 to 3.08 (group 8, and 34). However, deletion at *Glu-D1* locus did not affect tortilla flexibility relative to presence of 2+12 at this locus. This further illustrates that presence of 5+10 at *Glu-D1* locus has great contribution on dough strength which is necessary to maintain tortilla flexibility during storage.

As already mentioned, presence of 7+9 at *Glu-B1* contributes to dough strength. Therefore, wheat lines (group 4, 6, 7, 9, 32, 36, 39) possessing this allelic pair produced tortilla with superior flexibility (average of 3.36), and their average diameter value was acceptable with an average of 170.4 mm. In the presence of 2*/1 and 5+10 at *Glu-A1* and *Glu-D1* loci, respectively, presence of 17+18 instead of 7+9 at *Glu-B1* dramatically decreased tortilla flexibility scores over storage from 3.25 to 2.56 (group 33 and 32). Similarly, in the presence of 1 at *Glu-A1* and 5+10 at *Glu-D1*, presence of 7+8 instead of 7+9 decreased tortilla rollability scores from 3.85 to 3.31 (group 17 and 9).

In this study, it was also found that in the presence of 17+18 at *Glu-B1* and 2+12 at *Glu-D1*, presence of subunit 2* instead of subunit 1 at *Glu-A1* locus increase tortilla shelf stability from 1.63 to 2.88 (group 31 and 40). This can be attributed to dough strengthening effect of 2* at *Glu-A1*.

In general, tortillas from dough with strong gluten network had superior flexibility score over storage (> 3.0), but were smaller in diameter. Interestingly, however, most of the deletion lines tested in this study produced tortilla with good (acceptable) rollability scores on 16 days of storage and large diameter. Therefore, combination of deletions with right allelic composition of HMW-GS can be used to optimize wheat for good quality tortilla production

Two Dimensional Tortilla Extensibility

Tortilla texture was analyzed objectively with Two Dimensional (2D) tortilla extensibility test using TAXT2i texture analyzer. In this test, deformation modulus (N/mm), resistances to rupture (N), distance to rupture (mm), and work to rupture (N.mm) values were recorded objectively on the 0, 4, 8, 12 and 16 days of storage.

Tortilla Deformation Modulus

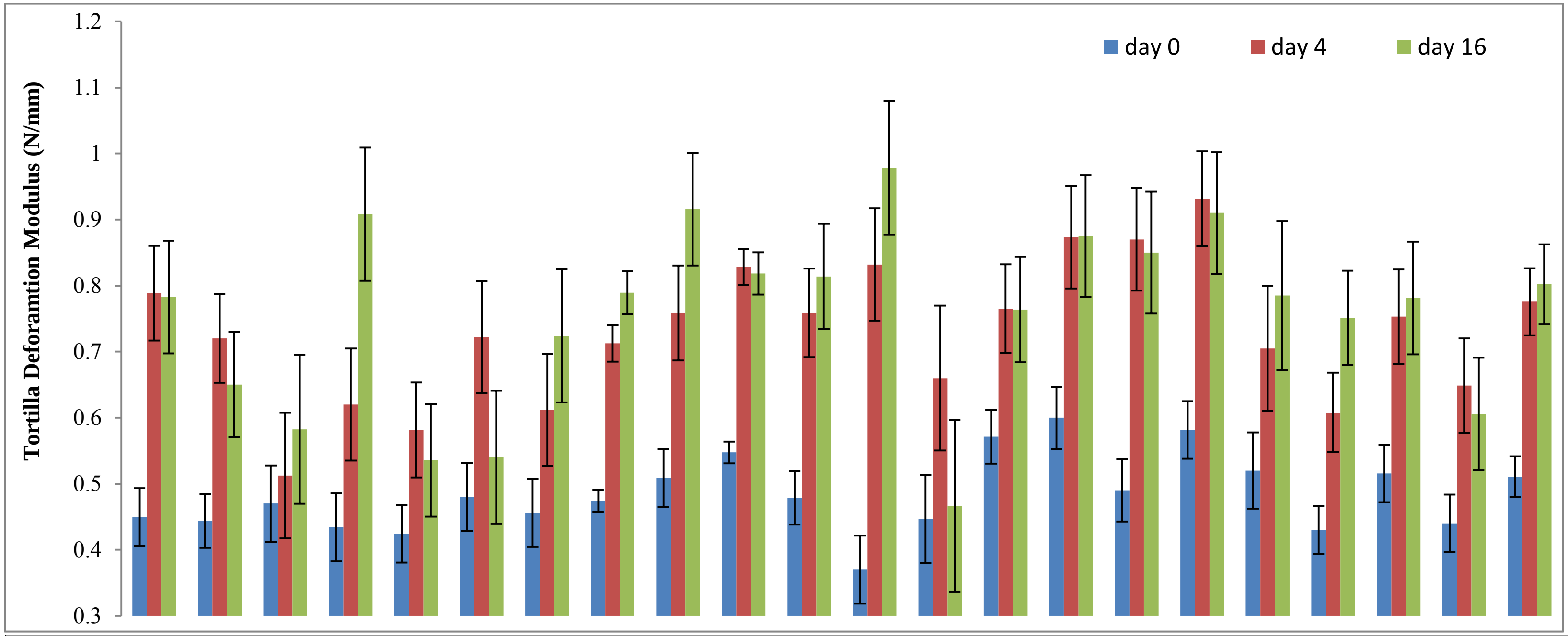
Deformation modulus was significantly affected ($p<0.05$) by deletions and variations in HMW-GS. Deformation modulus values generally increased when storage time increased and ranged from 0.37 to 0.60 N/mm on the day of tortilla production, whereas it ranged from 0.66 to 0.93 N/mm on day 4 of storage (Figure 6). Large textural differences occurred in the first 4 day of storage, whereas small textural differences were seen after 8 day of storage. This agrees with findings by Barros (2009) and Jondiko

(2010) and can be attributed to starch retrogradation over storage (Alviola and Waniska 2008).

Barros (2009) reported that deformation modulus is very important objective test to detect textural difference in wheat flour tortillas especially after 4 days of storage. Lower deformation modulus implies softer tortillas and vice versa. Tortillas from wheat lines possessing null genes at their HMW-GS generally had lower deformation modulus on both day 0 and day 16 of storage with averages of 0.45 and 0.67 N/mm, respectively, than tortillas with no deletion (average of 0.50 and 0.79 N/mm on day 0 and 16 of storage, respectively). This means that deletion lines produced softer tortillas than other lines. Flour IPP content correlated with tortillas deformation modulus with a correlation coefficient of 0.51. Additionally, deformation modulus of tortillas over storage was positively correlated with equilibrium force from stress relaxation test (r = 0.65) and dough resistance to extension (r = 0.47); meaning that tortilla deformation modulus increased when dough strength increased.

Wheat lines possessing (-, 7+9, 5) (group 6), (-, 7, 10) (group 7), and (2*, 17+18, 2+12) (group 31) at their *Glu-A1*, *Glu-B1*, *Glu-D1*, respectively formed highly extensible dough; thus, produced tortilla with the lowest deformation modulus values over time (0.54, 0.54, and 0.47 N/mm). This means that these tortillas were the softest at the 16 days of storage. These lines also produced large diameter tortillas (185.1, 181.3, and 177.1, respectively), but only group 6 tortillas had acceptable flexibility scores over storage (3.31). These results are also evidence that an increase in dough extensibility resulted in a decrease in tortilla deformation modulus over storage. On the other hand, tortillas from wheat lines possessing 5+10 at their *Glu-D1* locus generally had higher deformation modulus values over storage (average of 0.84 N/mm) compared to those without 5+10 (average of 0.66). Wheat lines possessing 1, 20a+20b/7+8, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci (group 29) produced tortillas with the highest deformation modulus (0.98 N/mm). These tortillas also had good flexibility scores over storage (3.83), but they were slightly smaller (164.6 mm) than other tortillas. Tortilla deformation modulus values over storage were positively correlated with tortilla flexibility on 16 days of storage ($r = 0.57$), but negatively correlated with tortilla diameter ($r = -0.73$), specific volume ($r = -0.55$), and tortilla lightness ($r = -0.53$). These indicate that dough strength has a great effect on tortilla deformation modulus and thus tortilla softness over storage.

In the presence of 2* at *Glu-A1* and 17+18 at *Glu-B1*, deletion at *Glu-D1* locus significantly decreased ($p < 0.05$) the deformation modulus at 16 days of storage from 0.85 to 0.72 N/mm, when compared to allelic pair of 5+10 (group 8, and 34), but when compared to allelic pair of 2+12, the deformation modulus at 16 days of storage significantly increased from 0.47 to 0.72 N/mm (group 8 and 31). Thus, the presence of 2+12 at *Glu-D1* has an overall weakening effect on dough compared to null allele at this locus; on the other hand, 5+10 has a strengthening effect on dough which may cause production of softer tortillas. Similarly, deletion of 2* at *Glu-A1*, in the presence of 7+9 and 2+12 at *Glu-B1* and *Glu-D1* loci, respectively significantly ($p < 0.05$) increased the deformation modulus from 0.78 to 0.91 N/mm at 16 days of storage compared to 2* at



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 6. Effect of deletions and variations in high molecular weight glutenin allelic composition on wheat tortilla deformation modulus. Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic composition. Error bars represent the ± standard error of means.
– refers to null alleles.

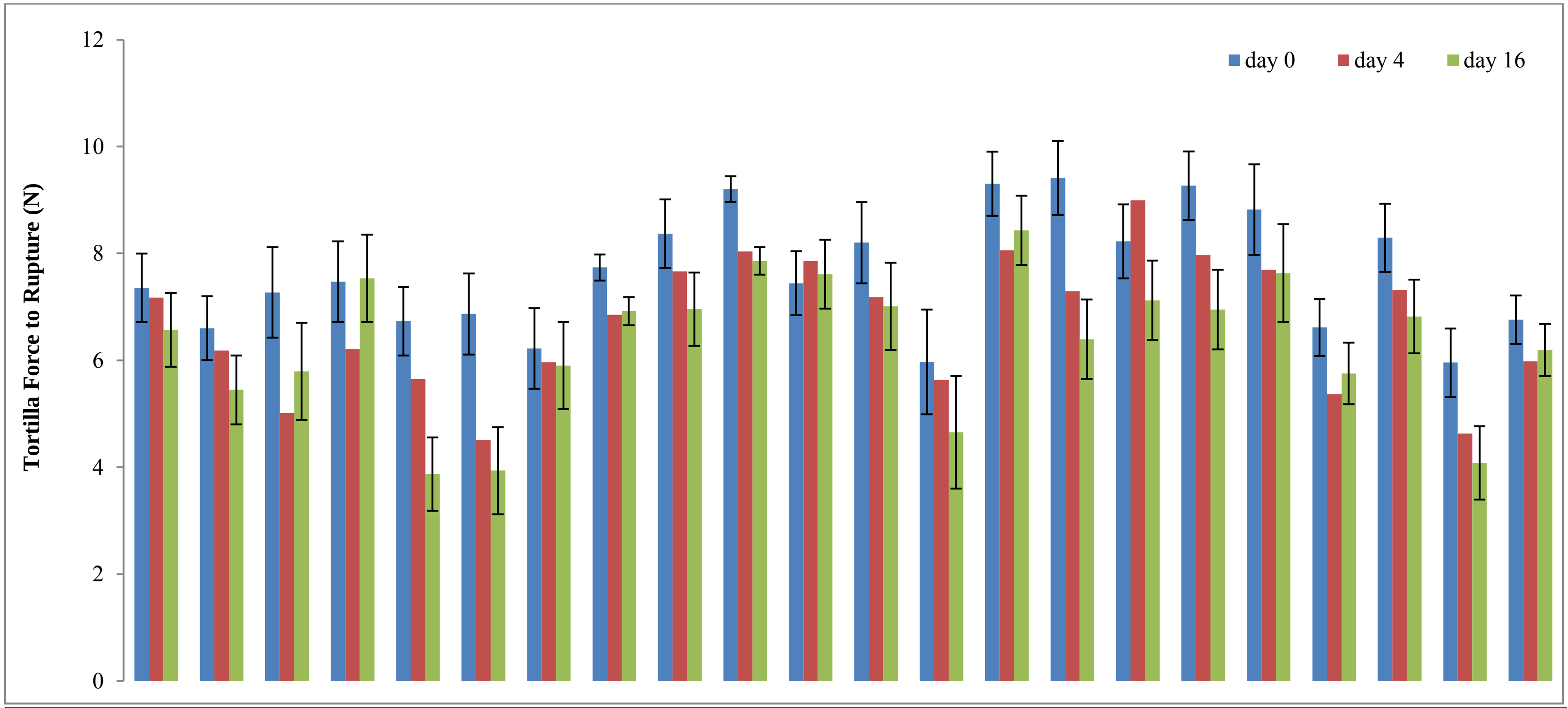
Glu-A1 locus (group 5 and 39). This shows that the presence of 2* at *Glu-A1* also decrease tortilla softness during storage.

Tortilla Force to Rupture

Force to rupture is a measure of firmness and hardness of tortilla, and it was significantly affected ($p < 0.05$) by deletions and variations in HMW-GS. Force required to rupture of tortilla was reduced over storage due to starch retrogradation and loss of moisture which make tortilla brittle. Force to rupture ranged from 5.97 to 9.41 N on the day of production, while it was between 3.94 and 8.43 N on day 16 of storage (Figure 7). Tortillas from deletion lines generally required less force to rupture on both day 0 and day 16 (average of 6.93 and 5.57 N, respectively) than tortillas from other lines (average of 7.97 and 6.69 N, respectively). Flour and dough properties altered tortilla force to rupture. The amount of IPP and dough development time were positively correlated with tortilla force to rupture values with correlation coefficients of 0.61, and 0.52, respectively. Similarly tortilla force to rupture was positively correlated with dough equilibrium force from stress relaxation test ($r = 0.70$), and dough resistance to extension ($r = 0.50$). These results also agreed with findings by Alviola and Awika (2010) that flour and dough properties can be used to predict tortilla texture during storage.

Tortillas from wheat lines possessing (-, 7+9, 5) (group 6) and (-, 7, 10) (group 7) required the lowest force to rupture over storage (3.87, and 3.93 N, respectively) meaning that tortillas from these lines were the least hard. These lines also produced the largest diameter tortillas. However, group 6 tortillas exhibited good flexibility at 16 days of storage (3.31), while group 7 tortillas had poor flexibility (2.42). On the other hand, tortillas from wheat line possessing 2*/1, 7+9, and 5+10 at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively required the greatest force to rupture. Tortillas from this line had also good flexibility scores over storage (3.25), but they were very small in diameter (153.8 mm), which can be attributed to dough strengthening effect of 7+9 at *Glu-B1* in combination with 5+10 at *Glu-D1* locus. Tortilla rupture force was positively correlated with tortilla moisture content (r = 0.63), and rollability scores over storage (r = 0.64), but negatively correlated with tortilla diameter (r = -0.86), and specific volume (r = -0.69), meaning that tortilla rupture force decreased, when dough strength decreased.

In the presence of 2* and 17+18 at *Glu-A1* and *Glu-D1*, respectively, deletion at *Glu-D1* locus decreased the tortilla force to rupture, when compared with tortillas from wheat lines possessing 5+10 at their *Glu-D1* locus; on the other hand, when compared to 2+12 at this locus, the force to rupture increased (group 8, 31, and 34). This confirms findings by Pierucci (2008) that presence of 5+10 at *Glu-D1* locus increased rupture force, while presence of 2+12 at *Glu-D1* locus decreased tortilla force requirement to rupture. These results can be attributed to the strengthening and weakening effects of 5+10 and 2+12 at *Glu-D1* on dough properties, respectively (Shewry et al 1992; Mondal et al 2008). Absence of either 5 or 10 at *Glu-D1* also dramatically decreased the force requirements to rupture (group 6 and 7). Presence of 7+9 instead of 7+8 at *Glu-B1* along with 1 at *Glu-A1* and 5+10 at *Glu-D1* did not affect force requirement of tortilla to rupture. This indicates that *Glu-A1* and *Glu-D1* loci have great effect on rupture force rather than *Glu-B1* locus.



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 7. Effect of deletions and variations in high molecular weight glutenin allelic composition on wheat tortilla force to rupture. Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic composition. Error bars represent the ± standard error of means.
– refers to null alleles.

Tortilla Distance to Rupture

Tortilla distance to rupture refers to tortilla extensibility. More extensible tortillas correspond to fresher and vice versa (Bejosano et al 2005). Tortilla distance to rupture values ranged between 19.1 and 24.6 mm on the day of production; tortillas from control flour exhibited the shortest distance to rupture (Figure 8). Deletion lines and non-deletion lines produced similar tortillas in terms of extensibility on the day of production with an average of 21.9 and 22.5 mm. Therefore, deletion of HMW-GS is not a major factor in determining tortilla extensibility. On the other hand, tortilla extensibility on day 16 was positively correlated with the amount of IPP of flour ($r = 0.62$), tortillas rollability scores on 16 days ($r = 0.78$), and moisture content ($r = 0.61$), but negatively correlated with tortilla diameter ($r = -0.57$), and specific volume ($r = -0.56$). This is an evidence of effect of dough strength on tortilla texture, meaning that tortilla flexibility increased, when dough strength increased. A strong correlation between tortilla distance to rupture on 16 days of storage and tortilla rollability scores over storage suggests that tortilla distance to rupture may be useful as an alternative objective method for subjective rollability test and agrees with findings by Alviola and Awika (2010).

Tortillas extensibility reduced when storage time increased, and this confirms that tortillas lose their flexibility over storage likely due to starch retrogradation. A sharp decrease in tortilla flexibility was observed on 4 day of storage with smaller decrease thereafter (Figure 8). This can be attributed to a big portion of starch retrogradation occurring in the first 4 days of storage, which contributes to loss of tortilla flexibility.

Therefore, this objective test is most useful for textural differences at the beginning of storage (Barros 2009).

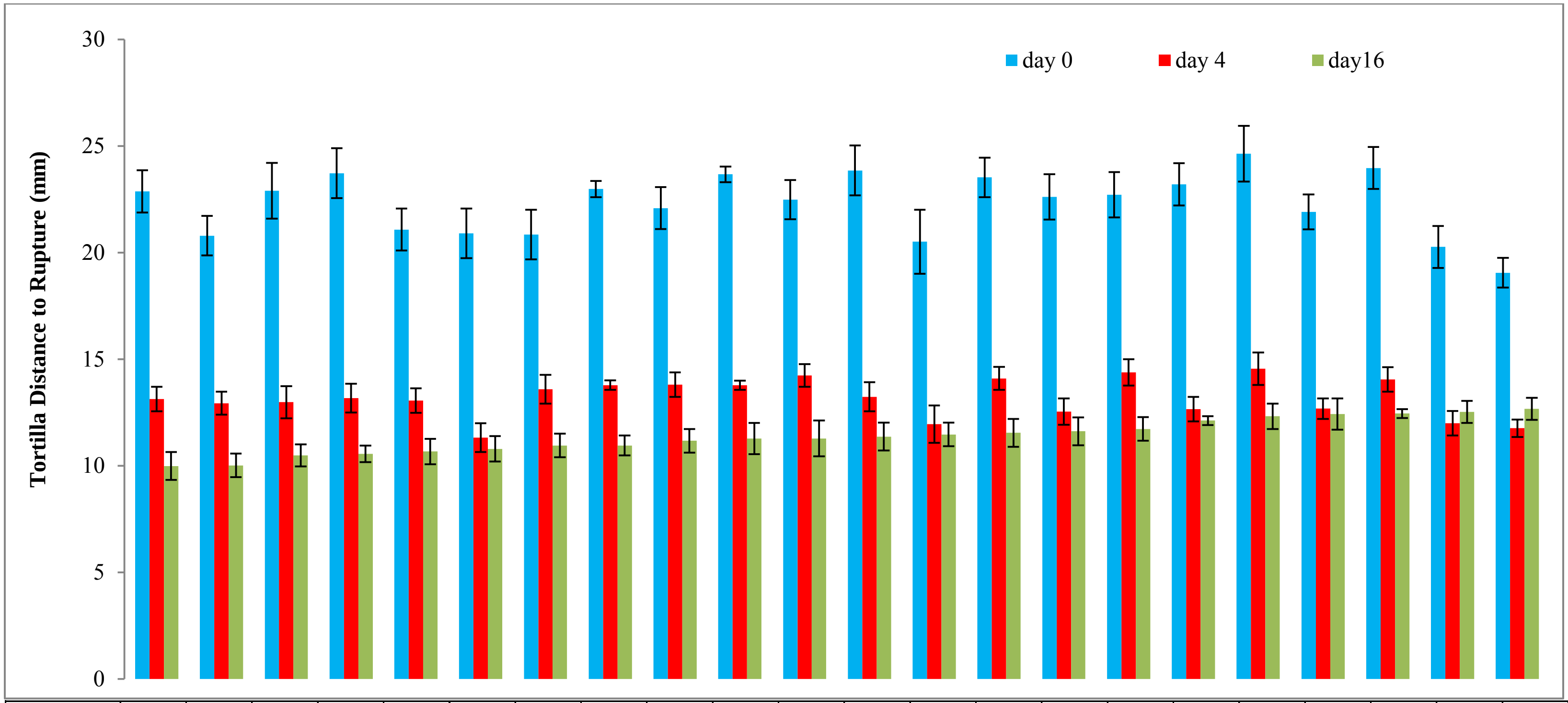
Tortillas made from flour possessing (1, 20a+20b/7+9, and 5+10) (group 36) and (2*/1, 7+9, and 5+10) (group 32) at (*Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively) required the longest distance to rupture on the day of production (24.6, and 23.5, respectively). These results also agree with subjective tortilla flexibility test (3.92, and 3.25, respectively). This can be attributed that interactive effect of 1, 7+9 and 5+10 formed strong gluten network which provides superior shelf stability to tortillas. On the other hand, wheat lines possessing (1, 17+18, and 2+12) (group 40) and (2*, 17+18, and 2+12) (group 31) produced tortilla which require the shortest distance to rupture on the day of production (20.3, and 20.5 mm, respectively). Tortillas from these lines had inferior flexibility scores over storage (1.63, and 2.88, respectively). This can be attributed to weakening effect of 2+12 at *Glu-D1* on dough.

Tortilla Work to Rupture

Work is a measurement of energy required to rupture tortillas. Work to rupture values were significantly affected ($p < 0.05$) by deletion and variations in HMW-GS. Work to rupture and distance to rupture had similar behavior, and it decreased when storage time increased. On the day of production work to rupture values ranged from 42.7 to 88.3 N.mm, while it was between 14.7 and 38.6 N.mm at 16 days of storage. Barros (2009) reported that work to rupture values on 16 days of storage show very good correlation with tortilla physical properties. This was confirmed by present data; work to rupture after 16 days of storage negatively correlated with tortilla diameter, and specific volume, with correlation coefficients of -0.81, -0.68, respectively, but positively correlated with tortilla moisture content (r = 0.70), and rollability scores on 16 days of storage (r = 0.70). These correlations are also indicators that work to rupture at 16 days of storage increased, when dough strength increased.

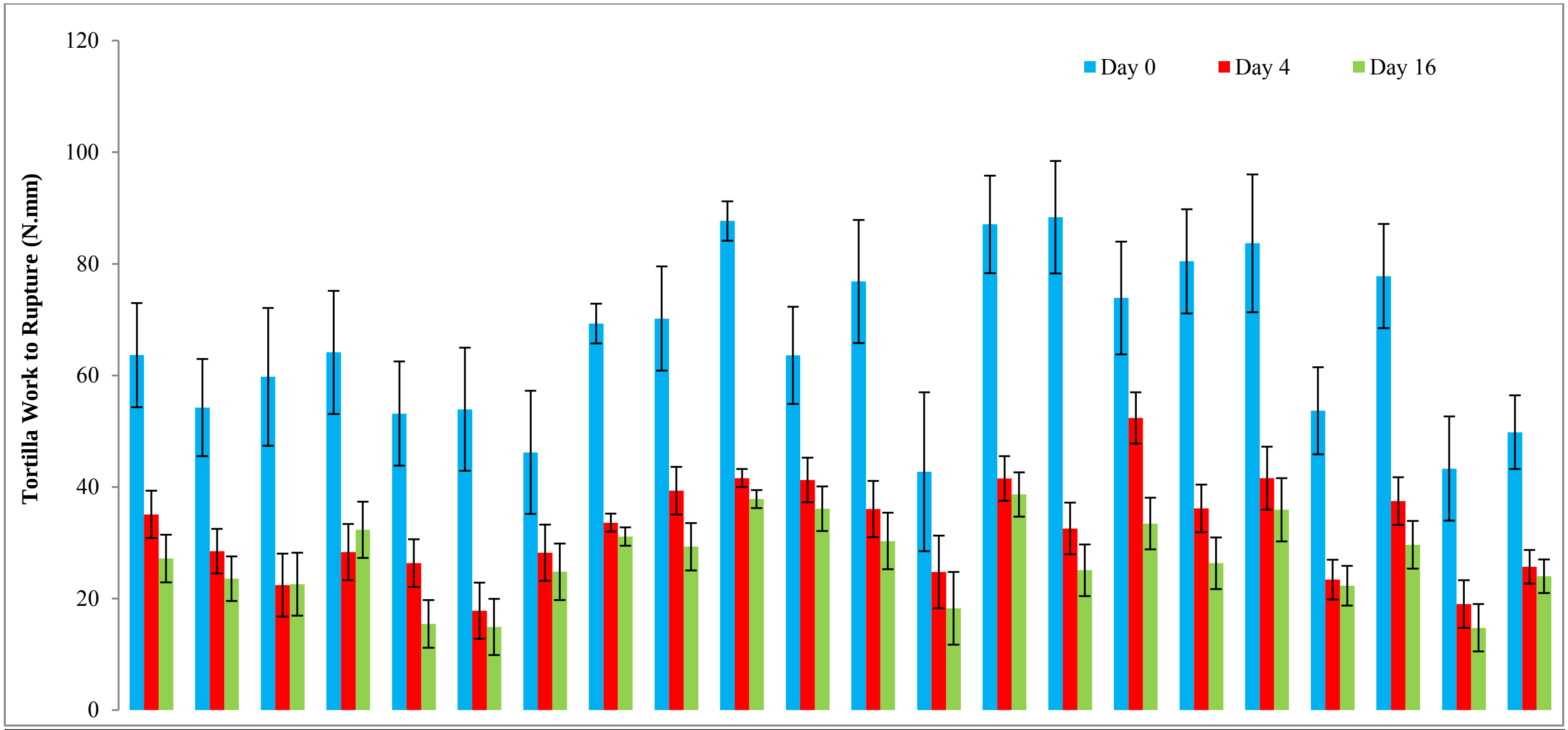
On 16 day of storage, tortillas from lines possessing (7+9, 5) (group 6) and (7, 10) (group 7) at their *Glu-B1* and *Glu-D1*, respectively along with null alleles at their *Glu-A1* locus exhibited the lowest work to rupture values (15.4, and 14.7 N.mm, respectively). These results suggest that deletion at *Glu-A1* and lack of either 5 or 10 at *Glu-D1* cause a reduction in tortilla force and distance to rupture values, and hence there is a reduction in work requirements to rupture these tortillas. As previously discussed, these lines produced tortilla with large diameter, but only group 6 tortillas had good shelf stability (3.31).

Tortillas from wheat line 2*/1, 7+9, and 5+10 showed the highest work to rupture (38.6 N.mm). This is attributed to strengthening effect of 5+10 at *Glu-D1* on dough structure which produce tortillas with higher force required to rupture, and hence tortillas from this line required higher work to rupture.



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	Unknown
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 8. Effect of deletions and variations in high molecular weight glutenin allelic composition on tortilla distance to rupture. Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic composition. Error bars represent the standard error of means. – refers to null alleles.



Group	1	2	4	5	6	7	8	9	17	18	27	29	31	32	33	34	35	36	37	39	40	50
Glu-A1	-	-	-	-	-	-	2*	1	1	2*	1	1	2*	2*/1	2*/1	2*	2*	1	2*	2*	1	
Glu-B1	-	7+8	20a+20b /7+9	7+9	7+9	7	17+18	7+9	7+8	7+8	20a+ 20b	20a+20b /7+8	17+18	7+9	17+18	17+18	17+18 /7+8	20a+20b /7+9	20a+20b	7+9	17+18	Unknown
Glu-D1	-	-	-	2+12	5	10	-	5+10	5+10	5+10	5+10	5+10	2+12	5+10	5+10	5+10	5+10	5+10	5+10	2+12	2+12	

Fig. 9. Effect of deletions and variations in high molecular weight glutenin allelic composition on wheat tortilla work to rupture. Glu= Glutenin. Numbers in the x axis refer to group of wheat lines with same allelic pair composition. Error bars represent the standard error of means.
– refers to null alleles.

CHAPTER III

CONCLUSION

HMW-GS in wheat are encoded by the loci *Glu-A1*, *Glu-B1*, and *Glu-D1* on the long arm of group 1 chromosomes, and their variants play significant roles in the functional properties of flour; hence dough properties and tortilla quality. Therefore, by careful selection of HMW-GS in the plant breeding program, it is possible to manipulate dough properties to produce good quality tortillas.

Presence of null genes in one or more of these loci generally resulted in a decrease in flour IPP content and likely amount of disulphide bonds presented in gluten network, causing the formation of weak dough which produced large diameter tortillas. Data from this study demonstrate that presence of 7+9 at *Glu-B1* locus along with null genes is important to obtain strong enough gluten network to maintain tortilla shelf stability. For example, deletion lines possessing the following HMW-GS (-, 20a+20b/7+9, -), (-, 7+9, 2+12), and (-, 7+9, 5) at their *Glu-A1*, *Glu-B1*, *Glu-D1* loci, respectively formed highly extensible dough, which produces large diameter tortillas with good flexibility scores (>3.0) after 16 days of storage. Tortillas produced from these lines also had lighter color (higher L value) than control and would be preferred by consumers for appearance. On the other hand, wheat lines possessing (-, 7+8,-) and (-, 7, 10) produced very extensible dough and large diameter tortillas, but they had inferior shelf stability (flexibility score on day 16 < 3.0).

Wheat line with complete HMW-GS deletions at the three loci; *Glu-A1*, *Glu-B1*, and *Glu-D1* (group 1) produced large diameter tortilla with good shelf stability. The absence of HMW glutenin subunits contributed to dough extensibility; therefore, the dough balls spread more during hot-pressing and retained a large diameter with minimal shrinkage. However, more studies need to be done to provide better understanding of the cause of good tortilla stability in the absence of HMW-GS at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci.

This study reveals that deletion of HMW glutenin genes from one or more loci of wheat can be used to develop wheat cultivars with optimum gluten functionality for tortillas.

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