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Use of Near-Isogenic Wheat Lines to Determine the Glutenin Composition and Functionality Requirements for Flour Tortillas

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In wheat (*Triticum aestivum* L), the synthesis of high molecular weight (HMW) glutenins (GS) is controlled by three heterologous genetic loci present on the long arms of group 1 wheat chromosomes. The loci Glu-A1, Glu-B1, and Glu-D1 and their allelic variants play important roles in the functional properties of wheat flour. This study focused on understanding the functionality of these protein subunits on tortilla quality. Near-isogenic wheat lines in which one or more of these loci were absent or deleted were used. Tortillas were prepared from each deletion line and the parent lines. The elimination of certain HMW-GS alleles alter distinct but critical aspects of tortilla quality such as diameter, shelf stability, and overall quality. Two deletion lines possessing HMW-GS 17 + 18 at Glu-B1 and deletions in Glu-A1 and Glu-D1 had significantly larger tortilla diameters, yet tortilla shelf life was compromised or unchanged from the parent lines used to develop the deletion lines or the commercial tortilla flour used as a control. Alternatively, a deletion line possessing Glu-A1 and Glu-D1 (HMW-GS 1, 5 + 10) and a deletion in Glu-B1 also significantly improved tortilla diameters. Whereas the increase in diameter was less than the line possessing only HMW-GS 17 + 18 at Glu-B1, the stability of the tortillas were, however, maintained and improved as compared to the parent lines containing a full compliment of HMW-GS. Thus, the presence of subunits 5 + 10 at Glu-D1 alone or in combination with subunit 1 at Glu-A1 appears to provide a compromise of improvement in dough extensibility for improved tortilla diameters while also providing sufficient gluten strength to maintain ideal shelf stability.

KEYWORDS: High molecular weight glutenins; tortillas; near-isogenic deletion lines

INTRODUCTION

The tortilla is a circular, light colored ethnic flat bread, once considered a Mexican specialty, that has moved into mainstream American diets. Tortillas are the second most popular bread after white bread (*1*) and are offered on two-thirds of restaurant menus nationwide (www.washingtonpost.com). As such, wheat flour tortillas contribute significantly to the wheat commodity market. Most of the U.S. tortilla industry is based in Texas and California, and according to the Tortilla Industry Association, represented a $6.1 billion dollar industry in the U.S. in 2004 (www.tortilla-info.com). Hard red winter wheat (HRWW), the major wheat class grown in Texas and across the Southern Great Plains has high protein levels and high gluten strength suitable for bread making. Whereas wheat gluten functionality is also important for tortilla quality, most HRWW cultivars produce poor quality tortillas (*2*). Because tortillas are not always consumed on the day they are baked, shelf stability is an important issue. As such, it has become a challenge for the tortilla producers to make tortillas with good consumer quality attributes while also maintaining extended shelf stability.

Wheat quality research in the last 50 years has focused on improving quality for bread production and its relationship to wheat gluten storage protein functionality. However, little research has focused on the gluten protein functionality requirements for optimal tortilla quality. The critical tortilla parameters are diameter, opacity, and rollability. Tortillas about 2 mm thick, evenly opaque, with an ample diameter, and at least a 3-week

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shelf-life are considered ideal. As in bread, wheat flour and gluten functionality contributes significantly to this shelf stability and the need for tortillas to resist breaking during consumption (*3–7*). However, the shelf-life of tortillas is longer than that of bread as tortillas retain their protein functionality and have decreased starch dispersion and firming as compared to bread (*8*). The diameter of tortillas also requires extensible dough that resists shrink-back during processing (*9*). The dough extensibility, in-turn, depends again on the gluten proteins and their interactions to form the gluten network. Thus, the dough extensibility during hot pressing and retention of tortilla flexibility after baking require a gluten functionality that is unique to the strong, viscoelastic gluten functionality needed for bread.

The tortilla industry currently uses bread wheat flours that require the addition of various reducing agents to reduce gluten strength, thereby producing tortillas with large diameters and good extensibility and shelf stability. L-Cysteine is widely used as a reducing agent because it competes with the disulfide bridge, forming cysteine residues in the gluten matrix. It is also a common perception by the industry that increasing the amount of these agents gives rise to good tortillas; however, recent studies observed small or insignificant improvements via increasing reducing agent concentration (*10*). The addition of these compounds also negatively affects the taste and quality of tortillas. A lack of knowledge of the gluten functionality requirements for tortillas is, thus, a problem for obtaining optimal tortilla quality.

Gluten is composed of high molecular weight (HMW) and low molecular weight (LMW) glutenins (GS) and gliadins and their allelic variants. The glutenins are the main component responsible for the end-use quality differences among wheat genotypes (*11*), and they are the primary determinants of bread-making quality and gluten elasticity (*12, 13*). The HMW-GS are located on the long arm of chromosome 1A, 1B, and 1D and represent 5–10% of the total seed protein. They are further subdivided into allelic pairs (x and y) on 1B and 1D and a single subunit on 1A. Each of these subunits affects dough quality. The allelic pairs encoded at the Glu-D1 locus (5 + 10, 2 + 12), followed by the single subunit at the Glu-A1 (1, 2*, null), and then those encoded at the Glu-B1 locus (20, 7 + 9, 17 + 18), are the principal components contributing to quality (*14*). It has also been hypothesized that the differences in the allelic pair on 1D locus (5 + 10 vs 2 + 12) are particularly important determinants of gluten strength, with 5 + 10 associated with gluten strength. In bread making, a strong gluten network is required, and it has been suggested that the introduction of the HMW glutenin allelic pair 5 + 10 can improve strength. Similarly, the Glu-B1 subunit 17 + 18 is strong, whereas subunit 20 is associated with weak dough strength.

Near-isogenic lines have been used to understand the relationships between the wheat protein composition and functional quality. Near-isogenic lines are developed to transfer a single gene or loci through backcrossing into a common genetic background. As such, they are also useful for understanding the functional contribution of that particular gene. For instance, the effects of the individual gliadin and glutenin alleles or allelic pairs on dough and bread-making quality can and have been studied without the confounding effects of the different genetic backgrounds (*15*).

Lawrence et al. (*16*) developed a set of near-isogenic deletion lines in which the number of HMW-GS varied from a full complement of five to zero. These lines, in which the loci for the glutenin and gliadin genes are absent, have been used to deduce their effects on bread dough functionality (*17*). Two important conclusions were derived from these studies: first, dough mixing strength and bread-making quality are dramatically reduced as HMW glutenins are deleted (*17*); and second, the allelic variation at the Glu-D1 locus is associated with dough strength and bread-making quality.

Table 1. Protein Composition of Parents and the HMW Glutenin Deletion Lines

	HMW glutenin alleles[a]		
wheat lines	Glu-A1	Glu-B1	Glu-D1
Olympic	1	20	5 + 10
Gabo	2*	17 + 18	2 + 12
FM2B	−	17 + 18	−
FM3	−	17 + 18	−
FM4	1	17 + 18	−
FM6	1	−	5 + 10
FM7	1	17 + 18	10
FM9	−	17 + 18	2 + 12
FM13	2*	17 + 18	2 + 12

[a] Deletions in a line are indicated by (−) in the chart.

The proportion of glutenins to gliadins also influence the dough properties and, thus, bread-making quality; however, the gluten functionality needed for tortilla making differs from that needed for bread making. Unlike bread, the specific protein composition and functionality required for tortillas is not yet known. Thus, there is a need to define the glutenin and gliadin composition and functionality requirements needed for optimal tortilla making. In this study, near-isogenic deletion lines were used to determine the effect that individual HMW-GS exert on tortilla quality.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The near-isogenic deletion lines were developed from mutant lines of the wheat cultivar "Olympic", null at the Glu-B1 locus, and an isogenic line of the cultivar "Gabo", null at the Glu-A1 and Glu-D1 loci. A set from this series of deletion lines was obtained from Dr. Finlay MacRitchie (Kansas State University, Manhattan, Kansas) (**Table 1**).

The wheat lines were grown in field plots at the Texas Agricultural Experiment Station at College Station and at McGregor, Texas in 2005. The lines were also grown at South Dakota State University, Brookings, South Dakota. The parent cultivars Gabo and Olympic were also grown along with the set of deletion lines.

Performances of these lines in both the locations were evaluated for protein composition and for tortilla-making ability. Laboratory-on-a-chip Capillary Electrophoresis was used to verify the HMW glutenin allelic composition of the field-grown deletion lines using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California) as previously described (*18*).

Polymeric Protein Analysis. Flours were extracted as described by Bean et al. (*19, 20*). Briefly, 100 mg of flour was extracted with 1 mL of 50% aqueous 1-propanol, and pellets were freeze-dried before protein determination (Nx5.7). Equal volumes of first and second extracts were pooled and analyzed by size exclusion HPLC using a Biosep SEC-4000 column (Phenomenex, Torrance, CA) on an Agilent 1100 HPLC system. Column temperature was maintained at 40 °C, and the mobile phase was 50% acetonitrile and 0.1% (w/v) trifluoroacetic acid at a flow rate of 0.5 mL/min. The injection volume was 20 μL, and UV-detection was done at 210 nm (*21*). The percent of insoluble polymeric proteins (IPP) was calculated from the weight and protein content of the freeze-dried pellet, and extractable proteins (EP) were calculated from the difference between flour protein and protein in the pellet.

Evaluation of Wheat Grain and Flour. A 300 kernel sample was used for determining kernel hardness, diameter, weight, and moisture content using the single kernel hardness test (SKHT) (Perten Single Kernel Characterization System SKCS 4100, Perten Instruments, Springfield, IL).

Table 2. The % Protein, % IPP, % Polymeric Protein (PPP), Dough Development Time (MDDT), and Peak Resistance (MU) of Grain or from Flour of Wheat Deletion Lines Grown in Texas (TX) and South Dakota (SD)

	% protein		% IPP		% PPP		MDDT (min)		MU	
line HMW-GS alleles (A,B,D)	TX	SD	TX	SD	TX	SD	TX	SD	TX	SD
FM2B (−, 17 + 18, −)	13.4	11.9	3.6	2.5	5.9	4.8	1.5	1.0	5.5	5.2
FM3 (−, 17 + 18, −)	12.8	11.4	4.6	3.3	6.3	5.0	2.0	2.0	5.0	4.6
FM6 (1, −, 5 + 10)	12.3	12.5	5.5	3.2	6.2	4.6	2.9	2.3	5.0	5.5
FM9 (−, 17 + 18, 2 + 10)	13.2	11.3	6.3	4.2	7.5	5.0	3.5	2.5	6.4	6.0
FM13 (2*, 17 + 18, 2 + 12)	12.7	11.6	6.7	5.3	7.5	6.0	3.6	2.8	6.5	6.4
Gabo (2*, 17 + 18, 2 + 12)	12.8	11.9	6.1	4.1	6.7	4.8	3.3	2.5	6.7	6.6
Olympic (1, 20, 5 + 10)	13.5	11.1	6.7	4.8	6.7	4.8	5.4	2.6	5.4	5.2

Cleaned grain was tempered to 14% moisture, allowed to rest, and milled to flour (Brabender Instruments, South Hackensack, NJ). Near-infrared reflectance spectrophotometry (NIR) was used to estimate the flour protein content and moisture content from the deletion and parent lines in three separate replicates (Perten PDA 7000 Dual Array with Grams Software) (*22*).

A 35 g sample of flour from each line was used for mixograph (Lincoln Manufacturing Company, Lincoln, NE) analysis to determine the dough mixing time and the dough strength of the flour. The dough mixing resistance (MU) and the dough mixing time (MDDT) were recorded from mixograms using standard procedures (*22*).

Tortilla Processing. The flour from each line grown in Texas and South Dakota were processed into tortillas. The tortillas were prepared according to a standard formulation (*23*) except that L-cysteine was not added. The formulation was standardized as 500 g of flour, 7.5 g of salt, 2.5 g of sodium stearoyl lactylate, 2 g of potassium sorbate, 2.3 g of encapsulated fumaric acid, and 30 g of shortening. The amount of water added was based on the mixograph water absorption. Commercially available tortilla flour (ADM Tortilla Flour, ADM Milling Company, Overland Park, Kansas) was used to compare the tortilla quality obtained from the commercial flour and the selected experimental lines. Two tortilla batches were made for each experimental wheat line. First, a smaller amount of flour was used to standardize the formulation and water requirement. This was followed by a second batch made from 500 g of flour. This sample was used for evaluation. Dry ingredients were mixed with the flour in a mixing bowl with a paddle at a low speed for 1 min placed over copper tubes through which heated water at 70 °C was pumped to control temperature (Model A-200, Hobart Corporation, Troy, OH). Shortening was then added, and the dough was paddle mixed for 2 min at low speed. Water (35 °C) was then added, and the dough was mixed for 1 min at low speed and then mixed at a medium speed for 6 min with a dough hook.

The dough was placed in a plastic tray for dough quality measurements. The dough was then proofed (Model 57638, National Manufacturing Company, Lincoln, NE) at 35 °C and 70% relative humidity for 5 min. The dough was pressed by hand and divided and rounded with Duchess Divider/Rounder (Bakery Equipment and Service Company, San Antonio, TX) into 36 dough balls of 43 g each. The dough balls were transferred to the plastic tray and rested in the proof chamber for 10 min at 35 °C and 65% relative humidity.

The dough balls were placed on a hot press (Micro-Combo model 0P01004–02, Lawrence Equipment Company Incorporation, South El Monte, CA) and pressed at 1100 psi. The tortillas were then baked in a three-tier oven (Micro-Combo Tortilla Oven, Model 0P01004–02, Lawrence Equipment, South El Monte, CA) set at a temperature of 350–360 °F. The dwell time was adjusted to 30 s. The tortillas were cooled on a three-tier cooling chain (Model 3106 INF, Superior Food Machinery Inc., Pico Rivera, CA), removed, and packed in low-density polyethylene bags and stored at 23 °C for quality evaluation.

Dough Evaluation. The dough was placed on a plastic tray, the temperature was measured using a thermometer, and the values were recorded. Dough extensibility and force to extend were evaluated subjectively (*24*).

Tortilla Evaluation. The tortillas were evaluated for their weights, diameters, heights, pHs, moistures, opacities, colors and rollabilities (*24, 25*). The pH and moisture content of individual tortillas from each line was determined as previously described (*22*).

The average opacity of 10 tortillas was subjectively evaluated using a continuous scale of 1–100% (1% being fully translucent and 100% being completely opaque). The color parameters, L* (lightness), ±a* (red−green), and ±b* (yellow−blue) were determined for each tortilla using a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan) by taking six measurements (three per side) of each tortilla. Tortilla shelf stability was evaluated using the rollability test (*23*), performed by wrapping a tortilla around a wooden dowel (1.0 cm in diameter). Ratings on a scale of 1−5 were recorded, with 1 being immediate breakage and 5 being no cracks or breakage. Rollabilities were determined on days 4, 10, and 14 of storage for each line using three tortillas from each line. The specific volume (cm^3/g), indicating fluffiness, was calculated for each line. The specific volume was calculated using the formula provided below.

$$\text{Specific volume} = \pi(\text{diameter}/2)^2 \times \text{height} \times (1000/\text{weight})$$

The quality index was then calculated based on the opacity, rollability (on day 14), and specific volume using the formula provided below.

$$\text{Quality index} = \text{opacity} \times \text{specific volume} \times \text{rollability score}$$

Method of Analysis. The data was analyzed using SPSS statistical software (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL) to estimate the correlations, compare the means, and test for significance by Tukey's HSD and ANOVA analysis. An all-fixed model was used to estimate the main effects and the interaction effects of the genotypes and the environments. The ANOVA analysis of the locations was determined separately for the main parameters; IPP, protein, diameter, rollability, and opacity. Results indicated significant genotypic effects (not shown). A combined analysis (fixed model) for both locations had significant genotypic, location, and genotype × location interaction effects (not shown). Because of the significance of the genotype × location effect for the major parameters, IPP, protein, diameter, rollability, and opacity, the quality parameters will be discussed separately for two locations in the results section.

RESULTS AND DISCUSSION

The flour protein content in deletion lines grown in Texas was almost 2% higher than those from South Dakota (**Table 2**). The higher temperatures in Texas during the grain filling period may have increased the protein accumulation via suppression of starch accumulation (*26*). As such, the % IPP was, on the whole, lower in South Dakota. Deletions in the glutenin loci resulted in significantly reduced IPP content in Texas (Tukey HSD = 0.06) and South Dakota (Tukey HSD = 0.11) (**Table 2**). Deletions in Glu-A1 and Glu-D1 reduced the % IPP in the near-isogenic lines FM2B and FM3 as compared to the parents in both locations, whereas FM6, which has a deletion in the Glu-B1 locus, had an intermediate IPP content. These results are in agreement with the decrease in % extractable polymeric protein (% EPP) and glutenin macropolymer (GMP) reported for these same deletion lines (*27, 28*). The lower % IPP has dramatic effects on the dough and end-use quality properties as described below.

Table 3. Pearson's Correlations of % IPP and % PPP with Dough and Tortilla Quality Parameters Developed from Near-Isogenic Deletion Lines Grown in Texas[a]

	% IPP	MDDT	extensibility	diameter	rollability	opacity
% protein	−0.459	−0.211	0.533[b]	0.146	0.272	0.067
% IPP		0.950[b]	−0.781[b]	−0.811[b]	0.749[b]	0.240
MDDT			−0.052	−0.211	0.034	0.021
extensibility				−0.48	0.203	0.149
diameter					−0.396	−0.216
rollability						−0.822
opacity						

[a] At the 0.05 level of significance. [b] Indicates high correlations.

Table 4. Pearson's Correlations of % IPP and % PPP with Dough and Tortilla Quality Parameters Developed from Near-Isogenic Deletion Lines Grown in South Dakota[a]

	% IPP	MDDT	extensibility	diameter	rollability	opacity
% protein	−0.514	−0.332	0.740[b]	0.072	−0.333	−0.351
% IPP		0.821[b]	−0.592	0.534	−0.291	0.121
MDDT			−0.266	−0.498	0.173	−0.377
extensibility				0.154	0.230	−0.242
diameter					−0.045	−0.379
rollability						−0.480
opacity						

[a] At the 0.05 level of significance. [b] Indicates high correlations.

Dough Strength. The effects of specific HMW-GS composition in the deletions on the dough mixing strength were significant and were independent of protein content in some cases. Mixograms from Texas and South Dakota grown lines produced similar curves, yet the mixograms from South Dakota flours were lower in intensity, presumably due to the lower flour protein content. Mixograph dough development time (MDDT) and peak dough resistance (MU) were used to assess the flour strength. Lines FM2B and FM3 had weak dough mixing tolerance, as indicated by the quick rise in peak and immediate fall, indicating poor dough stability (**Table 2**). The dough prepared from FM6 had intermediate strength and FM9, and FM13 had a strong mixing strength in both locations. The parent Gabo had higher % IPP values and produced a strong dough (**Table 2**) even with Glu-D1 2 + 12, which is associated with weak dough strength (*29*). The line FM9 has the same subunits at Glu-B1 and Glu-D1, but a deletion in Glu-A1 had a % IPP value almost similar to Gabo and a strong mixing strength (**Table 2**). Thus, 17 + 18 and 2 + 12 together can give rise to stronger dough mixing strengths. The line FM6, which has subunits 1 and 5 + 10 at Glu-A1 and Glu-D1, respectively, and a deletion in Glu-B1, had a significantly lower % IPP than FM9 and Gabo and had intermediate dough strength. The lines FM2B and FM3 have subunit 17 + 18 only at Glu-B1 and deletions at other loci. These two lines showed lower % IPP than FM6, FM9, and Gabo and reduced dough mixing time. Thus, the subunits at Glu-A1 and Glu-D1 are important in contributing to greater dough mixing strength. The strong correlations with of the % IPP and the dough mixing time support this statement (**Tables 3** and **4**).

Mixograph parameters along with breadmaking performance were the first functional properties reported for these deletion lines (*16*). The loss of tolerance and shortened mixing time coincides with deletions, particularly of the Glu-D1 locus, are confirmed here. The presence of the Glu-D1 HMW-GS have been well documented as contributing to breadmaking quality (Payne 1991), with flours deficient in this locus resulting in poor quality bread and with small loaf volume. The MDDT of this set of deletion lines has been shown to have strong correlations with rheological measurements, including elongational viscosity, G', and shear viscosity (*27, 30*). This can be explained by the changes in the % IPP in that the polymer size and concentration directly increases shear viscosity.

Table 5. Tortilla Quality Parameters of Tortillas Prepared from Flour of Wheat Deletion Lines Grown in Texas (TX)[a]

line HMW-GS alleles (A, B, D)	rollability (day 14)	diameter (mm)	specific volume (cm^3/g)	opacity (%)	Q. index
cont	2.5 b,c	163 c	1.42	80	284
FM2B (−, 17 + 18, −)	1.5 d	176 a	1.7	86	212
FM3 (−, 17 + 18, −)	2.3 b,c	171 b	1.7	86	300
FM6 (1, −, 5 + 10)	3.0 a,b	167 b	1.7	75	383
FM9 (−, 17 + 18, 2 + 10)	3.3 a	155 d	1.4	78	365
FM13 (2*, 17 + 18, 2 + 12)	2.5 b,c	165 c	1.5	78	300
Gabo (2*, 17 + 18, 2 + 12)	2.5 b,c	155 d	1.4	80	272
Olympic (1, 20, 5 + 10)	2.5 b,c	155 d	1.6	80	335

[a] Letters indicate Tukey's LSD significant difference groups.

Dough Quality. Dough extensibility is an important parameter that influences tortilla quality. Using a subjective method, where 3.0 on a scale of 1 (low) to 5 (high) is ideal, dough prepared from deletion lines FM2B and FM3 had high dough extensibility scores of 4.5 and 3.5 respectively (not shown). The other lines had extensibility scores of 3.0 that are near optimum. The elasticity scores indicate the force needed to extend, where 2.0 is ideal for tortillas on a scale of 1−5. Dough prepared from lines FM2B and FM3 had ideal elasticity scores of 2.0 whereas the score of line FM6 was 2.3. The other lines had higher elasticity scores of 3.0. The low elasticity scores with high extensibility scores indicated that these lines had dough that had good extensibilities without breaking and required less force to extend with little shrink-back. The commercially available tortilla control flour also had a good extensibility score but higher elasticity scores of 3.5 and 3.0, respectively.

Significant correlations were observed between the extensibility and the protein content (**Tables 3** and **4**). A significant correlation was observed in Texas. However, the extensibility was negatively correlated with increasing IPP content (**Table 3**). Good extensibility with high elasticity indicates that the dough extended readily but shrunk back from elasticity, which will result in a small diameter tortilla; this is observed when strong gluten is used (*31*). A certain amount of elasticity is required and has been dramatically demonstrated using a transgenic line where the HMW-GS genes were silenced. The resulting tortillas were excessively large with diameters approximately 20% larger than the parental wheat. The lack of HMW-GS results in the loss of elastic shrink-back and poor rollability of tortillas (*32*).

Tortilla Quality Evaluation. Tortilla quality was markedly affected by the deletions in the HMW glutenin loci. Because cysteine was not used in any of these experiments, the tortilla properties were due to the functionality of the glutenins present in the flour. Tortillas made from lines FM2B and FM3 had larger diameters, 1–2 cm larger than the control tortilla flour, and the parent cultivars (**Tables 5** and **6**). Both FM2B and FM4 had poor rollability scores in both locations, with FM4 producing smaller diameters (**Table 6**). In FM4, the interactions of subunit 1 from Glu-A1 with 17 + 18 on Glu-B1 altered the diameter versus FM3 and FM2B with only 17 + 18. The absence of the Glu-D1 loci also had a negative effect on the rollability of the tortillas. The line FM7, which has 1, 17 + 18, and 10, had a larger diameter than most of the lines, yet the rollabilities were poor with breakage by day 14. Thus, whereas the absence of

Table 6. Tortilla Quality Parameters of Tortillas Prepared from Flour of Wheat Deletion Lines Grown in South Dakota[a]

line HMW-GS alleles (A, B, D)	rollability (day 14)	diameter (mm)	specific Volume (cm^3/g)	opacity (%)	Q. index
cont	2.8 a,b	175 b	1.3	85	284
FM2B (−, 17 + 18, −)	1.0 b	181 a	1.5	91	136
FM3 (−, 17 + 18, −)	1.0 b	181 a	1.6	95	147
FM6 (1, −, 5 + 10)	3.0 a	174 b	1.3	82	330
FM4 (1, 17 + 18, −)	1.0 b	168 c	1.3	84	110
FM7 (1, 17 + 18, 10)	1.0 b	191 a	1.4	90	127
FM9 (−, 17 + 18, 2 + 10)	1.3 b	166 c	1.2	80	115
FM13 (2*, 17 + 18, 2 + 12)	1.0 b	175 b	1.4	84	115
Gabo (2*, 17 + 18, 2 + 12)	2.5 a,b	162 d	1.2	91	246
Olympic (1, 20, 5 + 10)	1.0 b	156 d	1.2	81	109

[a] Letters indicate Tukey's LSD significant difference groups.

Table 7. Contrasts between the Glutenin Alleles Absent in the Deletion Lines and Parent Cultivars

wheat lines	Glu-A1	Glu-B1	Glu-D1
FM2B	−	+	−
FM3	−	+	−
FM6	+	−	+
FM9	−	+	+
FM13	+	+	+
Gabo	+	+	+
Olympic	+	+	+
	Contrast		
protein	0.46	0.58	0.45
diameter	0.02[a]	0.01[a]	0.004[a]
rollability	0.00[a]	0.73	0.00[a]

[a] At the 0.05 level of significance.

subunit allelic pair 5 of Glu-D1 yielded larger diameter tortillas, the rollabilities were nonetheless lowered. Therefore, subunit 5 + 10 appears important for good tortilla shelf stability. Interactions between the subunits 2*, 17 + 18, and 2 + 12 did not seem to contribute to good tortilla diameter. Although similar in HMW-GS composition, FM13 and Gabo had differences between their diameters and rollabilities. The HMW-GS compositions are not able to account for all of the discrepancies in the lines, and the changes may have been due to other reasons such as variations in LMW-GS and gliadin alleles or starch composition (*31*). However, the presence of Glu-D1 HMW-GS subunits does confer a gain in function in tortilla rollability. The HMW-GS subunits at Glu-B1 alone are good for tortilla diameter but not good for rollabilities, yet when combined with the Glu-D1, better stability is observed. Tortillas made from lines possessing subunits 5 + 10 had better rollability scores than subunits 2 + 12. This could have been more thoroughly explained if a deletion line containing both the Glu-B1 17 + 18 and Glu-D1 5 + 10 subunits was available for comparison.

A contrast between the glutenin subunits supports the results obtained for the effects of the subunit composition on the tortilla quality parameters (**Table 7**). Deletions in Glu-A1 significantly affected the diameter and rollability of the tortillas. Deletions in Glu-B1 loci also improved the diameter significantly with no significant effect on rollability and flour protein content. Deletions in Glu-D1 loci significantly affected the diameter and the rollability of the tortillas while not altering the flour protein content. Thus, the interaction between Glu-A1 and Glu-D1 HMW-GS or Glu-D1 HMW-GS alone appears to be important for shelf stability.

Correlations were separately calculated between the tortilla quality parameters in both locations using SPSS software (**Tables 3** and **4**). The diameter of tortillas made from wheat grown in Texas had a strong negative correlation with the % IPP, while shelf stability (rollability at day 14) was strongly correlated with % IPP (**Table 3**), whereas those from South Dakota showed a significant correlation ($p < 0.05$) between dough extensibility and protein content (**Table 4**).

The results of this study demonstrate that, in addition to protein quantity, the presence of specific HMW-GS helps control the HMW/LMW glutenins and glutenin/gliadin ratios, which in turn controls the apparent size of the glutenin polymer isolated from wheat flour (*33*).

The HMW-GS composition found in FM2B and FM3, which has very good dough properties and diameter attributes, could be used in tortilla mixes. These tortilla mixes are usually used to make tortillas that are eaten fresh. The tortillas from these lines were fluffier, whiter in color, and would be preferred by consumers for appearance and light texture. Small businesses and households would appreciate the ease of mixing and dough processing attributes that these lines possess. Breeding for better environmental adaptation and/or also reducing the gliadin composition may also improve the stability of tortillas made from flour of cultivars with this composition.

Among all of the lines examined, FM6 had the best compromise of increased diameter with long shelf stability. It was better in quality than the commercial tortilla flour that contains L-cysteine for improved extensibility and performed better in both locations. This line has a subunit composition of 1 and 5 + 10 at Glu-A1 and Glu-D1 and has potential as a line with better tortilla quality attributes. It also has acceptable loaf volume reported in previous papers and may be compatible in a HRWW distribution system that targets bread quality (*17*).

ABBREVIATIONS USED

GS, glutenins; HMW-GS, high molecular weight glutenins; IPP, insoluble polymeric proteins; HRWW, hard red winter wheat; PPP, polymeric protein percent.

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