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Functionality of Gliadin Proteins in Wheat Flour Tortillas

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Gliadins are monomeric proteins that are encoded by the genes at the loci *Gli 1* and *Gli 2* present on the short arm of homologous wheat chromosomes 1 and 6, respectively. Studies have suggested that gliadins may play an important role in determining the functional properties of wheat flour. The main objective of this study was to understand the functionality of gliadins with respect to tortilla quality. The important tortilla quality attributes are diameter, opacity, and shelf stability, designated here as rollability or the ability to roll or fold the tortilla without cracking. In this study gliadin functionality in tortilla quality was studied using near-isogenic wheat lines that have deletions in either *Gli A1*, *Gli D1*, *Gli A2*, or *Gli D2* gliadin loci. The deletion lines are designated by the same abbreviations. Dough and tortillas were prepared from the parent line used to derive these deletion lines, each individual deletion line, and a control commercial tortilla flour. Quantitative and qualitative evaluations were performed on the dough and tortillas derived from the flour from each of these lines. None of the deletions in the gliadin loci altered the shelf stability versus that found for the parent to the deletion lines or control tortilla flour. However, deletions in the *Gli 2* loci, in particular *Gli A2* reduced the relative proportion of α- and β-gliadins with a greater cysteine amino acid content and gluten cross-link function versus the chain-terminating ω-gliadins in *Gli 1*, which were still present. As such, the dough and gluten matrix appeared to have greater extensibility, which improved the diameter and overall quality of the tortillas while not altering the rollability. Deletions in the *Gli 1* loci had the opposite result with increased cross-linking of α- and β-gliadins, polymeric protein content, and a stronger dough that decreased the diameter and overall quality of the tortillas. The data suggest that altering certain *Gli 2* loci through null alleles could be a viable strategy to develop cultivars improved for the specific functionality requirements needed for the rapidly growing tortilla market.

KEYWORDS: Gliadin; wheat; tortilla; *Gli 1*; *Gli 2*

INTRODUCTION

The tortilla is a circular light-colored ethnic flat bread once considered to be 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 industry in the United States 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 breadmaking. Although wheat gluten functionality is also important for tortilla quality, most hard red winter wheat 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 tortilla producers to make tortillas with good consumer quality attributes while also maintaining extended shelf stability.

Wheat quality research in the past 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

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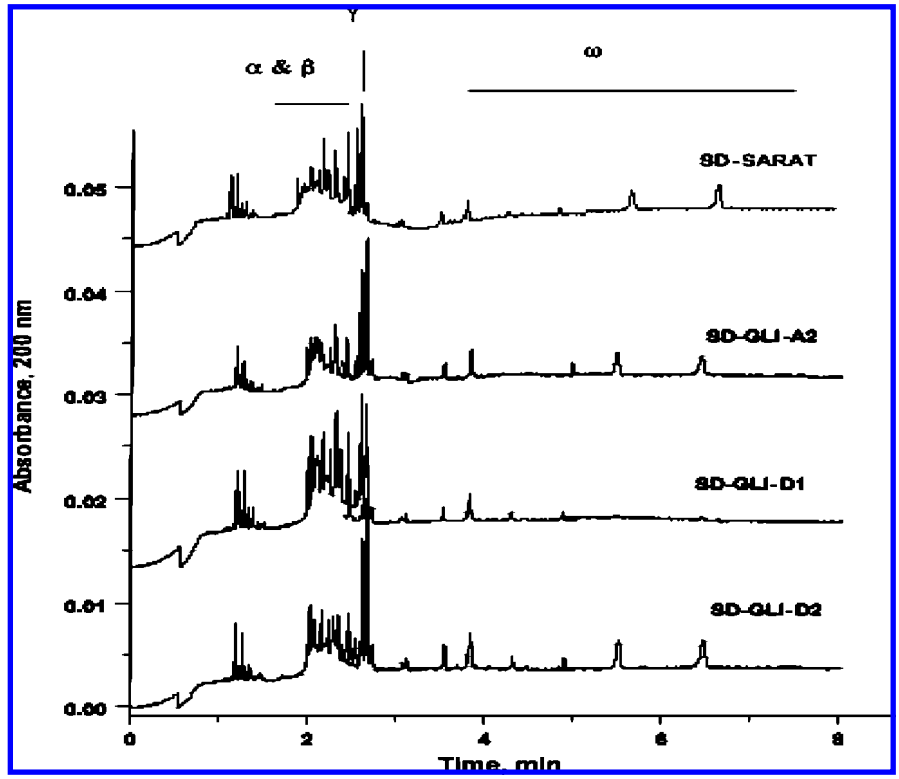

Figure 1. Capillary electrophoresis of the gliadin deletion lines and parent cultivar. The absence of ω-gliadin peaks in *Gli D1* indicates deletions in the *Gli 1* locus. The absence of some of the peaks in α- and β-subunits indicates deletions in *Gli A2* and *Gli D2*.

are diameter, opacity, and rollability. Tortillas about 2 mm thick that are evenly opaque, have an ample diameter, and have at least a 3-week shelf life are considered to be ideal (*3*). As in bread, wheat flour and gluten functionality contributes significantly to this shelf stability and the need for tortillas to resist breaking during consumption (*4*−*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 compared to bread (*8*). The diameter of tortillas also requires extensible dough that resists shrink-back during processing (*8*). The dough extensibility, in-turn, depends again on the gluten proteins. Thus, dough extensibility during hot pressing and retention of tortilla flexibility after baking requires 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, good extensibility, and shelf stability. L-Cysteine is widely used as a reducing agent as it competes with the disulfide bridge forming cysteine residues in the gluten matrix. It is also a common perception within the industry that increasing the amount of these agents gives rise to good tortillas; however, recent studies observed small or insignificant improvements via increase in reducing agent concentration (*9*). A lack of knowledge of the gluten functionality requirements for tortillas is, thus, a problem for obtaining optimal tortilla quality.

The rheological properties of dough from wheat flour are unique among cereal grains. The endosperm proteins that comprise the gluten network that derives the rheological properties of the dough are classified as glutenins or gliadins. Gluten is composed of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) and gliadins and their allelic variants. Glutenins and gliadins are differentiated on the basis of their polymeric or monomeric nature, respectively, and their solubility and functionality. These gluten proteins interact to develop a polymeric network that contributes to dough strength and quality. Gliadins are monomeric proteins and are classified into four groups, α-, β-, γ-, and ω-gliadins, on the basis of their electrophoretic mobility (*10*). The *Gli 1* and *Gli 2* genes encode for the gliadin proteins and are present on the short arm of chromosomes 1 and 6, respectively. They are tightly linked genes present in the three homologous loci of chromosome 1 as *Gli A1*, *Gli B1*, and *Gli D1*. In chromosome 6 they are present as *Gli A2*, *Gli B2*, and *Gli D2* (*10*). The *Gli 1* genes code for the ω- and γ-gliadins, and the *Gli 2* genes code for α- and β-gliadins. The *Gli 1* locus is tightly linked to the LMW glutenin loci *Glu 3* in chromosome 1 (**Figure 1**).

The α-, β-, and γ-gliadins have six to eight cysteine residues; as a result three to four intramolecular disulfide bonds occur (*11*). Although the HMW and LMW glutenins form the disulfide cross-linked gluten matrix, a small proportion (5−10%) of α-, β-, and γ-gliadins occupy the matrix cross-link function. The ω-gliadins also may take part in the polymer formation (*12*). Gliadins can also function as chain terminators such as the LMW glutenin subunits. Studies have suggested that an increase in relative gliadin content is associated with an increase in extensibility and loss of dough strength and reduced breadmaking quality (*13, 14*). Glutenin enrichment can circumvent this and increase dough strength.

The functionality of the gliadin alleles in the extensibility of the dough may thus contribute to good tortilla quality. Good-quality tortillas require an extensible and mellow gluten network that differs from the strong gluten network needed for breadmaking. High or low gliadin content, depending on the allele, may positively or negatively influence tortilla quality. In previous studies the protein composition and the dough mixing strength of the near-isogenic lines missing *Glu B3*/*Gli B1* loci with its recurrent parent were compared. A reduction in polymeric proteins was observed in association with a reduction

in dough strength (*15*). Deletions in the *Glu 1* loci reduced the glutenin to gliadin ratio and reduced dough strength (*10*) and also significantly altered breadmaking quality. Whereas bread firms and stales in a week, tortilla flexibility and rollability are exhibited for more than 3 weeks depending on flour properties, formulation, and processing (*16*). As such, the glutenin and gliadin functionality required to improve the quality of tortillas is different from that of bread. Research is thus needed to define the gliadin functionality requirements needed for optimal tortilla processing and quality. In this study, we have used near-isogenic wheat lines with deletions in *Gli 1* and *Gli 2* loci to relate qualitative and quantitative differences in gliadin composition to gliadin functionality in flour tortilla quality.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The Russian cultivar 'Saratovskaja' (Sarat) and the mutant deletion lines derived from 'Saratovskaja' were obtained from Dr. Finlay MacRitchie, Kansas State University. The mutant lines of Saratovskaja with deletions in *Gli 1* and *Gli 2* loci were selected to study the effect of gliadin functionality in tortilla quality. The parent cultivar Sarat and the mutant lines used in this study were grown in Brookings, SD, under the supervision of Dr. Karl Glover in 2005.

Protein Analysis. A gliadin protein analysis was conducted to confirm the absence of gliadin subunit in the deletion lines by using high-pressure capillary electrophoresis (HPCE) (*17*). The wheat kernels were ground in a mortar and pestle to produce flour. The gliadins were extracted from the flour using 70% ethanol (*18*). A 0.1 mM phosphate buffer was used as solvent for HPCE. The proteins were detected at an UV absorbance of 200 nm. Gold's software collected the sampling data, and the analysis was performed with Origin software (Microcal Software, North Hampton, MA).

Polymeric Protein Analysis. Flours were extracted as described in refs *19* and *20*. Briefly, 100 mg of flour was extracted with 1 mL of 50% aqueous 1-propanol, and pellets were freeze-dried before protein determination (N × 5.7). 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. Injection volume was 20 μL, and UV detection was done at 210 nm (*21*). The percent insoluble polymeric proteins (% IPP) was calculated from the weight and protein content of the freeze-dried pellet and extractable proteins (EP) from the difference between flour protein and protein in the pellet. The results are the mean of three technical repetitions.

Evaluation of Wheat Grain and Flour. Mixograph dough development time (MDDT) and peak dough resistance (MU) were used to assess the dough strength. 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 analysis to determine the dough mixing time and the dough strength of the flour (Lincoln Manufacturing Co., Lincoln, NE). MU and MDDT were recorded from mixograms using a standard procedure (*22*).

Tortilla Processing. The flours from each line grown in South Dakota were processed into tortillas. The tortillas were prepared according to a standard formulation (*23*) except that 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 mixograph of the water absorption. Commercially available tortilla flour (ADM Tortilla Flour, ADM Milling Co., Overland Park, KS) was used to compare the tortilla quality obtained from the commercial flour and the selected experimental lines. The tortillas from each experimental line were made in two batches, one using a smaller amount of flour to standardize the formulation and water requirement and second batch made from 500 g of flour used for evaluation.

Dry ingredients were mixed with the flour in a mixing bowl with a paddle at 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 Corp., Troy, OH). Shortening was then added and paddle mixed for 2 min at low speed. Water (35 °C) was then added and mixed for 1 min at low speed and then mixed at a medium speed for 6 min with a hook.

The dough was placed in a plastic tray for dough quality measurements. The dough was then proofed (model 57638, National Manufacturing Co., Lincoln, NE) at 35 °C and 70% relative humidity for 5 min. The dough was pressed by hand and divided and rounded with a Duchess Divider/Rounder (Bakery Equipment and Service Co., 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 Co. Inc., South El Monte, CA) and pressed at 1100 psi. The tortillas were then baked in the three-tier oven (Micro-Combo Tortilla Oven, model 0P01004-02, Lawrence Equipment) set at a temperature of 176−182 °C. The dwell time was adjusted to 30 s. The tortillas were cooled on a three-tier cooling chain (model 3106 INF, Food Machinery Inc., Pivo Machinery Inc., Pico Rivera, CA), removed and placed on a table for 1 min, flipped on the other side for cooling, packed in low-density polyethylene bags, and stored at 23 °C for quality evaluation.

Dough Evaluation. The dough quality properties were evaluated subjectively prior to proofing. The dough was placed on a plastic tray, the temperature was measured using a thermometer, and the values were recorded. The other dough properties such as softness, smoothness, extensibility, and force to extend were evaluated subjectively (*23*). These subjective tests were shown to correlate with objective tests using a texture analyzer.

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

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

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

Force to extend measures the elasticity of the dough, an indication of shrink-back of a tortilla that has been pressed. It is rated from 1 to 5, with 1 indicating less force required and 5 indicating extreme force required.

Tortilla Evaluation. The tortillas were evaluated for their weight, diameter, height, pH, moisture, opacity, color, and rollability (*23*, *24*). Using a balance, ruler, and digital caliper, respectively, the weights, diameters from two points, and height from 10 individual tortillas were averaged. The pH and moisture content of individual tortillas from each line were determined as described (*23*).

The opacity of 10 tortillas was subjectively evaluated using a continuous scale of 1−100% (1% being fully translucent and 100% being highly opaque). The values recorded averaged. The color parameters, L^* (lightness), $\pm a^*$ (red-green), and $\pm b^*$ (yellow-blue) were measured for each tortilla using a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan) using three measurements on each side of the tortillas. Tortilla shelf stability was evaluated using the rollability test (*23*) 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. The rollabilities were evaluated on the 4th, 10th, and 14th days following processing for each of the lines. Three tortillas from each of the lines were used for the measurements. The specific volume was then calculated for each of the lines (cm^3/g). The specific volume indicates the fluffiness of the tortillas. It ranges from 1.5 to 3.5 cm^3/g. The specific volume was calculated by the formula

$$\pi(\text{diameter} \times 2)/2 \times 1000/\text{weight}$$

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

$$\text{opacity} \times \text{specific volume} \times \text{rollability score (14th day of rotability)}$$

The index provides a collective quality score that takes into account the three important attributes in a good-quality tortilla. It serves as a tortilla quality ranking system for developing flour tortilla ingredient formulations and wheat cultivars that meet the specific attribute for good quality tortillas.

Method of Analysis. The data were analyzed using SPSS statistical software (SPSS 13.0 for Windows) to estimate the correlations, compare the means, and test for significance by Tukey's HSD and ANOVA.

RESULTS AND DISCUSSION

The HPCE analysis was used to verify the gliadin deletions in the mutant lines (**Figure 1**). The peaks corresponding to α- and β-gliadins at the *Gli 2* locus and to ω- and γ-gliadins in the *Gli 1* locus are shown. Note the reduction in high peak for ω- and γ-gliadins in Gli-D1 versus the parent line 'Saratovskaja' 'SARAT' (**Figure 1**) or *Gli 2* deletion lines, Gli-A2 or Gli-D2. Gli-A1 shows a similar pattern (not shown). Also, Gli-A2 or Gli-D2 shows a reduction in peak height of α- and β-gliadins versus the γ-gliadin peak in the same chromatogram versus 'SARAT" or Gli-D1 (**Figure 1**). The composition of each line used in the study is detailed in **Table 1**. Flour protein content analysis of the near-isogenic deletion lines revealed an increase in the relative percent of polymeric proteins (**Table 2**). This was expected because the reduction in monomeric proteins is thought to increase the polymeric proteins via an increase in the composition of monomeric chain terminators. One exception was observed in the line Gli-D2 that had no significant difference in its polymeric protein content from the parent of the deletion lines, 'Saratovsaya'. The deletions in the *Gli D2* loci may not have influenced the glutenin to gliadin ratios. The flour protein content of the parent cultivar as determined by NIR was 10.4%. The gliadin deletion lines had flour protein content of 10.5% (**Table 2**). The lower protein content may have resulted from the environmental conditions in South Dakota or poor adaptability. The flour protein content was not affected by the deletions in the gliadin alleles (**Table 2**).

Dough Strength. The behavior of the polymeric proteins in the gliadin lines was unique. Mixograph dough development time (MDDT) and peak dough resistance (MU) were used to assess the dough strength. The mixographs of the gliadin deletion lines lacked a defined peak, although they had higher dough resistance (**Table 2**). The increase in the % IPP resulted in stronger mixographs that lacked a defined peak mixing time, although an earlier peak time (MDDT) was recorded for each gliadin deletion line versus the parent (**Table 2**). That said, following this early peak the mixographs for each deletion line showed extreme resistance to breakdown versus the parent line with increased MU (**Table 2**). This was unique for the lines as an increase in the polymeric proteins was expected to produce a strong mixograph with a good peak and strong dough mixing resistance. The polymeric proteins in these lines did not behave as predicted and have similarity to mixographs from flours obtained from wheat lines overexpressing Glu-D1 gene pairs (*25*).

Dough Quality. Reduced dough mixing resistance and greater dough extensibility are related to good-quality tortillas (*16*). The extensibility measures the ability of the dough to stretch without breaking. The dough extensibility prepared from the gliadin deletion lines was counter to the results from the mixographs, indicating a very strong dough. The extensibility of the doughs was moderate at 3.0 on a 1 (low, breaks easily) to 5 (high, extends without breaking) scale. This ideal score of 3.0 occurred for all gliadin deletion lines including the parent 'Saratovskaja' even when the monomeric proteins were reduced and dough strength increased. The elasticity of all lines was also ideal at 2.0, where 1 is low and 5 is high elasticity. Both results are contrary to the mixograph results and earlier studies and expectations. The strong dough mixograph results from the deletion lines should have resulted in a lower extensibility rating and a high elasticity rating. 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.

Table 1. Gliadin Deletion Line Composition

	gliadin alleles			
line	*Gli A1*	*Gli A2*	*Gli D1*	*Gli D2*
Saratovskaja	ω,γ	α,β	ω,γ	α,β
Gli-A1	null	α,β	ω,γ	α,β
Gli-A2	ω,γ	null	ω,γ	α,β
Gli-D1	ω,γ	α,β	null	α,β
Gli-D2	ω,γ	α,β	ω,γ	null

Tortilla Quality Evaluation. The main tortilla quality parameters are diameter and rollability. Deletion in monomeric proteins increased the dough strength as indicated by the mixograph, yet deletion in the gliadin monomeric proteins maintained the ability of the dough to extend without increasing the elasticity or shrink-back of the dough, which would be expected to reduce tortilla diameters. Higher extensibilities would be desirable to obtain tortillas with larger diameter and good rollability. Whereas we recorded a higher % IPP in Gli-A2, the tortillas prepared from gliadin deletion line Gli-A2 were significantly larger (180 mm) than those prepared from the parent or control flour (**Figure 2**). The diameters of tortillas prepared from the gliadin deletion line Gli-D2 were similar to those of the parent at 175.2 mm. The gliadin deletion lines Gli-A1 and Gli-D1 behaved as expected; the tortillas prepared from these lines had a significantly ($p < 0.05$) smaller diameter than the parent cultivar and Gli-A2 and Gli-D2. The rollability scores of the gliadin deletion lines, parent cultivar, and control flour were not significantly different. The rollability scores were in a range similar to the control flour (2.3 versus 2.7, respectively) on the 14th day (**Figure 3**). The reduction in rollability was similar for all lines except that Gli-A2 and Gli-D2 had lower initial numerical rollability scores at day 4 (**Figure 3**). However, no significant differences for rollability were found between any of the gliadin deletion, parent, or control flours. The opacity scores were in an optimum range (**Table 2**).

There were significant correlations between the dough and tortilla quality parameters. The rollability or shelf life of the tortillas was positively correlated with protein content, yet negatively correlated with a reduced dough development time or a weaker dough ($p < 0.05$) (**Table 3**).

The % IPP increased in the gliadin deletion lines Gli-A1, Gli-A2, and Gli-D1 as expected due to deletions in the monomeric proteins (**Table 2**). The behavior of gliadin deletion line Gli-D2 was different from that of the other gliadin deletion lines. The % IPP in Gli-D2 was similar to the parent line 'Saratovsaya'; thus, deletion of Gli-D2 loci may not have affected the glutenin to gliadin ratios. The other gliadin deletion lines had an increased ratio of polymeric proteins. The mixo-

Table 2. Quality Evaluation Results of Dough and Tortilla Made from Flour from Gliadin Deletion Lines, Parents to the the Deletion Lines 'Saratovsaya', and the Commercial Tortilla Flour[a]

	gliadin allele composition											
genotype	A1	D1	A2	D2	% protein	% IPP	% PPP	MDDT	res MU	opacity %	sp vol (cm^3/g)	qual d14
control	?	?	?	?	12.0a	–	–	–	–	85	1.30b	304
Saratovskaja	+	+	+	+	10.4b	4.0	4.0	4.2	4.2	81	1.25b	201
Gli-A1	–	+	+	+	10.3b	5.7	5.1	3.2	5.4	82	1.19c	220
Gli-A2	+	+	–	+	10.7b	5.2	5.0	3.1	4.8	85	1.33a	253
Gli-D1	+	–	+	+	10.5b	5.3	5.0	3.4	5.4	79	1.19c	212
Gli-D2	+	+	+		10.5b	3.9	4.3	3.4	5.2	84	1.31b	248

[a] IPP, insoluble polymeric proteins; PPP, percent polymeric protein; MDDT, mid dough development time; MU, dough mixing resistance. IPP,PPP, MDDT, and MU are the mean of two technical repetitions. Letters a–d indicate significance ranks of the cultivars based on the significant differences (HSD = 2.02, $\alpha = 0.05$) obtained from Tukey-LSD analysis.

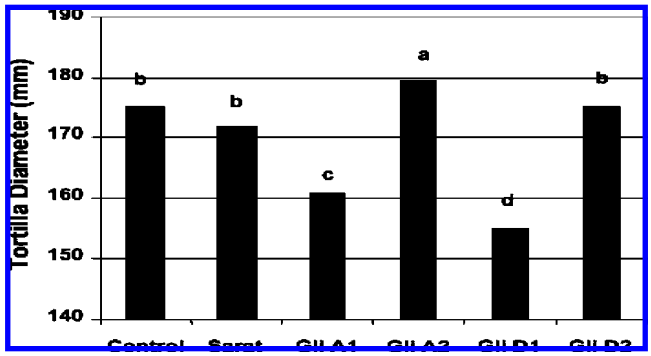

Figure 2. Average tortilla diameters (10 tortillas) of the gliadin deletion lines *Gli A1*, *Gli A2*, *Gli D1*, and *Gli D2*, parent cultivar 'Saratovskaja', and control tortilla flour. Letters a–d indicate significance ranks of the cultivars based on the significant differences (HSD = 2.02, $\alpha = 0.05$) obtained from Tukey-HSD analysis.

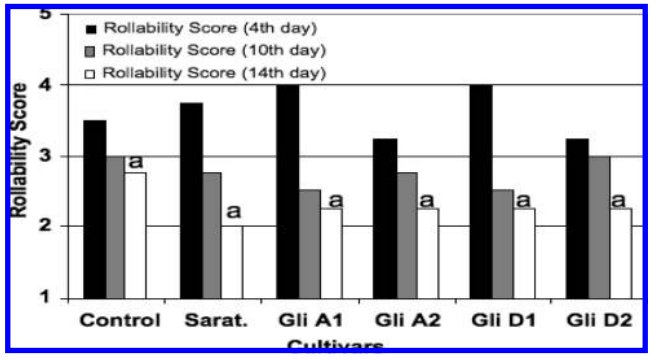

Figure 3. Rollability scores of the tortillas from made from flour from the gliadin deletion lines, parent to the deletion lines 'Saratovskaja', and the control flour on the 4th, 10th, and 14th days after processing of the tortillas. Ratings on a scale of 1–5 were recorded, with 1 being immediate breakage and 5 being no cracks or breakage. Although variations in means were found, as indicated by the letters above the bars for each line, no statistically significant differences between lines were observed at the 14th day after processing of the tortillas. No statistically significant differences were found between each line when analyzed on the 4th and 10th day (letters not shown).

graphs of the gliadin deletion lines were intermediate in dough strength with a short mix time, yet each maintained its resistance. The increase in the polymeric proteins should have produced a strong mixograph with a good peak and a strong dough mixing resistance. This may indicate that a loss in the gliadin content has unexpected consequences for the network. Furthermore, the tortillas prepared from the gliadin deletion lines are expected to have smaller diameters and better rollabilities. The HMW glutenins and gliadins are interlinked to form a cohesive mass of dough. The gliadins thus should facilitate the extensibility

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

	% IPP	MDDT	extensibility	diameter	rollability	opacity
% protein	–0.042	–0.953	0.403	0.378	0.919*[b]	0.559
% IPP		–0.675	–0.227	–0.493	0.557	–0.076
MDDT			0.060	–0.182	–0.953*[b]	–0.645
extensibility				–0.272	0.186	–0.475
diameter					0.176	0.820*[b]
rollabillity						0.563

[a] IPP, insoluble polymeric proteins; MDDT, mid dough development time. [b] *, at 0.05 level of significance.

of the network. The lines Gli-A2 and Gli-D2 had excellent tortilla properties. Gli-A2 had an increase in % IPP, yet the tortilla diameters were significantly larger (**Figure 2**). The increase in the HMW glutenins and the subsequent absence of the gliadin locus resulted in an increase in the polymeric proteins and high gluten strength. The high gluten strength resulted in abnormal mixing, and thus the mixographs obtained showed a very strong gluten network. The mixographs indicate that the proteins may have failed to form a homogeneous protein network. The glutenins may have formed aggregates rather than being a part of the network. When water was added to form the dough and the dough was processed into tortillas, these aggregated glutenin subunits provided the extensibility that resulted in good tortilla diameters with better overall quality in the Gli-A2 line (**Table 2**). This line required more water than estimated by the flour protein content; the excess water may have been another reason for the increased tortilla diameter in this line. Hydrogen bonds can affect the aggregation of the proteins (*26*). The glutenins in the absence of gliadins caused a restructuring of the glutenin polymer network that may have affected the behavior of these lines. The *Gli 1* genes code for the ω- and γ-gliadins and the *Gli 2* genes code for α- and β-gliadins. A small proportion (5–10%) of α- and γ-gliadins occupy the matrix cross-link function. The ω-gliadins also may take part in the polymer formation (*11*). In this study a decrease in *Gli A2* in particular appeared to increase the relative proportion of *Gli 1* ω- and γ-gliadins (**Figure 1**). Studies have suggested that an increase in relative gliadin content is associated with increased extensibility, loss of dough strength, and reduced breadmaking quality (*12*, *13*). Because gliadins can also function as chain terminators like the LMW glutenins, functioning to increase dough extensibility, the loss of *Gli A2* increased this functionality via increasing the relative concentration of matrix terminators to bridgers. The other lines *Gli A1* and *Gli D1* had an abnormal mixograph behavior and reduced tortilla diameters, indicating that during processing the polymeric proteins behaved as expected. In this case the relative concentrations of HMW-GS and gliadin bridgers were increased versus chain terminators. As such, an increase in the polymeric proteins in the absence

of gliadins that provide extensibility to the network resulted in an abnormally strong behavior of the network that is counter to the mellow network needed for tortillas (*25*).

Although some of the results were quite unique, the study revealed the importance of monomeric proteins in the gluten network formation. The absence of *Gli 2* gliadins increased the extensibility to the protein polymeric network even while increasing the polymeric protein content. This appeared to result from an abnormal network formation that positively altered tortilla quality parameters such as diameter while not altering shelf stability as measured by tortilla rollability. Whether the development of new cultivars null at the *Gli A2* with better adaptation and a higher overall protein content will help to improve dough extensibility for large-diameter tortillas while also maintaining a sufficient content of the appropriate polymeric gluten fraction to maintain shelf stability will require further studies.

ABBREVIATIONS USED

Gli, gliadin; Glu, glutenin; IPP, insoluble polymeric protein; PPP, polymeric protein percent; HMW-GS, high molecular weight glutenins.

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This paper is dedicated to our dear friend and colleague Ralph Waniska, who lost his battle with cancer in June 2007.

LITERATURE CITED

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