Research Article

Received: 6 January 2014 Revised: 14 February 2014 Accepted article published: 3 March 2014 Published online in Wiley Online Library: 31 March 2014

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6635

# Effect of high molecular weight glutenin subunit composition in common wheat on dough properties and steamed bread quality

**Pingping Zhang,[a,b] Tom O Jondiko,[a] Michael Tilley[c] and Joseph M Awika[a,d*]**

## Abstract

**BACKGROUND: Steamed bread is a popular staple food in Asia with different flour quality requirements from pan bread. Little is known about how glutenin characteristics affect steamed bread quality. This work investigated how deletions of high-molecular-weight glutenin subunits (HMW-GS) influence gluten properties and Chinese steamed bread quality using 16 wheat lines grown in Texas.**

**RESULTS: Although similar in protein content (134–140 mg $g^{-1}$), gluten composition and dough properties differed widely among the lines. Compared with non-deletion lines, deletion lines had lower ($P < 0.05$) unextractable polymeric protein (294 *vs* 470 mg $g^{-1}$), HMW-GS/low-molecular-weight glutenin subunit ratio (0.25 *vs* 0.41), dough force to extend (0.16 *vs* 0.44 N) and mixing peak time (2.03 *vs* 4.52 min). Deletion lines with HMW-GS composition of 2*/17+_/5+_ and 2*/17+_/2+12 showed moderate gluten strength (mixing peak time, 1.96–2.94 min; force to extend, 0.18–0.23 N) and high dough extensibility (106–129 mm). These lines also produced good steamed bread quality (score, 60.8–65.0) with good elasticity and crumb structure.**

**CONCLUSION: Deletion at *Glu-B1y* and/or *Glu-D1y* loci in high-strength hard wheat produced good dough properties for steamed bread. This suggests that wheat functionality for steamed bread can be improved by manipulating HMW-GS composition.**


**Keywords:** common wheat; glutenin subunits; dough; steamed bread, allele deletion

## INTRODUCTION

Steamed bread is a popular food in Asian markets, especially China, where it represents more than 40% of national wheat consumption. Although southern-style steamed bread and Guangdong steamed bread are consumed in southern China and Southeast Asian countries, northern-style Chinese steamed bread (CSB) is the most common and is consumed in northern China as daily staple food.[1,2] Wheat quality requirements for CSB are significantly different from those of pan bread, mainly owing to the different processing methods, i.e. steaming *versus* baking. Various studies have outlined variable gluten quality requirements for steamed bread. Steamed bread quality was positively correlated with protein content and gluten strength in a wheat population with weak to medium gluten strength.[2,3] However, the reverse relationship was observed in a strong gluten population.[4–6] Excessive elasticity caused difficulty in steamed bread processing and shrinkage and wrinkles of the finished product.[7] Rubenthaler *et al.*[5] observed a strong negative correlation between height after proving and height after steaming in strong flours owing to steamed product collapse after steaming, whereas soft wheat and weaker hard wheat flours could maintain their proved height. Medium protein content and gluten strength with good extensibility is desirable for both mechanized methods and handmade methods.[2,7]

Wheat processing quality is mainly controlled by gluten proteins, which consist of two types of protein, i.e. the monomeric gliadins and the polymeric glutenins. Glutenin is composed of high-molecular-weight (HMW-GS) and low-molecular-weight (LMW-GS) glutenin subunits that are coded by *Glu-1* and *Glu-3* loci respectively.[8] The quality and quantity of HMW-GS are critical components in gluten viscoelastic properties. HMW-GS account for only 10% of total gluten proteins but contribute more than 50% of the variation in bread-making quality.[9,10]

* *Correspondence to: Joseph M. Awika, Cereal Quality Lab, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX, USA. E-mail: jawika@ag.tamu.edu*

a *Cereal Quality Lab, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX, USA*

b *Provincial Key Lab for Agrobiology, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China*

c *USDA, Agricultural Research Service Center for Grain and Animal Health Research, Manhattan, KS, USA*

d *Nutrition and Food Science Department, Texas A&M University, College Station, TX, USA*

Various studies showed variable HMW-GS allele in single *Glu-1* loci or HMW-GS combination in three *Glu-1* loci related with special quality parameters.[9,11–13] Each *Glu-1* locus encodes two types of HMW-GS, i.e. *x*- and *y*-type, with different expression amount and amino acid structure. Regarding quantity, *x*-type HMW-GS contribute more to gluten strength compared with *y*-type HMW-GS.[14–16] However, *y*-type HMW-GS have a different cysteine number and distribution compared with *x*-type HMW-GS, which likely account for their different contribution to the building of a continuous gluten network by intermolecular disulfide bonds.[17]

The quantity of HMW-GS and the size distribution of glutenin polymeric protein are strongly associated with dough properties and bread-making quality.[14–16,18,19] The composition of HMW-GS is also closely correlated with the quantity and distribution of macro polymeric protein.[20,21] However, little is known about the relationship between glutenin characteristics and steamed bread quality. A positive correlation between macro polymeric protein and steamed bread quality was shown in a weak flour population in a previous study.[22] Zhu *et al.*[6] reported that the biotype with HMW-GS 5+10 showed higher steamed bread total score than that with HMW-GS 2+12 at *Glu-D1*, but this performance was only tested in one cultivar with high protein level. More information on HMW-GS contribution to steamed bread quality is needed.

Consistent product quality is becoming more important with rapid urbanization and mechanized/centralized processing, so identifying how alterations in HMW-GS impact Chinese steamed bread quality is important. The objective of this study was to investigate and compare the function of HMW-GS in determining dough properties and northern-style Chinese steamed bread quality among a set of American hard white wheat cultivars with variable HMW-GS composition or deletions.

## EXPERIMENTAL

### Wheat samples

The 16 hard white winter wheat genotypes (seven non-deletion and nine deletion lines) used in this study are listed in Table 1; entries 1–7 (designated N) were non-deletions and entries 8–18 (designated D) were deletions. They were grown in two locations in Texas (Texas A&M AgriLife Experiment Stations at McGregor and Bushland) in the 2011–2012 wheat season. Samples from each environment were bulked and analyzed separately.

### Quality testing

Grain hardness was recorded by the Single Kernel Characterization System (SKCS 4100, Perten Instruments North America Inc., Springfield, IL, USA) using AACC method (AACC 55–31.01).[23] All samples were classified as hard and were tempered to 150 g kg$^{-1}$ moisture content before milling. Grain samples were milled into flour using a Brabender Quadrumat Senior laboratory mill (C.W. Brabender Instruments, South Hackensack, NJ) following the standard procedure. The flour yield was about 70%. Flour protein content was determined by the near-infrared reflectance spectroscopy method (AACC 39–11.01) with a Perten DA7000.[23] A 10 g mixograph (National Mfg Co., Lincoln, NE, USA) was used to record dough mixing peak time and to calculate dough water absorption with correction based on mixograph curve (AACC 54–40.02).[23] Dough elasticity and extensibility were tested using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY, USA/Stable Micro Systems, Godalming, UK).

**Table 1.** HMW-GS composition of wheat lines

| Entry | Line | Deletion status | Glu-A1 | Glu-B1 | Glu-D1 |
|---|---|---|---|---|---|
| 1 | Fannin | N | 2* | 7+9 | 2+12 |
| 2 | TAM401 | N | 2* | 7+9 | 5+10 |
| 3 | TAM304 | N | 1 | 7+9 | 5+10 |
| 4 | TAM203 | N | 2* | 17+18 | 2+12 |
| 5 | TX11D3174 | N | 1 | 7+9 | 5+10 |
| 6 | TX11D3175 | N | 2* | 7+9 | 5+10 |
| 7 | T74-13X | N | 2*/1 | 7+9 | 5+10 |
| **8** | TX04CS00230 | D | _ | 17+18 | 5+10 |
| **9** | T68-21 | D | _ | 7+9 | 2+12 |
| **10** | T69-32 | D | _ | 7+9 | 5+_ |
| **11** | T76-11 | D | _ | 7+8 | _+_ |
| **12** | T78-34 | D | _ | 7+8 | _+_ |
| **13** | T82-1 | D | 2* | 17+18 | _+_ |
| **14** | T82-37 | D | _ | 7+_ | 10+_ |
| **15** | TX04CS00232 | D | 2* | 17+_ | 2+12 |
| **16** | TX04CS00231 | D | 2* | 17+_ | 5+_ |

N, non-deletion; D, deletion; /, heterozygous at HMW-GS gene locus; _, deleted at HMW-GS gene locus.

### Protein trait analysis

HMW-GS composition of experimental genotypes was identified by the Lab-on-a-Chip method in an Agilent 2100 bioanalyzer following the Protein 230 chip kit protocol (Agilent Technologies, Palo Alto, CA, USA) as described previously.[24] The standards used were Chinese Spring (null, 7+8, 2+12) and Jagger (1, 17+18, 5+10).

Size exclusion high-performance liquid chromatography (SE-HPLC) was employed to determine monomeric and polymeric protein distribution as described by Larroque *et al.*[25] A Biosep-SEC-S4000 column (300 mm × 7.8 mm; Phenomenex, Torrance, CA, USA) was used. Sodium dodecyl sulfate (SDS)-extractable polymeric protein in flour (EPP), SDS-unextractable polymeric protein in flour (UPP), per cent UPP in total polymeric protein (UPP%) and the ratio of glutenin to gliadin (Glu/Gli) were recorded and calculated as described by Larroque *et al.*[25] and Gupta *et al.*[18] Values of EPP and UPP were expressed as AU, which indicates $10^6$ absorbance units (peak area in chromatogram) of HPLC corresponding to 1 mg of flour.

Reverse phase high-performance liquid chromatography (RP-HPLC) was used to determine the ratio of HMW-GS to LMW-GS (HMW/LMW). Briefly, 100 mg of flour was suspended and shaken for 15 min in 1 mL of sodium iodate buffer (0.3 mol L$^{-1}$ sodium iodate, 75 g L$^{-1}$ isopropanol) and then for 5 min in 1 mL of water. After centrifugation at 17 000 × *g* for 5 min, the glutenin in the pellet was extracted in 1 mL of 500 g L$^{-1}$ isopropanol containing 20 g L$^{-1}$ 2-mercaptoethanol with shaking for 30 min. After centrifugation as above, 600 $\mu$L of the supernatant was collected and alkylated with 40 $\mu$L of 4-vinylpyridine for 15 min. The resulting sample was used for RP-HPLC analysis. A Biosep-C18 column (250 mm × 4.6 mm; Phenomenex) was used. The elution solvents were water containing 1 mL L$^{-1}$ trifluoroacetic acid (solvent A) and acetonitrile containing 0.5 mL L$^{-1}$ trifluoroacetic acid (solvent A). The gradient was as follows: 0–3 min, from 25 to 35% B; 3–24 min, from 35 to 53% B; 25–29 min, 25% B. The column temperature was 60 °C and the flow rate was 1 mL min$^{-1}$. The effluent was monitored at 210 nm and the absorbance areas corresponding to HMW-GS and LMW-GS were determined by manual integration using Agilent ChemStation software.



  

### Steamed bread processing and evaluation

CSB was processed and evaluated as described by Huang *et al.*[26] with modification. Briefly, instant dry yeast (10 g $L^{-1}$) and water (700–800 g $L^{-1}$ of optimal water absorption based on mixograph curve) were added to flour and mixed to optimal dough development using a 200 g mixer (National Mfg Co.). Fermentation followed for 60 min (35 °C, 85% relative humidity (RH)). Dough was then divided into three pieces, folded end to end and sheeted 20 times with a gap of 9/32 inch (National Mfg Co.), rounded and shaped into balls 5 cm high. Dough balls were then proved again for 15 min (35 °C, 85% RH) and steamed for 20 min. After cooling for 15 min in a bamboo tray at room temperature, CSB quality was evaluated. The CSB scoring scale was 100, including data for specific volume (volume/weight, 20), height (10), smoothness (10), skin color (10), crumb structure (15) and stress relaxation (35). Stress relaxation (SR) was tested using a TA-XT2i texture analyzer. A 28 mm thick slice of bun was cut from the center portion of the steamed bread using an electric knife. The slice was placed on a flat metal plate and compressed once to a distance of 14.3 mm at a speed of 1.7 mm $s^{-1}$ using an SMS P/35 metal probe. After compression, peak force 1 ($P_1$) was obtained, and after a 4 s dwell time, peak force 3 ($P_3$) was recorded; then the probe was returned to its initial position. $SR = [(P_1 - P_3)/P_1] \times 100$.

### Statistical analysis

Statistical analysis was done with SAS Version 9.0 (SAS Institute, Cary, NC, USA). One-way analysis of variance was used to detect significant differences among treatments, followed by the least significant difference test at $\alpha = 0.05$ significance level for mean separation among genotypes. Pearson's linear correlation coefficients between quality parameters and quantitative data were obtained.

## RESULTS AND DISCUSSION

### Wheat protein traits

Similar protein content averaged from the two environments was observed among the 16 genotypes, ranging from 134.2 to 139.9 g $kg^{-1}$ (Table 2). However, significant differences in relative and absolute amounts of glutenin fractions were observed because of variable HMW-GS composition and deletion status. HMW-GS deletion significantly decreased UPP% and HMW/LMW ratio compared with non-deletion lines, as described previous previously.[20,27,28] For example, line 9 with deletion at HWM-GS *1Ax* showed lower HMW/LMW ratio and UPP% compared with line 1 with *1Ax2** (Table 2). Lines 13 and 15 also had lower HMW/LMW ratio and UPP% compared with line 4 because of deletion at *1Dx+1Dy* and *1By* loci respectively (Tables 1 and 2).

Lines 11, 12 and 14 lost three HMW-GS and had the lowest Glu/Gli ratio, UPP% and HMW/LMW ratio. Lines 8, 9 and 15 only lacked one HMW-GS at *1A1x* or *1B1y* loci and showed relatively higher UPP% and HMW/LMW ratio than the other deletion lines. Both lines 13 and 16 lost two HMW-GS at variable *Glu-1* loci and had similar polymeric protein distribution. Further correlation analysis showed that HMW/LMW ratio correlated significantly with UPP ($R^2 = 0.91$) and UPP% ($R^2 = 0.88$). These results confirm that HMW-GS deletion influences the size distribution of polymeric protein by altering the HMW/LMW ratio.

### Dough properties

Broad variations in dough properties were observed in this genotype pool because of variable HMW-GS composition (Table 3).

**Table 2.** Effects of HMW-GS deletion on protein traits

| Entry | Protein (g $kg^{-1}$) | EPP | UPP | UPP% | Glu/Gli | HMW/LMW |
|---|---|---|---|---|---|---|
| 1 | 137.4a | 6.78e | 5.51bcd | 44.80b | 0.58cde | 0.37cd |
| 2 | 137.1a | 7.12bcde | 6.13abc | 46.31b | 0.64bcd | 0.40c |
| 3 | 134.5a | 6.27e | 6.62a | 51.16a | 0.67abc | 0.39c |
| 4 | 133.9a | 6.79e | 6.11abc | 47.35ab | 0.65bcd | 0.35de |
| 5 | 137.6a | 6.91de | 6.45a | 48.23ab | 0.69ab | 0.56a |
| 6 | 134.2a | 6.98cde | 6.22ab | 46.90b | 0.75a | 0.48b |
| 7 | 135.4a | 6.46e | 5.25cd | 44.54b | 0.62bcd | 0.34e |
| **8** | 138.5a | 7.17bcde | 4.31ef | 37.15c | 0.61bcde | 0.29fg |
| **9** | 136.4a | 7.93ab | 4.89de | 37.91c | 0.69ab | 0.32ef |
| **10** | 139.9a | 7.86abc | 3.41gh | 29.9de | 0.61bcde | 0.22h |
| **11** | 141.2a | 7.91abc | 2.93gh | 26.96ed | 0.61bcde | 0.22h |
| **12** | 135.8a | 7.05bcde | 2.56h | 26.44ed | 0.52b | 0.24h |
| **13** | 135.9a | 7.83abcd | 3.22gh | 29.17de | 0.64bcd | 0.27g |
| **14** | 135.9a | 7.74abcd | 2.80gh | 26.27e | 0.56de | 0.24h |
| **15** | 135.0a | 7.10bcde | 4.85de | 40.11c | 0.64bcd | 0.33e |
| **16** | 137.2a | 8.35a | 3.70defg | 30.51d | 0.61bcde | 0.27g |
| LSD | 7.8 | 0.94 | 1.71 | 4.14 | 0.10 | 0.03 |

Average from two environments. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). Entries in bold are deletion lines. EPP, SDS-extractable polymeric protein in flour; UPP, SDS-unextractable polymeric protein in flour; UPP%, per cent UPP in polymeric protein; Glu/Gli, ratio of glutenin to gliadin; HMW/LMW, ratio of HMW-GS to LMW-GS; LSD, least significant difference ($\alpha = 0.05$). Unit for EPP and UPP is $10^4$ absorbance units $mg^{-1}$ flour.

Mixograph peak time, dough resistance to extension and extensibility varied from 1.30 to 5.16 min, from 0.06 to 0.50 N and from 71.1 to 140.1 mm respectively. All deletion lines had lower mixing peak time (<3.0 min) and extension force (<0.26 N) and higher dough extensibility (>92.5 mm) compared with non-deletion lines (4.52 min, 0.44 N and 79.7 mm on average respectively). For example, line 15 had deletion at *Glu-B1y* compared with line 4; this increased its dough extensibility and decreased its mixograph peak time and resistance to extension relative to line 4. The more subunit deletions at *Glu-1* loci, the weaker the gluten strength was. Lines 10–12 and 14 had significantly lower resistance to extension ($P < 0.05$) than the other lines. Generally, deletions at *Glu-1* loci result in weak dough and inferior end-use functionality.[20,28]

Huang *et al.*[3] recommended that hard wheat with medium to strong strength is suitable to produce CSB. Addo *et al.*[4] compared hard and soft wheat for steamed bread processing quality and showed that high-strength flour, which is desirable in production of pan bread, was detrimental in production of steamed bread. For soft wheat, protein content was important in production of acceptable steamed bread quality. Other studies also showed that medium or medium to strong gluten with good extensibility is the desired target for all of kinds of breads regarding dough workability and product quality.[2,29] Acceptable dough extensibility should allow enough expansion of gas bubbles during proving and baking, and adequate elasticity is essential to avoid rupture of gas cells and produce a loaf with large volume and smooth and elastic inner structure.[30] Glutenin subunits differ in their functionality on dough rheological properties, and it is difficult to achieve a perfect combination of HMW-GS and protein content in combination with



  

**Table 3.** Effects of HMW-GS deletion on dough properties

| Entry | Mixograph peak time (min) | Resistance to extension (N) | Extensibility (mm) |
|---|---|---|---|
| 1 | 3.88c | 0.36cd | 75.46gh |
| 2 | 3.94c | 0.33cde | 92.0 defgh |
| 3 | 5.16a | 0.50b | 76.51fgh |
| 4 | 4.33bc | 0.43bc | 82.60efgh |
| 5 | 5.20a | 0.63a | 71.05h |
| 6 | 4.90ab | 0.43bc | 81.28fgh |
| 7 | 4.20c | 0.40bc | 78.91fgh |
| **8** | 2.67de | 0.25def | 102.3cdefg |
| **9** | 2.88d | 0.25def | 92.85defgh |
| **10** | 1.70fg | 0.08h | 143.0a |
| **11** | 1.67fg | 0.06h | 140.09ab |
| **12** | 1.68fg | 0.07h | 115.82abcd |
| **13** | 2.21ef | 0.11gh | 114.45abcd |
| **14** | 1.30g | 0.07h | 112.15bcde |
| **15** | 2.94d | 0.23efg | 106.25cdef |
| **16** | 1.96f | 0.18fgh | 129.2abc |
| LSD | 0.65 | 0.13 | 30.5 |

Average from two environments. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). Entries in bold are deletion lines. LSD, least significant difference ($\alpha = 0.05$).

good agronomic traits through traditional breeding systems.[10,31,32] The current study shows that deletion of some HMW-GS results in moderate gluten strength and high extensibility, a promising combination of traits for CSB production.

### Steamed bread quality

Steamed bread surface color is genetically controlled by several genes related to polyphenol oxidases and yellow pigments.[33] Samples used in this study were not selected under this criterion and thus showed low surface color scores (3.0–6.0 out of 10) (Table 4). Steaming provides higher air moisture than baking and gives a product with high moisture content. Excessively strong gluten usually causes too much expansion during steaming, which leads to shrinkage and wrinkling after steaming caused by partial collapse of the unstable crumb structure. For example, line 5 (1, 7+9, 5+10), which showed the highest gluten strength (Table 3), had the worst surface smoothness score (2.8) owing to wrinkles on the surface; it also had a partially collapsed crumb structure (lowest score, 4.1 out of 10). Steamed bread height is generally related to gluten elasticity and extensibility. Deletion lines 10–14 showed the lowest height compared with the other lines because of weak gluten strength and high extensibility, which resulted in dough spreading after proving. These five lines also had inferior structure score because of blisters on the surface and open crumb.

Stress relaxation is the most important parameter for steamed bread quality because it indicates overall eating quality.[34] Line 16 (2*, 17+_, 5+_) had the best stress relaxation score (22 out of 35), which meant the least loss of elasticity or stable physical structure when eaten. This attribute might be due to a good combination of dough resistance to extension (0.18 N) and extensibility (129.2 mm). Line 15 (2*, 17+_, 2+12) with deletion at *1By* also showed a relatively high steamed bread score (60.8). This line also had medium gluten strength (resistance to extension, 0.23 N) and good extensibility (106.3 mm). Lines 15 and 16 had the same composition at *Glu-A1* (2*) and *Glu-B1* (17+_), although line 16 has an additional subunit deletion, i.e. *Glu-D1y* 10. Thus it appears that this deletion at *D1y* weakened the dough enough to provide functionality similar to *Glu-D1* 2+12. Consequently, it seems that the deletion at *Glu-B1* resulting in 17+_ produced protein quality that was most favorable for CSB. For instance, line 4, which only differed from line 15 in lack of a deletion at *Glu-B1*, showed a markedly lower steamed bread score compared with line 15 (Table 4). The other lines with 17+18 at *Glu-B1* but deletions at *A1x* or *D1* (lines 8 and 13 respectively) also showed similarly poor overall CSB scores, further suggesting the important role of the specific deletion at *Glu-B1* (17+_) on protein attributes important for CSB quality.

Interestingly, lines 8 and 9, both characterized by *Glu-A1x* deletion, had similar dough properties to line 15 but inferior steamed bread stress relaxation score and crumb structure. This means that other factors besides gluten elasticity and extensibility, such as LMW-GS and gliadin composition and perhaps starch properties, also affected CSB quality. For example, Mondal *et al.*[35] found that deletion of specific gliadin subunits differentially impacted dough extensibility parameters and tortilla quality. Jin *et al.*[12] observed significantly different contributions to dough properties by allelic variation of HWM-GS and LMW-GS in a set of near-isogenic wheat lines, but no significant difference in most parameters of CSB.

Uthayakumaran *et al.*[28] investigated the pan bread and tortilla quality performance of genotypes null at all *Glu-1* loci and showed a non-expansive bread and poor tortilla rollability. However, individual deletion of specific HMW-GS seems a promising way to develop outstanding tortilla quality with good extensibility and shelf stability.[36] In the current study, partial HMW-GS deletions at *Glu-B1* and *Glu-D1*, lines 16 (2*, 17+_, 5+_) and 15 (2*, 17+_, 2+12), showed good overall CSB quality traits and the highest total score (65.0 and 60.8 respectively), with good texture and acceptable internal structure and appearance.

Zhang *et al.*[16] observed significant and positive correlations of polymeric protein content with steamed bread score and stress relaxation score among a population with weak gluten strength.[22] However, such a relationship was not observed in the current research. In this study, all deletion lines were created from genotypes with high gluten strength and high protein content (134.2–139.9 g $kg^{-1}$) rather than from a population with low protein content and weak gluten strength. The overall implication is that a minimum content of polymeric proteins may be required for optimal steamed bread quality, but other compositional parameters are also important. It is also important to point out that environmental effects cannot be fully accounted for in this study owing to the limited set of environments (two locations) used.

## CONCLUSIONS

Lines with HMW-GS deletions had lower HMW-GS amount and HWM-GS/LMW-GS ratio, which also resulted in significantly lower absolute and relative amounts of polymeric protein, compared with non-deletion lines. HWM-GS deletion did not significantly affect protein content but produced important variations in dough properties relevant to steamed bread quality. Deletion lines generally showed medium to weak gluten strength with higher gluten extensibility. Lines with one or two *Glu-1y* deletions (2*, 17+_, 5+_ and 2*, 17+_, 2+12) showed moderate gluten strength, high extensibility and good steamed bread quality. Specific deletions of



  

**Table 4.** Effects of HMW-GS deletion on scores of steamed bread quality traits

| Entry | Height | Smoothness | Color | Stress relaxation | Crumb structure | Specific volume | Total score |
|---|---|---|---|---|---|---|---|
| 1 | 9.0a | 5.0abcd | 3.0d | 13.0e | 9.8a | 14.5a | 54.3cd |
| 2 | 8.0abc | 4.0bcd | 3.0d | 13.0e | 9.4a | 13.5a | 50.9d |
| 3 | 7.5bcd | 3.5cd | 3.0d | 15.5d | 8.3ab | 14.5a | 52.3cd |
| 4 | 8.5ab | 3.8bcd | 4.0bcd | 15.0de | 7.9ab | 13.5a | 52.6cd |
| 5 | 7.0cd | 2.8d | 5.5ab | 16.0cd | 4.1d | 13.5a | 48.9d |
| 6 | 6.5d | 3.8bcd | 5.5ab | 20.0ab | 6.8abcd | 14.5a | 57.0bc |
| 7 | 8.5ab | 5.5abc | 5.0abc | 13.0e | 7.5abc | 14.0a | 53.5cd |
| **8** | 8.0abc | 5.5abc | 4.5abcd | 13.0e | 4.5cd | 16.0a | 51.5cd |
| **9** | 8.5ab | 5.5abc | 3.0d | 13.0e | 6.8abcd | 15.5a | 52.3cd |
| **10** | 7.0cd | 5.3abc | 5.5ab | 14de | 7.5abc | 14.0a | 53.3cd |
| **11** | 7.0cd | 7.0a | 3.5cd | 16.0cd | 6.3bcd | 14.5a | 54.3cd |
| **12** | 5.0e | 6.0ab | 5.5ab | 13.0e | 6.3bcd | 14.5a | 50.3d |
| **13** | 5.0e | 5.5abc | 5.0abc | 16.0cd | 6.0bcd | 15.0a | 52.5cd |
| **14** | 5.0e | 7.0a | 4.0bcd | 15.0de | 6.0bcd | 14.0a | 51.0d |
| **15** | 8.0abc | 5.5abc | 6.0a | 18.0bc | 8.3ab | 15.0a | 60.8ab |
| **16** | 8.0abc | 5.5abc | 5.5ab | 22.0a | 8.0ab | 16.0a | 65.0a |
| LSD | 1.3 | 2.3 | 1.9 | 2.1 | 3.1 | 2.8 | 5.8 |

Average from two environments. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). Entries in bold are deletion lines. LSD, least significant difference ($\alpha = 0.05$). For the stress relaxation (SR) score, the higher the SR measured by texture analyzer as described in 'Experimental', the lower the SR score is.

HMW-GS might be a viable strategy to develop new wheat genotypes for steamed bread processing.

## ACKNOWLEDGEMENTS

Financial support was kindly provided by Texas A&M AgriLife Research, the National Natural Science Foundation of China (31101146) and the Natural Science Foundation of Jiangsu Province (BK2011667).

## REFERENCES

1 Huang S, Quail K and Moss R, The optimization of a laboratory processing procedure for southern-style Chinese steamed bread. *Int J Food Sci Technol* **33**:345–357 (1998).
2 He ZH, Liu AH, Peña RJ and Rajaram S, Suitability of Chinese wheat cultivars for production of northern style Chinese steamed bread. *Euphytica* **131**:155–163 (2003).
3 Huang S, Yun SH, Quail K and Moss R, Establishment of flour quality guidelines for northern style Chinese steamed bread. *J Cereal Sci* **24**:179–185 (1996).
4 Addo K, Pomeranz Y, Huang ML, Rubenthaler GL and Jeffers HC, Steamed bread. II. Role of protein content and strength. *Cereal Chem* **68**:39–42 (1991).
5 Rubenthaler GL, Pomeranz Y and Huang ML, Steamed bread. IV. Negative steamer-spring of strong flours. *Cereal Chem* **69**:334–337 (1992).
6 Zhu J, Huang S, Khan K and O'Brien L, Relationship of protein quantity, quality and dough properties with Chinese steamed bread quality. *J Cereal Sci* **33**:205–212 (2001).
7 Lin ZJ, Miskelly DM and Moss HJ, Suitability of various Australian wheats for Chinese-style steamed bread. *J Sci Food Agric* **53**:203–213 (1990).
8 Gianibelli MC, Larroque OR and MacRitchie F, Biochemical, genetic, molecular characterization of wheat glutenin and its component subunits. *Cereal Chem* **78**:635–646 (2001).
9 Payne PI, Nightingale MA, Krattiger AF and Holt LM, The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J Sci Food Agric* **40**:51–65 (1987).
10 Liu L, He ZH, Yan J, Zhang Y, Xia XC and Peña RJ, Allelic variation at the *Glu-1* and *Glu-3* loci, presence of the 1B.1R translocation, and their effects on mixographic properties in Chinese bread wheats. *Euphytica* **142**:197–204 (2005).
11 Labuschagne MT and van Deventer CS, The effect of *Glu-B1* high molecular weight glutenin subunits on biscuit-making quality of wheat. *Euphytica* **83**:193–197 (1995).
12 Jin H, Zhang Y, Li GY, Mu PY, Fan ZR, Xia XC, *et al.*, Effects of allelic variation of HMW-GS and LMW-GS on mixograph properties and Chinese noodle and steamed bread qualities in a set of Aroona near-isogenic wheat lines. *J Cereal Sci* **57**:146–152 (2013).
13 HadiNezhad M and Butler F, Association of glutenin subunit composition and dough rheological characteristics with cookie baking properties of soft wheat cultivars. *Cereal Chem* **86**:339–349 (2009).
14 Wieser H and Kieffer R, Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. *J Cereal Sci* **34**:19–27 (2001).
15 Wieser H and Zimmermann G, Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality. *Eur Food Res Technol* **210**:324–330 (2000).
16 Zhang PP, He ZH, Zhang Y, Xia XC, Liu JJ, Yan J, *et al.*, Pan bread and Chinese white salted noodle qualities of Chinese winter wheat cultivars and their relationship with gluten protein fractions. *Cereal Chem* **84**:370–378 (2007).
17 Anjum FM, Khan MR, Din A, Saeed M, Pasha I and Arshad MU, Wheat gluten: high molecular weight glutenin subunits – structure, genetics, and relation to dough elasticity. *J Food Sci* **72**:56–63 (2007).
18 Gupta RB, Khan K and MacRitchie F, Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J Cereal Sci* **18**:23–41 (1993).
19 Zhu J and Khan K, Effects of genotype and environment on glutenin polymers and bread making quality. *Cereal Chem* **78**:125–130 (2001).
20 Don C, Mann G, Bekes F and Hamer RJ, HMW-GS affect the properties of glutenin particles in GMP and thus flour quality. *J Cereal Sci* **44**:127–136 (2006).
21 Naeem HA and MacRitchie F, Polymerization of glutenin during grain development in near-isogenic wheat lines differing at *Glu-D1* and *Glu-B1* in greenhouse and field. *J Cereal Sci* **41**:7–12 (2005).
22 Zhang PP, He ZH, Zhang Y, Xia XC, Chen DS and Zhang Y, Association between % SDS-unextractable polymeric protein (%UPP) and end-use quality in Chinese bread wheat cultivars. *Cereal Chem* **85**:696–700 (2008).
23 AACC International, *Approved Methods of Analysis* (11th edn). AACCI, St Paul, MN (2010) (available online only).

  

24 Mondal S, Tilley M, Alviola JN, Waniska RD, Bean SR, Glover KD, *et al.*, Use of near-isogenic wheat lines to determine the glutenin composition and functionality requirements for flour tortillas. *J Agric Food Chem* **56**:179–184 (2008).

25 Larroque OR, Gianibelli MC, Gomez SM and MacRitchie F, Procedure for obtaining stable protein extracts of cereal flour and whole meal for size-exclusion HPLC analysis. *Cereal Chem* **77**:448–450 (2000).

26 Huang S, Betker S, Quail K and Moss R, An optimized processing procedure by response surface methodology (RSM) for northern-style Chinese steamed bread. *J Cereal Sci* **18**:89–102 (1993).

27 Gupta RB, Popineau Y, Lefebvre J, Cornec M, Lawrence GJ and MacRitchie F, Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low $M_r$ or high $M_r$ glutenin subunits. *J Cereal Sci* **21**:103–116 (1995).

28 Uthayakumaran S, Lukow OM, Jordan MC and Cloutier S, Development of genetically modified wheat to assess its dough functional properties. *Mol Breed* **11**:249–258 (2003).

29 Nash D, Lanning SP, Martin JM, Blake NK, Souza E, Graybosch RA, *et al.*, Relationship of dough extensibility to dough strength in a spring wheat cross. *Cereal Chem* **83**:255–258 (2006).

30 Caffe-Treml M, Glover KD, Krishnan PG, Hareland GA, Bondalapati KD and Stein J, Effect of wheat genotype and environment on relationships between dough extensibility and breadmaking quality. *Cereal Chem* **88**:201–208 (2011).

31 Peterson CJ, Graybosch RA, Baenziger PS and Grombacher AW, Genotype and environment effects on quality characteristics of hard red winter wheat. *Crop Sci* **32**:98–103 (1992).

32 Kolster P, van Eeuwijk FA and van Gelder WMJ, Additive and epistatic effects of allelic variation at the high molecular weight glutenin subunit loci in determining the bread-making quality of breeding lines of wheat. *Euphytica* **55**:277–285 (1991).

33 Bagge M, Xia X and Lübberstedt T, Functional markers in wheat. *Curr Opin Plant Biol* **10**:211–216 (2007).

34 Huang S, Quail K, Moss R and Best J, Objective methods for the quality assessment of northern-style steamed bread. *J Cereal Sci* **21**:49–55 (1995).

35 Mondal S, Hays DB, Alviola JN, Mason RE, Tilley M, Waniska RD, *et al.*, Functionality of gliadin proteins in wheat flour tortillas. *J Agric Food Chem* **57**:1600–1605 (2009).

36 Jondiko TO, Alviola NJ, Hays DB, Ibrahim A, Tilley M and Awika JM, Effect of high-molecular-weight glutenin subunit allelic composition on wheat flour tortilla quality. *Cereal Chem* **89**:155–161 (2011).




  