

Effects of Incorporated Glutenins on Functional Properties of Wheat Dough

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ABSTRACT

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The development of reduction-oxidation methods to open the native gluten polymer, incorporate monomers, and repolymerize the modified polymer, has allowed new types of investigation of the effects of glutenin subunits on dough characters. These methods were used to incorporate bulk high molecular weight glutenin subunits (HMW-GS) and bulk low molecular weight glutenin subunits (LMW-GS) from four cultivars into the parent flours to modify the HMW-GS-to-LMW-GS ratio. In addition, the glutenins from the four cultivars were added to a single base flour of differing glutenin subunit composition. Finally, HMW-GS 7, 8, 5, and 10 were incorporated singly and in pairs into two base flours. The mixing time, peak resistance, maximum resistance to extension, and loaf height increased with increases in HMW-GS-to-LMW-GS ratio. There was a decrease in

resistance breakdown observed with increase in HMW-GS-to-LMW-GS ratio. The maximum resistance to extension slightly increased with increases in HMW-GS-to-LMW-GS ratio (strongly in cultivar Hartog). Dough extensibility decreased with increase in HMW-GS-to-LMW-GS ratio (except in Osprey derivative). In the incorporation studies of single and paired glutenin subunits, HMW-GS 5+10 gave a synergistic effect increasing mixing time, maximum resistance to extension, and loaf height as compared with HMW-GS 5 or 10 separately. In contrast, HMW-GS 7+8 showed an additive effect and HMW-GS 7 incorporated separately had a higher mixing time, maximum resistance to extension, and loaf height than those of HMW-GS 7+8 and HMW-GS 8.

Native glutenin is a polymer composed of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) subunits linked by disulfide bonds. A great deal of information has been derived from examining qualitative and quantitative differences between cultivars that correlate with differences between these protein subunits. Experimental manipulation of the subunits is complicated because simple addition of subunit proteins to flour does not result in them being incorporated into the glutenin polymer. Therefore, incorporation techniques were required. A technique for chemical incorporation of glutenin subunits into wheat dough by partial reduction followed by oxidation was developed by Bekes et al (1994) and has been used in subsequent studies of dough mixing properties. This method has been modified to make it suitable for use in studies of dough extension and baking properties (Uthayakumaran et al 2000). It is now possible to ask specific new questions about the functionality of glutenin subunits, individually and in combination.

Correlative studies have shown that higher ratios of HMW-GS to LMW-GS confer better breadmaking quality than lower ratios (Gupta et al 1992). The incorporation methods allow us to investigate this in an experimental fashion, without the confounding influence of other genetic differences.

Many correlative studies have shown that HMW-GS 5+10 confer stronger dough than the allelic combination 2+12 (Branlard and Dardevet 1985, Payne 1987). Bekes et al (1995) showed that incorporation of pairs of subunits 5+10 into a null-*Glu-D1* flour had a greater dough strengthening effect than the same amount of subunits 2+12.

For more detailed studies, it is desirable to have individual subunits in a pure form. For some glutenin subunits, mutant wheat lines are available which express only one HMW-GS (Payne and Seekings 1996). Purification of such single subunits is therefore much easier than when using conventional genotypes. Genes for the desired wheat proteins have been recombined into foreign genomes such as *Escherichia coli*, allowing the production of single polypeptides in modest quantities and facilitating protein purification and isolation. It is thus possible to have the gene product

expressed in a system free of other gluten proteins (Shewry et al 1992, Lee et al 1999). A further advantage of applying heterologous expression is the capability to produce polypeptides with chemical compositions that have been altered by genetic engineering. This article describes the effects of incorporated glutenins on the functional properties of wheat dough to demonstrate that heterologously expressed proteins function in the same fashion as native proteins. HMW-GS 7 and 8 prepared from mutant wheat lines and HMW-GS 5 and 10 which had been expressed in bacteria were used in incorporation experiments using the modified reduction-oxidation method (Uthayakumaran et al 2000).

MATERIALS AND METHODS

Flours of cultivars Banks, Hartog, and Sunbri were provided by BRI Australia Ltd., North Ryde, NSW. In addition, a sample identified as cultivar Osprey, but carrying the 1R translocation from rye, and therefore not authentic Osprey, was provided by BRI Australia Ltd. and labeled as "Osprey derivative". Samples of Galahad 7 and Galahad 8 were provided by P. I. Payne, PBI Cambridge Ltd., Trumpington, Cambridge, UK. Galahad 7 and Galahad 8 have null alleles for both the A and D genome (*Glu-1*) HMW-GS and express only the Bx7 or By8 subunit coded by the B-genome (*Glu-B1*) (Payne and Seekings 1996). Bulk HMW-GS and LMW-GS fractions were prepared from each of the six flours. Thus the Galahad 7 and 8 flour provided only HMW-GS Bx7 and HMW-GS By8, respectively, whereas the other flours provided a mixture (Table I). Bacterial lysates (expressed in *Escherichia coli*) containing HMW-GS 5 and 10 coded by *Glu-D1* were kindly donated by O. Anderson, USDA, Albany, CA.

Banks, Hartog, Osprey derivative, and Sunbri flours were used for studies on incorporating bulk HMW-GS and LMW-GS. To study the effect of incorporating HMW-GS 7, 8, 5, and 10, Banks

TABLE I
Glutenin Subunit Composition of Flours^a

| Sample | HMW-GS Composition | | | Allelic Composition of LMW-GS | | |
|-------------------|--------------------|---------------|---------------|-------------------------------|---------------|---------------|
| | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | <i>Glu-A3</i> | <i>Glu-B3</i> | <i>Glu-D3</i> |
| Banks | 2* | 7+8 | 2+12 | <i>b</i> | <i>b</i> | <i>c</i> |
| Hartog | 1 | 17+18 | 5+10 | <i>b</i> | <i>h</i> | <i>e</i> |
| Osprey derivative | 2* | 7+8 | 2+12 | <i>c</i> | <i>j</i> | <i>b</i> |
| Sunbri | 1 | 7+8 | 2+12 | <i>b</i> | <i>b</i> | <i>c</i> |

^a Glutenin subunit composition assessed by methods of Payne and Lawrence (1983) and Gupta and Shepherd (1990).

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and Hartog flours were used because they differed in D-genome HMW-GS composition (Table I) but had very similar protein contents (Table II).

Preparation of Bulk HMW-GS and LMW-GS Fractions and Single HMW-GS from Bacterial Extracts

The flour samples were defatted (MacRitchie and Gras 1973), and the glutenins were extracted by the method of Melas et al (1994) with modifications. Flour samples (16.0 g) were extracted three times for 30 min (agitating with a magnetic stirrer) with 190 mL of 50% propan-2-ol at room temperature ($24 \pm 1^\circ\text{C}$). The suspension was centrifuged at $20,600 \times g$ for 15 min and the

supernatant was discarded after each extraction. The resulting pellet was resuspended in 100 mL of 1% dithiothreitol (DTT) (1 g of DTT, 50 mL of propan-2-ol, 50 mL of 160 mM Tris-HCl, pH 8.0) and shaken for 30 min in an incubator at 65°C , then centrifuged at $29,600 \times g$ for 20 min. The supernatant was decanted and the volume was recorded. Acetone was added to the supernatant to a final concentration of 40% (v/v) and left to precipitate overnight at 4°C . After centrifugation at $29,600 \times g$ for 20 min, the resulting supernatant (enriched in LMW-GS) was collected, dialyzed against 0.1% (v/v) acetic acid at 4°C for 72 hr and freeze-dried. The pellet (enriched in HMW-GS) was resuspended in 100 mL of distilled water and centrifuged for 10 min at $20,600 \times g$. This was repeated twice. The resulting pellet was resuspended in 0.1% (v/v) acetic acid and freeze-dried. All fractions were then ground to a powder.

Each 1 g of sample of freeze-dried bacterial lysate, containing either HMW-GS 5 or 10, was extracted twice for 2 hr (agitating with an orbital shaker) with 5 mL of 50% propan-2-ol containing 1% β -mercaptoethanol. The first extraction was done at room temperature ($24 \pm 1^\circ\text{C}$) and the second at 60°C . The suspension was centrifuged at $29,600 \times g$ for 30 min at 4°C and the supernatant was decanted. Two volumes of 1.5M NaCl were added to the combined supernatant and left at 4°C for 48 hr. After centrifugation at $29,600 \times g$ for 1.5 hr at 4°C , the resulting supernatant was discarded and the pellet was resuspended in 5 mL of 8M urea, dialyzed against 0.1 mM acetic acid at 4°C for 72 hr and freeze-dried. The nitrogen contents of the fractions were determined by the Dumas total combustion method using a CHN-1000 elemental analyzer (Leco Inc., St. Joseph, MI). Protein (%) was estimated as $N \times 5.7$ (Table II). The protein components present in the isolated glutenin subunit fractions (Figs. 1 and 2) were determined by SDS-PAGE (Laemmli 1970).

Reversed-Phase HPLC

The peak area of the HMW-GS and that of the LMW-GS of each flour and of the isolated fractions was determined in triplicate by reversed-phase RP-HPLC (Marchylo et al 1989). Flour (25 mg)

TABLE II
Protein Contents of Experimental Material

| Sample | Protein Content % |
|-------------------------|-------------------|
| Banks flour | 13.0 |
| Glutenin-rich fraction | 90.3 |
| HMW-GS | 90.0 |
| LMW-GS | 92.4 |
| Hartog flour | 12.4 |
| Glutenin-rich fraction | 89.7 |
| HMW-GS | 90.0 |
| LMW-GS | 93.0 |
| Osprey derivative flour | 16.3 |
| Glutenin-rich fraction | 89.6 |
| HMW-GS | 89.0 |
| LMW-GS | 92.3 |
| Sunbri flour | 14.9 |
| Glutenin-rich fraction | 91.2 |
| HMW-GS | 91.0 |
| LMW-GS | 92.6 |
| HMW-GS 7 | 93.2 |
| HMW-GS 8 | 93.4 |
| HMW-GS 5 | 92.8 |
| HMW-GS 10 | 93.2 |

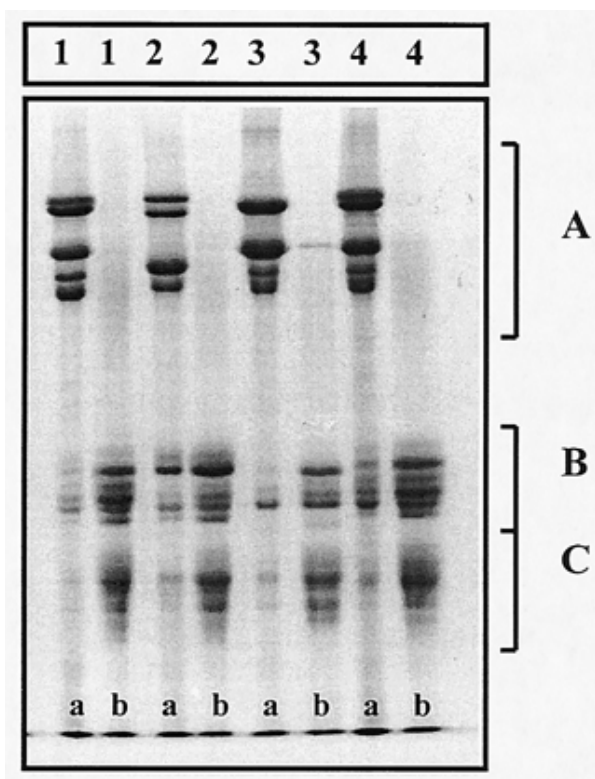


Fig. 1. SDS-PAGE of HMW-GS and LMW-GS extracted from Banks (1), Hartog (2), Osprey derivative (3), and Sunbri (4) flours. A, HMW-GS; B, LMW-GS (B-type); C, LMW-GS (C-type). a = HMW-GS, b = LMW-GS.

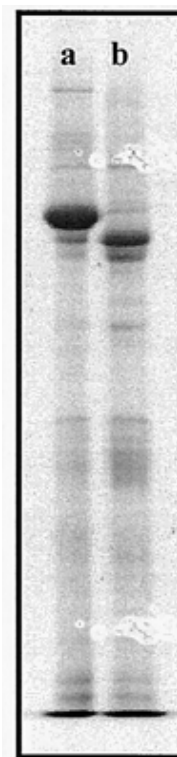


Fig. 2. SDS-PAGE of HMW-GS 7 and 8 extracted from flours Galahad 7 and Galahad 8. a = HMW-GS 7, b = HMW-GS 8.

was extracted with 1 mL of 70% ethanol for 30 min at room temperature, centrifuged at $17,000 \times g$ and the supernatant was discarded. The pellet was resuspended in 1 mL of 50% propan-1-ol, agitated for 30 min at room temperature, centrifuged at $17,000 \times g$ and the supernatant was discarded. The pellet was resuspended in 1 mL of 50% propan-1-ol containing 1% DTT (w/v), maintained for 1 hr in a water bath at 60°C and centrifuged at $17,000 \times g$. The supernatant, containing the reduced subunits of polymeric proteins, was alkylated with 10 μL of 4-vinylpyridine for 15 min in a water bath at 60°C , centrifuged at $17,000 \times g$ and filtered through a $0.45\text{-}\mu\text{m}$ PVDF filter before HPLC analysis. Aliquots of 20 μL were injected into a Vydac C 18 column, 300 \AA , $250 \times 4.6 \text{ mm}$ (The Separations Group, Hesperia, CA). Analysis was made at 70°C using two solvents: solvent A (0.07% trifluoroacetic acid in deionized water) and solvent B (0.05% trifluoroacetic acid in acetonitrile). A gradient of 24–48% of solvent B was used over a period of 55 min and the proteins were detected at 214 nm. The peak area of HMW-GS divided by the peak area of LMW-GS gave the HMW-GS-to-LMW-GS ratio.

Preparation of Blends

Blends of different HMW-GS-to-LMW-GS ratio were prepared by adding HMW-GS fraction and LMW-GS fraction (Table III) to the parent flours (2.0 g) which were Banks, Hartog, Osprey derivative, and Sunbri. The control was 30 mg of total glutenin-rich fraction isolated from the respective flours using the precipitation method (MacRitchie 1987) added to the parent flour. The HMW-GS fraction (30 mg) or LMW-GS fraction (30 mg) isolated from Banks, Hartog, Sunbri, and Osprey were also added to a single base flour (Banks). The control was 30 mg of Banks glutenin-rich fraction added to the base flour. Blends were prepared by adding 30 mg of HMW-GS fractions 7, 8, 5, or 10, or 15 mg of HMW-GS

7 plus 15 mg of HMW-GS 8 or 15 mg of HMW-GS 5 plus 15 mg of HMW-GS 10 to either Banks or Hartog flour. The controls were 30 mg of Banks and Hartog glutenin-rich fractions added to the respective flours.

Measurement of Functional Properties

For each cultivar, these experiments were made at a protein content equal to $1.2\times$ the protein content of the parent flour. The glutenin-to-gliadin ratio was held constant for experiments within each cultivar but was different for each cultivar. The reduction-oxidation procedure developed by Bekes et al (1994) was used for incorporating the different protein fractions into the flour for mixing and those developed by Uthayakumar et al (2000) for extension and baking. The total quantity of water to be used during mixing, extension, and baking was calculated using the protein and moisture contents of ingredients (AACC 2000). The water

TABLE III
HMW-GS-to-LMW-GS Ratios of Flours with Added Glutenin or Glutenin Subunits

| Additive to 2 g of Flour | HMW-GS-to-LMW-GS Ratio | | | |
|--------------------------------------|------------------------|--------|----------------------------|--------|
| | Banks | Hartog | Osprey deriv. ^a | Sunbri |
| None | 0.28 | 0.30 | 0.62 | 0.33 |
| 30 mg of glutenin | 0.28 | 0.30 | 0.62 | 0.33 |
| 30 mg of LMW-GS | 0.25 | 0.27 | 0.49 | 0.29 |
| 20 mg of LMW-GS + 10 mg of HMW-GS | 0.28 | 0.30 | 0.62 | 0.33 |
| 10 mg of LMW-GS + 20 mg of HMW-GS | 0.44 | 0.44 | 0.80 | 0.43 |
| 30 mg of HMW-GS | 0.55 | 0.48 | 1.08 | 0.62 |
| LSD ^b | 0.041 | 0.040 | 0.022 | 0.030 |

^a Osprey derivative.

^b Least significant difference ($P < 0.05$).

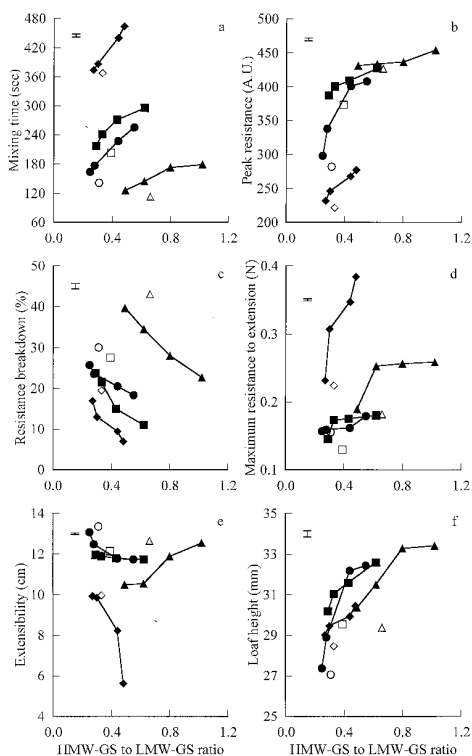


Fig. 3. Mixing, extension, and baking properties of four wheat cultivars with HMW-GS-to-LMW-GS ratio altered by incorporating HMW-GS, LMW-GS and mixtures of HMW-GS and LMW-GS. **a-f:** Mixing time (MT), peak resistance (PR), resistance breakdown (RB), maximum resistance to extension (R_{max}), extensibility (Ext), loaf height (LH). Banks (●), Hartog (◆), Osprey derivative (▲), and Sunbri (■). Open symbols are respective controls. Error bars ± 1 SE.

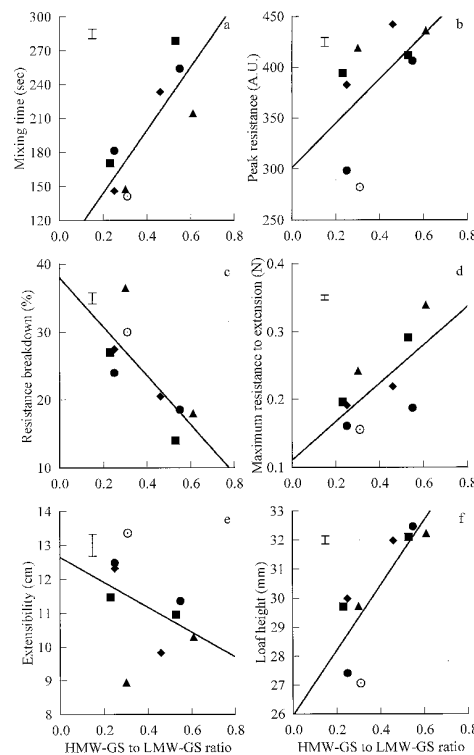


Fig. 4. Mixing, extension, and baking properties of Banks flour with HMW-GS-to-LMW-GS ratio altered by incorporating HMW-GS and LMW-GS from four different flours. **a-f:** Mixing time (MT), peak resistance (PR), resistance breakdown (RB), maximum resistance to extension (R_{max}), extensibility (Ext), loaf height (LH). Banks (●), Hartog (◆), Osprey derivative (▲), and Sunbri (■). Control (○). Error bars ± 1 SE.

absorptions were Banks 64.7%, Hartog 63.0%, Sunbri 65.0%, and Osprey derivative 65.4%. All formulations were mixed on a 2-g mixograph (TMCO, Lincoln, NE).

The blends (flour and the protein fraction), 450 μ L of reducing agent DTT (2 mg/mL in distilled water) and distilled water were mixed for 30 sec. The mixture was allowed to react for 4 min without further mixing. The partially reduced dough was treated with 250 μ L of oxidant (KIO_3 , 5 mg/mL in distilled water), mixed for 30 sec, rested for 5 min for the oxidizing reaction to take place and further mixed for 10 min. Mixing was done in triplicate and the parameters recorded were mixing time (MT, sec), peak resistance (PR, A.U.) and resistance breakdown (RB, %) (Gras et al 1990). All doughs were frozen in liquid nitrogen and freeze-dried. HMW-GS-to-LMW-GS ratios (Table III) of these doughs were determined using RP-HPLC.

Extension Testing

The flour (2 g), 450 μ L of DTT solution (0.2 mg/mL) and the remaining amount of distilled water were mixed for 30 sec. The mixture was allowed to react for 1 min without further mixing. The partially reduced dough was treated with 250 μ L of KIO_3 solution (5 mg/mL), mixed for 30 sec, rested for 5 min for the oxidizing reaction to take place and further mixed to reach 70% of

the optimum mixing time (including the initial two 30-sec mixes). Dough samples (1.7 g/test) were molded into cylinders \approx 6 mm diameter with a prototype mold. The mold consisted of a 153 mm diameter drum rotating at 20 rpm within a fixed 167 mm diameter partial outer drum. Dough pieces (1.7 g) were introduced and rolled around the annular space for \approx 300°. The molded pieces, \approx 45 mm long, were mounted on a sample carrier and rested at 30°C and >90% rh for 45 min before extension testing (Gras and Bekes 1996). Extension was performed in quadruplicate on a microextension tester with a 19 mm gap and 6 mm hook operating at 1 cm/sec. Recordings of the dough resistance and the sample carrier position were taken at 100 readings/sec and recorded by a personal computer, using LabTech Notebook software. Maximum resistance to extension (R_{max} , N) and extension before rupture (Ext, cm) were calculated (Rath et al 1994).

Microbaking

Flour, 450 μ L of DTT solution (2 mg/mL) and the water required were placed in the mixing bowl. The mixture was mixed for 30 sec and allowed to rest for 1 min. In the last few seconds of the resting period, 250 μ L of oxidant solution (KIO_3 , 2.5 mg/mL) was added along with the calculated amount of yeast suspension (10 g of compressed yeast + 8 g of sodium chloride salt + 2 g of improver in 100 mL of water). The improver used was a "rapid dough" formulation improver, which provides 100 ppm of ascorbic acid and 0.5 SKB units of cereal α -amylase per 100 g of flour. Mixing was resumed for 30 sec and the dough allowed to rest further for 5 min. The dough was then mixed to the peak dough development time (including the initial two 30-sec mixes). Loaves were prepared from 2.4 g of the resulting dough which was molded, rested for 20 min at 40°C in a small airtight container, then remolded, proofed for 45 min (40°C and 90% rh), and baked at 200°C for 17 min (Gras and Bekes 1996). Loaf height was measured with vernier calipers. Baking tests were made in triplicate.

Statistical Analyses

Statistical analysis of variance and analysis of covariance was made using Super Anova v 1.11 (Abacus Concepts Inc., Berkeley, CA).

TABLE IV
Molecular Size Distribution of Polymeric Protein in Doughs Incorporated with HMW-GS and LMW-GS

| Additive to 2 g of Flour | %UPP ^a | | | |
|-----------------------------------|-------------------|--------|----------------------------|--------|
| | Banks | Hartog | Osprey deriv. ^b | Sunbri |
| None | 67.3 | 70.6 | 63.2 | 63.2 |
| 30 mg of LMW-GS | 64.1 | 53.4 | 52.9 | 53.1 |
| 20 mg of LMW-GS + 10 mg HMW-GS | 67.9 | 70.5 | 63.0 | 63.4 |
| 20 mg of HMW-GS + 10 mg of LMW-GS | 73.6 | 77.6 | 77.8 | 64.0 |
| 30 mg of HMW-GS | 77.8 | 82.9 | 88.4 | 64.8 |
| LSD ^c | 0.80 | 0.90 | 0.62 | 0.70 |

^a Unextractable polymeric protein (%UPP) determined by size-exclusion HPLC (Singh et al 1990, Batey et al 1991).

^b Osprey derivative.

^c Least significant difference ($P < 0.05$).

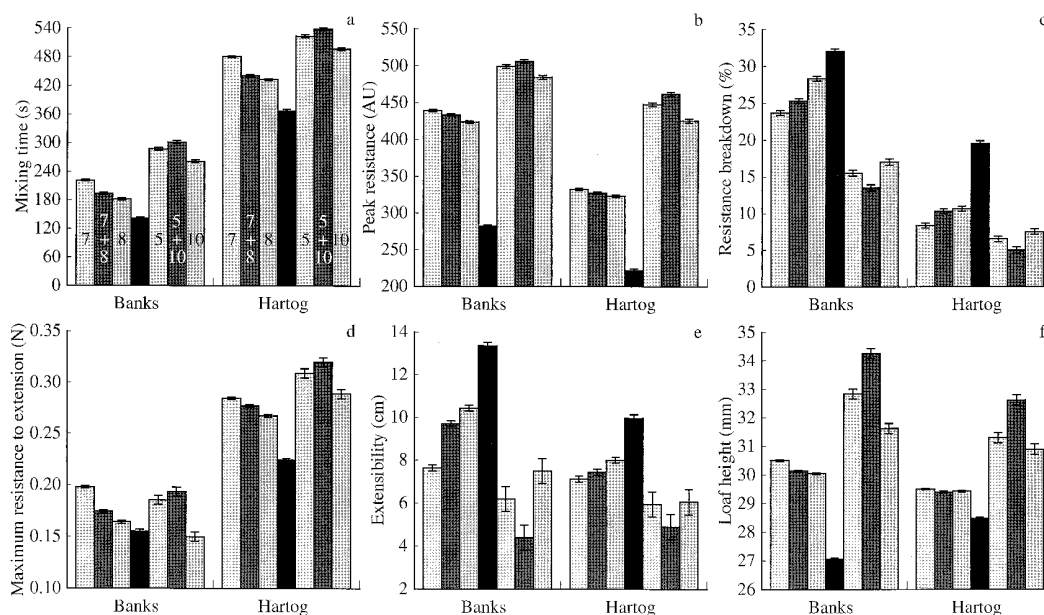


Fig. 5. Effect of HMW-GS Bx7, By8, Dx5, and Dy10 on mixing, extension, and baking properties of two base flours Banks and Hartog. **a-f:** Mixing time (MT), peak resistance (PR), resistance breakdown (RB), maximum resistance to extension (R_{max}), extensibility (Ext), loaf height (LH). From left to right: Bx7, Bx7 + By8, By8, control, Dx5, Dx5 + Dy10, Dy10. Error bars \pm 1 SE.

RESULTS

Incorporation of HMW-GS and LMW-GS to Parent Flour

Within each set of treated doughs, increasing the HMW-GS-to-LMW-GS ratio was associated with increased MT, PR, R_{max} , and LH, and decreased RB, while the direction of the effect on Ext depended on the cultivar (Fig. 3). Increases in the proportion of HMW-GS were associated with decreases in Ext in three flours (cvs. Banks, Hartog, and Sunbri) (Fig. 3e), but with increases in Ext in the fourth (Osprey derivative).

Incorporation of Various HMW-GS and LMW-GS into a Base Flour

In all cases, increases in the proportion of HMW-GS in the base flour irrespective of the source increased MT (Fig. 4a), as observed when HMW-GS from the same flour were used. The incorporation of LMW-GS from different wheat cultivars had a marginal effect on MT. The slope showed a positive relationship between increases in HMW-GS-to-LMW-GS ratio and mixing time. The PR increased significantly when HMW-GS from each of the four cultivars and LMW-GS from three were used (Fig. 4b). The incorporation of LMW-GS from Banks had a marginal effect on PR when compared with the control. There was a positive correlation between HMW-GS-to-LMW-GS ratio and PR. The addition of HMW-GS resulted in reduced RB (Fig. 4c) as observed when HMW-GS-to-LMW-GS ratio of the same flour was increased. The LMW-GS isolated from Osprey derivative showed the highest RB.

In every case, the increase in HMW-GS-to-LMW-GS ratio increased R_{max} (Fig. 4d). Incorporation of LMW-GS isolated from Banks had a marginal effect on R_{max} . Incorporation of both LMW-GS and HMW-GS decreased Ext (Fig. 4e). From three flours, HMW-GS had a greater effect than LMW-GS, but for Osprey derivative, the opposite was found, as in the previous experiment.

There was a general increase in LH when the HMW-GS isolated from different flours were incorporated to the base flour (Fig. 4f). The incorporation of LMW-GS also increased LH but not to the extent of HMW-GS. The LMW-GS isolated from Banks had the lowest effect. There was a positive overall correlation between HMW-GS-to-LMW-GS ratio and LH.

Incorporation of HMW-GS 7, 8, 5, and 10 to Two Base Flours

Incorporation of HMW-GS 7, 8, 5, and 10, singly or in combination, resulted in higher MT (Fig. 5a), greater peak resistance (Fig. 5b), lower resistance breakdown (Fig. 5c), higher maximum resistance to extension (Fig. 5d), lower extensibility (Fig. 5e), and greater loaf height (Fig. 5f) in both Banks and Hartog flours. HMW-GS 7 had a greater effect than subunit 8 in every case except for loaf height in Hartog. The effect of the combination of HMW-GS 7 and 8 was intermediate between the effects of the individual subunits in every case except loaf height in Hartog. HMW-GS 5 had a greater effect than subunit 10 in every case, except that both subunits had equivalent effects on extensibility of Hartog. The effect of subunit 10 on R_{max} in Banks was nonsignificant. The effect of the combination of HMW-GS 5 and 10 was always greater than the effects of the individual subunits, except that the margin was not significant for R_{max} over subunit 5 in Hartog and for extensibility over either subunit in Hartog.

DISCUSSION

The positive effects of incorporation of HMW-GS on dough stability and strength during mixing were highly pronounced in this study. The amount of the HMW-GS was more important than the composition in some experiments, whereas in others the composition had important effects.

Schropp et al (1995), Schropp and Weiser (1996) and Sapirstein and Fu (1996) showed that the concentration of the HMW-GS

contributed to an increase in R_{max} and a decrease in Ext, and the dough strengthening effect did not seem to be dependent on the HMW-GS composition. Results of the incorporation of various HMW-GS (total) from different flour sources to a single base flour showed this trend, as concentration rather than composition was the factor affecting functional properties. Thus, the effect of Hartog HMW-GS, including 5+10, was not significantly greater than other HMW-GS which included 2+12. The main exception was Osprey derivative HMW-GS which increased Ext. Results of these studies are also in agreement with those of Lawrence et al (1988), who showed that mixtures of different HMW-GS were similar in terms of their effect on quality. These authors further concluded that the effects of the different gene loci were attributable to quantitative differences, i.e., *Glu-B1* and *Glu-D1* had greater effects because they generally produce both subunits, whereas *Glu-A1* codes for only zero or one. Incorporating 30 mg of HMW-GS 5+10 into 2 g of 12% protein flour is adding one-eighth to the protein (or comprises one-ninth of the total protein). This probably is not enough to detect any specific differences. Hence adding 30 mg of HMW-GS to a flour that already contained HMW-GS would not give as distinct an effect as adding it to a base flour null for HMW-GS.

The experiment where the HMW-GS were incorporated to parent flours, confirmed previous observations (Payne et al 1987, Uthayakumaran et al 1999), that Hartog, the only flour with HMW-GS 5+10, had a longer MT and a higher R_{max} when compared with Banks, Osprey derivative, and Sunbri which have HMW-GS 2+12. The subunits coded by the A and B genome in all flours had similar scores according to Payne et al (1987).

Bekes et al (1995) showed that when individual HMW-GS 2, 12, 5, and 10 were incorporated into a *Glu-D1* null flour, their effects were smaller than when the pairs (2+12 or 5+10) were incorporated in a 1:1 molar ratio. The pairs thus clearly showed a synergistic effect. In the present study, this trend was observed with HMW-GS 5, 10, and 5+10 but not with subunits 7, 8, and 7+8, where the combination gave intermediate results. This observation was made in both flours studied and for most of the parameters. These variations in the results may be associated with the differences in loci (HMW-GS 2, 5, 10, and 12 are coded by *Glu-D1* whereas 7 and 8 are coded by *Glu-B1*) and also the much lower effect attributed to 7+8 in the glutenin score (Payne et al 1987). The effects were smaller than found by Bekes et al (1995), probably because these authors used a *Glu-D1* null line as the base flour.

Using genetic lines lacking one, two, or all three *Glu-3* loci, Gupta et al (1995) showed that LMW-GS contributed positively to dough strength, although far less than the HMW subunits. Sissons et al (1998), using incorporation of LMW-GS, reached the same conclusion. The present study confirms these observations where results indicate that HMW-GS had a far greater effect (higher MT, PR, R_{max} , and LH) than the LMW-GS. In contrast, Sapirstein and Fu (1996) stated that LMW-GS had little effect on dough properties but they used only 10 mg in 2 g, or one-third as much as the present experiments. Inspection of Fig. 4 shows that such a small amount in many cases would not have shown a detectable effect.

The size of the polypeptides added or incorporated seems to be one of the most important parameters defining the extent of the changes in mixing properties. The larger the subunit that is incorporated, the longer the MT (Bekes et al 1995). According to the observations on size distribution of polymeric proteins, we can say that the HMW-GS contributing to the highest average molecular size had the longest MT, highest PR, and highest R_{max} when compared with the smaller size LMW-GS. The Osprey derivative had the highest HMW-GS-to-LMW-GS ratio but a comparatively low unextractable polymeric protein (%UPP) when compared with the other flours (Table IV). The size distribution of polymeric proteins in doughs incorporated with various HMW-GS show that the dough with the highest average molecular size

showed the longest MT and highest R_{max} (HMW-GS 7 and HMW-GS 5+10 for the two sets of experiments). In vitro polymerization of mixtures of x - and y -type HMW-GS was significantly faster and resulted in a distribution richer in large polymers compared with the results of homopolymerization of either x - or y -type subunits (Szabo et al 1995). The size-distribution studies on HMW-GS 5, 10, or 5+10 followed this trend, but the HMW-GS 7, 8, or 7+8 did not. This contrasting behavior may be due to the reoxidation kinetics associated with the number and type of the subunits that are polymerized. In both experiments, where the HMW-GS-to-LMW-GS ratio was altered, and the HMW-GS were varied, the increase in molecular size distribution was associated with increased dough strength.

The incorporation studies of Osprey derivative HMW-GS and LMW-GS into the parent flour showed an atypical extensibility result. Extensibility and polymer size (determined by SE-HPLC) (Uthayakumaran 1999) both decreased with incorporated Osprey derivative LMW-GS, whereas in every other, these parameters moved in opposite directions. Incorporation of Osprey derivative LMW-GS into a base flour also reduced Ext. Among the four flours studied, Osprey derivative was the only one that had the 1B/1R translocation, where the short arm of the 1B chromosome is replaced by the 1R. Therefore, the LMW-GS normally coded by the *Glu-B3* are replaced by secalins. These secalins are reported to be similar to wheat gliadins in molecular weight, aggregation behavior, and amino acid composition (Tatham and Shewry 1991). The genes in the *Glu-B3* loci code for more than one-third of the LMW-GS (Gupta and Shepherd 1990). Some LMW-GS may be chain extenders and some may be chain terminators (Okita et al 1985, Sheets and Hedgcoth 1988). In this cultivar, the loss of some LMW-GS might be expected to have led to a reduction in chain terminators, and a consequent increase in overall average molecular weight of the polymeric protein in the dough. This would be consistent with the observed reduction in extensibility. However, the change seems to have led to reduction in %UPP of the dough, perhaps indicating that there may be some genes in the rye chromosome that are interacting with the LMW-GS giving the atypical behavior. The effect was also found when a base flour was used and no rye gene products were present. In these cases, some factor other than the size distribution may be contributing to the decrease in extensibility, and this conundrum may merit further investigation. It cannot be attributed to a failure of incorporation because the experiment was repeated three times with LMW-GS extracted at different periods.

For the first time, extension studies and baking studies were performed with direct incorporation of glutenin subunits which enabled understanding of the contribution of various glutenin fractions to dough extensibility and baking performances.

CONCLUSIONS

The results show that HMW-GS contribute positively to dough strength and stability. The LMW-GS contribute to dough strength to a lower extent. The increase in the proportion of LMW-GS increases extensibility. This effect is superimposed with the increase in protein content. When constant amounts of HMW-GS were added, 5+10 produced a greater effect than 5 or 10 separately, whereas 7+8 produced an intermediate effect to either 7 or 8 separately.

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