

Effect of Varying Protein Content and Glutenin-to-Gliadin Ratio on the Functional Properties of Wheat Dough

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ABSTRACT

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Gluten, starch, lipids, and water-soluble material were separated from seven wheat samples with a range of protein contents and breadmaking quality. The isolated glutes were further partitioned into gliadin- and glutenin-rich fractions using pH precipitation. Protein content and glutenin-to-gliadin ratio were systematically altered by blending these fractions into the original flours in calculated amounts. Mixing properties, extension-tester parameters, and baking performance of composite flours were determined using small-scale techniques. Results of dough testing with blends of constant glutenin-to-gliadin ratio showed increases in the mixing time,

mixograph peak resistance, maximum resistance to extension, extensibility, and loaf volume as the protein content increased. At constant protein content, increases in glutenin-to-gliadin ratio were associated with increases in mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume, and with decreases in extensibility. Thus, total protein content and glutenin-to-gliadin ratio independently affected dough and baking properties. The results have allowed the separation of the effects of flour protein quantity and composition on breadmaking properties.

Several approaches are available to answer the question “what constitutes the basis of baking quality in wheat flours?” One approach is to measure compositional parameters such as the amount of gluten, glutenin, or gliadin in a range of flours and to search for correlations with baking performance. Another approach is fractionation and reconstitution, where the functionality of each of the separated flour components is evaluated by varying its amount in a given flour or by interchanging separated fractions between flours of different baking quality. The results establish the roles of each component and show which are responsible for differences in quality. In one of the earliest of these studies, loaf volumes of flours from specific wheat types were correlated with protein content (Aitken and Geddes 1934). Low protein flour, enriched with dried gluten to give a range of protein contents, showed loaf volumes and dough strengths that were highly correlated with the levels of gluten protein (Aitken and Geddes 1938, 1939). When glutes prepared from different cultivars were tested in a standardized starch-gluten test system, loaf volumes were dependent on the source of gluten, that is, the properties of the wheat glutes were cultivar-dependent (Harris and Sibbitt 1942). The results of other systematic fractionation and reconstitution studies have implied that gluten protein was the component mainly responsible for inherent differences in baking quality of different wheat cultivars (Finney 1943).

More recently, it has also been shown that loaf volume depends on the composition of the protein. Glutenins and gliadins together represent ≈80% of the total protein in a typical wheat flour (Hoseney et al 1969, Bietz and Wall 1975, Pritchard and Brock 1994, Tatham and Shewry 1995). The contributions of gliadins and glutenins to dough properties have been long been recognized, and it has been suggested that the gliadins generally contribute to dough viscosity and glutenins contribute to dough elasticity (Khatkar and Schofield 1997). It is the unique combination of dough viscosity and dough elasticity that comprises the functional properties of dough. Major effects on loaf quality have been demonstrated due to the high molecular weight glutenin subunits (HMW-GS) present (Payne and Lawrence 1983), the glutenin-to-gliadin ratio (Doekes et al 1982, MacRitchie 1987, Gupta et al 1992, Blumenthal et al 1994, Pechanek

et al 1997), the molecular weight distribution (MacRitchie 1987, Gupta et al 1993), and overall protein content (MacRitchie 1992). In each of these studies, the importance of one of these parameters was established using sample sets where the other three parameters were largely uncontrolled. In the present work, we have developed a system where these other parameters can be kept constant.

In principle, experimental designs that incorporate the ability to vary only one aspect of composition at a time while maintaining the others as constants can be used to examine the relative importance of any of the aspects of gluten composition. This article describes the results of an improved approach that builds on the fractionation-reconstitution work on glutenins and gliadins previously reported (Hoseney et al 1969; MacRitchie 1985, 1987; Preston and Tipples 1980). This approach has been used to establish the relative effects of protein content and glutenin-to-gliadin ratio by varying the protein content and the glutenin-to-gliadin ratio independently.

MATERIALS AND METHODS

Samples

Wheat flours used in this study were derived from Australian cultivars of diverse breadmaking quality. Cultivars Banks, Hartog, Rosella, Sunbri, Yanac, and a derivative of Osprey containing the 1BL/1RS translocation (Osprey derivative) were obtained from BRI Australia Ltd., North Ryde, NSW. A commercial bakers' flour blend (Queensland Bakers') comprising cvs. Janz and Cunningham was obtained from Weston Milling, Enfield, NSW.

Nonstarch lipids were extracted with chloroform (MacRitchie and Gras 1973). Gluten, starch, and water-soluble material were separated from these defatted flours by manual washing followed by freeze-drying (MacRitchie 1985). Glutenin- and gliadin-rich fractions were isolated from each gluten using hydrochloric acid precipitation at pH 5.3 for gliadin and pH 3.9 for glutenin (MacRitchie 1985). These methods cause minimal disruption to the functionality of the extracted components (MacRitchie 1985, Skerritt et al 1996). The secalins extracted with the gliadins. The glutenin and gliadin contents of each flour, gluten, glutenin- and gliadin-rich fractions were determined in triplicate by size-exclusion HPLC (Batey et al 1991). For the purposes of this article, glutenin was defined as Peak I and gliadin as Peak II as described by these authors.

The nitrogen contents of the flours, glutes, and glutenin- and gliadin-rich fractions were determined by the Dumas total combustion method using an elemental analyzer (CHN-1000, Leco Inc., St. Joseph, MI). Protein (%) was estimated as $N \times 5.7$. The identity of HMW-GS was determined by SDS-polyacrylamide gel electrophoresis (Laemmli 1970). The protein contents and the allelic composition of the HMW-GS of the flours are given in Table I.

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Altering Protein Content and Glutenin-to-Gliadin Ratio

Blends of each of the base flours, with gluten and starch isolated from that flour, were prepared. For each cultivar, blends of flour and gluten isolated from it were prepared to have 110, 120, and 130% of the protein content in the base flour. Formulations containing 70, 80, and 90% of the protein of the parent flour were prepared by blending the flour with isolated starch. Each of the test mixtures was prepared so the glutenin-to-gliadin ratio was identical to the value of the parent flour.

Gluten, glutenin, or gliadin prepared from the parent flour was added to the flour to vary the glutenin-to-gliadin ratio, and protein content was kept constant at 120% of that of the parent flour.

The glutenins and gliadins extracted from Banks, Hartog, Osprey derivative, and Queensland Bakers' flours were added to a base flour (Queensland Bakers' flour) to vary both the glutenin-to-gliadin ratio and the subunit composition of the gliadin-glutenin. In this experiment, the protein content was kept constant at 120% of the base flour value.

Mixing

All formulations were mixed on a 2-g mixograph (TMCO, Lincoln, NE). Mixing trials were conducted using water absorptions estimated by Approved Methods (AACC 1995) using the calculated protein and moisture contents of the blend. The quantities of flour and water thus estimated were scaled to provide a constant 3.5 g of dough. Mixing was done in triplicate and the mean time to peak dough development was calculated (Gras et al 1990). Parameters recorded were mixing time (MT [sec]), mixograph peak resistance (PR [AU]), and resistance breakdown (RB [%]).

Extension Testing

Doughs for extension testing were mixed to peak dough development in a 2-g mixograph using 3.5 g of dough. Extension was done in duplicate on a microextension tester with a 19-mm gap and 6-mm hook operating at 1 cm/sec. Dough samples for extension testing (1.7 g/test) were molded into cylinders (\approx 6 mm diameter) with a prototype mold. They were then mounted on a sample carrier

and rested at 30°C and >90% rh for 45 min before extension testing (Gras and Bekes 1996). 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 (cm) were calculated using specially written software (Rath et al 1994).

Microbaking

Doughs were mixed to optimum development in the 2-g mixograph. The formulation used flour including the added fraction (100 parts), water (as calculated), salt (2 parts), fresh yeast (2.5 parts) and improver (0.5 parts). Loaves were prepared from 2.4 g of the resulting dough which was molded, rested for 20 min at 40°C, remolded, proofed for 45 min at 40°C and 90% rh, and baked at 200°C for 17 min (Gras and Bekes 1996). Loaf height (LH) was measured with vernier calipers. Baking tests were done in triplicate.

Statistical Analysis

Statistical analyses were made by analysis of variance, analysis of covariance, and regression analysis using the MSUSTAT package of computer programs (version 4.1) (Richard E. Lund, Montana State University, Bozeman, MT) and SuperAnova v1.11 (Abacus Concepts Inc., Berkeley, CA).

RESULTS AND DISCUSSION

Functionality of flour components isolated in this study has been checked by reconstitution and, as previously observed (MacRitchie 1985, Skerritt et al 1996), replacement of gluten by approximate mixtures of isolated gliadin and glutenin did not show significant differences in any dough characteristics, indicating that functional properties of the isolated components did not change significantly during sample preparation. Separation of the effect of protein quantity from the effect of glutenin-to-gliadin ratio required the isolated gluten to have the same glutenin-to-gliadin ratio as the parent flour, and within the limits of experimental error, this was found to be so (Table I).

TABLE I
Protein Content, Allelic Composition of HMW-GS, and Glutenin-to-Gliadin Ratios of Seven Flours

Sample	Protein Content (%)	Allelic Composition of HMW-GS			Glutenin-to-Gliadin Ratio		Ratio of HMW-GS to LMW-GS
		1A	1B	1D	Flour	Gluten	
Banks	13.0	2*	7+8	2+12	1.25	1.29	0.28
Hartog	12.4	1	17+18	5+10	1.25	1.27	0.30
Osprey derivative	15.8	2*	7+8	2+12	0.88	0.91	0.62
Queensland Bakers'	11.2	1	17+18	2+12	1.30	1.31	0.31
Rosella	8.2	2*	7+8	2+12	1.56	1.58	0.28
Sunbri	14.7	1	7+8	2+12	1.24	1.20	0.33
Yanac	9.2	2*	20	2+12	1.36	1.37	0.30
LSD ($P < 0.05$)					0.05	0.05	0.02

TABLE II
Mixing, Extension, and Baking Parameters^a for Mixtures of Queensland Bakers' Flour and Isolated Glutenins and Gliadins^b

Sample Added to Queensland Bakers' Flour	Glutenin-to-Gliadin Ratio	MT (sec)	PR (AU)	RB (%)	R_{max} (N)	Ext (cm)	LH (mm)
QGLUTEN	1.30	226.7d ^c	442.0cd	13.00b	0.270c	12.63b	35.13cd
QGLUTENIN	1.39	255.5e	419.5a	15.00c	0.368e	12.23b	36.39ef
QGLIADIN	0.97	153.3ab	441.7cd	22.00d	0.223b	14.44cd	34.33cb
BGLUTENIN	1.51	249.3e	428.0cb	13.50cb	0.580f	10.67a	35.73ed
BGLIADIN	0.94	150.7a	452.7ed	23.67ed	0.196ab	15.16d	34.56cb
HGLUTENIN	1.37	277.0f	424.3b	9.75a	0.560f	10.24a	36.47f
HGLIADIN	0.86	156.7ab	429.7cb	31.00g	0.171a	13.82c	32.71a
OGLUTENIN	1.37	192.0c	467.0e	25.00ef	0.322d	11.95b	35.47d
OGLIADIN	0.90	166.3b	451.7ed	26.67f	0.179a	12.67b	34.26b
LSD ($P < 0.05$)		13.18	17.03	1.88	0.035	1.01	0.84

^a Mixing time (MT), mixograph peak resistance (PR), resistance breakdown (RB), maximum resistance to extension (R_{max}), extensibility (Ext), loaf height (LH).

^b Source: Queensland Bakers' (Q), Banks (B), Hartog (H), Osprey derivative (O).

^c Values followed by the same letter are not significantly different ($P < 0.05$).

Protein Quantity

The variation of mixing time as a function of protein quantity was very dependent on the cultivar and on the protein level (Fig. 1a). In Queensland Bakers', Sunbri, and Rosella, increasing the protein content from a low value caused an increase in mixing time, but there was less effect at the highest protein contents. In Osprey derivative, Banks, and Yanac, increases in protein content had little effect at low protein content, but at the higher levels (120 and 130% of parent flour) mixing time did increase. Hartog flour showed much higher mixing time than the other flours.

Data points for all samples but Hartog showed a consistent linear-positive relationship between protein and mixograph peak resistance (Fig. 1b). For these six flours, the increase of mixograph peak resistance with protein content was slightly curved, with less effect seen at the highest protein contents. In Hartog, however, the effect of protein content on mixograph peak resistance was small and reached its maximum at intermediate protein content. This implies that there was cultivar-specific variation for mixograph peak resistance superimposed on a more general relationship between protein content and mixograph peak resistance.

The effect of protein content on resistance breakdown differed among the flours (Fig. 1c). Banks, Osprey derivative, and Rosella flours showed a general increase in resistance breakdown with increase in protein content. Queensland Bakers' and Sunbri showed an initial decrease in resistance breakdown followed by an increase, while Hartog and Yanac showed a consistent decrease in resistance breakdown with increasing protein content.

Both the maximum resistance to extension and extensibility (Ext) increased monotonically with increases in protein content (Fig. 1d and e). The slope and intercept values for the linear approximation of these relations varied from cultivar to cultivar, suggesting that mean resistance and response to total protein content were both genetically determined.

The quality of loaves as measured by loaf height showed a significant positive relationship with protein content for each sample (Fig. 1f and Fig. 2), in agreement with previous observations on loaf volume (Aitken and Geddes 1934, Harris and Sibbitt 1942, Finney 1943, and many subsequent articles).

Variation in Glutenin-to-Gliadin Ratio at Constant Protein Content

Increases in the proportion of glutenin in the blend generally increased mixing time (Fig. 3a), except in Sunbri at the highest glutenin level where the decrease was not significant. Changes in the glutenin-to-gliadin ratio had little or no effect on mixograph peak resistance (Fig. 3b), which seemed to depend solely on the cultivar and protein content, at least for the range in glutenin-to-gliadin composition used in this study.

Increasing the glutenin-to-gliadin ratio mostly led to reduced resistance breakdown (Fig. 3c) or increased tolerance to overmixing or stability. The size of this change varied widely and significantly from one cultivar to another (Fig. 3c). This may imply that resistance breakdown is genetically determined. It may also reflect increased transfer of mixing energy, and thus resistance break-

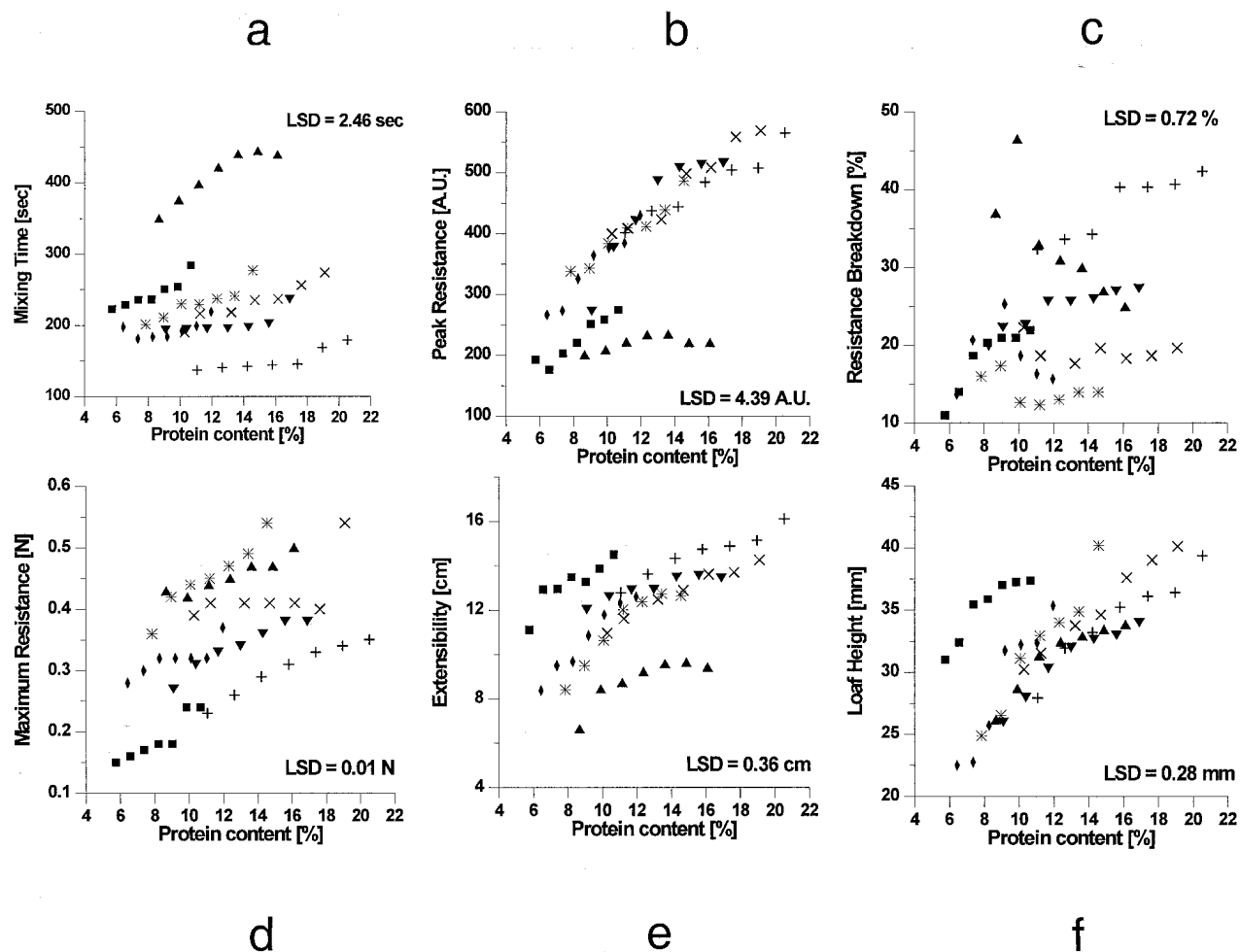


Fig. 1. Mixing, extension and baking properties of seven wheat cultivars with protein content altered by the addition of gluten or starch from the cultivar to provide 70, 80, 90, 100, 110, 120, and 130% of the protein in the parent flour. a) Mixing time; b) mixograph peak resistance; c) resistance breakdown; d) maximum resistance to extension; e) extensibility; and f) loaf height. Banks (▼), Hartog (▲), Osprey derivative (◆), Queensland Bakers' (*), Rosella (■), Sunbri (X), and Yanac (♦).

down, in the stronger doughs because the height of the mixograph curve represents a direct measure of the transfer of energy through the dough. Increases in glutenin-to-gliadin ratio were associated with increases in maximum resistance to extension in all cases (Fig. 3d).

The increase in resistance was consistent with the view that increases in net average molecular weight of dough protein increases dough strength (MacRitchie 1992). Increase in the glutenin-to-gliadin ratio was generally associated with a decrease in extensibility (Fig. 3e),

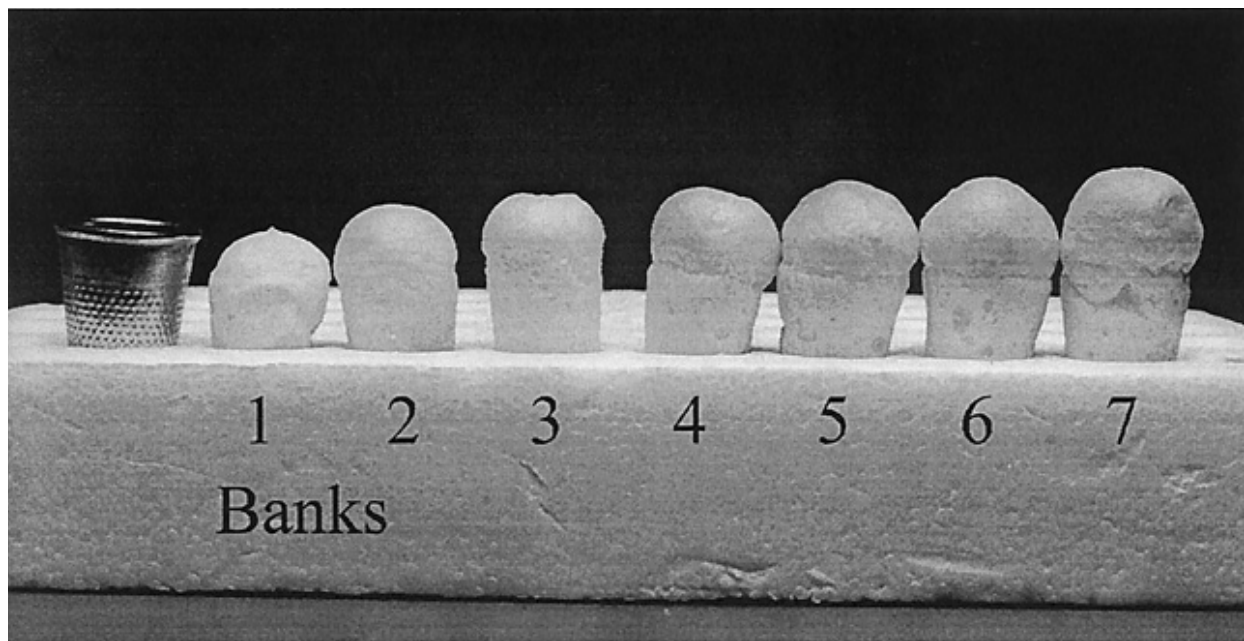


Fig. 2. Microbaked loaves from wheat cultivar Banks with protein content altered by the addition of gluten or starch from the cultivar to provide 70, 80, 90, 100, 110, 120, and 130% of the protein in the parent flour.

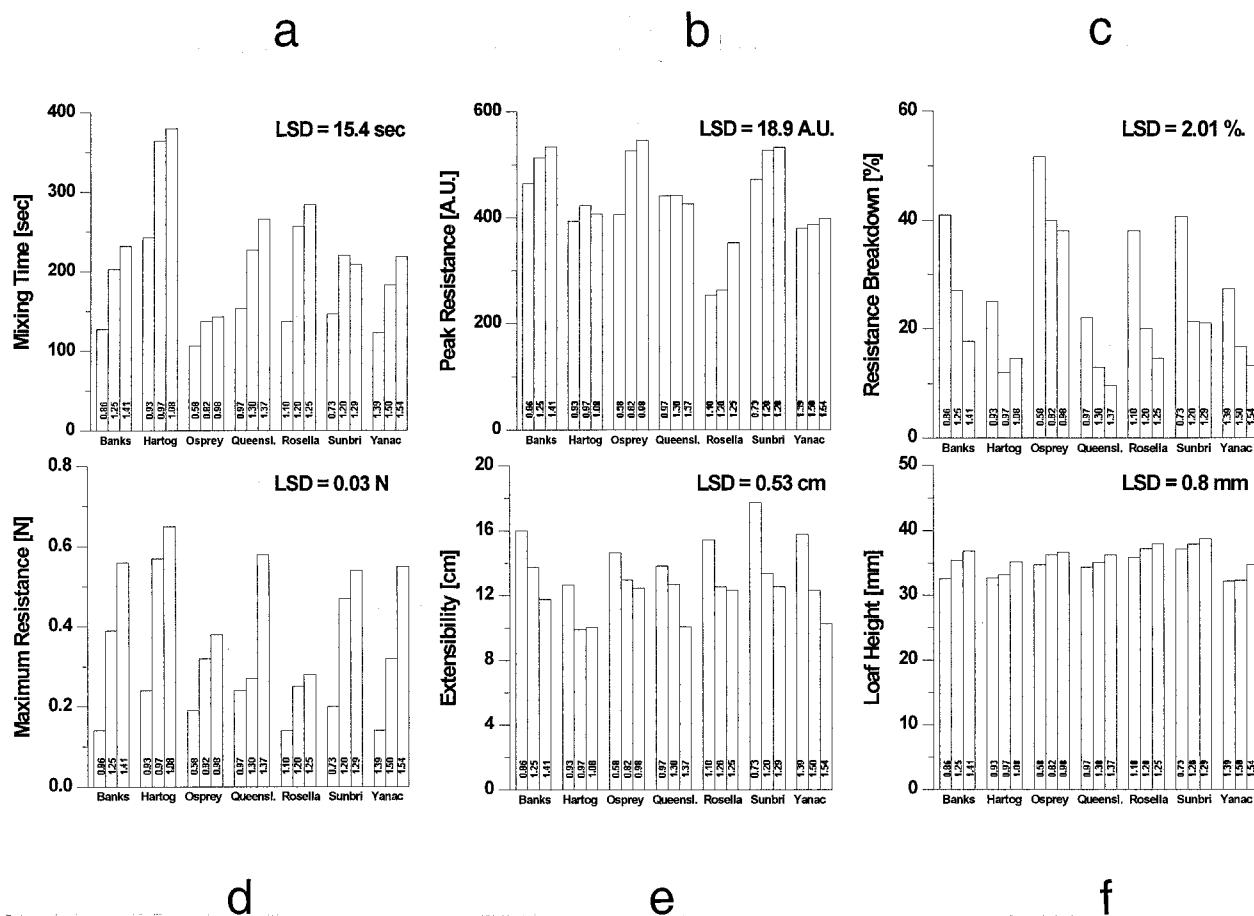


Fig. 3. Mixing, extension, and baking properties of seven wheat cultivars with glutenin-to-gliadin ratio altered by adding gluten, glutenin, or gliadin. a) Mixing time, b) mixograph peak resistance, c) resistance breakdown, d) maximum resistance to extension, e) extensibility, and f) loaf height. Values inside bars represent glutenin-to-gliadin ratios.

with the exception of Hartog at the highest glutenin level where the increase was not significant.

Increases in glutenin-to-gliadin ratio consistently showed significant increases in loaf height (Fig. 3f and Fig. 4) as previously reported for loaf volume (MacRitchie 1987). For the production of pan bread, the most important quality characteristic of any flour is its ability to produce bread of good volume. These results show that increases in glutenin-to-gliadin ratio consistently improved flour quality for this purpose, but if it is increased beyond the ranges used in this experiment (0.58–1.55), it may reach a point where the dough is too strong for breadmaking. From a practical viewpoint, the resulting increases in mixing time might make such large increases in glutenin proportion impractical.

Addition of Glutenins and Gliadins to a Base Flour

In this experiment, both the glutenin-to-gliadin ratio and the composition of the glutenin and the gliadin fractions were varied while keeping the absolute amount of protein constant. In almost every case, increases in the proportion of glutenin in the base flour increased mixing time (Table II), as observed when glutenins from the same flour were used. The extent of the change in mixing time was largely dependent on the final glutenin-to-gliadin ratio. As when gliadin and glutenin fractions were added to the parent flours, the relation between mixing time and glutenin-to-gliadin ratio was approximately linear, with the exception of the points corresponding to glutenin from Osprey derivative.

Mixograph peak resistance was slightly but significantly reduced when glutenin from Banks or Hartog was used, but increased when glutenin from the Osprey derivative was used (Table II). It is notable that this increase resulted from the addition of a fraction that had a higher amount of HMW-GS than glutenins from Banks, Hartog, and Queensland Bakers' flour (Table I). Differences in mixograph peak resistance resulting from admixture with gliadin fractions were not statistically significant.

Increases in the proportion of gliadins increased the resistance breakdown regardless of the gliadin source (Table II). The addition of the glutenins resulted in no change in resistance breakdown,

except for Osprey derivative glutenin, which was atypical and increased resistance breakdown. Thus, tolerance to overmixing or stability decreased with decreased glutenin-to-gliadin ratio as observed when the glutenin-to-gliadin ratio was altered with gliadin from the base flour.

In every case (Table II), increases in the proportion of glutenins in the test mixtures were negatively correlated with extensibility ($r = -0.69$) and positively correlated with maximum resistance to extension ($r = 0.84$). The trend line between maximum resistance and glutenin-to-gliadin ratio was nonlinear, and the point associated with Hartog glutenin appeared to be atypical. For extensibility, the relation appeared to be linear, although the points due to Hartog and Osprey derivative glutenins appeared atypical. The results imply that in wheat breeding, emphasis on the proteins that will increase the glutenin-to-gliadin ratio (or net molecular weight) at some desired protein level will result in flours that have stronger doughs but reduced extensibility.

Increases in glutenin contents at constant protein content were also associated with increases in loaf height (Table II), as observed when glutenin-to-gliadin ratio was altered with glutenin- and gliadin-rich fractions of the same flour.

The use of glutenin- and gliadin-rich fractions from various sources in one base flour (Queensland Bakers') confirmed the trends observed when glutenin- and gliadin-rich fractions were added to the parent flours. In particular, the relationships between glutenin-to-gliadin ratio and extensibility and maximum resistance to extension were strongly confirmed. It was also apparent that protein content and glutenin-to-gliadin ratio together were not sufficient to fully describe the observed behavior. It is perhaps no coincidence that those samples which behaved atypically in this study were those where either the quantity (ratio of HMW-GS to LMW-GS in Osprey derivative) or the quality (allelic composition of the HMW-GS in Hartog) in the glutenin fraction were particularly different from the remainder of the samples. It seems that both the monomeric and polymeric protein fractions have their own influence on functional properties. Investigation of the effects of the compositions of each of these monomeric and polymeric protein fractions

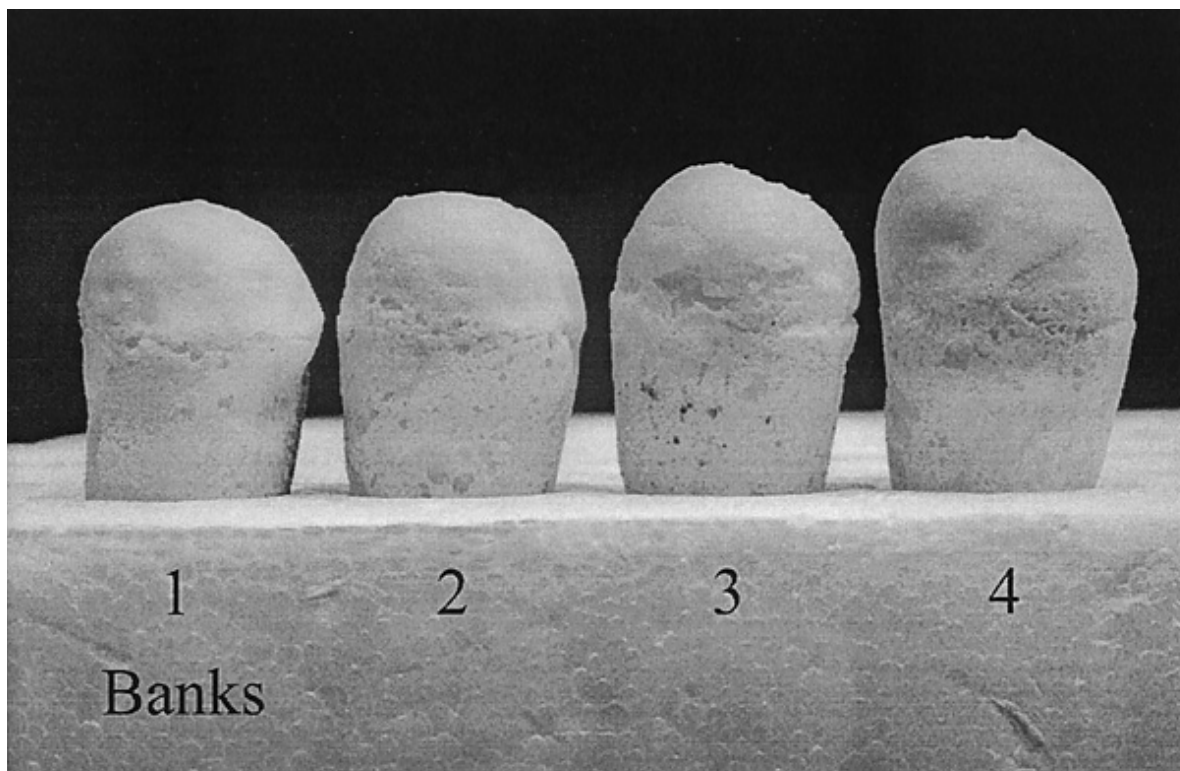


Fig. 4. Microbaked loaves from wheat cultivar Banks. 1) Parent flour; 2) flour plus gliadin; 3) flour plus gluten; 4) flour plus glutenin. Protein content of 2–4 equal to 120% of 1.

is in progress using the concepts described above (systematically changing one parameter at a time while keeping the remainder constant).

CONCLUSIONS

The protein content and glutenin-to-gliadin ratio (a measure of molecular weight distribution or protein size) have different roles in determining the various dough and bread quality parameters. Increases in the protein content at constant glutenin-to-gliadin ratio increased mixing time, mixograph peak resistance, extensibility, maximum resistance to extension, and loaf volume. Increases in glutenin-to-gliadin ratio at fixed protein content increased mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume. Increases in glutenin-to-gliadin ratio decreased resistance breakdown and extensibility. Differences in the quality observed from flour to flour are thus determined, in part, by a superimposition of the effects of protein content and glutenin-to-gliadin ratio. These two effects are not sufficient to fully describe the structure-function relationships in dough, and further work is required to determine the effects of the chemical composition on functional properties.

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