

## Effects of Gliadin Fractions on Functional Properties of Wheat Dough Depending on Molecular Size and Hydrophobicity

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### ABSTRACT

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The effects of  $\alpha$ - +  $\beta$ -,  $\gamma$ -,  $\omega$ - and total gliadins on mixing, extension baking, and techno-functional properties of doughs from hard and soft flours were measured using small-scale techniques. The addition of all gliadin fractions resulted in decreased mixing time, peak resistance, maximum resistance to extension, and loaf height, and in increased resistance breakdown and extensibility. The various gliadin fractions showed differences in functional properties, with  $\gamma$ -gliadin reducing the mixing

time and maximum resistance to extension to the greatest extent,  $\omega$ -gliadin contributing to the greatest reduction in loaf height, and  $\alpha$ - +  $\beta$ -gliadins having the least effect on reducing loaf height. The effects of gliadin fractions on loaf height were correlated with molecular mass, and effects on mixing time, maximum resistance to extension, and extensibility were correlated with hydrophobicity.

The gliadins, which comprise  $\approx$ 50% of the gluten, are alcohol-soluble monomeric proteins and interact by hydrogen bonding and hydrophobic interactions (Tatham and Shewry 1995). Gliadins can be classified into  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\omega$ -fractions based on mobility on an acid-polyacrylamide gel (pH 3.1) (Woychik et al 1961). The  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins are sulfur-rich and the  $\omega$ -gliadins are sulfur-poor (Shewry and Tatham 1997). Gliadin-supplemented doughs generally have a shorter mixing time, greater resistance breakdown, lower maximum resistance to extension, and decreased loaf volume (MacRitchie 1987; Fido et al 1997; Uthayakumar et al 1999).

Correlations between gliadin pattern and functionality are not as easily determined as in the high molecular weight glutenin subunits (HMW-GS), because gliadins are encoded by large multigene families that are inherited as tight linkage groups, so the effects overlap (Fido et al 1997). Furthermore, the genes for low molecular weight glutenin subunits (LMW-GS) are tightly linked with those for the  $\gamma$ - and  $\omega$ -gliadins. In correlation and reconstitution studies, gliadin fractions, in general, show the same effects on dough functionality as the total gliadin (Branlard and Dardevet 1985; Dong et al 1992; van Lonkhuijsen 1992; Fido et al 1997). Contrasting results have occasionally been found; for example,  $\gamma$ -gliadin had positive effects on loaf quality (van Lonkhuijsen et al 1992; Weegels et al 1994). The conflict in the results may be because most studies were correlative and, hence, possibly confounded by the LMW-GS composition. This problem is avoided by the direct approach of adding the gliadin to a base flour.

When gliadin is incorporated into a flour by first partially reducing the dough to allow the added proteins access into the gluten matrix, then reoxidizing it to include them in the polymer (Bekes et al 1994), it behaves the same way as when added directly into a

flour (Murray et al 1999). Thus, experiments with gliadin may be conducted by simple addition rather than a complex incorporation procedure. The aim of this study was to determine the roles of individual gliadin components on mixing, extension baking, and techno-functional properties of wheat dough.

### MATERIALS AND METHODS

The base flours were from cv. Banks, a medium-strength hard wheat with 13% protein, and cv. Rosella, a weak soft wheat with 8.2% protein, provided by BRI Australia Ltd., North Ryde, NSW.

Gliadin fractions (total,  $\alpha$ - +  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadins) were prepared as described by Fido et al (1997). Total gliadins were extracted from cv. Hereward by 70% aqueous ethanol as described by Shewry et al (1983). The fractions were separated by ion-exchange chromatography on carboxy-methyl cellulose, using 3M urea, 0.01M glycine acetate buffer, pH 4.6, and were eluted with a linear gradient of NaCl (Booth and Ewart 1969). The fractions were dialyzed against 1% (v/v) acetic acid at 4°C for 60 hr and freeze-dried.

Acid (pH 3.1) polyacrylamide gel electrophoresis (A-PAGE) was used to identify the gliadin fractions and to check purity (Tkachuk and Mellish 1980). The identification of the alleles followed the system proposed by Metakovsky (1991) and the *Gli-1* genotype of Banks was *Gli-A1g*, *Gli-B1b*, *Gli-D1a*, that of Rosella *Gli-A1g*, *Gli-B1b\**, *Gli-D1b*, and that of Hereward *Gli-A1b*, *Gli-B1a*, *Gli-D1b*. The *Gli-B1b\** allele differs from the *Gli-B1b* allele only by the absence of one band. The  $\alpha$ - and  $\beta$ -gliadins coded by *Gli-A2* were from the same family and the other two *Gli-2* loci were similar in the three cultivars. The HMW-GS composition of these cultivars has been given previously (Uthayakumar et al 1999). The purity of the gliadin fractions was also checked on SDS-PAGE (Laemmli 1970).

Three different gliadin components were determined based on hydrophobicity by reversed-phase high performance liquid chromatography (RP-HPLC) (Marchylo et al 1989). They corresponded to  $\omega$ -,  $\alpha$ - +  $\beta$ - and  $\gamma$ -gliadins, respectively.

The nitrogen contents of the flours and gliadins were determined by the Dumas total combustion method using a CHN-1000 elemental analyzer (Leco Inc., St. Joseph, MI). Protein (%) was estimated as  $N \times 5.7$ . The protein contents of the gliadins were  $89 \pm 2\%$ . The moisture contents were estimated using a flour-moisture calibrator (Infraalyzer 400, Technicon Instruments Corporation, Tarrytown, NY).

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Blends of each of the base flours were prepared with 10 mg of gliadin fraction and 2 g of flour. For the control, 10 mg of total gluten was added so the protein content remained constant in all blends in the same base flour. This amount was chosen to give a clearer effect than the 2–6 mg used by Fido et al (1997).

All formulations were mixed on a 2-g mixograph (TMCO, Lincoln, NE). Mixing trials were conducted using water absorptions estimated by Approved Methods (AACC 2000) from the protein and moisture contents of the blend. Mixing was done in triplicate, and the mean time to peak dough development was calculated (Gras et al 1990). Parameters recorded were mixing time (sec), peak resistance (AU), and resistance breakdown (%). Doughs for extension testing were mixed to peak dough development in a 2-g mixograph. Extension measurements were made in quadruplicate 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 sample carrier position were taken at 100 readings/s and recorded by a personal computer using Lab-Tech Notebook software. Maximum resistance to extension (N) and extensibility (cm) were calculated using specially written software (Rath et al 1994). Doughs for microbaking 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 (mm) was measured with vernier calipers. Baking tests were performed in triplicate.

Emulsifying activity index was measured by the turbidimetric method according to Pearce and Kinsella (1978). Emulsifying stability index was measured by the conductometric procedure (Kato et al 1985). Foaming properties were determined by the conductometric method of Tömösközi and Pungor (1993). Casein was included as a reference protein.

Statistical analyses of variance and covariance used MSUSTAT v 4.1 (Richard E. Lund, Montana State University, Bozeman, MT) and Super-Anova v 1.11 (Abacus Concepts Inc., Berkeley, CA).

## RESULTS

The electrophoretic mobility (A-PAGE) of the gliadin fractions isolated using ion-exchange chromatography (Fido et al 1997) was similar to that of the native fractions, indicating that the isolation procedure did not lead to major structural alteration of the gliadin fractions.

All gliadin fractions reduced mixing time, peak resistance, maximum resistance to extension, and loaf height, and increased resistance breakdown and extensibility, but the amount of change depended on the fraction (Fig. 1). The total gliadin extract generally gave an effect between the minimum and maximum given by individual fractions, as would be expected, except that it had less effect than the individual fractions on peak resistance in Banks and on loaf height in Rosella and more effect on maximum resistance to extension in Rosella.

The  $\gamma$ -gliadin fraction had the most effect on mixing time and extensibility in both cultivars and on peak resistance and maximum resistance to extension in Banks, but had the least effect on resistance breakdown in both cultivars. The  $\omega$ -gliadin fraction had the most effect on loaf height in both cultivars and on resistance breakdown in Rosella, while it had the least effect on maximum resistance to

TABLE I  
Emulsifying Properties of Gliadin Fractions

Fraction	Emulsion Activity (cm <sup>2</sup> /g)	Emulsion Stability Index (mS/min)	Foam Power (mS/min)	Foam Stability Index (mS/min)
Total gliadin	4.91	3.81	0.82	2.40
$\alpha$ - + $\beta$ - Gliadin	6.56	4.39	1.18	2.52
$\gamma$ -Gliadin	6.99	4.44	1.74	4.08
$\omega$ -gliadin	4.78	3.85	0.96	3.26
Casein	10.05	8.20	2.50	7.60
Standard error	0.09	0.42	0.003	0.28

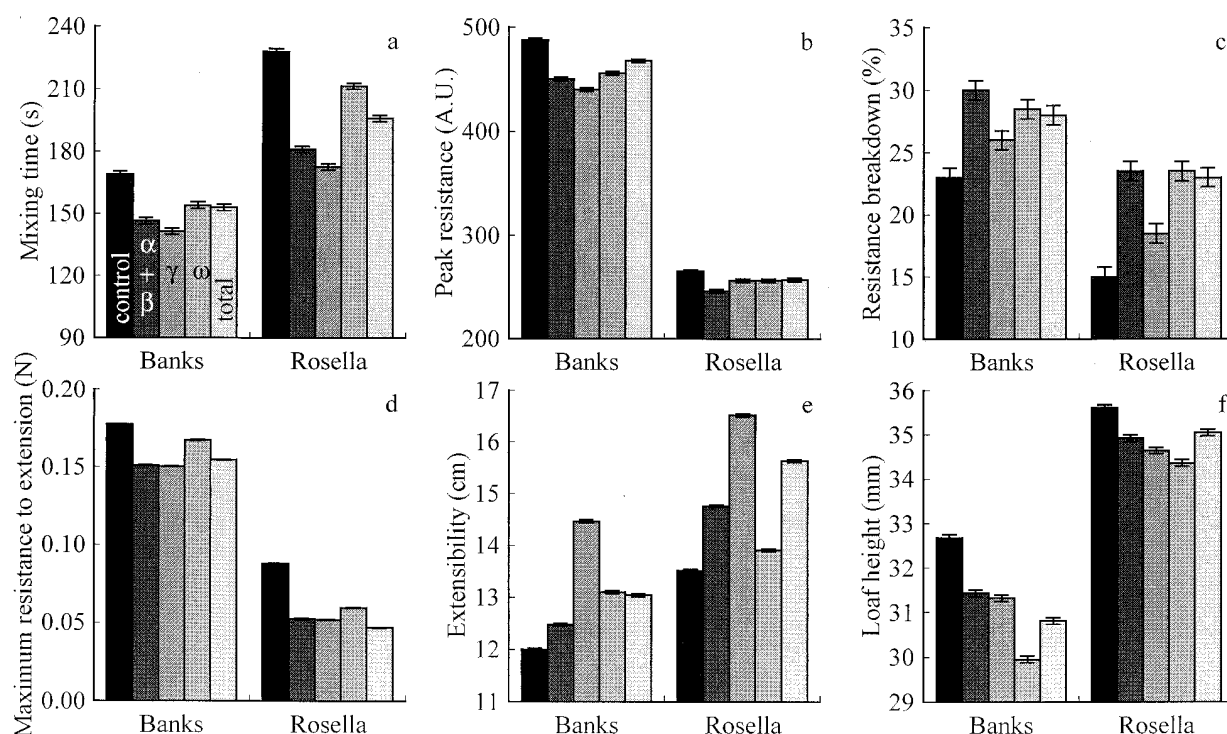


Fig. 1. Effect of gliadin fractions on a) mixing time, b) peak resistance, c) resistance breakdown, d) maximum resistance to extension, e) extensibility and f) loaf height of two base flours, Banks and Rosella. Left to right: control,  $\alpha$ - +  $\beta$ -,  $\gamma$ -,  $\omega$ - and total gliadin. Error bars  $\pm$  1 standard error.

extension and mixing time in both cultivars and on extensibility in Rosella. The  $\alpha$ - +  $\beta$ -fraction had the most effect on resistance breakdown in Banks and on both resistance breakdown and maximum resistance to extension in Rosella, whereas it had the least effect on loaf height in both cultivars and on extensibility in Banks.

The  $\gamma$ -fraction showed the greatest emulsion stability and activity, foam stability, and activity (Table I). The total gliadin had poorer emulsion stability, foam stability, and foam activity than its component fractions. Casein, a widely used reference protein, had foam and emulsion properties superior to any of the gliadin fractions.

Although there were only four points to test, a few correlations between foam or emulsion properties and mixing or extension properties were significant. Emulsion activity, emulsion stability, and foaming activity were all negatively correlated with mixing time in Banks ( $r^2 > 0.91$ ,  $P < 0.05$ ) and in Rosella ( $r^2 > 0.82$ ,  $0.05 < P < 0.10$ ). Foam stability was positively correlated with extensibility in Rosella ( $r = 0.964$ ,  $P < 0.05$ ) but not significantly in Banks ( $r = 0.872$ ).

## DISCUSSION

We have previously shown that the addition of total gliadin reduced mixing time, peak resistance, maximum resistance to extension, and loaf height, and increased resistance breakdown and extensibility (Uthayakumaran et al 1999). The results presented here showed that three fractions of the total gliadin acted in the same direction and that there were consistent features to the order of effects. The  $\alpha$ - +  $\beta$ -gliadin fraction was least detrimental to loaf height and gave a useful reduction in mixing time, but also introduced a great increase in resistance breakdown. Fido et al (1997) also showed that all gliadin fractions had a negative effect on mixing time and peak resistance and a positive effect on extensibility.

Each of the gliadin fractions was consistent in molecular weight range and in hydrophobicity. The approximate average molecular masses of the gliadins have been reported as 31,000 for  $\alpha$ - and  $\beta$ -gliadin, 35,000 for  $\gamma$ - gliadin, and 40,000–70,000 for  $\omega$ -gliadin (Fido et al 1997). The negative effects of the gliadin fractions on loaf height followed this sequence, in both base flours, but none of the other parameters did. When Khatkar and Schofield (1996) enriched Hereward flour with gliadin fractions isolated from the same flour, they also found that the negative effects of gliadins on mixing time increased with the size of the gliadin proteins. They further found that the peak dough resistance and loaf volume increased with the addition of different gliadin types, in contrast to most other results.

Banks and Rosella had the same *Gli-A1* and similar *Gli-B1* alleles but different *Gli-D1* alleles. The source of the gliadin fractions (cv. Hereward) had different alleles from both base flours at the *Gli-A1* and *Gli-B1* loci, but the same as Rosella at the *Gli-D1* locus. All three flours had similar *Gli-2* compositions. Hence, the gliadins of these flours were substantially similar and, in most cases, the addition of gliadin fractions gave similar results.

The order of gliadin hydrophobicity (measured by RP-HPLC) is  $\omega$ - <  $\alpha$ - and  $\beta$ - <  $\gamma$ -fraction (van Lonkhuijsen et al 1992; Weegels et al 1994). In the present study, as hydrophobicity increased, mixing time and maximum resistance to extension (in both cultivars) and peak resistance (in Banks) decreased, while extensibility increased (in Rosella). In addition, foam stability and emulsification properties followed this sequence. Fido et al (1997) found that the effects on mixing time followed the order of hydrophobicity, but in the opposite direction to that found here. Both Fido et al (1997) and the present study found that  $\gamma$ -gliadins were responsible for greatly reducing peak resistance and maximum resistance to extension and increasing extensibility. Fido et al (1997) used 2–6 mg of gliadin, extracted from Chinese Spring and added to two commercial flour blends, in contrast to the 10 mg added to two individual-cultivar flours used here. Differences in effect due to cultivar may be associated with the large differences in protein content (Gupta et al 1994) as well as the allelic composition of the gliadins (Metakovsky et al 1997).

Positive correlations of  $\gamma$ -gliadins with dough strength (Branlard and Dardevet 1985), dough resistance (Campbell et al 1987), mixing tolerance (Dong et al 1992), and breadmaking (van Lonkhuijsen et al 1992) have been reported, but the genotypes of the LMW-GS were not determined, which may have confounded these results. Weegels et al (1994) found positive effects when  $\gamma$ -gliadins were added to doughs, but the purity of the fractions has been questioned (Fido et al 1997) and the gliadin fractions used in the present study were similar to those of Fido et al (1997). Some individual  $\alpha$ - and  $\beta$ -gliadin bands also correlated positively with several quality traits (Branlard and Dardevet 1985), but again, that study relied on correlations rather than adding fractions to a base or reconstituted flour.

Biscuit making requires highly extensible soft wheats (Lupton and Derera 1981). In the present study, gliadins, especially the  $\gamma$ -fraction, contributed to higher extensibility. Therefore, flours with enhanced levels of these components may be expected to perform better as biscuit flours. The extensive literature on biscuit (cookie) making makes little reference to gliadin composition. This may be a promising new direction for experimentation. In Japan, palatability of boiled noodles was positively correlated with resistance to extension and extensibility (Eguchi et al 1981). Lin et al (1994) showed that extensibility was related to quality of Chinese dried noodles but resistance was not. Increasing the amount of gliadin in the flour, especially the  $\omega$ -gliadin, would increase extensibility, but reduce maximum resistance to extension to a lesser extent.

Previous emulsion studies on gliadins have also shown that the  $\omega$ -fraction exhibited the poorest emulsifying properties and that there was little difference between the emulsion properties of the  $\alpha$ - +  $\beta$ -fraction and  $\gamma$ -fraction (Popineau and Pineau 1993). This comparison mirrored the effect on loaf height and raises the question of a common basis. Foaming properties of the  $\alpha$ - +  $\beta$ -fraction and  $\gamma$ -fraction were well distinguished in the present study. Nevertheless, because there are only four classes of gliadin, it is difficult to draw conclusions from correlations. Examination of individual gliadin bands, or of classes of gliadins with widely differing properties (e.g.,  $\omega$ -gliadins from highly divergent wheats), may give the additional degrees of freedom necessary to detect a common basis between the surface activity demonstrated in foaming or emulsification and that shown in breadmaking.

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