Effects of Increased High Molecular Weight Glutenin Subunits Content of Flour on Dough Mixing Behavior and Breadmaking

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The level of high molecular weight glutenin subunits (HMW-GS) in Soissons flour was increased with isolated Soissons HMW-GS (1.0−4.0 mg/g of flour) by either simple addition or using a previously developed method (Békés et al. *Cereal Chem*. **1994**, *71*, 44−50) for incorporation of monomeric glutenin subunits into flour endogenous glutenin polymers. It was demonstrated that the incorporation method, originally developed for Mixograph tests, is equally well-suitable for straight dough breadmaking tests, allowing direct extension of the effect of incorporated glutenin subunits on dough Mixograph mixing properties to their effect on loaf volume. Although the expected dough strengthening effect (increased mixing requirement and tolerance to overmixing) upon incorporation of HMW-GS was indeed clear from the Mixograms, the improved dough properties did not lead to higher loaf volumes. Furthermore, simple addition of unalkylated HMW-GS also strengthened the dough. On the other hand, alkylated Soissons HMW-GS did not improve dough mixing properties. The different effects of alkylated and unalkylated HMW-GS may suggest that even with simple addition unalkylated HMW-GS can be incorporated into flour endogenous glutenin polymers or may point to the importance of free sulfhydryl groups during dough mixing.

Keywords: *Breadmaking; dough mixing; high molecular weight glutenin subunits*

INTRODUCTION

Hydrated wheat gluten proteins have viscoelastic properties that are essential for breadmaking. The viscous properties are generally ascribed to the monomeric gluten proteins (gliadins), while the polymeric gluten proteins (glutenin) are responsible for strength and elasticity. Glutenin polymers consist of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) linked by interchain disulfide bonds. Indirectly, the effect of different protein fractions on breadmaking performance can be evaluated by correlation studies, whereby the quantity and/or presence of a certain fraction is correlated with a quality parameter. In addition, direct assessment of the importance of a particular protein fraction is possible by supplementing a base flour with such protein fraction and evaluating its effect on dough mixing properties and/or breadmaking performance. However, in the case of the glutenin subunits, it is reasonable to assume that simple addition does not suffice, since they do not exert their functional role as monomeric proteins but as part of a polymeric structure. To solve this problem, Békés and co-workers (1994a) devised a method to incorporate glutenin subunits in the native glutenin polymers of flour during the initial stages of dough mixing. Thus, the glutenin structure is first reversibly disrupted by reduction. This allows added glutenin subunits to be incorporated during oxidative restoration. It seems indeed logical that a partially disrupted glutenin structure is an ideal matrix for incorportion of glutenin subunits. Reducing agent (first) and oxidant (second) are mixed with the flour and glutenin subunits during the initial stages of a Mixogram recording with sufficient resting times for reduction and reoxidation (Békés et al., 1994a,b). In such an 'incorporation' protocol, HMW-GS increased dough mixing requirement and tolerance to overmixing significantly (Békés and Gras, 1994; Békés et al., 1994b; Sapirstein and Fu, 1996). The effect of LMW-GS is, however, less clear. LMW-GS were also found to strengthen dough, although their effect was somewhat lower than that of HMW-GS (Sissons et al., 1998). On the other hand, Sapirstein and Fu (1996) observed only very little effect on dough mixing properties upon incorporation of LMW-GS.

In this study, we extended the proposed incorporation protocol to straight dough breadmaking experiments in order to evaluate the effect of an increased level of HMW-GS on breadmaking performance.

MATERIALS AND METHODS

Flour. Grain from wheat cultivar Soissons (1995 harvest) was conditioned 24 h at 14.5% moisture and milled (75% extraction rate) on a Bühler MLU-202 laboratory scale mill (Uzwil, Switzerland) according to AACC Method 26−31 (AACC, 1994). Soissons flour was used both as base flour for Mixograph recordings and breadmaking and for isolation of HMW-GS. The HMW-GS composition of Soissons was 2*, 7+8, and 5+10 [nomenclature according to Payne and Lawrence (1983)].

Isolation and Alkylation of HMW-GS. Flour (100 g) was extracted twice with chloroform (500 mL). The extract was removed by filtration, and residual flour was washed with small amounts of chloroform. Air-dried defatted flour was extracted with 50% (v/v) *n*-PrOH. In procedure 1, two 30-min extractions (500 mL) at 60 °C were used. In procedure 2, defatted flour was extracted at room temperature first with 500 mL (1 h), second with 300 mL (30 min), and third with 200 mL (30 min). This extraction sequence removes all

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monomeric proteins at room temperature (Fu et al., 1996). Residues were recovered by centrifugation (1500*g*, 10 min, 25 °C) and subsequently extracted with 50% (v/v) *n*-PrOH containing 5% (v/v) β-mercaptoethanol (β-ME), either once for 30 min at 60 °C with 500 mL (procedure 1) or twice for 1 h at room temperature with 333 mL (procedure 2). Suspensions were centrifuged (10000*g*, 30 min, 10 °C), and the *n*-PrOH concentration of the supernatants was adjusted to 60% (v/v) by slowly and under continuous stirring adding *n*-PrOH containing 5% (v/v) β-ME. HMW-GS were allowed to precipitate for 1 h at 7 °C and were recovered by centrifugation (10000*g*, 30 min, 10 °C). To prepare alkylated HMW-GS, precipitated HMW-GS were dissolved (1 h, 60 °C) in 0.082 M Tris-HCl buffer (pH 7.5) containing 50% (v/v) *n*-PrOH and 5% (v/v) β-ME (60 mL) and alkylated overnight at room temperature with 4-vinylpyridine (7.0 mL). Alkylated HMW-GS were recovered by increasing the *n*-PrOH concentration to 60% (v/v), precipitation at 7 °C for 1 h, and centrifugation (30 min, 10000*g*). Precipitated HMW-GS were washed three times with deionized water and subsequently dissolved in 0.01 M acetic acid (procedure 1) or dialyzed for 3 days at 7 °C against 0.01 M acetic acid (procedure 2). Finally, HMW-GS were lyophilized.

Analytical Methods. Moisture contents were determined (in duplicate) according to AACC Method 44-19 (AACC, 1994). The flour Farinograph water absorption was determined with a Farinograph (Brabender, Duisberg, Germany) equipped with a 50-g bowl according to AACC Method 54-21 (AACC, 1994). Salt-soluble protein, gliadin, and glutenin fractions were prepared according to a modified Osborne fractionation procedure (Chen and Bushuk, 1970).

Protein contents (N × 5.7) were determined (at least in duplicate) by the Kjeldahl method (Jones, 1991). The HMW-GS/LMW-GS ratio of Soissons flour was estimated from the weight fraction of glutenin subunits in the glutenin subunit extract that precipitated at 60% (v/v) *n*-PrOH. Purity of HMW-GS was checked with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) according to Laemmli (1970). HMW-GS (1.0 mg) were dissolved in 0.125 M Tris-HCl (pH 6.8) containing 1% (w/v) dithiothreitol (DTT), 2% (w/v) SDS, 30% (w/v) glycerol, and 0.002% (w/v) bromophenol blue (600 μL). For SDS−PAGE under nonreducing conditions, DTT was omitted from the buffer. Aliquots (30 μL) were separated on 10% (w/v) acrylamide, 0.1% (w/v) *N*,*N*′-bis-methyleneacrylamide slab gels (160 × 140 × 1.5 mm) using SE 600 Hoefer (Pharmacia Biotech., San Fransisco, CA) vertical electrophoresis equipment. Gels were stained overnight in deionized water containing 40% (v/v) methanol, 7.0% (v/v) acetic acid, and 0.025% (w/v) Coomassie brilliant blue R-250 and were destained in deionized water containing 5.0% (v/v) methanol and 7.0% (v/v) acetic acid. Carbohydrate contents/compositions of isolated HMW-GS were determined by gas−liquid chromatography (Englyst and Cummings, 1984). HMW-GS preparations (10.00 mg) were hydrolyzed for 1 h in 2.0 M sulfuric acid (270 μL) at 100 °C. Internal standard solution (β-D-allose, 1 mg/mL) (50 μL) was added to the cooled hydrolysates. Alditol acetates were prepared as described by Roels and Delcour (1996) and separated on a Supelco SP-2380 column (30-m length, 0.32-mm internal diameter, 0.2-μm film thickness) (Belleforte, PA) with He as carrier gas, using a Chrompack 9011 gas chromatograph (Middelburg, The Netherlands) equipped with a flame ionization detector. Separation temperature was 225 °C; injection and detection temperature was 275 °C.

Mixograph Tests. Mixograms were recorded (at least in duplicate) with a 10-g Mixograph (National Manufacturing, Lincoln, NE) according to a scaled up and slightly modified version of a method developed previously for the 2-g Mixograph (Békés et al., 1994a). Soissons flour (10.00 g, 14% moisture base), with or without added HMW-GS (10 or 40 mg), was mixed for 45 s with deionized water (4.59 mL) and DTT solution (0.5 mL) of varying concentrations. After 5 min resting to ensure sufficient reduction, KIO_3 solution (0.5 mL) of varying concentrations was added to the partially reduced dough and mixed with the dough for 30 s. The predeveloped dough was rested for 5 min to allow reoxidation before final mixing for 10 min. The total amount of added water (5.59 mL) was thus that corresponding to a maximum Farinograph dough consistency of 500 Brabender units. For 'incorporation' of HMW-GS into the glutenin polymers, levels of DTT and KIO_3 were chosen so that without HMW-GS a Mixogram was obtained that closely resembled that of the dough with additions of water instead of DTT and KIO_3 solutions. When the effect of simple 'addition' of HMW-GS was investigated, DTT and KIO_3 solutions were replaced with water. HMW-GS were mixed with the base flour with mortar and pestle and overnight shaking. The Mixogram parameters determined included (1) mixing time (MT), i.e., time to peak dough resistance (PR), (2) PR, (3) bandwidth at PR (BWPR), (4) resistance breakdown (RBD), i.e., ratio of the difference between PR and dough resistance at 3 min after MT and PR, and (5) bandwidth breakdown (BWBD), i.e., ratio of the difference between BWPR and bandwidth at 3 min after MT and BWPR (Békés et al., 1994a).

Table 1. Composition of Soissons Flour

		protein fractions (%)[b]				
moisture (%)	protein (%)[a]	salt-soluble	gliadin	acetic acid-soluble glutenin	acetic acid-insoluble glutenin	HMW-GS/LMW-GS wt ratio
13.98	11.89	17.3	35.3	8.7	38.7	0.37

[a] On a dry matter basis. [b] Expressed as percent of total protein.

Breadmaking Tests. Breadmaking quality was assessed (in triplicate) with a straight-dough breadmaking test for 10 g of flour (Shogren and Finney, 1984). Ingredients were Soissons base flour (10.00 g), sucrose (0.6 g), salt (0.15 g), shortening (Crisco, Procter and Gamble, Cincinnati, OH) (0.3 g), lyophilized yeast (Fermipan, Gist-Brocades, Delft, The Netherlands) (0.076 g), and water (5.59 mL). HMW-GS (0−40 mg) were mixed with flour as above. Dough mixing with a 10-g pin-mixer (National Manufacturing, Lincoln, NE) was halted twice with addition of solutions of DTT and KIO_3 in 0.5 mL of deionized water as above. After the last resting period, doughs were mixed to PR, fermented, and baked according to Shogren and Finney (1984). Loaf volumes (LV) were measured by glass bead displacement (Vanhamel et al., 1991).

RESULTS AND DISCUSSION

Oxidative Restoration of Partially Reduced Doughs. Békés et al. (1994a) demonstrated that doughs can be partially reduced with DTT and subsequently reoxidized with KIO_3 with restoration of dough mixing properties as evaluated by the Mixograph. These workers reported levels of 2.5 (Békés et al., 1994a,b) and 450 μg of DTT/g of flour (Gras and Békés, 1996) for partial dough reduction with Australian breadmaking flours. However, with the Soissons flour that we used (composition in Table 1), the lower level of DTT proved insufficient to produce any significant effect on dough rheological properties as evaluated by the Mixograph (results not shown). Using the higher level of reducing agent with Soissons flour, the Mixogram curve showed a completely reduced dough. Although we were still able to restore more or less dough mixing properties by using the recommended level of oxidizing agent (625 μg of KIO_3/g of flour) (Gras and Békés, 1996), we preferred not to induce such a strong breakdown of glutenin structure as we believe that only a partial disruption of the glutenin polymer network allows for incorporation of HMW-GS into a structure that resembles that of the original flour. Following screening the effect of several levels of DTT on the Mixogram curve, we seleceted 50 μg of DTT/g of flour to produce a partially reduced

Table 2. Mixogram Parameters and Loaf Volume for Control, Reduced (50 μg of DTT/g of flour), and Reduced and Subsequently Optimally Reoxidized (30 μg of KIO_3/g of flour) Doughs[a]

treatment	MT (s)	PR (% FS)	BWPR (% FS)	RBD (%)	BWBD (%)	LV (cc)
control ($n = 8$)	211 ± 6	50.5 ± 0.6	23.0 ± 0.9	8 ± 2	30 ± 5	55.0 ± 1.4
reduced ($n = 3$)	123 ± 5	47.0 ± 0.9	13.0 ± 1.6	17 ± 2	53 ± 6	43.2 ± 0.9
optimally reoxidized ($n = 6$)	202 ± 7	49.0 ± 1.2	21.5 ± 1.6	8 ± 2	36 ± 7	54.9 ± 1.2

[a] Abbreviations used: MT, mixing time; PR, peak dough resistance; BWPR, bandwidth at peak resistance; RBD, resistance breakdown; BWBD, bandwidth breakdown; LV, loaf volume; % FS, percent of full scale.

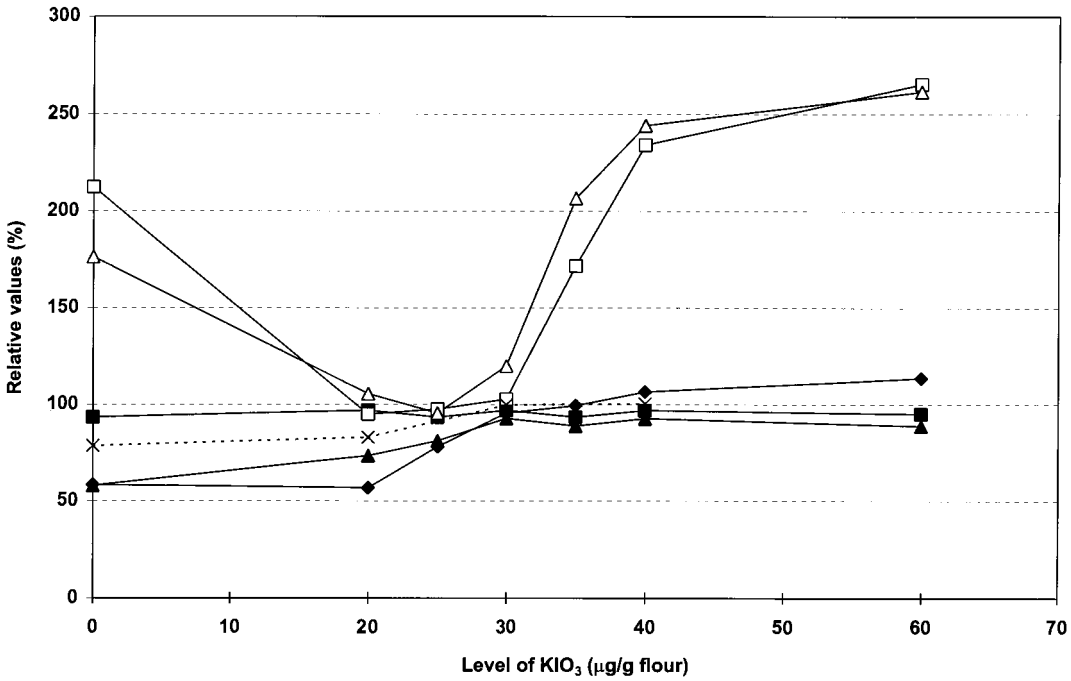

Figure 1. Effect of the level of KIO_3 for reoxidation of partially reduced (50 μg of DTT/g of flour) doughs on Mixograph parameters [mixing time (◆), peak dough resistance (■), bandwidth at peak dough resistance (▲), resistance breakdown (□), bandwidth breakdown (△)] and loaf volume (×). Values are relative to the values of unreduced control doughs.

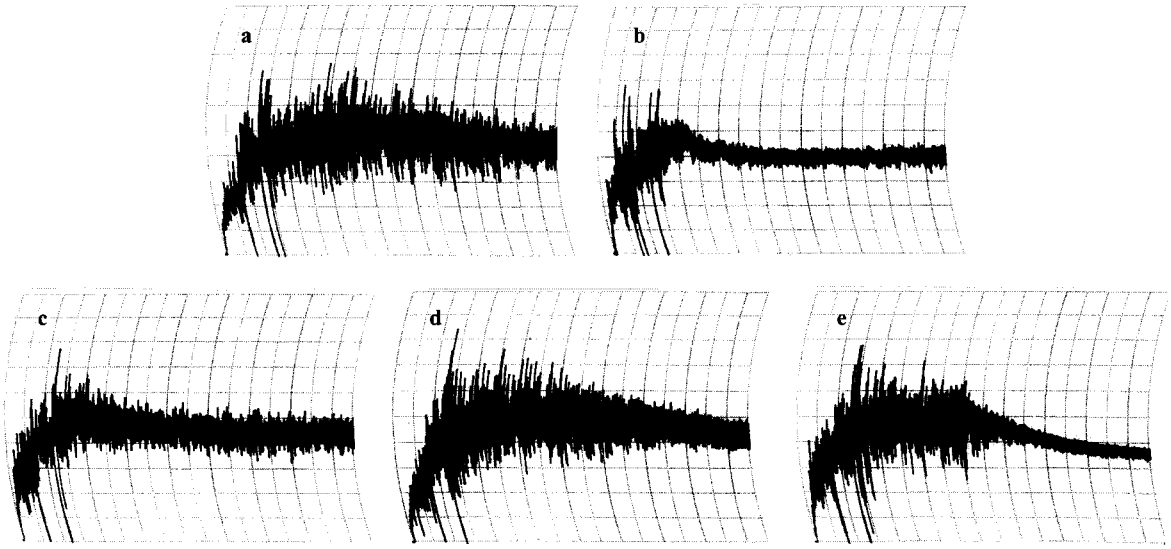

Figure 2. Effect of dough reduction and subsequent reoxidation on the Mixogram curve of Soissons flour [unreduced (a), reduced with 50 μg of DTT/g of flour (b), reduced and reoxidized with 20 (c), 30 (d), and 40 (e) μg of KIO_3/g of flour].

glutenin structure as a matrix for the incorporation of HMW-GS. The changes in the absolute values of Mixograph parameters and loaf volume upon dough reduction and subsequent reoxidation are presented in Table 2. The data in Table 2 further provide a good indication of the experimental error associated with the different experimental results presented in this manuscript (Figures 1 and 4). The level of 50 μg of DTT/g of flour drastically decreased Mixograph MT and BWPR and increased dough breakdown as evaluated by the increases of RBD and BWBD (Figures 1 and 2, Table 2). Furthermore, LV was considerably lower as well (Figures 1 and 3, Table 2). Mixograph PR was affected to a lesser extent (Figure 1, Table 2). The effects of reduction on the Mixograph parameters were similar to those reported by Békés et al. (1994a) except for

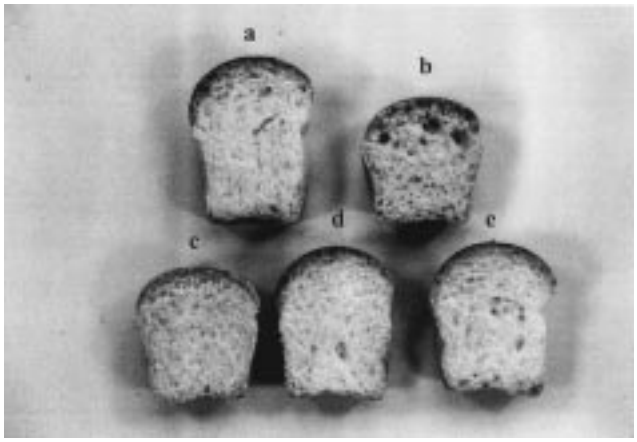

Figure 3. Effect of dough reduction and subsequent reoxidation on the volume and appearance of loaves of bread baked with Soissons flour [unreduced (a), reduced with 50 μg of DTT/g of flour (b), reduced and reoxidized with 20 (c), 30 (d), and 40 (e) μg of KIO_3/g of flour].

BWBD that, in their study, decreased indicating a higher stability of bandwidth after reduction.

Figure 1 shows the extent of restoration of different Mixograph parameters for different levels of KIO_3. Although restoration of MT was best with 35 μg of KIO_3/g of flour, best overall restoration was with 30 μg of KIO_3/g of flour. A lower level of KIO_3 resulted in lower MT and BWPR and a higher level in a faster decrease of resistance (increase of RBD) and an even more pronounced faster decrease of bandwidth (increase of BWBD) during overmixing. Interestingly, optimal reoxidation requires a 1.28-fold excess of KIO_3 over DTT compared with what can be predicted on the basis of the reaction mechanism of DTT and KIO_3 (Fitchett and Frazier, 1986). Optimal restoration with the conditions described by Gras and Békés (1996) requires an even larger excess of 2.96 for optimal reoxidation after reduction with 450 μg of DTT/g of flour. Obviously, reactions of KIO_3 other than with free sulfhydryl groups take place.

Figures 1 and 3 show that 30 μg of KIO_3/g of flour also restored LV. While 20 μg of KIO_3/g of flour still resulted in considerably smaller (about 80% of the control) loaves with inferior texture and shape (Figure 3), 30 μg of KIO_3/g of flour restored LV as well as texture and shape. Higher levels (40 μg of KIO_3/g of flour) did not further improve LV. These observations show for the first time that, with appropriate oxidation conditions, not only dough mixing properties can be restored, but also breadmaking performance, making it possible to directly extend the effect of incorporated HMW-GS on dough mixing properties to their effect on LV.

Effect of HMW-GS on Dough Mixing Properties and LV. Both procedures used for isolation of HMW-GS were based on their selective precipitation from a glutenin subunit extract at 60% (v/v) *n*-PrOH (Marchylo et al., 1989) after previous removal of monomeric gliadins with 50% (v/v) *n*-PrOH. Protein contents (Table 3) and SDS−PAGE profiles (results not shown) indicated high purity of the HMW-GS isolated by both procedures. SDS−PAGE under nonreducing conditions revealed a minor (less than 5%) presence of HMW-GS dimers in the HMW-GS preparations. Carbohydrate contaminations, although very minor (2.4−2.8%), appeared to be mainly arabinogalactans with an arabinose/galactose ratio of ca. 0.7 (Loosveld et al., 1997) (Table 3).

Isolated unalkylated HMW-GS were added or incorporated in Soissons base flour at levels of 1.0 and 4.0 mg/g of flour using the described protocol. The applied levels represent estimated increases in glutenin concentration of 2% and 8% (in the case of incorporation) and estimated increases in HMW-GS concentration of 7% and 28%, respectively. The effects of HMW-GS isolated at high (procedure 1, Figure 4a) or low (procedure 2, Figure 4b) temperature were essentially the same. Incorporation of 4.0 mg of HMW-GS/g of flour considerably increased MT and BWPR (Figure 4). Incorporation of 1.0 mg of HMW-GS/g of flour caused a smaller increase in these parameters or had almost no effect. PR was not strongly affected by incorporation of HMW-GS, although small increases could be observed. The effects of incorporation of HMW-GS on dough stability during overmixing were very pronounced (Figure 4). A large increase in stability (decrease of RBD and BWBD) was observed even with 1.0 mg of HMW-GS/g of flour. Extremely stable doughs resulted from incorporation of 4.0 mg of HMW-GS/g of flour. In general, the effects of incorporation of HMW-GS on dough mixing were comparable with those observed by Békés et al. (1994b) and Sapirstein and Fu (1996) indicating an increased mixing requirement and increased tolerance to overmixing. However, simple addition of HMW-GS produced similar increases in MT as incorporation (Figure 4), while Békés et al. (1994b) observed a decrease upon addition of HMW-GS. Similarly, although smaller than the effect in the case of incorporation, we also observed a dough stabilizing effect (decrease of RBD and BWBD) upon simple addition of HMW-GS. Békés et al. (1994b) observed a slightly decreased tolerance to overmixing upon addition of HMW-GS. To eliminate completely the possibility of incorporation of HMW-GS, the free sulfhydryl in the latter was blocked with 4-vinylpyridine. When using 4.0 mg of alkylated HMW-GS/g of flour in the incorporation protocol, no significant effects on MT, PR, or BWPR (Figure 4a) were observed. In contrast with the observed increased tolerance to overmixing upon incorporation of unalkylated HMW-GS, tolerance to overmixing was slightly decreased (Figure 4a). Addition of alkylated HMW-GS at a level of 4.0 mg/g of flour produced a slight decrease of MT and slight increases in PR and BWPR (Figure 4a). Dough tolerance to overmixing was unaffected by the addition of alkylated HMW-GS (Figure 4a). This contrasts with the observation that addition of unalkylated HMW-GS increases dough stability dramatically. The differences in the effect of addition of unalkylated versus alkylated HMW-GS suggest some incorporation of unalkylated HMW-GS even with simple addition or point to the importance of free sulfhydryl on monomeric proteins during dough mixing.

Unexpectedly, no improvement in breadmaking performance could be observed by either addition or incorporation of HMW-GS (Figure 4) that accompanied the improved dough mixing properties observed in this and earlier studies (Békés et al., 1994b; Sapirstein and Fu, 1996) upon increasing the HMW-GS content of flour. Our results are therefore different from those described by Uthayakumaran et al. (1997). The latter authors in a 2.4-g breadmaking procedure found an increase in loaf height upon incorporation of HMW-GS. Regretfully, the lack of experimental detail in the latter paper, such as

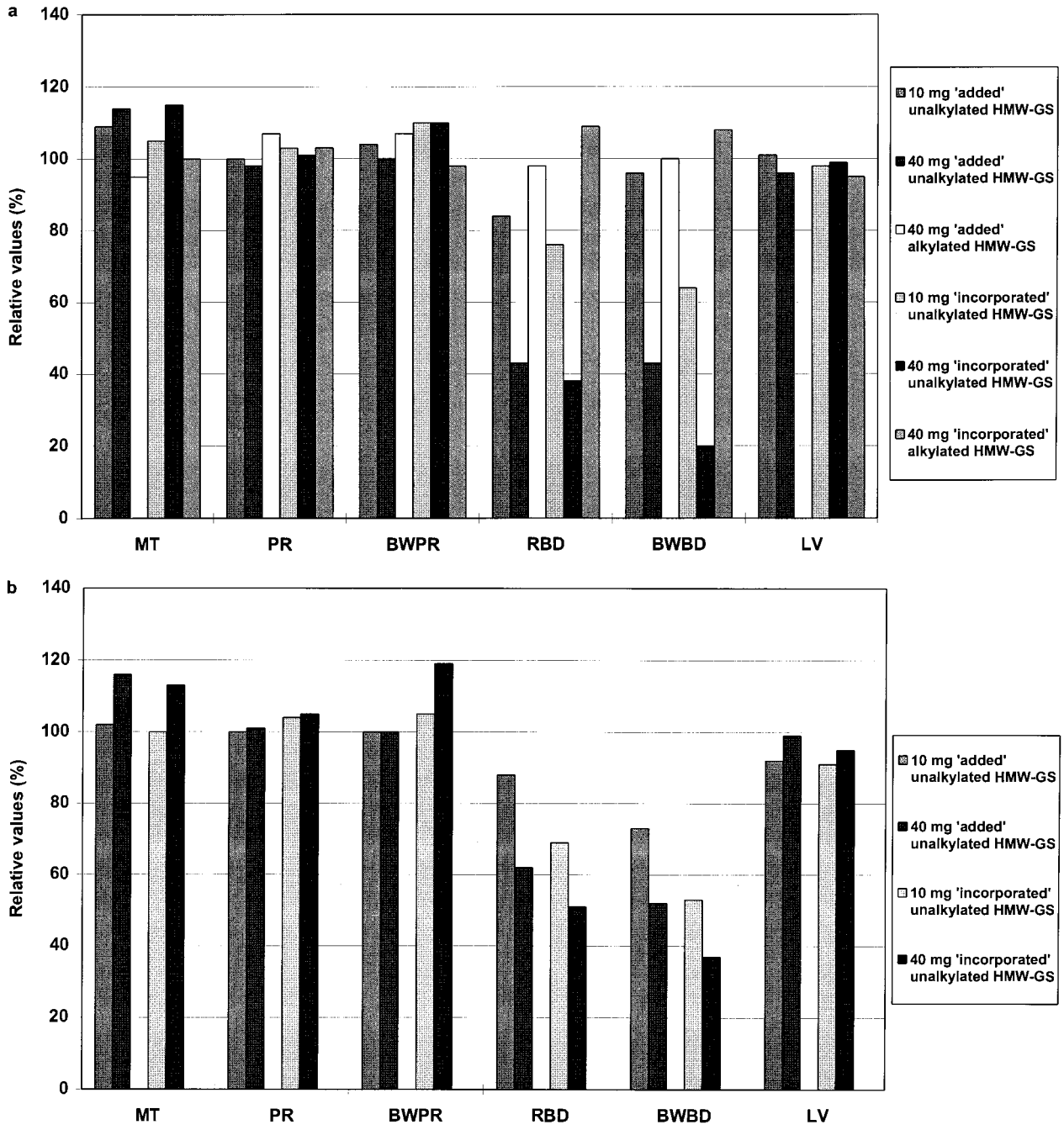

Figure 4. Effect of addition or incorporation of unalkylated and alkylated HMW-GS of Soissons flour [isolated according to procedure 1 (a) or 2 (b)] on Mixogram parameters (MT, mixing time; PR, peak dough resistance; BWPR, bandwidth at peak resistance; RBD, resistance breakdown; BWBD, bandwidth breakdown] and loaf volume (LV) of Soissons flour. Values for addition are relative to the values of unreduced control doughs, and values for incorporation are relative to the values for reduced and optimally reoxidized control doughs.

Table 3. Composition of HMW-GS

isolation procedure	protein content (%)[a]	carbohydrate content (%)[a]	carbohydrate composition (%)[b,c]				
			Ara	Xyl	Man	Gal	Glc
1	88.6	2.4	37.0	2.2	0.3	52.4	8.1
2	91.9	2.8	29.5	4.7	0.3	41.7	23.8

[a] On an as is basis. [b] Expressed as percent of total monosaccharides after acid hydrolysis. [c] Abbreviations used: Ara, L-arabinose; Xyl, D-xylose; Man, D-mannose; Gal, D-galactose; Glc, D-glucose.

the type of flour used, the specific HMW-GS that produced the effect as well as their incorporation level, a quantitative expression of the loaf height increase, and the extent of restoration of loaf volume in control experiments, makes an interpretation of the different results totally impossible.

CONCLUSIONS

This study shows that the incorporation protocol designed by Békés et al. (1994a) for evaluating the effect of glutenin subunits on dough mixing properties can also be used for breadmaking experiments. However, although the previously observed (Békés et al., 1994b; Sapirstein and Fu, 1996) dough strengthening effect (longer mixing requirement and higher tolerance to overmixing) upon incorporation of HMW-GS was also obvious in this study, breadmaking performance remained largely unaffected.

ABBREVIATIONS USED

BWBD, bandwidth breakdown; BWPR, bandwidth at peak resistance; DTT, dithiothreitol; HMW-GS, high molecular weight glutenin subunits; LMW-GS, low molecular weight glutenin subunits; LV, loaf volume; β-ME, β-mercaptoethanol; MT, mixing time; *n*-PrOH, *n*-propanol; PR, peak dough resistance; RBD, resistance breakdown; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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