

# Effects of High and Low Molecular Weight Glutenin Subunits on Rheological Dough Properties and Breadmaking Quality of Wheat

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## ABSTRACT

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High molecular weight (HMW) or low molecular weight (LMW) subunits of different chemical state (reduced, reoxidized with  $\text{KBrO}_3$ , or  $\text{KIO}_3$ ) or gliadins were added in 1% amounts to a base flour of the wheat cultivar Rektor and mixed with water. The corresponding doughs were then characterized by microscale extension tests and by microbaking tests and were compared to doughs from the base flour without additives. The maximum resistance of dough was strongly increased by HMW subunits in a reduced state and by HMW subunits reoxidized with  $\text{KBrO}_3$ . A moderate increase of resistance was caused by HMW subunits reoxidized with  $\text{KIO}_3$  and by LMW subunits reoxidized with  $\text{KBrO}_3$  or  $\text{KIO}_3$ . This resistance was strongly lowered by LMW subunits in a reduced state and by gliadins. The extensibility of dough was significantly increased only by gliadins and reduced HMW subunits; HMW

subunits reoxidized with  $\text{KBrO}_3$  had no effect, and all other fractions had a decreasing effect. In particular, glutenin subunits reoxidized with  $\text{KIO}_3$  induced marked decrease of extensibility, resulting in bell-shaped curve extensigrams, which are typical for plastic properties. The effect of reoxidized mixtures (2:1) of HMW and LMW subunits on maximum resistance depended on the oxidizing agent and on the conditions (reoxidation separated or together); extensibility was generally decreased. Bread volume was increased by addition of HMW subunits (reduced or reoxidized with  $\text{KBrO}_3$ ) and decreased by LMW subunits (reoxidized with  $\text{KBrO}_3$  or  $\text{KIO}_3$ ) and by a HMW-LMW subunit mixture (reoxidized with  $\text{KBrO}_3$ ). The volume was strongly decreased by addition of reduced LMW subunits. A high bread volume was related to higher values for both resistance and extensibility.

The unique functional properties of wheat dough are due to the storage proteins of the endosperm (Pomeranz 1988). After flour is mixed with water, storage proteins form a rubbery mass (the gluten) that can be fractionated with aqueous alcohols into the soluble, predominantly monomeric gliadins and the insoluble, aggregated glutenins. Both fractions are cohesive, but their contribution to other functional properties of dough is different. Gliadins determine viscosity, while glutenins regulate strength and elasticity. The glutenin fraction consists of two main protein subgroups: high molecular weight (HMW) and low molecular weight (LMW) subunits, which occur in flour in proportions from  $\approx 1:2$  to  $1:3$  on a weight basis depending on the cultivar (Wieser et al 1994). The amounts of both HMW and LMW subunits showed good correlations with the maximum resistance of dough and gluten, but twice as many LMW subunits were necessary to get the same effects on resistance as from HMW subunits. The extensibility of dough and gluten was mainly dependent on the ratios of gliadins to both HMW and LMW subunits (Wieser et al 1994). Previous studies on the effects of gluten proteins after addition to flours have been focused on the HMW subunits and on the rheological properties of gluten (Schropp et al 1996). The results demonstrated that the extensibility of gluten was increased by monomeric subunits and was decreased by reoxidized subunits. The maximum resistance of gluten was increased by reoxidized HMW subunits, when the major portion of the product was in an aggregated state (reoxidation with  $\text{KBrO}_3$ ). These investigations were continued by studying the effects of both HMW and LMW subunits on the rheological properties of dough and on bread volume.

## MATERIALS AND METHODS

### Preparation of Protein Fractions

Gliadin and glutenin subunits were prepared according to the procedure described previously (Wieser et al 1998). Defatted flour (10 g) of the cultivar Rektor (REK) was extracted twice with a salt solution (67 mM  $\text{HKNaPO}_4$ , pH 7.6, + 0.4M NaCl, 100 mL) at room temperature (RT  $\approx 20^\circ\text{C}$ ). Subsequently, the residue was

extracted three times with 60% (v/v) ethanol (50 mL) at RT. After centrifugation ( $40,000 \times g$ , 5 min,  $20^\circ\text{C}$ ), the combined supernatants (gliadins) were dialyzed and freeze-dried. To isolate glutenin subunits, the residue obtained was extracted twice with 50% (v/v) 2-propanol containing Tris/HCl (0.08M, pH 8.0, 50 mL) and dithioerythritol (1.0%, w/v) at  $60^\circ\text{C}$  under nitrogen. After centrifugation ( $40,000 \times g$ , 5 min,  $20^\circ\text{C}$ ), supernatants were combined, and HMW subunits were precipitated by addition of acetone (40%, v/v) (Melas et al 1994). Supernatant (LMW subunits) and precipitate (HMW subunits) were dialyzed against nitrogen-saturated acetic acid and freeze-dried. The protein contents ( $N \times 5.7$ ) of the products were determined by the Dumas method. Thiol contents of protein fractions were determined using Ellman's reagent (Schropp et al 1995).

### Reoxidation of HMW and LMW subunits

The HMW subunits (114 mg = 100 mg of protein) and the LMW subunits (117 mg = 100 mg of protein) were dissolved under nitrogen in trifluoroacetic acid (TFA, 0.1% v/v, 10 mL). After stirring for 1 hr at RT, 200  $\mu\text{L}$  (HMW subunits) and 320  $\mu\text{L}$  (LMW subunits) of  $\text{KBrO}_3$  solution (1.8 mg of  $\text{KBrO}_3/\text{mL}$  of 0.1% TFA) or 200  $\mu\text{L}$  (HMW subunits) and 320  $\mu\text{L}$  (LMW subunits)  $\text{KIO}_3$  solution (2.4 mg of  $\text{KIO}_3/\text{mL}$  of 0.1% TFA) were added, representing a molar ratio of halates to Cys of 0.25. The suspension was stirred for 20 hr, the reoxidized fractions were dialyzed against nitrogen-saturated TFA (0.1 %, v/v) until they were free of oxidizing agent, then they were lyophilized.

### Extension Tests on Dough

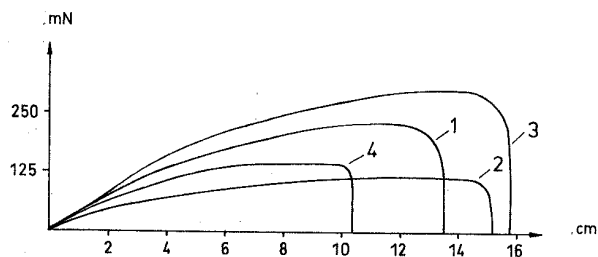
Base flours (10 g) of REK were mixed with lyophilized gliadins, HMW, and LMW subunits using an IKA mill ( $2 \times 5$  sec). In the reference tests without additives, base flour was treated in the same manner. Flours (10 g) and NaCl (0.2 g) were mixed with distilled water up to 550 farinogram units using a microfarinograph (Brabender) at  $22^\circ\text{C}$  and 60 rpm. Constant water addition (6.8 mL) was used for all experiments. Mixing was continued to maximum consistency ( $\approx 4.5$  min). Microextension tests on dough were performed as described by Kieffer et al (1981, 1998). Doughs were pressed into a Teflon mold prewarmed to  $22^\circ\text{C}$  and were allowed to stand for further 40 min at  $22^\circ\text{C}$  under a water-saturated atmosphere. Maximum resistance (mN) and extensibility (cm) of doughs were measured using an SMS/Kieffer dough and gluten extensibility rig with the texture analyzer TA-XT2 (Stable Micro Systems, Surrey, UK).

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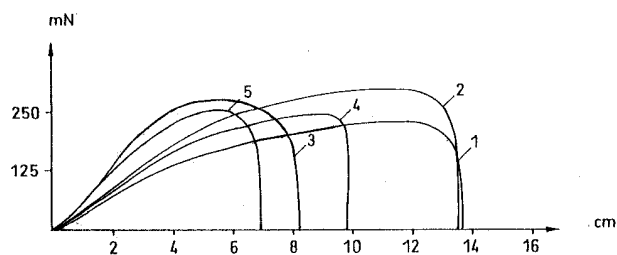
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## Microbaking Tests

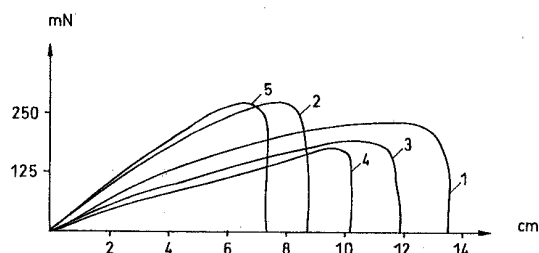
Dough production followed the same procedure as for extension tests, but with the addition of sucrose (0.1 g), coconut fat (0.1 g), fresh yeast (0.7 g), and L-ascorbic acid (0.2 mg) to 10 g of flour. Doughs were mixed in a mixer for 1 min at 22°C and 1,250 rpm



**Fig. 1.** Extensigram of dough from base flour of cultivar Rektor without additive (1), with addition of 1% gliadin (2), 1% reduced HMW subunits (3), and 1% reduced LMW subunits (4).



**Fig. 2.** Extensigram of dough from base flour of cultivar Rektor without additive (1); with addition of 1% HMW subunits reoxidized with KBrO<sub>3</sub> (2), reoxidized with KIO<sub>3</sub> (3); with addition of 1% LMW subunits reoxidized with KBrO<sub>3</sub> (4), and reoxidized with KIO<sub>3</sub> (5).



**Fig. 3.** Extensigram of dough from base flour of cultivar Rektor without additive (1), with addition of 1% HMW-LMW subunits (1:2.5) reoxidized separated with KBrO<sub>3</sub> (2), reoxidized together with KBrO<sub>3</sub> (3), reoxidized separated with KIO<sub>3</sub> (4) and reoxidized together with KIO<sub>3</sub> (5).

(Kieffer et al 1993), and proofed at 30°C for 20 min and a further 35 min. Microbaking tests were performed as described by Kieffer et al (1998) at 230°C for 10 min.

## RESULTS AND DISCUSSION

### Isolation of Protein Fractions

Albumins, globulins and gliadins were extracted from REK flour with a salt solution and 60% (v/v) ethanol, respectively. The residue was extracted with buffered 50% 2-propanol under reducing conditions and at increased temperature to obtain glutenin subunits (Wieser et al 1998). HMW subunits were then separated from LMW subunits by precipitation with acetone (Melas et al 1994). The protein content ( $N \times 5.7$ ) of the dialyzed and lyophilized fractions was 87% (gliadins), 88% (HMW fraction), and 86% (LMW fraction), respectively; the residual material was probably salt and water. HMW and LMW subunits were reoxidized with either KBrO<sub>3</sub> or KIO<sub>3</sub> according to the procedure described previously (Schropp et al 1995). The size distribution of reoxidized proteins has been described previously (Schropp et al 1995; Antes and Wieser 2001).

### Microextension Tests

The rheological investigations were performed with dough according to the method of Kieffer et al (1998). Different protein fractions (Table I) were added to the base flour at 1% (dry protein according to total flour weight). For comparison with HMW and LMW subunits, gliadins from REK were added to the base flour. In agreement with previous studies on gluten (Kim et al 1988, Schropp et al 1996), the addition of gliadins resulted in a marked decrease of the maximum resistance (MR) and in an increase of the extensibility (EX) (Fig. 1, Table I). In contrast to the monomeric gliadins, the addition of reduced HMW subunits, which were also monomeric but had free thiol groups, led to a significant increase of MR (226–303 mN) and EX (13.5–15.7 cm). This result did not agree with the studies of Schropp et al (1996), who found a weaker and more extensible gluten after adding reduced HMW subunits. Different preparation of gluten and dough may be the reason for this. Where dough was allowed to stay over 40 min at 30°C after adding the reduced HMW subunits, gluten was immediately washed out after mixing. Obviously, there was not enough time for the HMW subunits to be incorporated into the gluten network. According to Graveland et al (1980) and Hamer and Lichtendonk (1987), the resting period of dough is particularly important for the formation of glutenin macropolymers. Addition of reduced LMW glutenin subunits, however, resulted in a completely different effect (Fig. 1). Both MR and EX were clearly decreased. It can be supposed that reduced LMW subunits destroy the gluten network. This result is reflected by a very weak dough.

**TABLE I**  
Maximum Resistance (MR) and Extensibility (EX) of Dough and Bread Volume Obtained from Different Flour Mixtures

Additive (1 %)	Extensigram Reference		Extensibility Values <sup>a</sup>		Baking Test <sup>b</sup> Loaf Volume (mL)
	Fig.	Curve	MR (mn)	EX (cm)	
None (base flour from cv. Rektor)	1–3	1	226 ± 14	13.5 ± 0.4	62 ± 1.5
Gliadins	1	2	112 ± 15	15.2 ± 0.3	nd <sup>c</sup>
HMW subunits reduced	1	3	303 ± 7	15.7 ± 0.7	66 ± 1.7
LMW subunits reduced	1	4	145 ± 8	10.4 ± 0.5	44 ± 0.2
HMW subunits reoxidized with KBrO <sub>3</sub>	2	2	295 ± 7	13.6 ± 0.6	66 ± 0
HMW subunits reoxidized with KIO <sub>3</sub>	2	3	262 ± 13	8.2 ± 0.7	nd
LMW subunits reoxidized with KBrO <sub>3</sub>	2	4	245 ± 8	9.8 ± 0.4	51 ± 1.5
LMW subunits reoxidized with KIO <sub>3</sub>	2	5	254 ± 24	6.7 ± 0.3	47 ± 0.5
HMW-LMW subunits (1:2.5) reoxidized with KBrO <sub>3</sub> <sup>d</sup>	3	2	276 ± 14	8.8 ± 0.3	57 ± 1.2
HMW-LMW subunits (1:2.5) reoxidized with KBrO <sub>3</sub> <sup>e</sup>	3	3	193 ± 15	12.0 ± 0.5	nd
HMW-LMW subunits (1:2.5) reoxidized with KIO <sub>3</sub> <sup>d</sup>	3	4	184 ± 16	10.2 ± 0.8	nd
HMW-LMW subunits (1:2.5) reoxidized with KIO <sub>3</sub> <sup>e</sup>	3	5	279 ± 35	7.3 ± 1.1	nd

<sup>a</sup> Mean of four determinations ± standard deviation.

<sup>b</sup> Mean of three determinations ± standard deviation.

<sup>c</sup> Not determined.

<sup>d</sup> Reoxidation separated.

<sup>e</sup> Reoxidation together.

For further experiments, HMW and LMW subunits of REK were reoxidized with  $\text{KBrO}_3$  (Schropp et al 1995). Chromatographic analysis of the products by gel-permeation chromatography (Antes et al 2001) showed that  $\approx 51\%$  of reoxidized HMW subunits were in a polymerized state and  $\approx 49\%$  were in a monomeric state. Of LMW subunits reoxidized with  $\text{KBrO}_3$ , 65% were in a polymeric state. The analysis with Ellman's reagent revealed that 6.7% (HMW subunits) and 14.3% (LMW subunits) of total Cys were in the thiol forms. The thiol content was important because, according to Bekes et al (1994) and Schropp et al (1996), small amounts of free thiol groups should be present for the incorporation of reoxidized HMW subunits into the gluten network. As shown in Table I and Fig. 2, the addition of the reoxidized HMW fraction led to a significant increase of MR (226–295 mN); EX did not change. Addition of reoxidized LMW fraction resulted in a major decrease of EX (13.5–9.8 cm); MR was only slightly increased. This indicated that reoxidized LMW subunits reacted with the gluten network quite differently than reoxidized HMW subunits.

Previous studies revealed that reoxidation of HMW and LMW subunits with  $\text{KIO}_3$  resulted in different molecular weight distributions. HMW subunits formed 43% polymeric proteins and LMW subunits formed 84% (Antes and Wieser 2001). Free thiol groups were absent in both fractions. The addition of HMW subunits reoxidized with  $\text{KIO}_3$  decreased EX (13.5–8.2 cm); MR was increased. Adding reoxidized LMW subunits also led to a significant decrease of EX (13.5–6.7 cm) and an increase of MR (226–254 mN). In both cases, the elastic properties of dough were changed to a plastic character, well documented by the bell-shaped curves of the extensigrams (R. Kieffer, *personal communication*). Possibly, the absence of free thiol groups prevented incorporation into gluten network.

Further experiments were performed with HMW and LMW subunits mixed 1:2.5 and oxidized separately or together. The addition of 1% HMW-LMW subunit mixture, which was reoxidized separately with  $\text{KBrO}_3$  and mixed after oxidation, showed an MR increase of 226–276 mN, while EX decreased 13.5–8.8 cm (Fig. 3). The comparison with the samples of reoxidized HMW subunits ( $\text{KBrO}_3$ ) and reoxidized LMW subunits ( $\text{KBrO}_3$ ) (Fig. 2) indicated that HMW subunits were responsible for the increase of MR, and LMW subunits were responsible for the decrease of EX. A different effect was obtained by adding a mixture of HMW-LMW subunits (1:2.5) reoxidized with  $\text{KBrO}_3$ , where both EX and MR decreased (Fig. 3). The dough strengthening effect of reoxidized HMW subunits was abolished by reoxidation together. The addition of a HMW-LMW subunit mixture, which was reoxidized separately with  $\text{KIO}_3$  significantly decreased MR (226–184 mN) and EX (13.5–10.2 cm). Adding a mixture that was reoxidized together, also decreased EX, but MR was slightly increased. The dough showed a plastic behavior, similar to that of HMW and LMW subunits reoxidized with  $\text{KIO}_3$  (Fig. 2).

### Microbaking Tests

Baking tests were performed on a microscale (10 g of material) according to Kieffer et al (1998). Glutenin subunits in a reduced or reoxidized state were added to the base flour at 1% (dry protein to total flour weight), and the loaf volume of the small breads was regarded as a measure for the effects of additives (Table I). The loaf volume obtained with 10 g of base flour (REK) without additive reached 62 mL. The addition of HMW subunits, either in reduced state or reoxidized with  $\text{KBrO}_3$ , increased the loaf volume up to 66 mL. This reflected very well the correlation between MR and loaf volume (Kieffer et al 1998). The addition of LMW subunits reoxidized with  $\text{KBrO}_3$ , however, resulted in a completely different effect. Probably because of the enormous decrease of extensibility of dough, the loaf volume dropped from 62 to 51 mL. LMW subunits reoxidized with  $\text{KIO}_3$ , forming a dough with plastic properties, led to an even smaller loaf volume (47 mL). The smallest volume (44 mL) was obtained after addition of reduced LMW subunits. This result was also in accordance with the curve of the extension test (Fig. 1). A mixture of HMW-LMW subunits (1:2.5) reoxidized separately with  $\text{KBrO}_3$  also decreased the volume from 62 to 57 mL. The

values lay between the samples of reoxidized HMW subunits ( $\text{KBrO}_3$ ) and reoxidized LMW subunits ( $\text{KBrO}_3$ ). The results obtained in this study showed that dough properties measured by microextension tests were related to loaf volumes and thus to breadmaking quality.

## CONCLUSIONS

The addition of various gluten protein fractions to a standard flour influences the rheological properties of corresponding doughs in a different manner and to a different extent. The effect of HMW and LMW subunits in a reduced state or reoxidized with  $\text{KBrO}_3$  is generally different. EX particularly is much higher after addition of HMW subunits compared with LMW subunits. This demonstrates that the reactions of both protein types with the gluten network of dough differ completely; only after reoxidation with  $\text{KIO}_3$  the effects are similar and lead to a plastic dough. Free thiol groups appear to be important for the incorporation of proteins into the gluten network and thus for the effects on dough. When HMW and LMW subunits were mixed together, reoxidation before or after mixing greatly influences the effect on dough. Low bread volume is caused by the addition of LMW subunits reoxidized with  $\text{KBrO}_3$  or  $\text{KIO}_3$  and is related to a decreased EX of the dough. HMW subunits reoxidized with  $\text{KBrO}_3$  and also in a reduced state have a positive effect on bread volume.

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