# Effects of High Molecular Weight Subunits of Glutenin on the Rheological Properties of Wheat Gluten

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## ABSTRACT

High molecular weight (HMW) glutenin subunits of different chemical state (reduced, S-alkylated, reoxidized with $KBrO_3$ or $KIO_3$) and of different compositions (cv. Rektor [HMW subunits 5, 7, 9, 10], cv. Apollo [HMW subunits 2, 6, 8, 12]) were added, mostly in 1% amounts, to base flours of Rektor and Apollo. The corresponding glutens were then characterized by microscale extension tests and compared to glutens from the flours without additives. The extensibility of gluten was increased by monomeric proteins (gliadin, reduced HMW subunits) and was decreased by reoxidized HMW 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 $KBrO_3$). A maximum level of resistance was reached with the addition of 1.5–2.0% of reoxidized HMW subunits. HMW subunits reoxidized with $KIO_3$ had an decreasing effect on gluten resistances. Main differences to HMW subunits reoxidized with $KBrO_3$ were the lower portion of polymerized proteins and the absence of free thiol groups. Monomeric proteins (reduced or S-alkylated HMW subunits, gliadin) generally decreased maximum resistance. The effect of reoxidized HMW subunits on gluten properties did not seem to depend on the HMW subunit composition.


It is commonly accepted that high molecular weight (HMW) subunits of glutenin play an important role for gluten formation and properties and thus, for the breadmaking quality of wheat (Shewry et al 1992). Since the studies of Payne and coworkers (Payne et al 1981, 1984) describing correlations between subunit compositions and breadmaking quality, investigations have been mainly focused on a qualitative point of view, namely the presence or absence of specific subunits. More recently, however, it was suggested that, to predict quality, it is not sufficient to characterize HMW subunits in purely qualitative terms, and moreover, that the absolute quantity and the relative proportions of HMW subunits are also considered important (Marchylo et al 1989, Sutton et al 1989, MacRitchie et al 1991, Seilmeier et al 1991, Sutton 1991, Kolster et al 1993).

Previous quantitative determinations of gluten protein types in flours from different wheat cultivars have revealed a high correlation between the amount of HMW subunits and the maximum resistance of dough and gluten and also between the ratio of gliadin to HMW subunits and extensibility (Wieser et al 1994a,b). To confirm such relationships, HMW subunits of different state and combination were mixed into reference flours, and the corresponding glutens were characterized by microscale extension tests.

## MATERIAL AND METHODS

### Preparation of Protein Fractions

Gluten, gliadin, and HMW subunits were prepared according to the procedures described previously (Köhler et al 1991, Schropp et al 1995). Flours of the cultivars Rektor (REK) and Apollo (APO) were mixed with distilled water in a farinograph (Brabender) for 1 min, and the resulting doughs were washed by hand under tap water; the glutens obtained were lyophilized. A portion of gluten was extracted three times with 70% (v/v) aqueous ethanol and centrifuged. The combined supernatants (gliadins) were lyophilized.

The residue was dissolved at 60°C under reducing conditions in 50% 1-propanol, and HMW subunits were then precipitated by increasing the propanol concentration to 60% (Marchylo et al 1989, Schropp et al 1995). After centrifugation, the precipitates were dialyzed and lyophilized (HMW fraction).

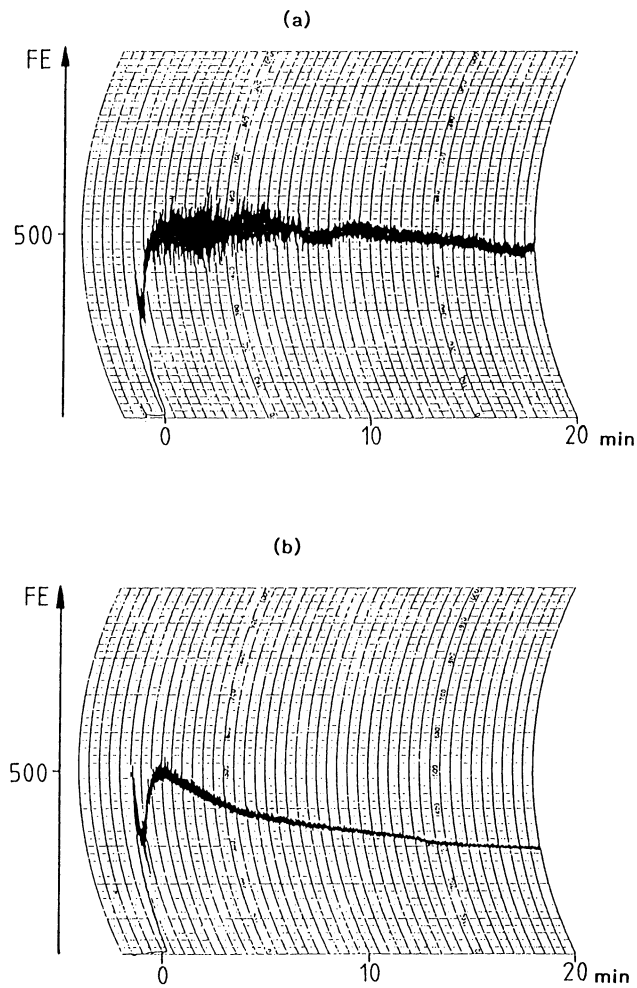


**Fig. 1.** Farinograms of the base flours of the cultivar Rektor **(a)** and the cultivar Apollo **(b)**.

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### Reoxidation of HMW Fractions

HMW fractions (240 mg) (192 mg of protein according to amino acid analysis) were dissolved under nitrogen in 9.6 ml of trifluoroacetic acid (TFA, 0.1%) (2% protein solution). After stirring for 1 hr at room temperature, 0.77 mg of $KBrO_3$ or 0.98 mg of $KIO_3$ (molar ratio halates/Cys = 0.25) were added and stirred for 20 hr more. The samples were then dialyzed under nitrogen against 0.1% TFA until they were free of oxidizing agent and then lyophilized.

### Alkylation of HMW Fractions

HMW fractions (120 mg) (96 mg of protein) were dissolved in 15.0 ml of 50% aqueous 1-propanol containing urea (2 mol/L), dithioerythritol (0.05 mol/L) and tris HCl (0.082 mol/L, pH 7.5). 0.71 ml of freshly distilled 4-vinylpyridine was added and heated at 60°C for 15 min. The samples were then dialyzed against acetic acid (0.01 mol/L) and lyophilized.

### Rheological Studies

Base flours (10 g) of REK and APO were mixed with the different additives using an IKA mill (2 × 5 sec). In the reference tests without additives, base flours were treated in the same manner. Material (10 g) was mixed with distilled water up to ≈550 farinogram units using a 10-g farinograph (PL 997, Brabender) at 30°C. Mixing continued for a total of 6 min. The resulting dough was cooled for 20 min in ice water and then washed (Glutomatic 2100, Falling Number) with distilled water for 10 min. Rheological properties of the glutens obtained were characterized by microscale tests described by Kieffer et al (1981).

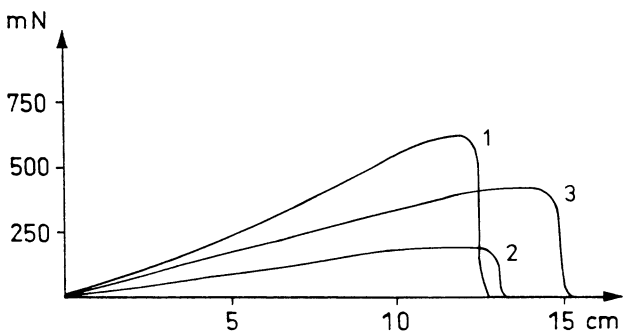


**Fig. 2.** Extensigrams of gluten from base flour of the cultivar Rektor without additive (1), with addition of 1% gliadin (3), and from base flour of the cultivar Apollo without additive (2).

**TABLE I**
**Maximum Resistance (MR) and Extensibility (EX) of Gluten Obtained from Different Flour Mixtures[a]**

| Curve and Base Flour[b] | | Additive[c] | MR (mN) | EX (cm) |
|---|---|---|---|---|
| 1 | REK | – | 610 ± 26 | 12.5 ± 0.7 |
| 2 | APO | – | 168 ± 21 | 13.3 ± 0.5 |
| - | REK | 1 % gluten REK | 590 ± 28 | 12.6 ± 0.7 |
| 3 | REK | 1 % gliadin REK | 422 ± 21 | 15.0 ± 0.4 |
| 4 | REK | 0.5 % HMW subunits REK* | 694 ± 27 | 11.8 ± 0.7 |
| 5 | REK | 1.0 % HMW subunits REK* | 759 ± 19 | 10.8 ± 0.5 |
| 6 | REK | 1.5 % HMW subunits REK* | 806 ± 26 | 10.6 ± 0.6 |
| 7 | REK | 2.0 % HMW subunits REK* | 825 ± 30 | 10.7 ± 0.6 |
| 8 | REK | 1.0 % HMW subunits REK** | 462 ± 31 | 11.6 ± 0.5 |
| 9 | REK | 1.0 % HMW subunits REK† | 465 ± 28 | 15.6 ± 0.8 |
| 10 | REK | 1.0 % HMW subunits REK†† | 492 ± 19 | 12.8 ± 0.8 |
| 11 | REK | 1.0 % HMW subunits APO* | 753 ± 30 | 11.6 ± 0.5 |
| 12 | APO | 1.0 % HMW subunits REK* | 400 ± 27 | 10.5 ± 0.8 |
| 13 | APO | 1.0 % HMW subunits APO* | 390 ± 19 | 11.6 ± 0.4 |

[a] Mean of four determinations ± standard deviation.
[b] See Figs. 2–5 for curves. Wheat cultivars Rektor (REK) and Apollo (APO).
[c] * = Reoxidized with $KBrO_3$, ** = reoxidized with $KIO_3$, † = reduced; †† = S-alkylated.

## RESULTS AND DISCUSSION

The rheological studies on gluten were performed according to previous investigations regarding the influence of various cereal prolamins on gluten rheology (Kim et al 1988). Flours from two widely different wheat cultivars were used as base material. REK flour had 14.6% protein (N × 5.7); an HMW subunit composition of 5, 7, 9, 10; and a high breadmaking quality. APO flour had 13.3% protein; subunit composition of 2, 6, 8, 12; and a poor breadmaking quality. The mixing curves of the base flours were representative for good (REK) and poor (APO) dough mixing properties (Fig. 1). The rheological properties of corresponding glutens are shown in Figure 2. Gluten from REK (curve 1) had a maximum resistance (MR) of 610 mN and an extensibility (EX) of 12.5 cm, the curve of a strong gluten. In contrast, gluten from APO (curve 2) was weak (MR = 168 mN, EX = 13.3 cm).

As controls for mixing conditions and measuring systems, gluten and gliadin from REK were added to the base flour at a level of 1% (dry protein to total flour weight). As expected, the addition of its own gluten did not significantly change the extensigram (curve not shown) of the gluten from the base flour (Table I). The addition of gliadin, however, resulted in a drastic decrease of MR and increase of EX (curve 3). This result agrees with previous studies (Kim et al 1988) and reflects very well the correlations between gluten properties and the proportion of gliadins and glutenins (Wieser et al 1994b).

For further experiments, the HMW subunits of REK were reoxidized with $KBrO_3$ according to the method described previously (Schropp et al 1995). The chromatographic analysis of the product by gel chromatography showed that ≈53% were in a polymerized state and ≈47% were in a monomeric state; 15% of the thiol groups were free and not disulfide-bonded. Four different amounts (0.5, 1.0, 1.5, and 2.0%) were added to the REK base flour. As shown in Table I and Figure 3, the addition of the reoxidized HMW fraction led to a significant increase of MR and decrease of EX. This effect was clearly dependent on the amount added. With 0.5 and 1.0% additions, MR was increased almost linearly from 610 mN (curve 1) to 694 mN (curve 4) and 759 mN (curve 5). By the addition of higher amounts (1.5 and 2.0%), MR approached a maximum level of ≈830 mN (curves 6 and 7). Contrary to MR, EX was decreased from 12.5 cm (without addition) to 10.7 cm (2.0%). These results confirm the importance of the amount of HMW subunits for gluten strength (Wieser et al 1994b).

The reoxidation of HMW subunits with $KIO_3$, however, resulted in a completely different effect (Fig. 4). By addition of

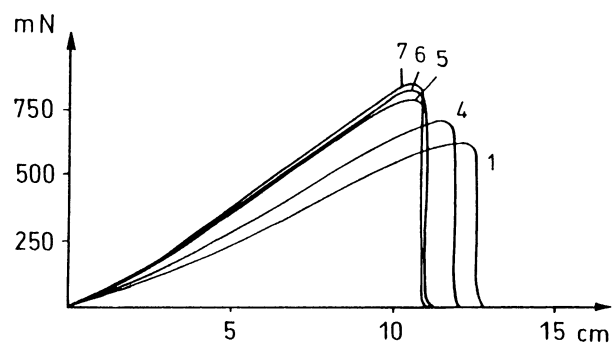


**Fig. 3.** Extensigrams of gluten from base flour of the cultivar Rektor without additive (1), with addition of 0.5% (4), 1.0% (5), 1.5% (6), and 2.0% (7) high molecular weight subunits of the cultivar Rektor reoxidized with $KBrO_3$.

1% of HMW subunits to the REK base flour, the MR of gluten dropped from 610 to 462 mN and EX from 12.5 to 11.6 cm (curve 8). Major differences when compared with $KBrO_3$ oxidation have been shown to be, both, the lower portion of polymerized subunits (≈31%) and the complete oxidation of thiol groups to intra- and intermolecular disulfide bonds (Schropp et al 1995). The importance of small amounts of free thiol groups for the incorporation of HMW subunits into the gluten network was also suggested by Bekes et al (1994). When, however, total thiol groups were free, as in reduced and nonalkylated subunits, the addition resulted in a weak (MR = 465 mN) and extensible (EX = 15.6 cm) gluten (curve 9). This affect appears to be comparable to those obtained with cysteine or glutathione. The amount of thiol groups of reduced subunits mixed to the base flour was equivalent to ≈90 ppm of the amino acid cysteine, and thus, much more than the amount usually used for weakening dough or gluten. Also monomeric, but S-alkylated, HMW subunits caused a similar reduction of MR (492 mN), but EX was in a range (12.8 cm) comparable with that of gluten of the base flour (curve 10).

For the comparison of HMW subunit compositions with different quality scores (Payne et al 1987), the HMW subunits of APO (2, 6, 8, 12; quality score 4) were oxidized with $KBrO_3$ under the same conditions as for REK (5, 7, 9, 10; quality score 7). Molecular weight distribution and thiol content of the reoxidized subunits from both cultivars have not shown any significant differences (Schropp et al 1995). As shown in Figure 5, the addition of 1% of HMW subunits to the base flour of REK had the same effect on gluten MR (759 and 753 mN respectively, curves 1, 5, 11), whereas EX appeared to be somewhat different (10.8 and 11.6 cm). Using flour from APO as base material caused a strong increase of MR, and a decrease of EX could be observed after addition of reoxidized REK and APO subunits (curves 2, 12, 13). Again, the effects of reoxidized subunits of both cultivars were only slightly different.

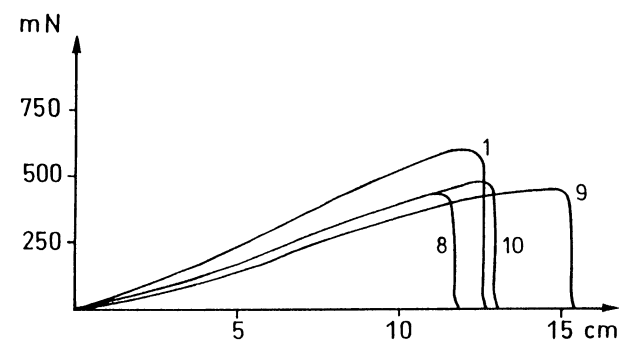


**Fig. 4.** Extensigrams of gluten from base flour of the cultivar Rektor without additive (1), with addition of 1% high molecular weight subunits of the cultivar Rektor reoxidized with $KIO_3$ (8), reduced (9), and S-alkylated with 4-vinylpyridine (10).

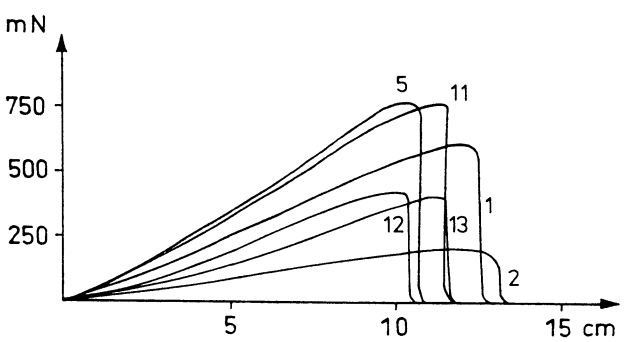


**Fig. 5.** Extensigrams of gluten from base flour of the cultivar Rektor without additive (1), with addition of 1% high molecular weight (HMW) subunits of the cultivar Rektor reoxidized with $KBrO_3$ (5), of 1% HMW subunits of the cultivar Apollo reoxidized with $KBrO_3$ (11) and from base flour of the cultivar Apollo without additive (2), with addition of 1% HMW subunits of the cultivar Rektor reoxidized with $KBrO_3$ (12) and with addition of % HMW subunits of the cultivar Apollo reoxidized with $KBrO_3$ (13).

## CONCLUSION

The addition of various gluten protein fractions to a standard flour influences the rheological properties of corresponding glutens in a different manner and to a different extent. The extensibility of gluten is increased by monomeric proteins (gliadins, reduced HMW subunits) and is decreased by aggregated proteins (reoxidized HMW subunits). The maximum resistance of gluten is increased by reoxidized HMW subunits, when the major portion is in a polymerized state (reoxidized with $KBrO_3$). In addition to molecular weight distribution, the thiol content of the reoxidation product appears to be important. The effects are strongly dependent on the amount of reoxidized subunits added to the base flour. A significant influence of differences in HMW subunit composition on gluten rheology could not, however, be established by the present study.

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