Reoxidation of High Molecular Weight Subunits of Glutenin

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ABSTRACT

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Reoxidation behavior of high molecular weight (HMW) subunits of glutenin was studied with regard to subunit composition (Rektor cultivar: 5, 7, 9, 10; Apollo cultivar: 2, 6, 8, 12), protein concentration (1, 2, and 3%), solvent composition and pH (2.0 and 8.0), oxidant ($KBrO_3$; KIO_3; and O_2) and reaction time (0–20 hr). The characterization of reoxidized products was achieved by the determination of the thiol content, the molecular weight distribution, and the subunit proportions. The results demonstrated that HMW subunits could be reoxidized to polymers with molecular weights up to several million. The different combinations of subunits did not influence the kinetic of oxidation and polymerization, and the proportions of subunits were independent of the degree of polymerization. Yield and molecular weight distribution of polymers were related both to the protein concentration and to the molar ratio of oxidants to thiol groups. Reoxidation with $KBrO_3$ and oxygen proceeded much slower than with KIO_3 and led to higher proportions of polymerized proteins. Obviously, more inter- and less intramolecular disulfide bonds were formed by oxidation with $KBrO_3$ and oxygen compared with KIO_3. The kinetics of reoxidation with the halates were independent of the pH values, whereas reoxidation with oxygen took place only at pH 8, and not at pH 2. The addition of urea did not affect reoxidation, whereas the addition of sodium dodecyl sulfate prevented any polymerization.

Gluten proteins are the most important components of the unique rheological properties (cohesivity, viscoelasticity) of wheat dough. According to their solubility in aqueous alcohols, they can be divided into the soluble, chiefly monomeric, gliadins and the insoluble aggregated glutenins. Both fractions differ in their contribution to dough properties: gliadins are cohesive but with low elasticity, whereas glutenins are both cohesive and elastic (Wrigley and Bietz 1988). Variations in dough properties of wheat cultivars are often ascribed to different amounts of large glutenin aggregates, which are stabilized by covalent and noncovalent forces. Disulfide bonds are of special importance, and the actions of dough improvers such as bromate, iodate, cysteine, and ascorbic acid are closely related to disulfide and thiol groups of gluten proteins.

High molecular weight (HMW) subunits of glutenin are proposed to play a key role in the formation of glutenin aggregates, and most models presented in the literature contain linear polymers of HMW subunits as a backbone linked by intermolecular disulfide bonds. Furthermore, numerous studies on wheat cultivars have stressed the importance of structure and amount of HMW subunits for dough properties and breadmaking quality. Nevertheless, only a few studies describe the oxidation behavior of HMW subunits. Recently, it has been demonstrated that both HMW and low molecular weight (LMW) subunits of glutenin delivered high portions of large polymers by reoxidation with oxygen, whereas reduced gliadins remained monomeric to a high degree (Werbeck and Belitz 1993). Detailed studies on the oxidation behavior of HMW subunits dependent on oxidizing agents and conditions are described here. Two different combinations of HMW subunits isolated from two cultivars with good and poor baking qualities, respectively, are compared.

MATERIALS AND METHODS

Preparation of HMW Fractions

According to the procedure described by Köhler et al (1991), the flours of the cultivars Rektor and Apollo 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 and then exhaustively extracted with 70 % aqueous ethanol to remove gliadins. Using the extraction-precipitation method of Marchylo et al (1989), the residues (glutenins) were extracted twice with 50% aqueous 1-propanol containing 0.05 mol/L of dithioerythritol at 60°C under nitrogen. After centrifugation, supernatants were combined, and HMW subunits precipitated by addition of 1-propanol to a final concentration of 60%. The precipitates were collected by centrifugation, dialyzed under nitrogen against acetic acid (0.01 mol/L) and lyophilized.

Characterization of HMW Fractions

Amino acid compositions of HMW fractions were determined after acid hydrolysis (Wieser et al 1987) using a Biotronic amino acid analyzer LC 5001. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Krause et al (1988). Thiol groups were quantified with Ellman's reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman 1959) using a calibration curve determined with reduced glutathione.

Reoxidation of HMW Fractions

A typical reoxidation assay was: 60 mg of HMW fraction of Rektor (48 mg of protein $\triangleq$ 4.6 μmol of Cys) were dissolved under nitrogen in 4.8 ml of trifluoroacetic acid (TFA; 0.1%, pH 2.0). After being stirred for 1 hr at room temperature (~20°C), 192 μg of $KBrO_3$ ($\triangleq$ 1.15 μmol) were added (molar ratio of $KBrO_3$ to Cys = 0.25) and stirred for another 20 hr. After 5, 15, 30, 60, 120, 240, and 1,200 min, 100- and 250-μl aliquotes were taken for the immediate determination of thiol groups and for gel-permeation chromatography (GPC), respectively. In the latter case, the reaction was stopped by the derivatization of thiol groups with 4-vinylpyridine at pH 8.4. In additional assays, the molar ratio of $KBrO_3$/Cys (0.125; 0.625, 1.25; 62.5), protein concentration (2 or 3%), and solvent (6 mol/L of urea plus 0.5 % triethylamin and 0.05 mol/L TRIS adjusted to pH 8.0 with acetic acid) were modified. Urea (6 mol/L) or SDS (1.5%) were added to two further assays.

The reoxidation with KIO_3 was performed using the molar ratios 0.0625 and 0.25. Additional 200-μl aliquotes were taken from the assay with the ratio 0.0625 to determine the amount of cysteic acid residues formed by reoxidation with KIO_3. These samples were reduced with $NaBH_4$ according to Henschen (1986) and then analyzed with Ellman's reagent.

The reoxidation with oxygen was achieved by supplying the samples with oxygen under a constant pressure of 120 mbar. Two further assays using TFA (pH 2.0) or urea plus buffer (pH 8.0) as solvent were analyzed.

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Analytical Gel-Permeation Chromatography

Before chromatography, cysteine residues were derivatized with a 10-fold molar excess of 4-vinylpyridine in a TRIS buffer at pH 8.4 containing 1.5% SDS. After 2 hr, the samples were filtered through a 0.45-μm membrane. The chromatography was performed using: precolumn, Ultropac G SWP (7.5 × 75 mm, Pharmacia); column, Ultropac TSK 4000 SW (7.5 × 300 mm, Pharmacia); elution solvent, TRIS HCl (62.5 mmol/L, pH 7.0) plus SDS (1.5%); flow rate, 0.3 ml/min; detection, UV absorbance at 220 nm.

Preparative Gel-Permeation Chromatography

A 60-mg HMW fraction from the cultivar Rektor (48 mg of protein, according to amino acid analysis) was dissolved in 4.8 ml of urea plus buffer (pH 8.0) and oxidized with oxygen under a pressure of 120 mbar and magnetic stirring. After 20 hr, the pH was adjusted to 3.5 with acetic acid. The solution was filtered through a 0.45-μm membrane and then chromatographed: column, Sephacryl S-500 HR superfine (3 × 95 cm, Pharmacia); elution solvent, urea (6 mol/L) plus NaCl (0.1 mol/L) adjusted to pH 3.5 with acetic acid; flow rate, 30 ml/hr; detection, UV absorbance at 280 nm. The eluate was divided into six fractions, which were collected, dialyzed against acetic acid (0.01 mol/L), and lyophilized. The protein content of each fraction obtained was determined by amino acid analysis.

Reversed-Phase HPLC

Fractions (1 mg) of I–V obtained by GPC on Sephacryl S-500 were dissolved under nitrogen in 1 ml of 50% (v/v) aqueous 1-propanol containing urea (2 mol/L), dithioerythritol (0.05 mol/L), and Tris HCl (0.082 mol/L, pH 7.5), and heated at 60°C for 15 min (Marchylo et al 1989). After the samples were filtered through a 0.45-μm membrane, 400-μl aliquotes were separated by reversed-phase (RP) HPLC on C_8 silica gel under the conditions described by Seilmeier et al (1991).

RESULTS

Isolation and Characterization of HMW Fractions

Glutens from the cultivars Rektor (good baking quality) and Apollo (poor baking quality) were extracted with 70% aqueous ethanol to remove gliadins. The remaining glutenins were dissolved under nitrogen in 50% aqueous 1-propanol containing dithioerythritol as a reducing reagent. HMW subunits were then precipitated selectively by increasing the propanol concentration to 60% (Marchylo et al 1989). After centrifugation, the precipitates were dialyzed under nitrogen against dilute acetic acid and lyophilized (HMW fractions).

The protein contents of both HMW fractions determined by amino acid analysis was ~80% (Table I). Their amino acid compositions were nearly identical and in good agreement with data from the literature (Moonen et al 1985, Wieser et al 1990). Total glutenin fractions and HMW fractions were compared by SDS-PAGE (Fig. 1). The protein patterns showed the different combinations of HMW subunits (Rektor: 5, 7, 9, 10; Apollo: 2, 6, 8, 12) already described by Krause et al (1988). The HMW fraction from the cultivar Apollo appeared to be free of contamination by other types of gluten proteins. That from the cultivar Rektor contained small amounts of ω-type gliadins, but was also free of LMW subunits. The thiol content of both fractions analysed with Ellman's reagent was similar and corresponded to 1.17 and 1.07% cysteine, respectively. The cysteine-cystine content determined by amino acid analysis indicated that the subunits were in a completely reduced form.

TABLE I
Amino Acid Composition (mol%) and Content of Cysteine and Protein (% by weight) of High Molecular Weight Fractions[a]

	Cultivar	
	Rektor	Apollo
Asx	1.2	1.2
Thr	3.3	3.4
Ser	6.7	6.4
Glx	36.6	36.2
Pro	12.7	12.1
Gly	17.1	18.0
Ala	3.3	3.4
Cys	1.1	1.0
Val	1.9	2.0
Met	0.5	0.6
Ile	1.0	0.9
Leu	3.8	4.1
Tyr	5.6	5.6
Phe	0.9	0.6
His	1.2	1.4
Lys	1.0	1.1
Arg	2.1	2.0
Cysteine		
A[b]	1.14	1.03
E[c]	1.17	1.07
Protein[b]	80.6	79.5

[a] Uncorrected values (mean of two determinations); Trp was not determined.
[b] Derived from amino acid analysis.
[c] Determined with Ellman's reagent.

Reoxidation with $KBrO_3$

Figure 2 presents the thiol content in dependence on the reaction time, when the HMW fraction from the cultivar Rektor (1% protein in 0.1% TFA) was reoxidized with different amounts of $KBrO_3$ (0.125–62.5, molar ratio of $KBrO_3$/Cys). With high amounts (62.5), the thiol groups were totally oxidized within 5 min. The decrease of thiol content was slower with lower amounts, and in the case of lowest amounts (0.25 and 0.125), considerably higher portions of thiol groups were present even after a reaction time of 20 hr. Almost the same results were obtained with a 3% protein solution, with urea buffer (pH 8.0) as solvent or with the HMW fraction from the cultivar Apollo (curves not shown).

The molecular weight distribution of reoxidized proteins was determined by GPC on TSK G 4000 (separation range for proteins:

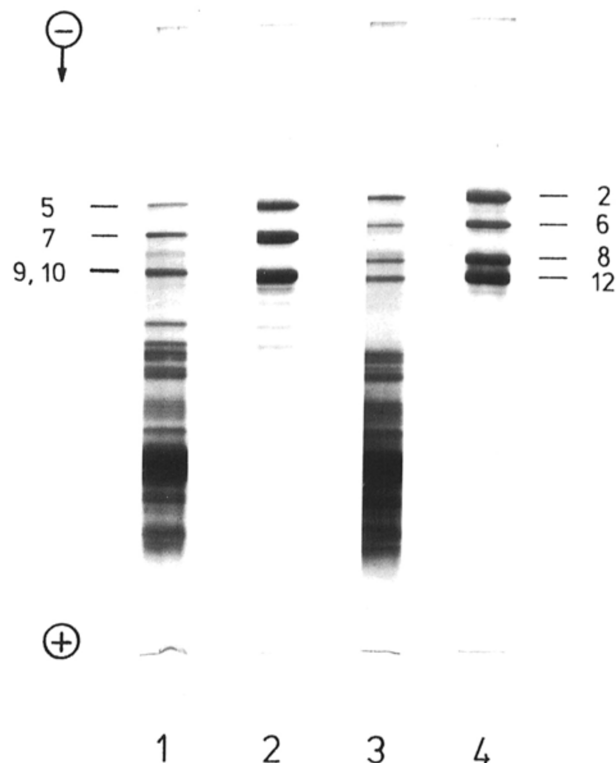

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoregrams of glutenin fractions: 1) whole glutenin, Rektor; 2) high molecular weight fraction, Rektor; 3) whole glutenin, Apollo; 4) HMW fractions, Apollo.

$M_r = 5 \times 10^3 - 10^6$). After reoxidation and before chromatography, thiol groups were derivatized with 4-vinylpyridine. As an example, the chromatograms of different HMW fractions from the cultivar Rektor (2% protein in 0.1% TFA) reoxidized with $KBrO_3$ (0.25) are presented in Figure 3. For quantification, the absorbance areas of the chromatograms were divided into five fractions according to peaks I–V. During the first hour of reaction, fraction IV corresponding to the monomeric HMW subunits was dominating; afterwards, the amount of polymerized proteins (fractions I–III) increased remarkably. The proportions of the different fractions are compiled in Table II. Assay 1 (chromatograms shown in Fig. 3) contained ~43% proteins in a monomeric form and ~53% in a polymeric form after 20 hr of reaction time. Using a 1% protein solution (assay 2), the proportion of the monomeric fraction IV was somewhat higher, and those of fractions I and II were lower. Because the thiol contents were the same as in assay 1, less intermolecular and more intramolecular disulfide bonds were apparently formed. The addition of high amounts of urea (assay 3) did not affect the results significantly. Obviously, hydrogen bonds do not influence the reoxidation behavior of HMW subunits. In contrast, the proteins remained chiefly in a monomeric form when SDS was added (assay 4). Thus, the polymerization was prevented when the proteins were covered with SDS. The comparison of assay 1 (Rektor) and assay 5 (Apollo) revealed that different combinations of subunits do not affect the degree of polymerization.

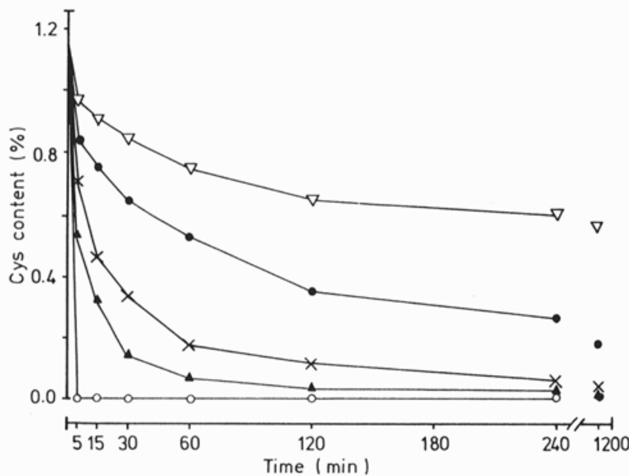

Fig. 2. Cysteine content of high molecular weight subunits (cv. Rektor, 1% protein, 0.1% trifluoroacetic acid) reoxidized with different amounts of $KBrO_3$. ▽ = 0.125; ● = 0.25; × = 0.625; ▲ = 1.25; ○ = 62.5.

Reoxidation with KIO_3

The complete oxidation of the thiol groups of HMW subunits with KIO_3 (0.25) was achieved within 5 min (Fig. 4). Even with very low amounts (0.0625), the content of thiol groups decreased very fast and remained constant after the oxidizing agent was consumed. In theory, the molar ratio KIO_3/Cys (0.17) was necessary to oxidize all thiol groups in the solution (Tkachuk and Hlynka 1961). The pH values of the solvents used (2.0 or 8.0) did not influence the kinetics of the reoxidation (curve not shown). The oxidation products obtained with KIO_3 were fully reducible with sodium borohydride (Fig. 4); consequently, thiols were exclusively oxidized to disulfides, but not to cysteic acid as supposed by Hird and Yates (1961) and Graveland (1983).

The molecular weight distribution of HMW fractions oxidized with KIO_3 (0.25) (Table III, assay 6) reflects very well the thiol contents determined. After 5 min, the maximum proportion of polymerized proteins (fractions I–III: 42.9%) was obtained, this decreased to 30.6% after 20 hr. The degree of polymerization was lower in a 1% than in a 2% protein solution (assays 6 and 7). Compared with $KBrO_3$ in equimolar amounts, KIO_3 was by far the faster and more effective oxidizing agent, and obviously, more intramolecular and less intermolecular disulfide bonds were formed.

Reoxidation with Oxygen

The reoxidation with oxygen was performed under a constant pressure of 120 mbar. Figure 5 shows the thiol content of HMW subunits (Rektor, 1%protein) oxidized at different pH values (2.0 or 8.0). The decrease of thiol content at pH 8.0 was similar to that with $KBrO_3$ (0.25) during the first 180 min. Afterwards, the decrease was more pronounced using oxygen, and the thiol groups were completely oxidized after 20 hr. The reason for these different effects may be that oxygen was added permanently, whereas the amount of $KBrO_3$ was constantly diminished. At pH 2.0, however, remarkable differences between the reactivity of oxygen and the halates were observed: the thiol groups were not at all oxidized by oxygen (Fig. 5).

The influence of the pH value on the reoxidation behavior was also demonstrated by GPC (Table IV). The molecular weight distribution did not change significantly during oxidation at pH 2.0 (assay 8). The proportion of the monomeric fraction IV, however, dropped from 83.5 to 27.8% after 20 hr of reaction time, oxidizing at pH 8.0 (assay 9). Comparing all assays per-

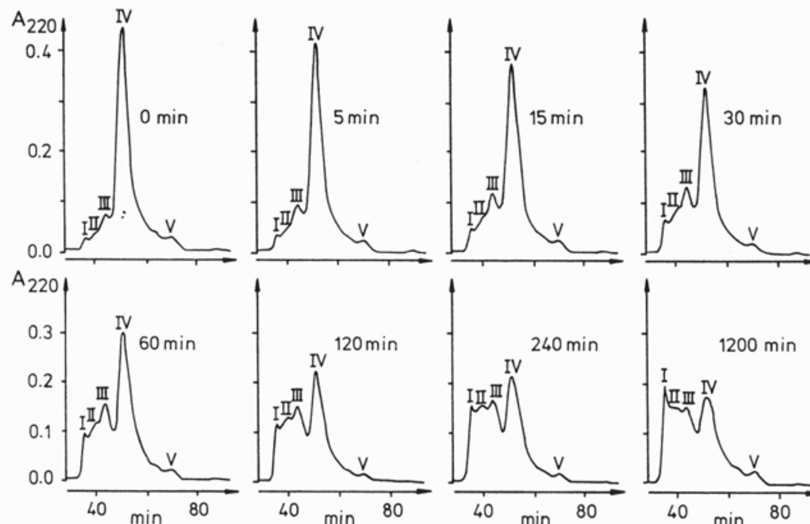

Fig. 3. Analytical gel-permeation chromatography (TSK G 4000) of high molecular weight fractions (cv. Rektor, 2% protein, 0.1% trifluoroacetic acid) reoxidized with $KBrO_3$ (0.25) for different time periods.

formed, reoxidation with oxygen at pH 8.0 lead to the highest proportions of the highly polymerized fractions I and II (21.8 and 24.6%, respectively).

For the quantitative analysis of single subunits, the HMW fraction of Rektor was reoxidized at pH 8.0 for 20 hr and preparatively separated on Sephacryl S-500 (separation range for proteins $M_r = 10^4 - 2 \times 10^7$). The eluate was divided into six fractions (Fig. 6), which were dialyzed and analyzed for protein content by amino acid analysis. The protein yield of single fractions according to the total reoxidized fraction is presented in Table V. In accordance with the analytical GPC on TSK 4000 (Table IV), there was only a minor portion of monomeric proteins (fraction V = 20.4%) in the reoxidized sample. The molecular weights of polymerized subunits were distributed rather regularly over the whole separation range up to the void volume ($M_r = 2 \times 10^7$). The proportions of single subunits of the fractions I–V were determined by RP-HPLC on C_8 silica gel using the conditions described by Seilmeier et al (1991). The results did not reveal any significant difference and were in agreement with a previous analysis of the total HMW fraction from Rektor (Seilmeier et al 1991).

TABLE II
Proportion (%) of Fractions Obtained by Gel-Permeation Chromatography for Reoxidation with $KBrO_3$ (0.25)[a]

Conditions	Fractions	0	5	15	30	60	120	240	1200
		Reaction Time (min)							
Assay 1:									
2% protein,	I	1.6	2.1	3.0	4.6	5.9	8.7	11.4	13.7
0.1% TFA,[b]	II	3.7	4.9	6.7	8.8	11.6	16.4	17.9	18.7
cv. Rektor	III	7.7	11.1	15.2	19.0	20.7	21.8	21.7	20.9
	IV	83.5	78.9	72.2	64.9	59.1	50.9	46.5	43.1
	V	3.5	3.0	2.9	2.7	2.7	2.2	2.5	3.6
Assay 2:									
1% protein,	I	1.8	2.0	2.6	3.1	4.5	5.5	6.8	9.4
0.1% TFA,	II	3.9	4.2	6.2	7.1	9.8	12.6	16.4	14.9
cv. Rektor	III	8.0	12.1	13.5	17.1	19.8	22.3	22.7	22.3
	IV	83.1	78.8	74.6	69.7	62.7	56.7	51.2	50.0
	V	3.2	2.9	3.1	3.0	3.2	2.9	2.9	3.4
Assay 3:									
2% protein,	I	1.6	1.7	1.7	2.2	3.4	4.2	7.2	13.2
0.1% TFA,	II	4.0	4.3	5.0	6.5	9.4	12.4	16.7	20.5
cv. Rektor	III	8.1	9.9	11.4	14.1	16.7	18.9	19.5	17.4
+ urea	IV	83.8	81.0	78.8	74.2	67.6	61.5	54.1	46.6
(6 mol/L)	V	2.5	3.1	3.1	3.0	2.9	3.0	2.5	2.3
Assay 4:									
2% protein,	I	1.7	1.8	1.7	1.5	1.8	1.5	1.7	2.2
0.1% TFA,	II	3.9	3.6	3.4	3.3	4.0	3.6	3.6	3.9
cv. Rektor	III	8.1	8.0	7.7	7.7	7.8	8.0	7.2	8.3
+ SDS	IV	83.0	83.4	83.6	84.0	83.0	83.4	84.0	82.0
(1.5%)	V	3.3	3.2	3.6	3.5	3.4	3.5	3.5	3.6
Assay 5:									
2% protein,	I	1.6	2.5	3.3	5.2	7.6	9.7	13.7	16.0
0.1% TFA,	II	3.9	5.6	7.2	10.5	13.3	15.4	18.0	19.8
cv. Apollo	III	8.1	11.6	14.2	15.9	15.5	16.8	16.2	15.9
	IV	82.4	76.9	72.2	66.0	61.1	55.7	49.8	46.1
	V	4.0	3.4	3.1	2.4	2.5	2.4	2.3	2.2

[a]Results of single experiments on TSK G 4000.
[b]Trifluoroacetic acid.

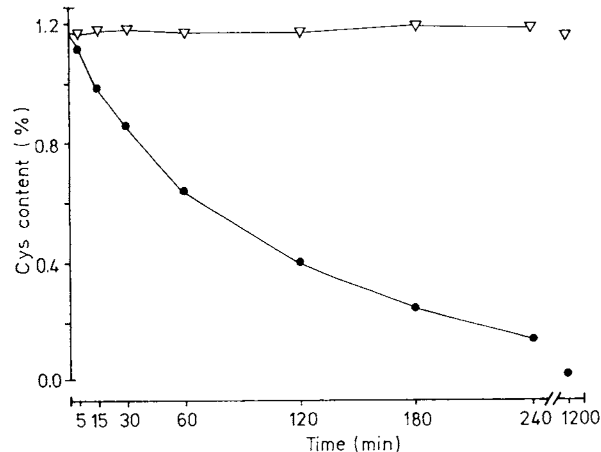

Fig. 5. Cysteine content of high molecular weight subunits (cv. Rektor, 1% protein) reoxidized with O_2 at different pH values (∇ = pH 2.0; ● = pH 8.0).

TABLE III
Proportion (%) of Fractions Obtained by Gel-Permeation Chromatography for Reoxidation with KIO_3 (0.25)[a]

Conditions	Fractions	0	5	15	30	60	120	240	1200
		Reaction Time (min)							
Assay 6:									
2% protein,	I	1.8	7.1	7.4	7.1	6.5	5.9	6.0	6.2
0.1% TFA,[b]	II	4.0	13.2	12.1	11.9	11.3	9.6	8.8	7.9
cv. Rektor	III	7.9	22.6	21.8	22.2	20.8	20.2	19.0	16.5
	IV	82.9	54.7	55.8	55.7	58.4	60.3	61.9	64.5
	V	3.4	2.4	2.9	3.1	3.0	4.0	4.3	4.9
Assay 7:									
1% protein,	I	1.9	6.6	6.3	6.5	6.2	5.8	5.6	5.8
0.1% TFA,	II	4.2	8.9	9.5	9.0	7.9	8.1	7.0	6.8
cv. Rektor	III	7.7	16.5	16.8	16.5	15.5	15.0	14.2	12.5
	IV	83.5	65.6	65.1	65.4	67.3	67.8	69.4	70.6
	V	2.7	2.4	2.3	2.6	3.1	3.3	3.8	4.3

[a]Results of single experiments on TSK G 4000.
[b]Trifluoroacetic acid.

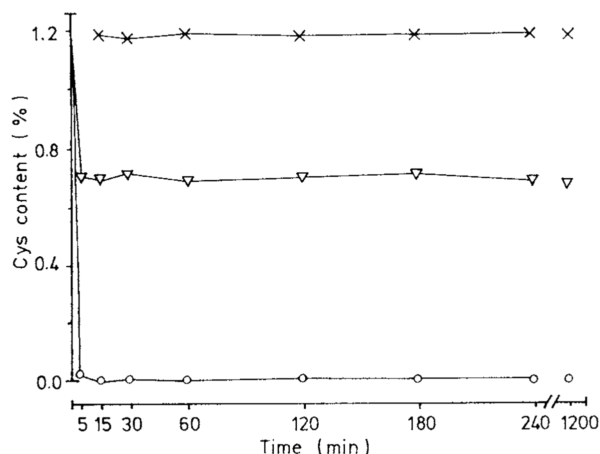

Fig. 4. Cysteine content of high molecular weight subunits (cv. Rektor, 1% protein, 0.1% trifluoroacetic acid) reoxidized with different amounts of KIO_3. ∇ = 0.0625; ○ = 0.25; × = assay (0.0625) reduced with $NaBH_4$.

TABLE IV
Proportion (%) of Fractions Obtained by Gel-Permeation Chromatography for Reoxidation with O_2[a]

Conditions	Fractions	0	5	15	30	60	120	240	1200
		Reaction Time (min)							
Assay 8:									
2% protein,	I	1.7	1.8	1.8	1.6	1.9	1.6	1.8	2.0
0.1% TFA,[b]	II	3.8	3.8	4.2	3.9	3.8	3.5	3.7	3.9
cv. Rektor	III	7.9	7.9	8.1	8.1	7.8	7.6	8.0	8.4
	IV	83.3	83.0	82.9	83.5	83.3	84.0	83.7	82.6
	V	3.3	3.5	3.0	2.9	3.2	3.3	2.8	3.1
Assay 9:									
2% protein,	I	1.6	2.1	3.1	4.4	6.0	9.3	13.7	21.8
urea buffer	II	3.7	6.2	8.2	10.6	11.4	15.5	18.9	24.6
(pH 8.0),	III	7.8	9.4	12.6	17.5	21.0	25.5	30.0	22.4
cv. Rektor	IV	83.5	79.0	73.2	64.4	59.0	46.8	34.4	27.8
	V	3.4	3.3	2.9	3.1	2.6	2.9	3.0	3.4

[a]Results of single experiments on TSK G 4000.
[b]Trifluoroacetic acid.

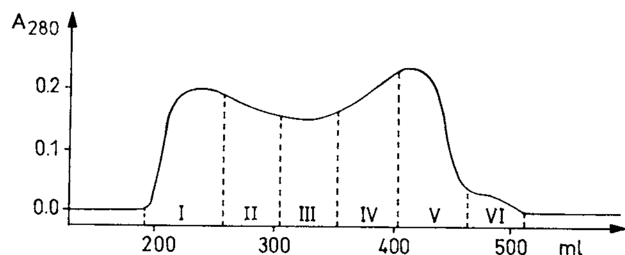

Fig. 6. Preparative gel-permeation chromatography (Sephacryl S-500) of the high molecular weight fraction of cv. Rektor reoxidized with O_2 for 20 hr.

DISCUSSION

The wheat cultivars Rektor and Apollo, with different combinations of HMW subunits, were selected for the present study. The combination 5, 7, 9, 10 of Rektor has been related to good breadmaking quality and the combination 2, 6, 8, 12 of Apollo has been related to poor quality (Payne et al 1984). The preparation of subunits from gluten in amounts and purity necessary for reoxidation experiments was achieved by a specific extraction-precipitation procedure described by Marchylo et al (1989). Amino acid and thiol content analysis showed that the subunits produced were in a fully reduced state before reoxidation. The effect of three oxidizing agents were compared: $KBrO_3$ and KIO_3, which are applied as dough improvers, and oxygen acting as usual oxidant during dough mixing. The oxidation products obtained under varied conditions were characterized by the determination of thiol contents and molecular weight distributions. Generally, the products remained soluble in the solvent used, even when high molecular weights ($>10^6$) were reached.

In agreement with previous studies (Werbeck and Belitz 1993), the amount of polymerized proteins was correlated positively to the concentration of HMW subunits; obviously, more intermolecular disulfide bonds were formed by using higher concentrations. This effect was also demonstrated for reduced glutenin by Beckwith and Wall (1966). The decrease of thiol content and the increase of average molecular weight was additionally related to the molar ratio of oxidant to the thiol content of HMW subunits. Reoxidation under identical conditions was significantly faster with KIO_3 than with $KBrO_3$; even applied in very low amounts, the oxidation with KIO_3 was finished within 5 min. These observations agree with data from the literature as to which iodates are classed as fast-acting. Bromates are slow-acting improvers and the optimum amount of KIO_3 is much lower than that of $KBrO_3$ (Kamman 1984). Moreover, the reoxidation with KIO_3 yielded chiefly monomers with intramolecular disulfide bonds, whereas the reoxidation with $KBrO_3$ led to a much higher proportion of polymerized proteins probably linked by intermolecular disulfide bonds. The kinetics of reoxidation with $KBrO_3$ and oxygen were similar within the first hours; afterwards, the continuous supply of oxygen resulted in lower thiol contents and higher average molecular weights. In contrast to the halates, the effect of oxygen was strongly dependent on the pH value of the solution; at pH 8.0, the thiol groups were completely oxidized within 20 hr and at pH 2.0, however no reaction could be observed. A low reoxidation rate of reduced glutenin with oxygen under acidic conditions (pH 3.5) was also described by Beckwith and Wall (1966). The reason for this dependence on pH remains unclear.

Dough properties and breadmaking quality of wheat cultivars have often been associated with the amount of large glutenin aggregates and, on the level of protein components, with the presence of certain HMW subunits. With respect to the reoxidation behavior, however, the present study did not reveal any difference between the two combinations of HMW subunits representative for good and poor quality. Moreover, quantitative RP-HPLC indicated that the single subunits were incorporated into polymers in the same proportions as in the reduced HMW fraction. Further investigations are currently in progress to demonstrate the reoxidation behavior of single HMW subunits, also in combination with LMW subunits.

TABLE V
Protein Yield (% of protein applied on the column) of Fractions Obtained by Gel-Permeation Chromatography

	Fractions						
R[a]	I	II	III	IV	V	VI	Σ
3.1	14.6	15.2	13.8	17.9	20.4	0.6	85.6

[a] Insoluble after reoxidation.

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