

Reoxidation Behavior of Wheat and Rye Glutelin Subunits

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ABSTRACT

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The ability of HMW and LMW subunits of wheat glutelin to form a polymeric gluten network by intermolecular disulfide bonds is responsible for the unique rheological properties and baking quality of wheat dough. Because the mechanism of gluten formation is not fully understood, the reoxidation behavior of HMW and LMW subunits of wheat glutelin and HMW subunits of rye glutelin was studied. The subunits were isolated from wheat flour cv. Rektor (REK) and from rye flour cv. Danko (DAN) with a selective extraction and precipitation method. For reoxidation, different oxidants (KBrO₃ and KIO₃), protein concentrations (0.5, 1.0, and 2.0%), solvent compositions, pH values (2.0 and 8.0), and reaction times (0–360 min) were compared. The characterization of reoxidized products was achieved by the determination of the thiol content with the Ellman's reagent, and of the *M_r* distribution by gel-permeation chromatography. The results demonstrated that both HMW and LMW subunits could be slowly reoxidized with KBrO₃ to polymers with *M_r* up to several millions. Yield and *M_r* distribution of polymers were dependent both on the protein concentration and on the molar ratio of oxidants to thiol groups. The HMW

subunits of wheat glutelin (HMW-REK) yielded slightly higher quantities of polymeric proteins than did the HMW subunits of rye (HMW-DAN). Reoxidation with KIO₃ proceeded much faster than with KBrO₃ and led to lower proportions of polymerized proteins for HMW-REK and HMW-DAN. Obviously, more intra- and fewer intermolecular disulfide bonds were formed by reoxidation with KIO₃ compared with KBrO₃. In contrast, LMW-REK was reoxidized with KIO₃ to higher amounts of polymeric aggregates, which indicated that LMW subunits formed intermolecular disulfide bonds with both KIO₃ and KBrO₃. Independent of the protein type and the oxidant used for reoxidation, more inter- and fewer intramolecular disulfide bonds were formed when the protein concentration was increased. Single subunits 5, 7, and 10 were isolated from HMW-REK by preparative acid-PAGE and were reoxidized with KBrO₃ for 360 min. The *M_r* distribution indicated that *x*-type subunit 5 had a greater tendency to form polymers than *x*-type subunit 7. The *y*-type subunit 10 was characterized by a lower proportion of polymers after reoxidation than *x*-type subunits 5 and 7.

HMW subunits of wheat glutelin are assumed to play a key role in the formation of gluten aggregates, and numerous studies have demonstrated the importance of both structure and quantity of HMW subunits for dough rheology and breadmaking quality of wheat cultivars (Weegels et al 1996). LMW subunits also are integrated into gluten aggregates and are important for dough and gluten properties (Wieser et al 1994b). Neither HMW and LMW subunits occur in flour and gluten as monomers but are polymerized by means of intermolecular disulfide bonds (Shewry and Tatham 1997, Müller and Wieser 1997). For a better understanding of the mechanism of gluten formation, it is of interest to investigate the reoxidation behavior of different types of glutelin subunits. Reoxidation studies have already been described for HMW subunits of wheat glutelin (Schropp et al 1995, Szabo et al 1995, Candler et al 1996). Previous experiments demonstrated that the reoxidation of HMW subunits with KBrO₃ and oxygen proceeded much slower than with KIO₃ and led to higher proportions of polymerized proteins (Schropp et al 1995). Obviously, more intermolecular and fewer intramolecular disulfide bonds were formed by oxidation with KBrO₃ and oxygen compared with KIO₃. The present work deals with reoxidation of the total LMW subunit fraction and single HMW subunits of wheat, as well as with the total HMW subunit fraction of rye. The latter subunits are homologous to wheat HMW subunits (Kipp et al 1996) but are not involved in the formation of a gluten-like material. For comparison and for the demonstration of reproducibility, the HMW subunits of wheat were included again.

MATERIALS AND METHODS

Isolation and Characterization of HMW and LMW Subunits

Kernels of the wheat cultivar Rektor (REK) and of the rye cultivar Danko (DAN) were milled to flours using a laboratory mill (Brabender Quadrumat Jr). Flours were sieved through a 0.2-mm sieve and 500 g of flour was defatted twice with light petroleum (40–60°C boiling range; 2 L) for 30 min at room temperature (≈20°C).

The solvent was removed by filtration and the flour was dried overnight at rt. Different procedures (Marchylo et al 1989, Melas et al 1994) were tested and optimized for the selective extraction and precipitation of HMW and LMW subunits. The purest fractions of wheat were obtained when defatted flour (24 g) was extracted three times with 60% (v/v) ethanol (600 mL) at rt for 30 min. Subsequently, the residue was extracted twice with 50% (v/v) 2-propanol containing 80 mM Tris-HCl (pH 8.0; 120 mL) and 1.0% (w/v) dithioerythritol (DTE) for 30 min at 60°C under nitrogen. After centrifugation (5 min, 20°C, 40,000 × *g*), supernatants were combined and HMW subunits were precipitated by the addition of acetone to a final concentration of 40% (v/v). Supernatant (LMW subunits) and precipitate (HMW subunits) were dialyzed against nitrogen-saturated 0.01M acetic acid and freeze-dried. The HMW subunits of rye glutelin were obtained according to the method described by Kipp et al (1996), whereby flour was directly extracted with 50% 1-propanol containing 1% (w/v) DTE, and the HMW subunits were precipitated by giving a final concentration of 60% 1-propanol.

Amino acid compositions of HMW and LMW subunits were analyzed after acid hydrolysis (6M HCl, 110°C, 24 hr) using a Biotronic amino acid analyzer LC-5001. The protein content of HMW and LMW subunits was determined according to the Dumas method ($N \times 5.7$) using a protein-nitrogen analyzer (Leco FP-328). 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 established with reduced glutathione. The procedure used was described by Schropp et al (1995). Concentrations of HMW and LMW subunits in the isolated fractions were determined by RP-HPLC according to Wieser et al (1998).

Reoxidation of HMW and LMW Subunits

Glutelin subunits (18 mg of protein) were dissolved in 0.1% (v/v) trifluoroacetic acid (TFA) (1.8 mL, pH 2.0). After stirring for 1 hr at rt, 25 μL (for HMW subunits) and 40 μL (for LMW subunits) of KBrO₃ solution (1.8 mg KBrO₃/1 mL of 0.1% TFA) were added, representing a molar ratio of KBrO₃ to Cys of 0.625; the mixture was stirred for another 6 hr. After 5, 15, 30, 60, 120, 180, 240, 300, and 360 min, 160-μL aliquots were taken and immediately mixed with 0.1% TFA (340 μL), 50% 1-propanol and NaKHPO₄ (0.05M, pH 8.0, 1 mL) and Ellman's reagent (39.6 mg

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of DTNB/10 mL of NaKHPO₄ [0.5M, pH 7.0], 0.5 mL). The samples were allowed to stand over 30 min at rt; then they were centrifuged (10 min, 3,000 × g; 20°C) and measured at 412 nm against a blank. In additional assays, the molar ratio of KBrO₃ to Cys (1.25, 62.5) and the type of solvent (6M urea + 0.5% [v/v]triethylamine + 0.05M Tris-HCl, pH 8.0) were modified (Schropp et al 1995). The reoxidation with KIO₃ was performed using molar ratios of KIO₃ to Cys of 0.0625 and 0.25.

Reoxidation Before Gel-Permeation Chromatography

Glutelin subunits (25 mg of protein) were dissolved in 0.1% (v/v) TFA (2.5 mL). After stirring for 1 hr at rt, 50 µL (for HMW subunits) and 80 µL (for LMW subunits) of KBrO₃ solution (1.8 mg of KBrO₃/1 mL of 0.1% TFA) or 100 µL (HMW subunits) and 160 µL (LMW subunits) of KIO₃ solution (1.2 mg of KIO₃/1 mL of 0.1% TFA) were added to obtain a molar ratio of oxidant to Cys of 0.25. For assays with a molar ratio of 0.5, the amounts of oxidizing agents were duplicated. After 5, 15, 30, 60, 120, 180, 240, 300, and 360 min, 250-µL aliquots were taken for derivatization with 4-vinylpyridine and subsequent gel-permeation chromatography (GPC) analysis. The derivatization of cysteine residues was performed with a 10-fold molar excess of 4-vinylpyridine in 125 mM Tris-HCl (pH 8.4) containing 1.5% (w/v) SDS at rt for 2 hr (Schropp et al 1995).

Isolation and Reoxidation of Single HMW Subunits

Defatted flour of REK was extracted with 50% (v/v) 1-propanol containing 50 mM DTE, and the HMW subunits were precipitated by addition of 1-propanol to a final concentration of 60% (v/v) following the procedure of Marchylo et al (1989).

The precipitated subunits were dissolved in a solvent containing 6M urea, 25 mM acetic acid, 30% (v/v) glycerine, and 0.5% (w/v) DTE, and incubated for 15 min at 60°C. Samples (15 µL) were separated by acid-PAGE using a 10.5% acrylamide gel (Khelifi

and Branlard 1991, Morel 1994). Single HMW bands were cut out of the gel and the gel strips were extracted with 50% (v/v) 1-propanol. The suspension was centrifuged (15 min, 750 × g, 20°C), and the supernatants were filtered through a 0.45-µm membrane, dialyzed against nitrogen-saturated 0.01M acetic acid and freeze-

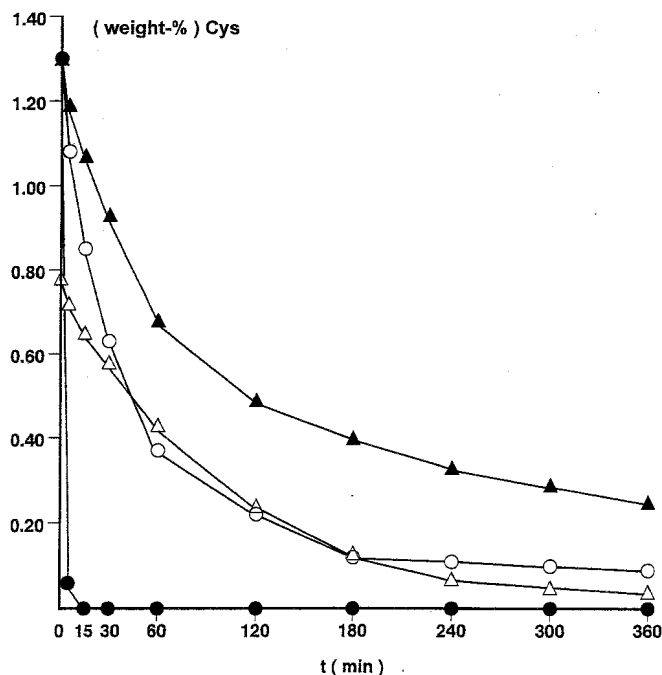


Fig. 1. Cysteine content of LMW Rektor (1% protein) reoxidized with different ratios of KBrO₃ to Cys (▲ = 0.625, pH 2.0; ○ = 1.25, pH 2.0; ● = 62.5, pH 2.0; △ = 0.625, pH 8.0) depending on reoxidation time.

TABLE I
Proportions (%) of Fractions Obtained by Gel-Permeation Chromatography After Reoxidation of HMW and LMW Subunits with KBrO₃ at pH 2.0

Conditions ^a	Fraction	Time (min)				
		0	5	30	180	360
Assay 1	I	0.0	0.0	0.7	2.4	2.7
	II	10.4	13.4	17.2	24.3	29.9
	III	86.2	84.8	79.4	70.9	64.0
	IV	3.3	1.8	2.7	2.4	3.4
Assay 2	I	0.0	0.0	2.4	3.9	4.7
	II	8.3	10.3	21.4	36.8	46.2
	III	84.0	80.7	63.6	49.8	43.9
	IV	7.7	9.0	12.6	9.5	5.2
Assay 3	I	0.0	0.0	0.0	3.6	4.0
	II	0.0	0.0	12.7	40.0	43.8
	III	7.8	16.3	23.0	13.5	13.9
	IV	92.2	83.7	64.3	42.9	38.3
Assay 4	I	0.0	0.0	1.3	4.3	4.4
	II	0.0	0.0	24.2	43.4	44.9
	III	7.4	25.7	25.1	15.5	15.5
	IV	92.6	74.3	49.4	36.8	35.2
Assay 5	I	0.0	2.2	3.2	4.5	5.0
	II	0.0	14.3	35.9	56.1	57.3
	III	8.7	17.3	11.0	6.5	9.4
	IV	91.3	66.2	49.9	32.9	28.3
Assay 6	I	0.0	0.0	4.2	4.9	4.8
	II	2.5	10.0	30.0	42.3	46.2
	III	20.1	34.9	27.8	21.0	19.3
	IV	77.4	55.1	38.0	31.8	29.7
Assay 7	I	1.5	2.0	2.6	2.3	2.8
	II	26.0	28.7	34.4	45.5	42.2
	III	58.0	56.0	51.9	43.2	45.5
	IV	14.5	13.7	11.1	9.0	9.5

^a Assay 1 = 0.5% HMW-Rektor (REK) KBrO₃ (0.25); assay 2 = 1% HMW-REK KBrO₃ (0.25); assay 3 = 0.5% LMW-REK KBrO₃ (0.25); assay 4 = 1% LMW-REK KBrO₃ (0.25); assay 5 = 1% LMW-REK KBrO₃ (0.5); assay 6 = HMW/LMW-REK KBrO₃ (0.25); assay 7 = HMW-Danko KBrO₃ (0.25) [numbers in parentheses molar ratio of oxidant to cysteine content].

dried. The purified HMW subunits (6 mg) were mixed with 2.5% (w/v) NaBH₄ solution (400 μL) to reduce disulfide bonds which possibly were formed during extraction. The reduction was performed at 50°C for 60 min. Nonconsumed NaBH₄ was destroyed by the addition of 1.0M HCl (200 μL). Reoxidation with KBrO₃ (molar ratio, 0.25) was performed as described above.

GPC

The samples were filtered through a 0.45-μm membrane. GPC conditions were precolumn = aqua-OH-8-P (50 × 7.7 mm; Machery-

Nagel); main column = aqua-OH-50-8 (300 × 7.7 mm; Machery-Nagel) (separation range for proteins: $M_r = 2 \times 10^4 - 100 \times 10^4$); elution solvent = 62.5 mM Tris HCl (pH 7.0) containing 1.5% (w/v) SDS; flow rate = 0.6 mL/min; detection = UV absorbance at 220 nm.

Because of limited amounts of proteins available for reoxidation, only single experiments were performed. The comparison of results with a previous study on the reoxidation behavior of HMW-REK (Schropp et al 1995) indicated a good reproducibility of the distribution into monomeric and polymeric fractions. Reproducibility

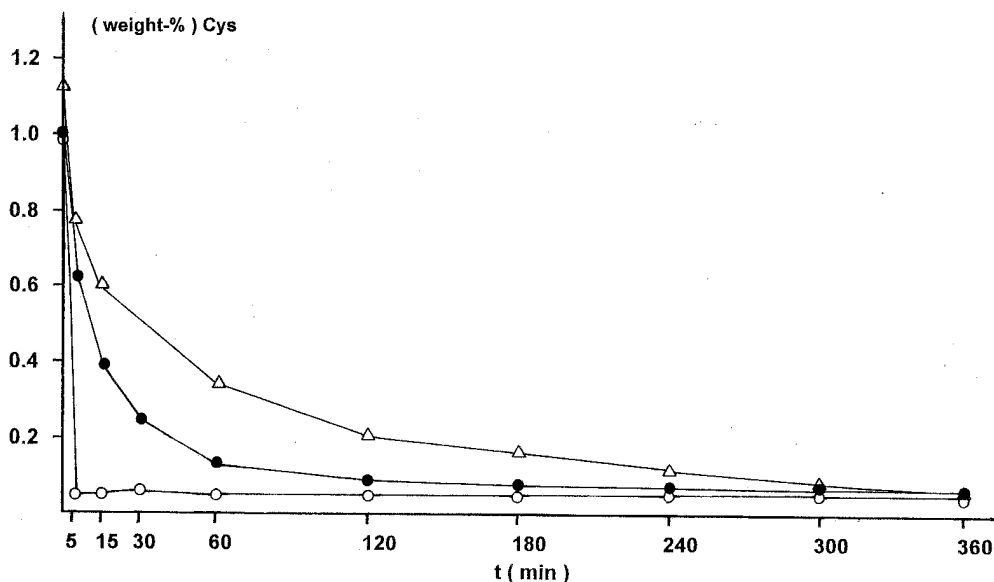


Fig. 2. Cysteine content of HMW Danko (1% protein) reoxidized with KBrO₃ (● = molar ratio to Cys 0.5, pH 2.0) and with KIO₃ (○ = molar ratio to Cys 0.25, pH 2.0) and of HMW Rektor (1% protein) reoxidized with KBrO₃ (Δ = molar ratio to Cys 0.625, pH 2.0) depending on reoxidation time.

TABLE II
Proportions (%) of Fractions Obtained by Gel-Permeation Chromatography After Reoxidation of HMW and LMW Subunits with KIO₃ at pH 2.0

Conditions ^a	Fraction	Time (min)				
		0	5	30	180	360
Assay 8	I	0.0	4.0	3.4	3.3	3.4
	II	17.8	40.1	41.8	39.9	39.9
	III	77.1	51.9	50.6	52.4	52.1
	IV	5.1	4.0	4.2	4.4	4.6
Assay 9	I	0.0	4.9	5.0	4.9	5.0
	II	10.3	42.4	41.7	42.5	42.5
	III	79.2	45.2	45.2	45.4	45.4
	IV	10.5	7.5	8.1	7.2	7.1
Assay 10	I	0.0	3.9	4.2	5.2	5.6
	II	0.0	42.5	46.1	53.6	58.2
	III	8.0	14.3	12.6	9.9	8.4
	IV	92.0	39.3	37.1	31.3	27.8
Assay 11	I	0.0	6.6	8.6	10.3	9.9
	II	0.0	55.5	62.1	61.0	67.2
	III	7.3	10.9	8.0	9.6	7.3
	IV	92.7	27.0	21.3	19.1	15.6
Assay 12	I	0.0	6.4	8.4	9.9	10.4
	II	0.0	67.9	68.3	71.6	69.3
	III	5.5	5.7	6.0	4.3	4.8
	IV	94.5	20.0	17.3	14.2	15.5
Assay 13	I	0.0	4.2	4.1	5.4	6.2
	II	7.4	43.4	42.6	45.7	48.8
	III	32.5	28.7	28.0	23.6	22.7
	IV	60.1	23.7	25.3	25.3	22.3
Assay 14	I	0.0	2.2	2.7	2.1	1.9
	II	21.3	38.4	39.0	36.2	35.8
	III	67.3	49.2	47.5	49.5	51.0
	IV	11.4	10.2	10.8	12.2	11.3

^a Assay 8 = 1% HMW-Rektor (REK) KIO₃ (0.25); assay 9 = 2% HMW-REK KIO₃ (0.25); assay 10 = 0.5% LMW-REK KIO₃ (0.25); assay 11 = 1% LMW-REK KIO₃ (0.25); assay 12 = 2% LMW-REK KIO₃ (0.25); assay 13 = 1% HMW/LMW-REK KIO₃ (0.25); assay 14 = 1% HMW-Danko KIO₃ (0.25) [numbers in parentheses molar ratio of oxidant to cysteine content].

bility was also demonstrated by different assays with nonreoxidized LMW-REK (0 min, Tables I and II). However, when HMW-REK or HMW-DAN, were analyzed before reoxidation was started (0 min), reproducibility was poor. Obviously, slight oxidation took place during storage of the freeze-dried proteins, and results were influenced by the storage time.

RESULTS AND DISCUSSION

Isolation and Characterization of HMW and LMW Subunits

For the preparation of total HMW and LMW glutelin subunits of wheat cv. Rektor (HMW-REK and LMW-REK), the procedure of Melas et al (1994) was optimized so that both fractions could be obtained with relatively high purity and yield, as evaluated by SDS-PAGE and RP-HPLC. The quantification by RP-HPLC (Wieser et al 1998) indicated a purity of 80 and 88% for HMW-REK and LMW-REK, respectively. HMW glutelin subunits of rye cv. Danko (HMW-DAN) were isolated as previously described (Kipp et al 1996). The yields of HMW-REK (1.0% by flour weight), HMW-DAN (0.32%), and LMW-REK (2.3%) were in agreement with the yields obtained previously (Schropp et al 1995, Kipp et al 1996, Wieser et al 1998). The protein contents were 88.0, 82.8, and 85.5% for HMW-REK, HMW-DAN, and LMW-REK, respectively.

The thiol contents of HMW-REK, HMW-DAN, and LMW-REK determined with Ellman's reagent corresponded to 1.12, 1.02, and 1.31% cysteine by weight, respectively. Cys contents determined by amino acid analysis were 1.09, 1.40, and 2.19% for HMW-REK, LMW-REK, and HMW-DAN, respectively, and were in good agreement

with data from the literature (Melas et al 1994, Schropp et al 1995, Kipp et al 1996). For HMW-DAN, amino acid analysis showed a considerable difference compared with the determination with Ellman's reagent. The large degree of polymerization of the preparation (Table I, assay 7; Table II, assay 14) might be an explanation.

Reoxidation of Fractions with KBrO_3

The effect of variations in protein concentrations (0.5, 1.0, and 2.0%), amounts of oxidant (0.625–62.5 molar ratio of KBrO_3 to Cys), pH values (2.0 and 8.0), and reaction times (0–360 min) on the reoxidation of glutelin subunits with KBrO_3 was studied. In agreement with Schropp et al (1995), thiol groups of HMW-REK were completely oxidized within 5 min when high amounts of KBrO_3 (62.5) were added, and the decrease of thiol groups was slower with lower amounts. The kinetics of reoxidation were independent of pH and protein concentration (results not shown). LMW-REK behaved similarly to HMW-REK with regard to the oxidation with KBrO_3 (Fig. 1): the decrease of thiol content was dependent on the oxidant concentration, and most significant changes occurred within the first 3 hr. The only difference with HMW-REK was that the thiol content of LMW-REK decreased faster and to a larger extent during the total period of oxidation in a urea-containing buffer at pH 8.0 than in 0.1% TFA (pH 2.0) without urea (Fig. 1). The reoxidation curves of HMW-DAN (Fig. 2) were comparable to those of HMW-REK, except that there was a faster decrease of thiol groups, even when HMW-DAN was oxidized with a slightly lower concentration of KBrO_3 (molar ratio of KBrO_3 to Cys of 0.5 instead of 0.625).

TABLE III
Proportions (%) of Fractions Obtained by Gel-Permeation Chromatography After Reoxidation (360 min) of Single HMW Subunits of cv. Rektor with KBrO_3 (0.25)^a

Subunit	Fractions		
	I	II	III
5	3.9	41.5	54.6
7	3.5	23.3	73.2
10	2.9	10.3	86.8

^a Molar ratio of oxidant to cysteine content 0.25.

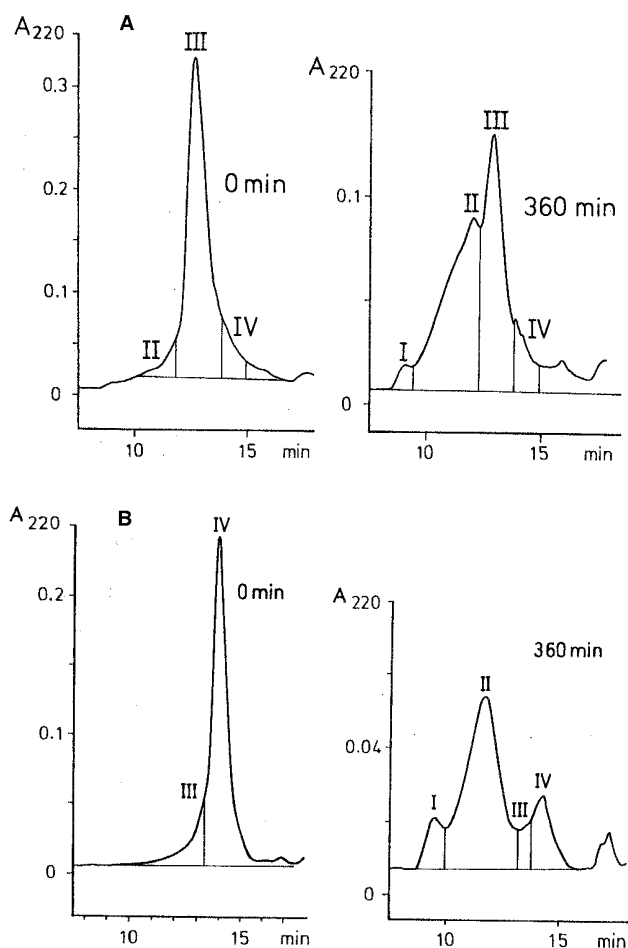


Fig. 3. Gel-permeation chromatography of **A**, HMW Rektor (1% protein) reoxidized with KBrO_3 (molar ratio to Cys 0.25, pH 2.0); **B**, LMW Rektor (1% protein) reoxidized with KIO_3 (molar ratio to Cys 0.25, pH 2.0) for reaction times at 0 and 360 min.

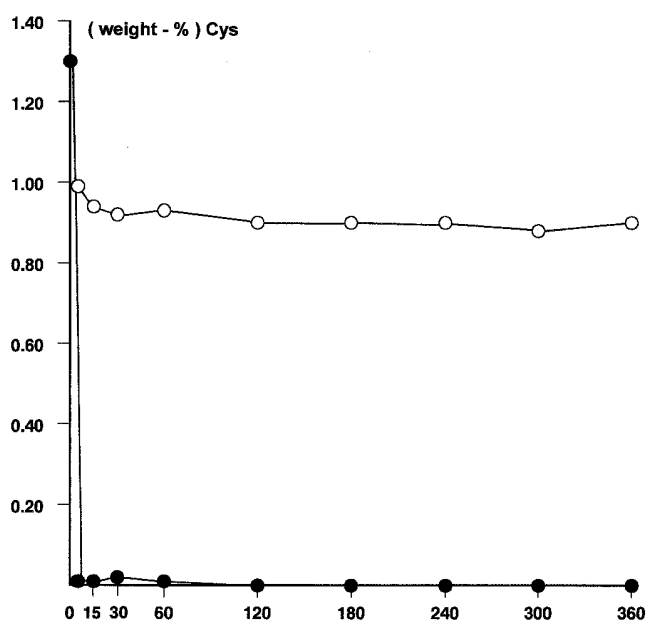


Fig. 4. Cysteine content of LMW Rektor (1% protein) reoxidized with KIO_3 (● = molar ratio to Cys 0.25, pH 2.0; ○ = molar ratio to Cys 0.0625, pH 2.0) depending on reoxidation time.

The M_r distribution of reoxidized proteins was determined by GPC after derivatization of thiol groups with 4-vinylpyridine. The elution profiles were divided into four fractions (Fig. 3, I–IV). In reoxidized HMW subunits, fraction III contained monomeric proteins and fractions II and I polymeric proteins (Fig. 3A). The monomeric LMW subunits occurred in fraction IV, and the corresponding oligomeric and polymeric proteins in fractions I to III (Fig. 3B). The relative proportions of these fractions for different oxidation conditions are shown in Table I. In agreement with Schropp et al (1995), fraction III of HMW-REK decreased during oxidation with KBrO_3 (0.25), predominantly in favor of fraction II (assay 1 and 2). A higher protein concentration (1% in assay 2 instead of 0.5% in assay 1) led to larger amounts of high-molecular aggregates, which were located in fraction I and II.

The monomeric fraction IV of LMW-REK also decreased during oxidation (assay 3). Initially, only oligomers of fraction III were formed. Later on, fraction III decreased, while fraction II became the major fraction. As with HMW-REK, higher protein concentrations of LMW-REK (assay 4) led to larger amounts of the polymeric fractions I–III and lower amounts of the monomeric fraction IV. The same effect was observed with an increased ratio of KBrO_3 to Cys (assay 5). Remarkably, this effect of protein concentration was far less pronounced with LMW subunits than with HMW subunits. The oxidation of a mixture of HMW-REK and LMW-REK at a ratio of 1:2.5 (as it occurred approximately in REK flour), reflects very well the oxidation behavior of the separate fractions (assay 6).

Already, at the beginning of the oxidation (0 min), fractions I and II of HMW-DAN contained considerably more protein (assay 7) than those of HMW-REK (assay 2). At the end (360 min), HMW-DAN contained slightly fewer polymeric proteins (fractions I + II) than HMW-REK.

Reoxidation of Fractions with KIO_3

In contrast to the slow but continuous reoxidation with KBrO_3 , a complete oxidation of the thiol groups of HMW-REK within 5 min was observed with KIO_3 (Schropp et al 1995).

Identical results were obtained with HMW-REK and HMW-DAN in the present study. When LMW-REK (1%) was oxidized at a KIO_3 to Cys ratio of 0.25, the thiol groups were also totally oxidized within 5 min (Fig. 4). Even at a low ratio of KIO_3 to Cys of 0.0625, the content of thiol groups decreased very quickly and remained constant after the oxidant was consumed (after 15 min).

The proportions of the different GPC fractions are summarized in Table II. All changes took place within the first 5 min of reoxidation of HMW-REK with KIO_3 (assay 8 and 9). Compared with the oxidation with KBrO_3 (assay 2), oxidized HMW-REK (assay 8) contained more monomeric (fraction III) and fewer polymeric proteins (fractions I–II), suggesting that formation of intramolecular disulfide bonds was favored by KIO_3 . The same was observed with HMW-DAN (assays 7 and 14). However, large amounts of polymerized proteins (fractions I–III) were obtained with LMW-REK (assays 10, 11, and 12).

Apparently, many more intermolecular disulfide bonds were formed during the fast oxidation with KIO_3 compared with the slow oxidation with KBrO_3 and compared with the KIO_3 oxidation of HMW-REK. Remarkably, the oxidation behavior of the mixture of HMW and LMW-REK (1:2.5) with KIO_3 (assay 13) was only slightly different from that with KBrO_3 (assay 6). The effect of protein concentration was comparable with the effect observed with KBrO_3 oxidation, in that increasing the concentration of both HMW-REK (assays 8 and 9) and LMW-REK (assays 10 and 11) led to larger amounts of polymeric proteins.

Reoxidation of 2% LMW-REK (assay 12) did not show the expected large increase of polymeric proteins compared with 1% (assay 11) because proteins were not completely soluble in that concentration.

Isolation and Reoxidation of Single HMW Subunits of Wheat

Three, four, or five HMW subunits are usually present in cultivars of bread wheat. According to differences in the primary structure, they can be grouped into x - and y -type subunits (Shewry et al 1992). With the exception of subunit 5, which has an additional Cys residue at the beginning of the repetitive domain, x -type subunits contain three Cys residues in the N-terminal domain and one Cys residue in the C-terminal domain (Köhler et al 1993, Shewry and Tatham 1997). The y -type subunits contain five Cys residues in the N-terminal domain, one residue in the repetitive domain, and one residue in the C-terminal domain. The x -type HMW subunits had a strong effect on the strength of dough and gluten, and y -type subunits had a weak effect (Wieser et al 1994a,b). One reason could be the greater tendency of x -type subunits to form intermolecular disulfide bonds (Shani et al 1992).

To study the reoxidation behavior of single HMW subunits, subunits 5, 7, and 10 were initially isolated from HMW-REK by RP-HPLC. However, GPC analysis indicated only monomers present after reoxidation with KBrO_3 . Obviously, the conformation of the subunits was completely changed by RP-HPLC, such that intermolecular disulfide bonds could not be formed. Therefore, acid-PAGE was chosen as a more gentle separation method (Khelifi et al 1991, Morel 1994). The proteins were extracted from the gel, dialyzed, and freeze-dried. Major subunits 5, 7, and 10 were reoxidized with KBrO_3 (at a KBrO_3 to Cys ratio of 0.25) and studied by GPC.

The proportions of the monomeric fraction III and the polymeric fractions I and II are presented in Table III. Remarkably, reoxidation of single subunits with KBrO_3 did not produce the same high proportion of polymeric proteins as was produced by reoxidized HMW-REK (assay 2, 50.9%). This reflected very well the results of Szabo et al (1995), who showed that the oxidation of mixtures of x - and y -type subunits was significantly faster than homopolymerization of either x - or y -type subunits and resulted in a distribution richer in larger polymers. The x -type subunit 5 showed a much greater tendency to form intermolecular disulfide bonds (fraction I + II: 45.4%) than x -type subunit 7 (I + II: 26.8%). This was also reported by Szabo et al (1995), who found that subunit 5 formed larger polymers than 7 by oxidation with KBrO_3 . The differences could be explained by different secondary structures and different positions of Cys residues within the N-terminal domain of both subunits (Köhler et al 1997) and by the additional Cys residue at the beginning of the repetitive domain of subunit 5. Thus, the ability of Cys residues in subunit 5 to form intermolecular disulfide bond may be increased. The y -type subunit 10 revealed the lowest tendency to form polymeric products (fraction I + II, 13.2%). This result is in agreement with the conclusion of Shani et al (1992) that Cys residues in the repetitive and C-terminal domains of y -type subunits tend to form an intramolecular disulfide bond that may reduce the ability to polymerize.

CONCLUSIONS

The determination of thiol groups and the separation by GPC both demonstrated that HMW-REK, HMW-DAN, and LMW-REK were reoxidized much faster by KIO_3 than by KBrO_3 . The reoxidation behavior of HMW-REK and HMW-DAN was similar, and small differences were not sufficient to explain why rye doughs do not form a gluten network like wheat dough. Both HMW-REK and HMW-DAN produced more polymeric proteins during oxidation with KBrO_3 than with KIO_3 . The most remarkable result, however, was that LMW-REK formed very high amounts of polymeric proteins when oxidized with KIO_3 . Independent of the oxidant used, higher protein concentrations led to larger amounts of polymeric proteins. Reoxidation of single HMW subunits of REK with KBrO_3 , and determination of the M_r distribution by GPC, indicated that x -type HMW subunits form higher amounts of polymers than y -type HMW subunits. The x -type subunit 5 has a greater tendency to form polymeric proteins than x -type subunit 7.

LITERATURE CITED

- Candler D., Szabo C., Murray D., and Bekes F. 1996 In vitro polymerisation of glutenin subunits using protein disulfide isomerase. Pages 133-136 in: *Gluten 96*. C. W. Wrigley, ed. RACI: Melbourne, Australia.
- Ellman, G. L. 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82:70-77.
- Khelifi, D., and Branlard, G. 1991. A new two step electrophoresis method for analyzing gliadin polypeptides and high and low molecular weight subunits of glutenin of wheat. *J. Cereal Sci.* 13:41-47.
- Kipp, B., Belitz, H.-D., Seilmeier, W., and Wieser, H. 1996. Comparative studies of high M_r subunits of rye and wheat. I. Isolation and biochemical characterization and effects on gluten extensibility. *J. Cereal Sci.* 23:227-234.
- Köhler, P., Belitz, H.-D., and Wieser H. 1993. Disulphide bonds in wheat gluten: further cystine peptides from high molecular weight (HMW) and low molecular weight (LMW) subunits of glutenin and from γ -gliadins. *Z. Lebensm. Unters. Forsch.* 196:239-247.
- Köhler, P., Keck-Gassenmeier, B., Wieser, H., and Kasarda, D. D. 1997. Molecular modeling of the N-terminal regions of high molecular weight glutenin subunits 7 and 5 in relation to intramolecular disulfide bond formation. *Cereal Chem.* 74:154-158.
- Krause, I., Müller, U., and Belitz, H.-D. 1988. Charakterisierung von Weizensorten durch SDS-Polyacrylamidgel-Elektrophorese (SDS-PAGE) und zweidimensionale Elektrophorese (2D-PAGE) der Glutenine. *Z. Lebensm. Unters. Forsch.* 186:398-406.
- Marchylo, B. A., Kruger, J. E., and Hatcher, D. W. 1989. Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. *J. Cereal Sci.* 9:113-130.
- Melas, V., Morel, M.-H., Autran, J.-C., and Feillet, P. 1994. Simple and rapid method for purifying low molecular weight subunits of glutenin from wheat. *Cereal Chem.* 71:234-237.
- Morel, M. H. 1994. Acid-polyacrylamide gel electrophoresis of wheat glutenins: A new tool for the separation of high and low molecular weight subunits. *Cereal Chem.* 71:238-242.
- Müller, S., and Wieser, H. 1997. The location of disulphide bonds in monomeric γ -type gliadins. *J. Cereal Sci.* 26:169-176.
- Schropp, P., Belitz, H.-D., Seilmeier, W., and Wieser, H. 1995. Reoxidation of high molecular weight subunits of glutenin. *Cereal Chem.* 72:406-410.
- Shani, N., Steffen-Campbell, J. D., Anderson, O. D., Greene, F. C., and Galili, G. 1992. Role of the amino- and carboxy-terminal regions in the folding and oligomerization of wheat high molecular weight glutenin subunits. *Plant Physiol.* 98:433-441.
- Shewry, P. R., Halford, N. G., and Tatham, A. S. 1992. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* 15:105-120.
- Shewry, P. R., and Tatham, A. S. 1997. Disulphide bonds in wheat gluten proteins. *J. Cereal Sci.* 25:207-227.
- Szabo C., Bekes F., and Murray, D. 1995. In vitro polymerisation studies on glutenin subunits. Pages 63-68 in: *Proc. 45th Aust. Cereal Chem. Conf.* Y. Williams and C. W. Wrigley, eds. RACI: Melbourne, Australia.
- Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-18.
- Wieser, H., Antes, S., and Seilmeier, W. 1998. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chem.* 75:644-650.
- Wieser, H., Seilmeier, W., and Belitz, H.-D. 1994a. Use of RP-HPLC for a better understanding of the structure and the functionality of wheat gluten proteins. Pages 235-272 in: *HPLC of Cereal and Legume Proteins*. J. E. Kruger and J. A. Bietz, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Wieser H., Seilmeier W., and Kieffer R. 1994b. Relationship between the amount of gluten protein types and the rheological properties of different wheat cultivars. Pages 141-150 in: *Gluten Proteins 1993*. Assoc. Cereal Res.: Detmold, Germany.

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