Effect of Added Pentosans on Some Physical and Technological Characteristics of Dough and Gluten[1]

J. MICHNIEWICZ, C. G. BILIADERIS, and W. BUSHUK

ABSTRACT

The effect of added water-soluble and water-insoluble pentosans of wheat (WS-W and WI-W, respectively) and water-soluble pentosans of rye (WS-R) on farinograph properties of wheat doughs, on the yield of wet and dry gluten, and on the solubility of doughs and glutens in acetic acid (HAc) and acetic acid-urea (AU) solvents was investigated. Additionally, the HAc and AU fractions were examined by gel-filtration chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All three pentosan preparations had a marked effect on farinograph properties; water absorption and dough development time increased. On an equal weight basis, the WS-R pentosans produced the largest changes in these parameters. The effect of pentosans on gluten yield varied according to the characteristics of the base flour and the type and amount of pentosans added. In general, the yield of wet gluten decreased, particularly at the lower of the two levels (1 and 2%) of pentosan supplementation. Added WI-W and WS-R had a significant effect on the extractability of proteins from the dough and gluten of Katepwa (Canada western red spring wheat variety) with HAc and AU solvents. Adding pentosans decreased the amount of protein soluble in HAc and increased the amount in AU extracts for the dough samples; opposite trends were observed for the gluten. The results of gel filtration and electrophoresis show that WS-W and WI-W pentosans affect the aggregation-disaggregation processes of the high molecular weight proteins and produce minor changes in the electrophoretic patterns of the HAc- and AU-soluble proteins.

Cereal Chem. 68(3):252-258

The role of pentosans in the formation and properties of dough has been the subject of many investigations (D'Appolonia and Gilles 1971, Hoseney 1984, Amado and Neukom 1985, Meuser and Suckow 1986, Kühn and Grosch 1989), but it is still not fully understood. Because of their high water-absorbing capacity, pentosans strongly influence the breadmaking properties of wheat flours (Jelaca and Hlynka 1972, Hoseney 1984). Despite the similarities in chemical composition, the water-soluble (WS) and water-insoluble (WI) pentosan fractions seem to affect the properties of dough (and probably of bread) differently (Amado and Neukom 1985, Meuser and Suckow 1986). Furthermore, the functional effect of pentosans on wheat dough and bread seems to depend on the technological characteristics of the base flour (Jelaca and Hlynka 1972, Jankiewicz and Michniewicz 1976, Izydorczyk et al 1990, Michniewicz et al 1990).

The present study was undertaken to compare the effect of WS and WI pentosans isolated from one variety of wheat and WS pentosans from rye on the farinograph characteristics of dough and the gluten yield washed out from doughs of several wheat flours supplemented with various amounts of pentosan preparations. We also examined the involvement of WI wheat and WS rye pentosans in the aggregation-disaggregation processes of gluten proteins of a wheat cultivar of the Canada western red spring class, using gel-filtration chromatography and electrophoresis.

MATERIALS AND METHODS

Flour

Flour was milled from the grain of pure cultivars of Canada western red spring wheat (Katepwa), Canada prairie spring wheat (HY 320), Canada western soft white spring wheat (Fielder), and an unregistered U.S. hard red spring variety (Marshall) on a Bühler pneumatic laboratory mill after tempering to a moisture content appropriate for each wheat class. The protein (N × 5.7) content of the flours and glutens was determined by the Kjeldahl procedure (AACC 1983). Total and WS pentosan contents of the flours were determined by the phloroglucinol method of Douglas (1981). Other chemical and technological characteristics of these flours were reported elsewhere (Michniewicz et al 1990).

[1]Paper No. 179 of the Food Science Department, University of Manitoba, Winnipeg, MB R3T 2N2.

Chemicals

Reference proteins, bovine serum albumin, 2-mercaptoethanol, and salivary α-amylase (Type IXA) were obtained from Sigma Chemical Co. (St. Louis, MO). The glucoamylase was a product of Boehringer Mannheim Canada (St. Laurent, PQ). Acrylamide, bisacrylamide, and sodium dodecyl sulfate (SDS) were of electrophoresis grade and were obtained from Bio-Rad (Richmond, CA). All other chemicals were of analytical reagent grade.

Preparation of Pentosans

WS and WI wheat pentosans (WS-W and WI-W, respectively) were obtained from Katepwa flour according to Izydorczyk et al (1990) and Michniewicz et al (1990), respectively. In these preparations, salivary α-amylase and glucoamylase were used to remove starch contaminants. WS rye pentosans (WS-R) were isolated from a Polish rye variety (Dankowskie Zlote) using the procedure of Jankiewicz and Michniewicz (1976).

Fractionation of Dough and Gluten

Doughs (10 g) were prepared by mixing for 15 min in the microfarinograph with and without the addition of a specified amount of WS-W, WI-W, and WS-R. The dry pentosan preparations were blended with flour in dry form by mixing for 10 min. Water addition was varied, as required, to give a dough with a consistency of 500 BU. Wet gluten was prepared according to the glutomatic (Falling Number AB, Stockholm, Sweden) procedure (ICC 1980). A portion of each gluten was dried at 65°C overnight to estimate the yield of dry gluten. All doughs and remaining glutens were freeze-dried, ground with a mortar and pestle, and stored at 4°C for further analysis.

Dry gluten and dough samples from Katepwa flour (the control and WI-W and WS-R supplemented flour) were subjected to a sequential two-step extraction using solvents of 0.05*M* acetic acid (HAc) and acetic acid-urea (AU) (0.1*M* acetic acid and 3*M* urea). Freeze-dried gluten (400 mg) or dough (2 g) were mixed with 20 ml of 0.05*M* HAc, shaken at 4°C for 1 hr, and centrifuged at 4°C (5,000 × *g*) for 20 min. The supernatants were collected, and residues were resuspended in another 20-ml portion of HAc solution. The total procedure involved four extractions. Up to 100 ml of combined supernatants were made with 0.05*M* HAc. The residues were then suspended in 20-ml portions of AU solvent and extracted twice, as indicated above. Up to 50 ml of combined AU extracts were made with AU solvent. Extracts and residues after extraction of HAc and AU were dialyzed exhaustively against 0.01*M* HAc (4°C), frozen, and freeze-dried (McMaster and

Bushuk 1983). Fractionations were performed three times, and the results were averaged. Student's t test was used to assess differences in dry matter in HAc-soluble, AU-soluble, and residue fractions. Total carbohydrate, pentosan, and protein contents of the dry fractions were determined, respectively, by the phenolsulfuric acid method (Dubois et al 1956), phloroglucinol method (Douglas 1981), and the Pierce BCA Protein Assay Reagent (Pierce, Rockford, IL), using bovine serum albumin as a standard. The presence of urea in the AU extracts did not interfere with the results of protein determination as assessed by preliminary experiments with known amounts of gluten in which various concentrations of urea (up to 2.0M) were included.

Gel-Filtration Chromatography

Freeze-dried HAc extracts of doughs and glutens were dissolved in 0.05M HAc and fractionated on a Sephacryl S-300 column (2.6 × 80 cm) at 22°C. The column was equilibrated and eluted with 0.05M sodium acetate buffer, pH 4.1, at a flow rate of 20 ml/hr. Fractions of 5 ml were collected. Freeze-dried AU-soluble material from dough and gluten was dissolved in AU solvent and subjected to gel filtration on a Sephacryl S-300 column (2.6 × 60 cm), using AU solvent as the eluant, at a flow rate of 15 ml/hr. Fractions of 4 ml were collected. The void and total volumes (V_o and V_t, respectively) were determined by chromatography of Blue Dextran 2,000 and xylose, respectively. Eluted fractions were pooled to give two fractions, I and II, dialyzed against 0.01M HAc, frozen, and freeze-dried. Total carbohydrates in the fractions were determined by the phenolsulfuric acid method, and protein content was monitored by direct absorbance readings at 280 nm.

Electrophoresis

SDS-PAGE was performed on an LKB 2001 electrophoresis unit according to Ng and Bushuk (1987). Molecular weights were estimated from migration distances of bovine serum albumin, egg albumin, and carbonic anhydrase (mol wt 66,000, 45,000, and 29,000, respectively) and of the glutenin subunits of Neepwa wheat (Ng and Bushuk 1989).

Amino Acid Analysis

Amino acid analyses were performed on an LKB Alpha Plus 4151 automatic amino acid analyzer. Samples were hydrolyzed with 6M HCl in Pierce vacuum hydrolysis tubes for 24 hr at 110°C, neutralized with 25% NaOH, and diluted with sodium citrate buffer (Rotter et al 1989). Values for aspartic and glutamic acids included asparagine and glutamine, respectively. Tryptophan was not determined.

RESULTS AND DISCUSSION

Chemical Composition of Flours and Gluten Samples

Yields of straight grade flours were 67.1–75.1%. The protein content of glutens prepared from control doughs varied from 75.6% for HY 320 to 79.3% for Marshall (Table I). Adding pentosans to doughs caused only slight changes in the protein content of the glutens washed from the supplemented dough. Comparisons between the pentosan contents of flours and the corresponding glutens (Table I) show that about 50% of pentosans (depending on wheat variety) remained in the gluten. D'Appolonia and Gilles (1971) reported higher pentosan contents and very similar protein contents in gluten samples prepared from various wheat varieties. The differences in pentosan content might be a consequence of the different ways of washing out gluten (manual versus mechanical). Adding pentosans to dough did not cause a proportional increase in the pentosan content of the corresponding gluten samples; the 2% addition of WI-W to dough caused only a slight increase in pentosan content, whereas the 2% addition of WS-R had no effect.

TABLE I
Protein and Pentosan Contents of Flours and Gluten Samples Washed from Doughs Supplemented by WI-W[a] or WS-R Pentosan Preparations

	Dough					
	Katepwa		Marshall		HY 320	
	Pentosan (%, db)	Protein[b] (%, db)	Pentosan (%, db)	Protein (%, db)	Pentosan (%, db)	Protein (%, db)
Flours	2.06±0.04[c]	12.1	1.76±0.07	12.7	1.96±0.05	10.0
Gluten samples						
Control	0.78±0.07	78.0	0.82±0.07	79.3	0.79±0.08	75.6
2% WI-W	1.02±0.02	81.0	1.11±0.09	77.1	1.00±0.06	79.2
2% WS-R	0.71±0.03	79.9	0.93±0.08	76.5	0.78±0.02	78.4

[a] WI-W = water-insoluble wheat, WS-R = water-soluble rye.
[b] Protein (N × 5.7) content determined by Kjeldahl method.
[c] Mean ± standard deviation.

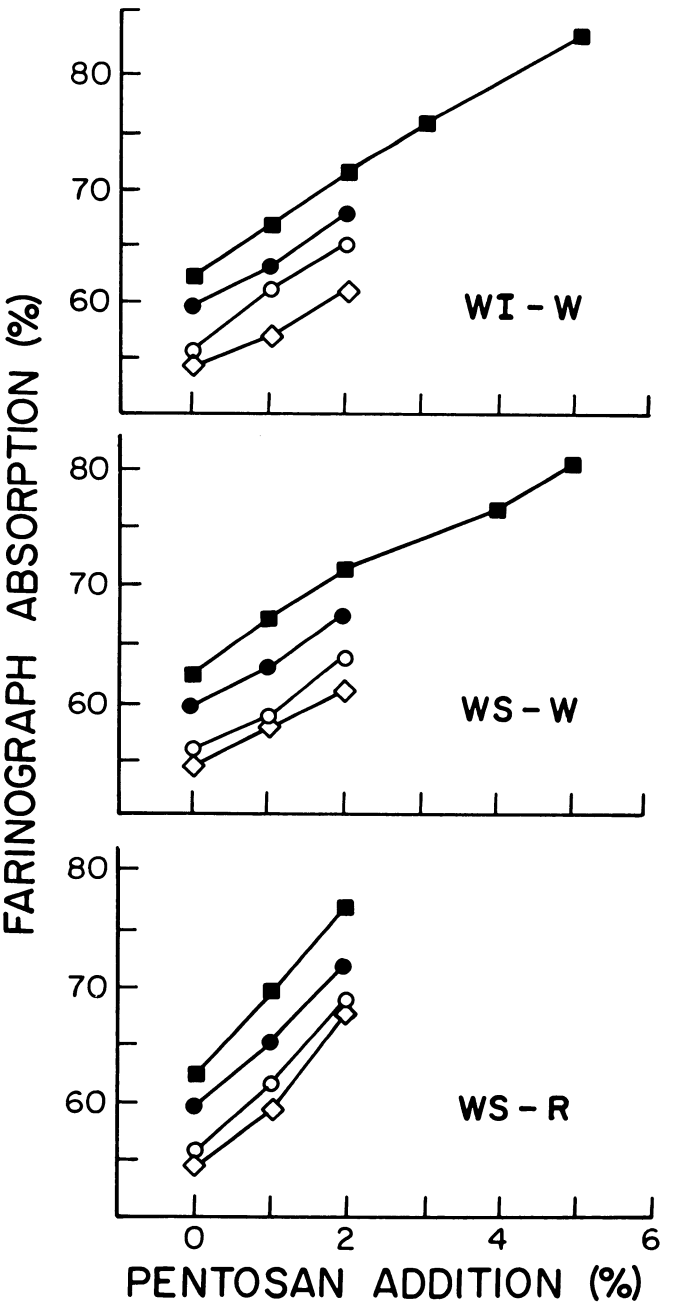

Fig. 1. Farinograph water absorption of wheat flours supplemented with pentosan preparations. WI-W = water-insoluble wheat, WS-W = water-soluble wheat, WS-R = water-soluble rye, ■ = Katepwa, ● = Marshall, ○ = HY 320, □ = Fielder. Data represent means of triplicate analyses; standard deviations were less than 1.1% in all cases.

Effect of Pentosan Preparations on Farinograph Characteristics of Wheat Doughs

The values for farinograph water absorption of doughs from four widely different wheat varieties supplemented with various pentosan preparations are shown in Figure 1. Absorption increased linearly with the amount of added pentosan for all four flours tested. For example, for Katepwa base flour, $r = 0.999$ ($P < 0.01$) for WS-R; $r = 0.994$ ($P < 0.01$) for WS-W; and $r = 0.998$ ($P < 0.01$) for WI-W). The WS-R pentosans caused the largest rate of increase in water absorption for all base flours. Of the wheat pentosan preparations, WI-W exhibited a slightly higher potential to increase the water absorption than did WS-W. Kühn and Grosch (1989) reported that both WS and WI rye pentosans had a similar effect on water absorption of wheat flour. Apparently, the purity, composition, and varietal origin of pentosan preparations can affect their contribution to the water absorption of doughs from supplemented flours.

The added pentosans also affected dough development time (DDT) in the microfarinograph. The most distinct change was the increase observed within the 0–2% range. The WS-R pentosan was the most effective in increasing DDT; the 2% addition of WS-R to dough of Katepwa increased DDT to 10 min, compared with 5.5 min for the control. The corresponding values for doughs supplemented with WS-W and WI-W were 8 and 7.5 min, respectively. Differences were also observed among the base flours. A substantial increase in DDT was also noted for the base flours HY 320 and Fielder; the 2% addition of WS-R prolonged DDT 4–9.5 min for HY 320 and 1.5–3.5 min for Fielder. The increased DDT for these flours brought about by WS-W and WI-W were similar but less than the increase brought about by WS-R supplementation.

Effect of Added Pentosans on Gluten Yield

The effect of pentosans on the yields of wet and dry gluten depended on the amount and type of pentosan added and on the characteristics of the base wheat flour (Fig. 2). Adding WI-W at 1% caused a slight increase in the amount of gluten washed from doughs. On the other hand, adding WS-R pentosans resulted in a decrease in both wet and dry glutens from all three flours used in this experiment. The changes brought about by WS-W were approximately intermediate between those of WI-W and WS-R.

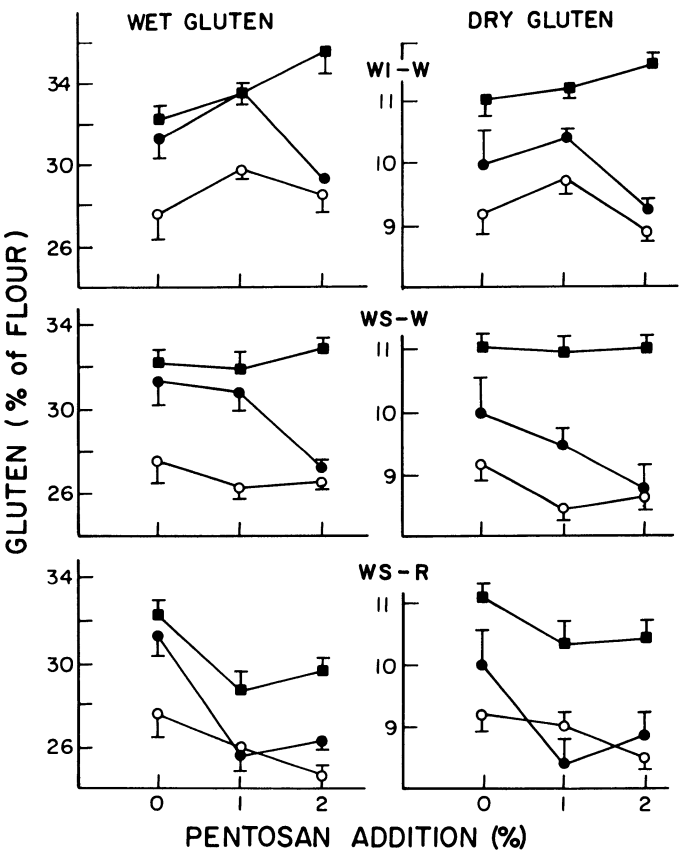

Fig. 2. Yield of wet and dry gluten washed from doughs supplemented with pentosan preparations. WI-W = water-insoluble wheat, WS-W = water-soluble wheat, WS-R = water-soluble rye, ■ = Katepwa, ● = Marshall, ○ = HY 320.

The response to increasing amounts of pentosans differed among varieties. Although for Katepwa the yield of gluten increased continuously with increased WI-W, for Marshall and HY 320 an increase occurred at the 1% level of WI-W, followed by a decrease at 2%. As anticipated from the lower protein content of the base flours, gluten yields from HY 320 and Marshall doughs were much lower than those for Katepwa for all three types of pentosans and both levels of supplementation.

Fractionation of Dough and Gluten

After interactions were allowed to take place during mixing, the doughs were extracted sequentially with HAc and AU solvents. Fractionation was used to investigate the possibility of the pentosans interfering with the interactions between gliadin and glutenin to form gluten. Fractionation studies were performed for Katepwa flours supplemented with WS-R and WI-W.

For the dough samples, HAc extracted the highest amount of dry matter, whereas AU dissolved only about 1%. (Table II) The results for doughs showed no statistically significant effect from pentosan addition, except that the amount of dry matter extracted by AU from flour supplemented with WI-W was significantly higher than that from the control dough. Adding pentosans reduced the dialysis losses, indicating that solubles become complexed with other fractions, mostly with the HAc-extractable fraction and the residue.

The amounts of dry matter extracted from the gluten samples were markedly different from the amounts extracted from dough. Adding pentosans decreased the amount of solids extracted with HAc and increased the amount extracted with AU by an approximately equal magnitude. The dry matter of the residue increased slightly. Compared with the data for doughs, which indicate that a considerable amount of total solids were lost during dialysis, more than 90% were recovered in fractionation of gluten; the dialysis losses were significantly lower for gluten than for dough. The amount of dry matter extracted from the control gluten was higher than that reported by Zawistowska et al (1985) and Arakawa et al (1977). This can be attributed to the four-step extraction procedure used in this work, compared with the single-extraction procedure (using 0.05*M* HAc) used in previous studies.

Protein and carbohydrate contents of the dough and gluten fractions are given in Table III. HAc extracts of WS-W or WI-R doughs supplemented with pentosans had lower protein content than did extracts of the control samples. The opposite was observed for the AU extracts. Different trends were observed for the glutens derived from doughs supplemented with pentosans.

TABLE II
Yield of Dough and Gluten Fractions (%) Obtained by Acetic Acid and AU[a] Extractions

	HAc-Soluble	AU-Soluble	Residue	Solids Recovered	Dialysis Loss
Dough					
Control	17.5 a[b] ±1.8	0.9 a±0.1	65.8 a±0.7	84.2	15.8
WI-W, 2%	18.0 a ±1.0	1.2 b±0.1	68.6 b±0.5	87.8	12.2
WS-R, 2%	21.8 a ±0.3	0.9 ac±0.1	69.6 b±0.1	92.3	7.7
Gluten					
Control	80.7 a ±0.9	4.4 a±0.3	8.9 a±1.0	94.0	6.0
WI-W, 2%	77.4 b ±0.8	7.0 b±0.8	10.4 a±1.6	94.8	5.2
WS-R, 2%	74.1 b ±3.4	7.3 b±0.4	10.5 a±0.6	91.9	8.1

[a]AU = acetic acid-urea, WI-W = water-insoluble wheat, WS-R = water-soluble rye.
[b]Means with different letters within columns differ significantly ($P = 0.05$).

Adding pentosans led to a slightly higher protein content of HAc extracts and lower protein content of AU extracts compared with control samples. The effect of pentosans on the extractability of HAc-soluble protein was the opposite of that reported for mixed doughs (Johansson et al 1971, Tanaka and Bushuk 1973, Jankiewicz and Michniewicz 1976). The results of this work suggest that pentosans affect the aggregation processes of wheat proteins in different ways, depending on the presence of other constituents.

Adding WS-R to dough caused a slight increase in the amount of pentosans extracted with HAc and AU, but large amounts still remained in the residue after extraction. Similar changes in pentosan distribution of the extracts were also observed for the gluten fractions. Glutens from doughs fortified with WI-W contained fewer pentosans in the HAc extract and slightly more in the AU extract than did the control samples.

Gel Filtration and Electrophoresis

The gel-filtration profiles of HAc extracts of doughs and glutens are compared in Figures 3 and 4, respectively. In general, the elution profiles had three peaks corresponding to molecular weights (calculated from the K_{av} values) above 300,000, 60,000–70,000, and about 30,000. Extracts from doughs with added pentosans and the glutens had profiles with peaks of similar elution volume but with varying proportions of eluted protein and carbohydrate. The relatively small amount of protein eluting in the first peak fraction for control dough and gluten, as previously reported (Jankiewicz and Pomeranz 1965, McMaster and Bushuk 1973, Bushuk and MacRitchie 1989), appears to be a direct consequence of cryoaggregation processes that occur during freeze-drying and impede protein solubility. Hashizume et al (1974) suggested that cryoaggregation of glutenin proteins results from intermolecular interactions involving disulfide bonds and free sulfhydryl groups that occur as a result of increasing protein concentration during freezing. On the other hand, Okada et al (1987) reported that cryoaggregation results from interactions involving hydrophobic groups of high molecular weight proteins that are exposed during dough mixing and before freezing.

The addition of pentosans (both WI-W and WS-R) to dough resulted in increased protein in the extract that eluted in the first peak fraction at the expense of the lowest molecular weight peak. Furthermore, a concomitant increase occurred in the coeluting carbohydrates. This was particularly evident for the dough supplemented with WS-R (Fig. 3). Gel-filtration profiles for the gluten samples (Fig. 4) showed smaller changes than those observed for dough, indicating that most of the soluble carbohydrate is washed out during gluten preparation. In these samples, a small amount of carbohydrate coeluted with fraction II (third peak) proteins.

Gel-filtration profiles of AU extracts from doughs and corresponding glutens (data not shown) were characterized by one slightly asymmetric peak for protein, eluting in the V_o and two carbohydrate peaks. The first peak eluted with V_o proteins, whereas the second eluted in the vicinity of V_t.

TABLE III
Carbohydrate and Protein Contents of HAc[a] and AU Extracts of Dough and Gluten Samples

Sample	Extract		Pentosan	
	Protein (%, db)	Carbohydrate (%, db)	Total Carbohydrate[b] (%)	Pentosans in Dough[c] (%)
Dough				
Control				
HAc-soluble	59.7 ± 4.9	17.7 ± 1.3	17.7	24.6 ± 0.8
AU-soluble	65.9 ± 5.9	23.2 ± 1.7	7.9	1.2 ± 0.1
2% WI-W addition				
HAc-soluble	53.9 ± 5.6	16.5 ± 1.3	20.2	16.0 ± 1.1
AU-soluble	75.7 ± 8.9	21.9 ± 1.0	18.0	1.2 ± 0.1
2% WS-R addition				
HAc-soluble	48.1 ± 2.1	22.7 ± 0.8	46.6	58.5 ± 0.5
AU-soluble	69.0 ± 4.8	25.7 ± 3.6	11.7	0.7 ± 0.1
Gluten				
Control				
HAc-soluble	83.6 ± 4.5	1.2 ± 0.1	36.2	46.5 ± 3.0
AU-soluble	94.0 ± 3.5	4.6 ± 0.3	11.9	3.2 ± 0.1
2% WI-W				
HAc-soluble	87.2 ± 7.3	1.3 ± 0.1	31.8	30.4 ± 0.7
AU-soluble	79.5 ± 10.0	3.5 ± 0.5	21.3	4.9 ± 0.2
2% WS-R				
HAc-soluble	86.2 ± 6.3	1.1 ± 0.1	49.0	56.3 ± 3.1
AU-soluble	85.5 ± 5.3	2.4 ± 0.1	19.9	4.9 ± 0.6

[a]HAc = acetic acid, AU = acetic acid-urea, WI-W = water-soluble wheat, WS-R = water-insoluble rye.
[b]Pentosan content as a percent of total carbohydrates extracted in each particular fraction.
[c]Amount of extracted pentosans as a percent of the total pentosans in dough or gluten.

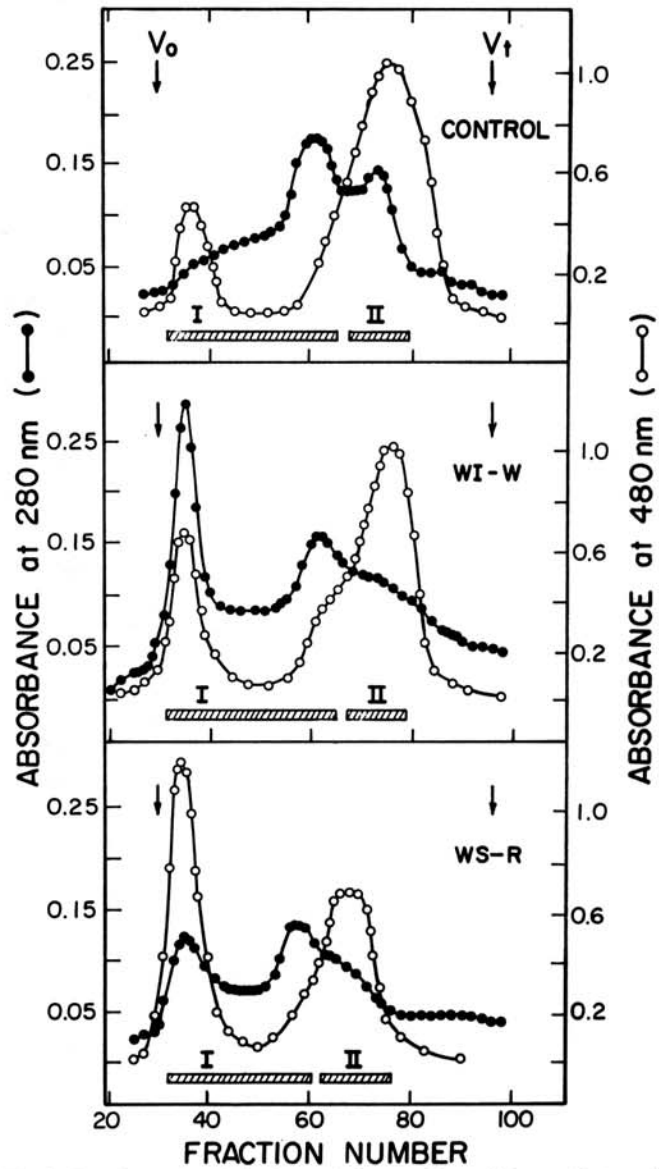

Fig. 3. Protein and carbohydrate elution profiles of freeze-dried acetic acid extracts of Katepwa doughs supplemented with pentosan preparations. V_o = void volume, V_t = total volume, control = the control dough, WI-W = dough with 5% water-insoluble wheat, WS-R = dough with 2% water-soluble rye, ○ = carbohydrates, ● = proteins, horizontal bars = collected fractions for analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Gel-filtration results for the HAc and AU extracts of gluten and dough are in general agreement with previous reports (McMaster and Bushuk 1973, Jankiewicz and Michniewicz 1976, Zawistowska et al 1985), which show that some carbohydrate material is associated with the aggregated proteins of flour fractionated by gel filtration. Overall, the gel-filtration data presented here indicate that adding pentosans to dough increases the aggregation tendency of wheat proteins.

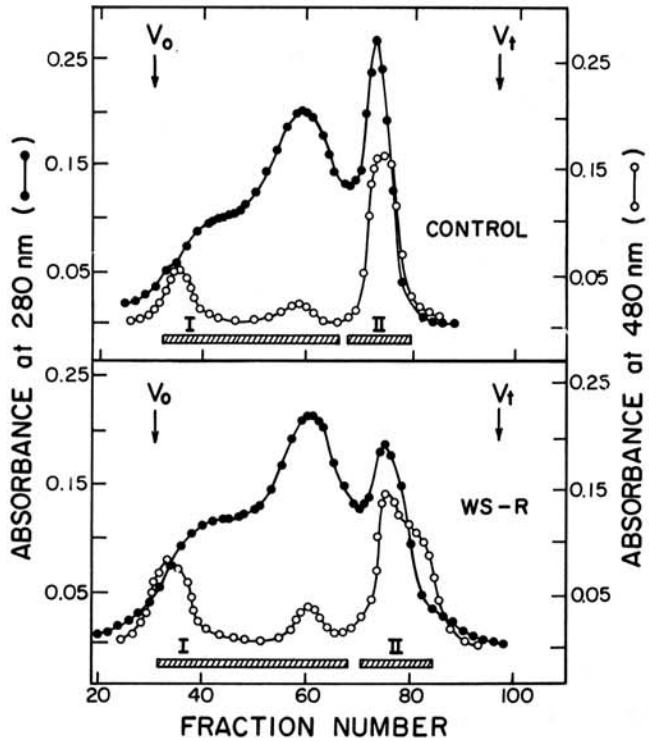

Fig. 4. Protein and carbohydrate elution profiles of freeze-dried acetic acid extracts of Katepwa glutens washed from doughs supplemented with pentosan preparations. V_o = void volume, V_t = total volume, control = the control gluten, WS-R = gluten washed from the doughs with 2% addition of water-soluble rye, ● = proteins, ○ = carbohydrates, horizontal bars = collected fractions for analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

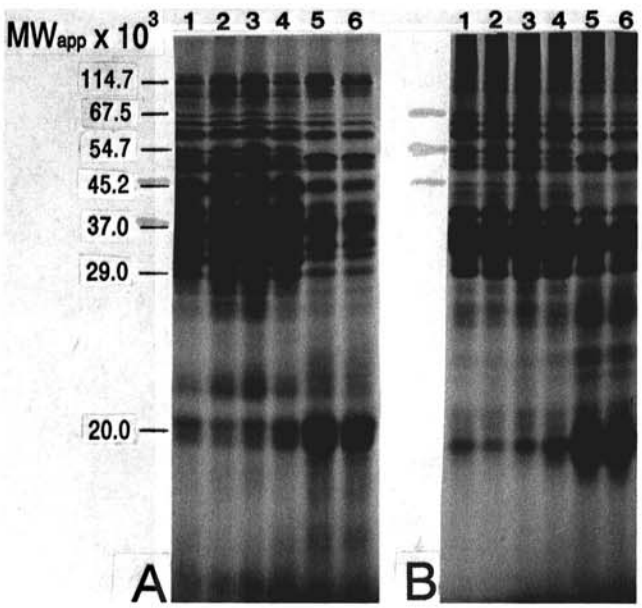

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams of reduced and unreduced protein fractions obtained by gel filtration of acetic acid-soluble protein extracted from dough. **A,** reduced samples **B,** unreduced samples. Lanes 1–3, fraction I (control, water-insoluble wheat, and water-soluble rye, respectively), lanes 4–6, fraction II (control, water-insoluble wheat, and water-soluble rye, respectively). MW app. = apparent molecular weight of Katepwa wheat subunits, listed on the left.

The SDS-PAGE analysis of reduced and unreduced subfractions collected by gel filtration of dough and gluten HAc extracts revealed some changes in the patterns caused by pentosan supplementation. Patterns of reduced fraction I of the HAc extracts of dough (Fig. 5A, lanes 1–3) show that after pentosans were added, the bands in the region of subunits of low molecular weight (LMW, mol wt 24,000–30,000) became more intense. The patterns of the same fraction under unreduced conditions (Fig. 5B, lanes 1–3) were similar, except for the amount of protein that did not enter the gel. In extracts of doughs supplemented with WI-W or WS-R (Fig. 5B, lanes 2 and 3), somewhat less protein entered the gel and seemed to cause streaking in the gel. It appears that the tendency of the HAc-soluble proteins to aggregate is influenced by the presence of pentosans. Adding WI-W or WS-R pentosans to dough affected the SDS-PAGE patterns of gel-filtration fraction II of the HAc-soluble protein of dough. Patterns under reducing conditions (Fig. 5A, lanes 4–6) show that added WI-W or WS-R caused a disappearance of a minor protein band with an apparent molecular weight of 54,700. Also, the intensity of the bands in the region of 35,000–45,000 decreased, whereas that of bands in the region of 20,000 increased.

Similar changes were observed for the same fractions examined by SDS-PAGE under unreduced conditions (Fig. 5B, lanes 4–6). Most of the bands in the electrophoregrams of fraction II of dough migrated at the same mobility before and after reduction, except those with a molecular weight of about 45,000, which did not appear as resolved bands for the unreduced sample. Similar but less distinct patterns were obtained for gel-filtration fractions of HAc extracts of gluten (results not shown).

The SDS-PAGE results of gel-filtration fractions I and II for the HAc-soluble proteins suggest that pentosans increase the tenacity of the aggregation of proteins in the HAc extracts of dough and gluten. Further work is required to determine precisely how pentosans affect the association phenomena among the LMW proteins eluted in fraction II.

The SDS-PAGE patterns of reduced AU-soluble protein from gluten and dough (Fig. 6) show the five high molecular weight glutenin subunits of Katepwa (Ng and Bushuk 1987); in addition, the patterns show numerous bands representing the LMW subunits and gliadin proteins. The patterns of the control dough and its gluten (Fig. 6, lanes 4 and 1, respectively) differ slightly, especially in the region of mol wt 60,000–70,000.

Adding pentosans to doughs also caused some changes in subunit patterns of the AU-soluble protein of dough and gluten. In the gluten samples (Fig. 6, lanes 2 and 3), WI-W and WS-R modified the protein pattern in the range of 60,000–70,000 mol wt. The pattern of the WI-W sample (Fig. 6, lane 2) contained the subunit of 54,700 mol wt, which is not present in the patterns of the other AU-soluble fractions. Patterns of AU-soluble fractions of dough show that WI-W caused a partial disappearance of protein bands in the range of 60,000–70,000 mol wt (Fig. 6, lane 5), whereas WS-R did not affect those subunits (Fig. 6, lane 6).

Overall, the results of experiments with gel filtration and electrophoresis suggest that during dough mixing, added pentosans can alter the composition of specific protein fractions of dough and gluten. Therefore, we can conclude that pentosans are actively involved in the interactions of flour proteins in dough and gluten and thereby contribute directly to the technological characteristics of bread dough.

Amino Acid Composition

The amino acid composition of flour, gluten, and HAc- and AU-soluble fractions of dough and gluten (Table IV) were determined to provide additional information on changes in the protein composition of fractions brought about by adding pentosans. The composition of the HAc-soluble fractions from dough and gluten was quite similar to that of flour and

TABLE IV
Amino Acid Compositions of Katepwa Flour, Gluten, and Gluten and Dough Fractions Obtained by Two-Step Extraction Procedure[a]

Amino Acid	Flour	Gluten	Gluten Fractions				Dough Fractions	
			HAc[b]	AU	AU/WI-W[c]	AU/WS-R[c]	HAc	AU
Aspartic acid[d]	3.47	2.66	2.52	4.05	3.29	4.62	2.92	5.20
Threonine	2.35	2.06	1.93	2.79	2.36	3.17	2.02	3.32
Serine	4.11	3.69	3.61	4.63	4.07	5.43	3.69	5.47
Glutamic acid[e]	48.37	51.06	51.66	42.00	45.97	33.05	51.70	31.31
Proline	10.78	10.51	10.78	8.68	9.88	10.19	10.72	9.33
Glycine	2.96	2.74	2.51	4.28	3.34	4.82	2.49	5.10
Alanine	2.36	1.97	1.80	2.94	2.43	3.27	1.95	3.64
Cysteine	0.99	1.49	1.57	1.37	1.45	1.48	1.51	1.39
Valine	2.97	2.82	2.77	3.53	3.27	3.58	2.70	4.19
Methionine	1.74	1.73	1.65	2.01	2.06	2.64	1.57	2.47
Isoleucine	2.07	2.07	2.19	2.27	2.32	2.42	1.96	2.59
Leucine	4.82	4.48	4.42	5.20	4.83	5.82	4.38	5.99
Tyrosine	1.69	2.55	2.47	3.31	2.80	3.79	2.33	3.81
Phenylalanine	4.50	4.15	4.20	3.97	4.18	4.63	4.30	4.48
Histidine	1.78	1.59	1.58	1.94	1.91	2.67	1.60	2.46
Lysine	2.32	1.73	1.64	3.21	2.58	3.93	1.56	4.41
Arginine	2.75	2.70	2.71	3.82	3.27	4.49	2.58	4.85

[a]Molecular percent of the total proteins; tryptophan was not determined.
[b]HAc = acetic acid, AU = acetic acid-urea, WI-W = water-insoluble wheat, WS-R = water-soluble rye.
[c]Pentosans added at 2%, dry flour basis.
[d]Includes asparagine.
[e]Includes glutamine.

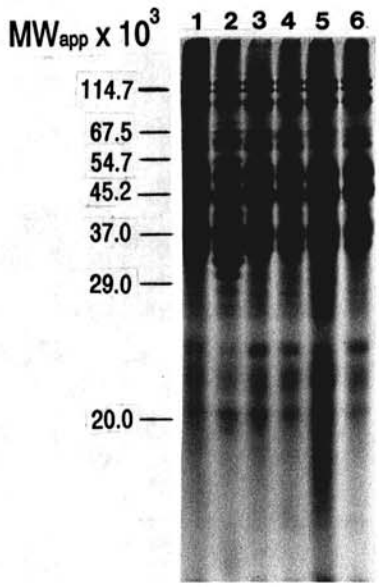

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams of reduced acetic acid-urea-soluble proteins extracted from gluten and dough. Lanes 1–3, gluten extracts (control, water-insoluble wheat, and water-soluble rye, respectively), lanes 4-6, dough extracts (control, water-insoluble wheat, and water-soluble rye, respectively). MW app. = apparent molecular weight of Katepwa wheat subunits, listed on the left.

unfractionated gluten. Adding pentosans to the flour before dough mixing did not change the composition of these fractions (data not shown). Amino acid composition of the proteins in all AU-soluble fractions is typical of glutenin (McMaster and Bushuk 1983). These fractions contained lower proportions of glutamic acid and proline and higher proportions of basic (Lys, Arg, His) amino acids, aspartic acid, and threonine than did the HAc-soluble fractions. AU-soluble fractions derived from gluten contained higher amounts of glutamic acid and lower proportions of basic amino acids than did the same fractions from dough.

Pentosans had a significant effect on the amino acid composition of the AU-soluble fraction of gluten, indicating a definite effect on the redistribution of different proteins among fractions. WS-R pentosans caused a decrease in the proportion of glutamic acid and an increase in the proportions of hydrophobic (Pro, Leu, Ile, Phe) and basic (Lys, Arg, His) amino acids. WI-W pentosans caused a slight increase in the proportions of glutamic acid and proline and a small decrease in proportions of basic amino acids (Lys, Arg).

The data on amino acid composition suggest that different proteins are aggregated in the AU-soluble fraction when WS and WI pentosans are incorporated. These results may be related to the differences observed in the electrophoretic patterns of AU-soluble gluten proteins (Fig. 6, lanes 2 and 3). Further studies are required to completely unravel the role of pentosans in the aggregation-disaggregation processes of gluten proteins during dough mixing.

ACKNOWLEDGMENT

Financial support from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

LITERATURE CITED

AMADO, R., and NEUKOM, H. 1985. Minor constituents of wheat flour: The pentosans. Page 249 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 46-12, approved October 1976, revised October 1986. The Association: St. Paul, MN.

ARAKAWA,T., YOSHIDA, M., MORISHITA, H., HONDA, J., and YONEZAWA, D. 1977. Relation between aggregation behavior of glutenin and its polypeptide composition. Agric. Biol. Chem. 41:995.

BUSHUK, W., and MacRITCHIE, F. 1989. Wheat proteins: Aspects of structure that determine breadmaking quality. Pages 345-369 in: Protein Quality and the Effects of Processing. R. D. Phillips and J. W. Finley, eds. Marcel Dekker: New York.

D'APPOLONIA B. L., and GILLES, K. A. 1971. Pentosans associated with gluten. Cereal Chem. 48:427.

DOUGLAS, S. G. 1981. A rapid method for the determination of pentosans in wheat flour. Food Chem. 7:139.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

HASHIZUME, E., KAKIUCHI, K., KOYAMA, E., and WATANABE, T. 1974. Denaturation of soybean protein by freezing. Agric. Biol. Chem. 35:44.

HOSENEY, R.C. 1984. Functional properties of pentosans in baked goods. Food Technol. 1:114.

INTERNATIONAL ASSOCIATION FOR CEREAL SCIENCE AND TECHNOLOGY (ICC) 1980. Standard 137. Mechanical determination of the wet gluten content of wheat flour. The Association: Vienna.

IZYDORCZYK, M., BILIADERIS, C. G., and BUSHUK, W. 1990. Oxidative gelation studies of water-soluble pentosans from wheat. J.

Cereal Sci. 11:153.
JANKIEWICZ, M., and MICHNIEWICZ, J. 1976. The changes of some properties of wheat dough protein complex as affected by addition of rye pentosan preparation. Ann. Poznan Agric. Univ. 89:75.
JANKIEWICZ, M., and POMERANZ, Y. 1965. Isolation and characterization of wheat flour proteins. I. Separation of salt- and acid-dispersible proteins by gel filtration, polyacrylamide gel electrophoresis, and sucrose gradient ultracentrifugation. J. Sci. Food Agric. 16:644.
JELACA, S. L., and HLYNKA, I. 1972. Effect of wheat-flour pentosans in dough, gluten, and bread. Cereal Chem. 49:489.
JOHANSSON, H., HALL, O., SELLERGREN, E., and THULIN, L. 1971. Solubility and machinability of dough proteins of wheat, rye and rye-wheat. Sver. Utsaedesfoeren. Tidskr. 81:262. Chem. Abstr. 75(17):108660c.
KÜHN, M.C., and GROSCH, W. 1989. Baking functionality of reconstituted rye flours having different nonstarchy polysaccharide and starch contents. Cereal Chem. 66:149.
McMASTER, G.J., AND BUSHUK, W. 1983. Protein-carbohydrate cornplexes in gluten: Fractionation and proximate composition. J. Cereal. Sci. 1:71.
MEUSER, F., and SUCKOW, P. 1986. Non-starch polysaccharides. Pages 42-61 in: Chemistry and Physics of Baking. J. M. V. Blanshard, P. J. Frazier, and T. Galliard, eds. Royal Society of Chemistry: London.
MICHNIEWICZ, J., BILIADERIS, C. G., and BUSHUK, W. 1990. Water-insoluble pentosans of wheat: Composition and some physical properties. Cereal Chem. 67:434.
NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324.
NG, P. K. W., and BUSHUK, W. 1989. Concerning the nomenclature of high molecular weight glutenin subunits. J. Cereal Sci.9:53.
OKADA, K., NEGISHI, Y., and NAGAO, S. 1987. Studies on heavily ground flour using roller mills. II. Chemical alteration of proteins, particularly globulin, during dough mixing. Cereal Chem. 64:3.
ROTTER, R. G., FRØHLICH, A. A., MARQUARDT, R. R., and MILLS, P. A. 1989. Estimation of fungal contamination of cereal grains determined by measuring glucosamine concentration. Can. J. Anim. Sci. 69:235.
TANAKA, K., and BUSHUK, W. 1973. Changes in flour proteins during dough mixing. I. Solubility results. Cereal Chem. 50:590.
ZAWISTOWSKA, U., BEKES, F., and BUSHUK, W. 1985. Involvement of carbohydrates and lipids in aggregation of glutenin proteins. Cereal Chem. 62:340.

[Received March 6, 1990. Revision received December 4, 1990. Accepted December 7, 1990.]