Pentosans from Gluten-Washing Wastewater: Isolation, Characterizations, and Role in Baking[1]

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ABSTRACT

Water-soluble pentosans were isolated from commercial gluten-washing wastewater. Compared with extracts prepared in the laboratory from bread flour, this pentosan extract had a higher pentosan concentration and a lower protein content. The pentosans were fractionated on a Sepharose 4B column. The molecular size distribution results indicated that the pentosans had apparently been modified by microbial enzymes in the wash water. Addition of the pentosans significantly increased dough water absorption and decreased dough mixing time. The pentosans reduced shear thinning and decreased the pasting peak viscosity of starch. Baking tests demonstrated that the addition of pentosans positively affected bread-baking performance and significantly decreased crumb firmness during storage at room temperature. Addition of the pentosans had no significant effect on loaf volume.

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The major nonstarchy polysaccharides of wheat flour are the pentosans, which are classified as hemicelluloses. Although flour contains only small amounts of water-soluble pentosans (about 0.5–0.8%), their chemical nature and physical characteristics significantly influence flour performance (Yeh et al 1980). Many researchers have studied these chemical and physical properties (Lineback et al 1977; Izydorczyk et al 1991a,b) and their role in breadmaking (Jelaca and Hlynka 1972, Michniewicz et al 1991).

In some commercial wheat starch-gluten production, the excess water that contains most of the soluble portion of the flour and some insoluble solids is discharged as effluent. Although some studies (Finley et al 1973, Oomah and Mathieu 1987) have considered pretreatment as the most economic method for controlling wastewater effluents, little research has been published on the use of pentosans from wash wastewater.

Oomah and Mathieu (1987) studied functional properties of commercially produced wheat flour solubles. They reported that the pentosan contents in four spray-dried, wheat flour, soluble concentrates varied between 8.82 and 15.03%. This indicates that, because some of the wash water is recycled, the pentosan yield from dried solids of gluten-washing wastewater is much higher than that from water-soluble extracts prepared directly from flour. Therefore, collection and use of gluten-washing wastewater might not only effectively reduce pollution but also provide a large source of pentosans.

The purpose of this study was to isolate and purify the pentosans from commercial gluten-washing water, to characterize them, and to evaluate their functionality in bread.

MATERIALS AND METHODS

Sources of Gluten-Washing Wastewater, Flour, Starch, and Chemicals

The samples of gluten-washing wastewater were obtained from Midwest Grain Products, Atchison, KS, and prepared by either of the following two ways. A small amount (9×10^{-4}%) of sodium azide (NaN_3) was added for microbial inhibition and the water sample was kept at room temperature; or, after collection, the water sample was placed immediately in the refrigerator until it could be freeze-dried. Commercial bread flour (bleached and bromated with 11.5% moisture, 12.5% protein, and 0.5% ash on a 14% moisture basis) was obtained from Cargill, Wichita, KS. Unmodified wheat starch (Mid sol 50) was obtained from Midwest Grain Products. Most chemicals were from Fisher Scientific Ltd., Winnipeg, Manitoba, or Sigma Chemical Co., St. Louis, MO. Pancreatin (P1625, Sigma) was from porcine pancreas, and α-amylase (EC 3.2.1.1, Sigma) was from *Bacillus* species.

Isolation and Purification of Water-Soluble Pentosans

Water-soluble pentosans were isolated on the basis of the procedures developed by Pence et al (1950), D'Appolonia and Gilles (1971), Yeh et al (1980), and Antoniou et al (1981). Freeze-dried gluten-washing water or bread flour (236.2 g) was slurried with distilled water (1:2, w/v) for 10 min at room temperature, or the gluten-washing water (600 ml) was used directly. The supernatant was collected after centrifugation at $1,000 \times g$ for 20 min at room temperature. Then the extract was heated to 93°C, held at that temperature for 10 min, cooled, and centrifuged at 30,000 $\times g$ for 30 min. The supernatant was stirred for 30 min with 5 g of special Filtrol (activated, strong acidic clay, Fluka, Ronkonkoma, NY) per 100 ml of solution at pH 5.5–6.0. The Filtrol was then removed by centrifugation at $13,000 \times g$ for 20 min.

Next, the supernatant was adjusted to pH 7.2–7.4 with NaOH (6*N*) solution and digested using a 2% centrifuged solution of pancreatin (0.8 g) (activity at least equivalent to 3× USP specifications) in 0.1% sodium chloride. The digestion was carried out at 32–34°C for 24 hr, with continuous stirring in the presence of 0.05% NaN_3 for microbial inhibition. Residual NaN_3 was removed by subsequent dialysis and washings. After digestion for 24 hr, the solution was heated to 100°C, held at that temperature for 10 min, cooled, and centrifuged at $13,000 \times g$ for 15 min.

The supernatant was dialyzed against distilled water by using a membrane tube with a molecular weight cutoff of 12,000–14,000) (Fisher Scientific Ltd.). The dialysis water was left running continuously and was periodically checked by the phenolsulfuric acid method (Dubois et al 1956) for the presence of sugars. Dialysis was stopped when no detectable sugar was present in the water. The solution was then added to absolute ethanol in a 1:4 ratio (i.e., adjusted to 80% alcohol) and kept at 4°C overnight. The precipitate was collected by filtration through Whatman no. 541 filter paper. The pentosan fraction was then washed sequentially with 100 ml of 95% ethanol, acetone, and ethyl ether and dried with N_2 at room temperature under reduced pressure.

Protein Estimation

Protein concentrations in the freeze-dried wash water and in the purified pentosans from gluten-washing wastewater and flour were determined by the method of Smith et al (1985) with crystalline bovine serum albumin as a standard.

Quantitation of Pentosans

Quantitative estimations of the amounts of pentosans in the freeze-dried gluten-washing wastewater and of the purified soluble pentosans from the wash water and flour were based on total pentose content. The orcinol-HCl method of Hashimoto et al (1987) was used with D-xylose as a standard.

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Gel Filtration

The molecular size distributions of the pentosans from the gluten-washing wastewater and the flour extracts were studied by using a Sepharose 4B column chromatograph according to the methods of Fincher and Stone (1974) and Izydorczyk et al (1990). Gel-permeation chromatography was performed on the column (2.5 × 85.0 cm) packed with Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified pentosans (15 mg) were dissolved in 4 ml of 0.01*M* phosphate buffer, pH 6.8, and loaded onto the column. The samples were eluted at a flow rate of approximately 20 ml/hr with a degassed solution of 0.3% (w/v) NaCl containing 0.05% (w/v) NaN_3 at room temperature. Fractions (7.1 ml) were collected on a FRAC-100 fraction collector (Pharmacia Fine Chemicals). Each effluent fraction was analyzed for carbohydrate by the phenolsulfuric acid procedure (Dubois et al 1956). The void and total bed volumes of the column were determined by using a mixture of 10 mg of blue dextran-2,000,000 and 2 mg of D-xylose in 1 ml of 0.01*M* phosphate buffer on the column. The elution volume of blue dextran determined the void volume of the column, whereas D-xylose measured the total bed volume. The effluents were assayed by the phenolsulfuric acid method (Dubois et al 1956).

Mixograph Study

To determine the effect of the pentosan extracts on dough rheological properties, the commercial bread flour was used on the 2-g prototype mixograph (Rath et al 1990) with a computerized data acquisition and analysis system. All samples were run at the same absorption level, which was the optimum absorption for the bread flour used as a control, and were determined in quadruplicate. All additives were added directly to the flour immediately before mixing. The results of the mixogram characteristics, with or without addition of the pentosans, from all of the experiments were analyzed together as a randomized complete block design.

Pasting of Wheat Starch and Bread Flour

The pasting properties of wheat starch and flour in the presence of the water-soluble pentosans were determined by using the Rapid Visco-Analyser (RVA) (Newport Scientific, Narrabeen, NSW, Australia) with an OmniScribe recorder (Curken, New Milford, CT) according to the procedure described by Deffenbaugh and Walker (1989). The dried pentosan powder was rehydrated overnight in the refrigerator before being used to disperse the 3 g of starch or 4 g of flour in the RVA sample can. An 18-min procedure was used to analyze the samples. All measurements were made on duplicate samples. Other materials (such as ash, residual chemical reagents, etc.) possibly present in the pentosan extract also were added to the starch or flour samples to evaluate their effects on starch and flour pasting properties.

Determination of α-Amylase Activity

The residual α-amylase activity in the pentosan extracts was determined by using the Nelson colorimetric copper method (Robyt and Whelan 1953) with a modification on the blank solution. The blank was prepared by using 1.0 ml of the pentosan solution and 1.0 ml of water. A standard curve was prepared by using maltose hydrate.

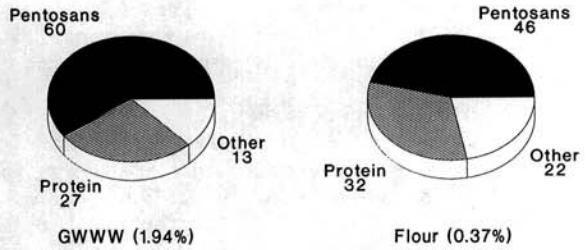

Fig. 1. Compositions and yields of solids extracted from gluten-washing wastewater (GWWW) and from flour.

Baking Test

The optimum water absorption for the flour was predicted by using a 10-g computerized mixograph. A series of water amounts (56, 60, 63, 64, 65, and 66%) was assayed by addition to 10-g (14% moisture basis) flour portions. The straight-dough pup loaf procedures of Neufeld and Walker (1990) were used. In this system, optimum water absorption and dough development time were employed. Bread without pentosan addition was treated as a control. The loaf volumes and weights were measured immediately after baking. All samples were run in quadruplicate each time.

Bread Firmness Measurements

After cooling for 2 hr, the bread samples were stored in sealed plastic bags at room temperature for one to seven days. The control bread was used for each measurement. All bread measurements were performed in triplicate. Firmness of the bread crumb was determined by using a Voland-Stevens-Lfra texture analyzer with a 25-mm-diameter indenter (Voland Corp., Hawthorne, NY) fitted with a Sargent-Welch chart recorder (model XKR, Sargent-Welch, Skokie, IL) (AACC method 74-09, AACC 1983).

Other Analyses

The quantities of protein, ash, total lipid, and crude fiber in the freeze-dried wastewater were determined according to AACC methods 46-09, 08-01, 30-20, and 32-10, respectively (AACC 1983). All analyses were performed in duplicate.

RESULTS AND DISCUSSION

The average chemical composition of the freeze-dried gluten-washing wastewater before purification was 23.78% protein, 7.71% ash, 0.14% fat, 0.46% crude fiber, 4.90% soluble pentosans, and 63.01% insoluble materials and soluble starch.

Comparative Water-Soluble Pentosans from Wheat Flour and Gluten-Washing Wastewater

Water-soluble pentosans were isolated from gluten-washing wastewater and wheat flour by the same procedure. The extracts from gluten-washing wastewater had a higher yield in percent (w/w), a greater concentration of pentosans, and a lower protein content (Fig. 1) than the extracts from bread flour. The higher yield indicated that the pentosans from the gluten-washing wastewater became concentrated during gluten washing, probably because the water was recycled. The result was similar to that reported by Oomah and Mathieu (1987) for pentosans isolated from commercial wheat flour solubles. Therefore, wash wastewater could be a source of pentosans. The higher purity with a lower total protein content of pentosans from the gluten-washing wastewater agrees with results reported by Yeh et al (1980). The difference of the pentosan compositions between the wastewater and

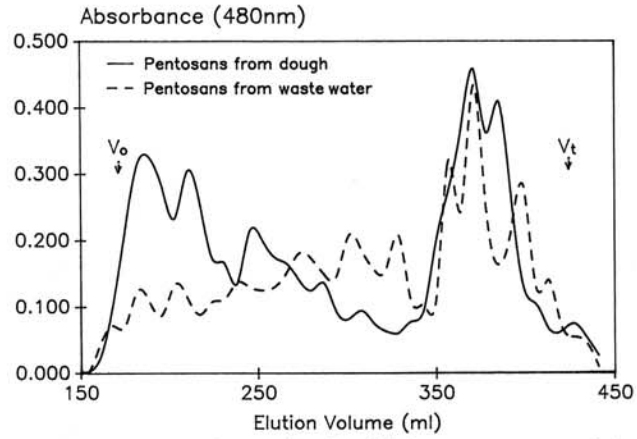

Fig. 2. Sepharose 4B elution profile of purified pentosans extracted from the gluten-washing wastewater and from bread flour. V_o = void volume; V_t = total bed volume.

TABLE I
Effects of Pentosans on Mixograph Dough Mixing Properties

Sample	Pentosan Concentration (%)	Peak Time (min)	Peak Height (%)	Mix Tolerance (°)	Curve Width,[a] %			
					L	P	R	T
Control	0.0	4.91 ± 0.35	42.03 ± 0.82	163.5	22.57	21.69	21.95	9.94
With PW[b]	0.3	3.99 ± 0.52	46.29 ± 0.98	161.0	22.69	21.38	20.34	9.08
	1.0	3.55 ± 0.39	47.13 ± 1.04	147.0	24.68	25.07	20.02	8.20
With PF[c]	0.3	3.95 ± 0.59	45.84 ± 1.84	159.0	24.05	23.12	20.28	9.31
	1.0	3.69 ± 0.77	47.75 ± 3.43	157.0	25.64	24.85	21.67	8.89

[a] L, left of peak width; P, peak width; R, right of peak width; T, curve tail width.
[b] Pentosans extracted from gluten-washing wastewater.
[c] Pentosans extracted from bread flour in the laboratory.

flour may be attributed to the different sources of the solubles. For flour, the soluble fraction was collected from a flour slurry with no gluten formation; for the wash water, the soluble fraction was collected after the separation of gluten.

Molecular Distribution of the Pentosans on Sepharose 4B

Figure 2 shows the elution curves for the pentosans extracted from the wastewater and flour passed through the Sepharose CL-4B. Peaks were found between the void volume and total bed elution volume, indicating that pentosans from both sources had similar molecular weight ranges. The elution profile for the pentosans from wheat flour was similar to the profiles reported by Fincher and Stone (1974), Yeh et al (1980), and Izydorczyk et al (1990). The pentosans from wheat flour were fractionated into two obvious, but not completely separated, fractions by gel filtration on Sepharose 4B. According to Fincher and Stone (1974), the first broad peak (between about 150 and 250 ml) exhibited a high molecular weight arabinoxylan and the second exhibited a lower molecular weight arabinogalactan. However, the elution profile for the pentosans from the gluten-washing wastewater showed that the high molecular weight arabinoxylan content decreased noticeably and that the content of lower molecular weight fractions, which were between arabinoxylan and arabinogalactan fractions, increased. The arabinogalactan fraction (between about 350 and 450 ml) was similar to the fraction in the pentosans from flour. These results indicate that the arabinoxylan pentosans from gluten-washing wastewater had been degraded. Because of the presence of bacteria (4.7×10^6 organisms per milliliter) in the wastewater before collection (*unpublished,* analyzed by V. Proctor), the degradation of the pentosans could be due to the action of enzymes produced by bacteria in the wash wastewater, as mentioned by Simpson (1954). Kulp (1968) showed that the pentosan fractions modified by the enzyme (cellulase) of *Trichoderma reesei* were superior in baking quality to those isolated from flour without enzyme treatment. McCleary et al (1986) reported that loaf volume was increased approximately 12% with addition of 16 or more units per 25 g of flour of *endo*-1,4-β-D-xylanase, which was required to remove the functional properties of the pentosans. Kuhn and Grosch (1988) indicated that modification of the nonstarchy polysaccharide fraction of rye flour decreased crumb firmness (i.e., retarded bread staling).

Effect on Mixogram Characteristics

Incorporation of pentosans from gluten-washing wastewater and from wheat flour into the dough had the same significant effect on the mixogram characteristics. The pentosans extracted from both sources were extremely hydrophilic. Adding pentosans resulted in stiffer doughs, in agreement with the observation of Jelaca and Hlynka (1972). Data on the effect of the pentosan preparation on dough mixing properties (Table I) clearly show this phenomenon. The water-soluble pentosans (1.0%) from the wash wastewater and wheat flour absorbed water at 5.32 and 5.70 times their weight, respectively. The smaller increase in water absorption from wastewater pentosans was caused by the degradation of those pentosans. The water absorption capacity is higher than that reported by Kim and D'Appolonia (1977), i.e., water-soluble pentosans absorbing water at 4.4 times their weight. The differences observed in the water absorption capacity may be partly due to the methods of isolation and purification employed in the investigations.

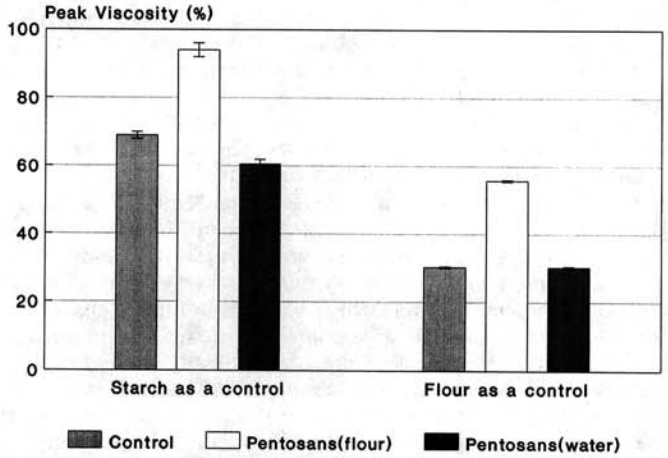

Fig. 3. Comparison of the effects of pentosans (10% addition on dry starch basis) from two sources on the Rapid Visco-Analyser pasting peak viscosities of starch and flour.

The peak times of the doughs in the presence of the pentosans from both the wash wastewater and wheat flour were all lower than that of the control (Table I). These results are in agreement with previous observations of Kulp (1968) and Kim and D'Appolonia (1977). The decrease in peak time may be due to active interactions between proteins and pentosans through weak, secondary bonds, which could be beneficial to dough development (Udy 1957, Michniewicz et al 1991). The mixing tolerance of the pentosan-containing doughs was lower than that of the control dough, in agreement with results reported by Kim and D'Appolonia (1977). Statistical analysis showed that incorporation into the dough of pentosans from both wash wastewater and wheat flour had the same significant effect on the mixogram characteristics. A number of other compounds possibly present in the pentosan extracts (residual chemical reagents and small sugars) were tested to see whether they also would have the same influence on the mixogram characteristics. The results of the comparisons indicated that only the pentosans were responsible for increasing water absorption and decreasing dough mixing time.

Effect on RVA Pasting Peak Viscosity

Comparisons using the RVA demonstrated that the pentosans extracted from the gluten-washing wastewater lowered starch peak viscosity, whereas the pentosans from wheat flour increased the peak viscosity (Fig. 3). A number of other compounds possibly present in the pentosan extracts from the gluten-washing wastewater were tested to see whether they also could reduce the peak viscosity of wheat starch. Ash samples extracted from the wash

water and xylose + arabinose were found to have no decreasing effect. Ether, which was used for drying the pentosan extract during isolation, was found to have an effect on the curve shape that did not occur with the addition of the pentosans. Chemical analysis of α-amylase activity confirmed that no measurable bacterial α-amylase residue, which was presumed to be residual heat-stable bacterial enzyme in the pentosan extract, existed in the pentosans isolated from the gluten-washing wastewater. It was thought that the lower protein content of the pentosans from the gluten-washing wastewater might have been responsible for the decrease in starch pasting viscosity. However, water-soluble pentosans isolated from dough with a lower protein content than that from flour (made in the laboratory) increased the pasting viscosity. The results indicate that isolating the pentosans either from flour slurry or from dough, i.e., with different protein contents in the pentosan extract, does not change their influence on starch pasting viscosity.

The RVA pasting studies suggested that the nature of individual pentosans within the water-soluble pentosan fraction could be responsible for either a decrease or an increase in starch peak paste viscosity.

The pentosans from both the wash wastewater and the flour also were added to wheat flour to see whether pentosans from the wash wastewater still decreased the peak viscosity in the presence of flour proteins. Figure 3 shows that the addition of the pentosans from flour increased the peak viscosity of flour by the same amount as in starch, whereas inclusion of the pentosans from the wash wastewater had no influence on the peak viscosity of flour.

Effect on Breadmaking Characteristics

Table II presents the effects of the water-soluble pentosans from the gluten-washing wastewater on baking absorption, mixing time, loaf weight, and loaf volume. The discrepancy between the observed pentosan water-binding capacity and the amount of additional water needed upon addition of pentosans could be due to the addition of various ingredients to a dough during baking. With 1.0% pentosan addition, 2.0% extra water could be added and mixing time could be reduced by 30 sec for the preparation of a dough of the desired consistency. The increase in the weight of the loaf observed was about 5%. Inclusion of the pentosans produced more relaxed doughs than the control formula. Commercially, the water-binding capacity of the pentosans could be an advantage for their use in bread. Besides absorbing 2.0% extra water, they would also retain 3% extra moisture during baking.

Even though the average volume of the pentosan-containing loaves was higher, according to the results of *t*-test analysis, addition of the pentosans had no statistically significant effect on loaf volume. The results from the frequency histogram show that 80% of the bread volume measurements lie below 909.7 and 897.9 cm^3 and above 851.6 and 839.2 cm^3 for breads with and without pentosans, respectively. Pence et al (1950) attributed the slight increase in loaf volume with the addition of water-soluble pentosans in their baking experiments to the small amounts of protein remaining in their pentosan preparations. Using a gluten-starch baking system, D'Appolonia et al (1970) reported that the slight increase in loaf volume of the gluten-starch loaves was due to the protein or to a protein-carbohydrate complex and not to the pentosans themselves. However, Hoseney et al (1969) indicated that the water-soluble proteins of albumin and globulin did not improve loaf volume, but removing water-soluble pentosans reduced loaf volume. The reasons for detrimental or beneficial effects on loaf volume with the addition of water-soluble pentosans are still not clear. The overall quality of bread incorporating pentosans from the gluten-washing wastewater, based on volume, crumb score, crumb softness, crumb grain, and shread, showed a slight improvement that was attributed mainly to the improvement in crumb softness.

TABLE II
Effects of Pentosans from Gluten-Washing Wastewater on Breadmaking Characteristics

Dough	Water Absorption (%)	Mixing Time (sec)	Loaf Volume (cm^3)	Loaf Weight (g)
Control	66.3	250	852.0 ± 14.9	140.0 ± 1.1
With 1.0% pentosans	68.3	220	871.0 ± 16.5	146.5 ± 1.3

Effect on Bread Firmness

The results of compression measurements made for the crumb of breads stored at room temperature are presented in Figure 4. It was evident that bread containing the pentosan preparation from the gluten-washing wastewater was lower in firmness than the control, especially after two days of storage. After six days, mold growth occurred in all samples. Kim and D'Appolonia (1977) studied the effect of pentosans on bread staling rate. They indicated that adding pentosans decreased the amount of starch components available for crystallization and, thus, decreased the bread staling rate. Jankiewicz and Michniewicz (1987) suggested that the process of retrogradation was restrained by formation of the starch-pentosan complex.

Potential Commercial Applications

Pentosans have several possible applications in the food industry. According to Anderson and Andon (1988), corn flour tortilla doughs are a perfect example for the need of water binding, yet they have little or no need for viscosity. Ice cream and frozen desserts are other possible applications. Pentosans with high water-binding properties might be used to give the desired viscosity in the unfrozen blend and a smooth texture in the finished product. Salad dressings, flan-type puddings, cookie mixes, and weak flour breads all need humectants or viscosity builders and represent a potential market.

CONCLUSIONS

Several characteristics of the pentosans from the gluten-washing wastewater can be described. 1) The pentosans apparently had been partially modified by bacterial enzymes in the wash wastewater before collection. 2) The pentosans exhibited positive effects on dough rheological properties, i.e., increased dough water absorption and decreased dough development time. 3) The pentosans decreased peak viscosity of the starch pasting curve and reduced shear thinning, but they showed no influence on flour pasting properties.

The addition of pentosans had a significant effect on decreasing the firmness of bread, but there was no statistically significant effect on bread loaf volume. The pentosan molecular size and various complex formations were important for the effects of pentosan fractions on the stach pasting properties and bread-

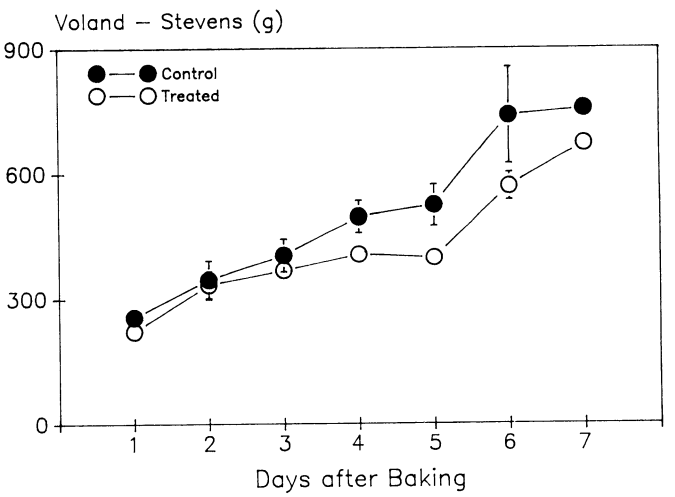

Fig. 4. Firmness comparison between control and breads with 1% pentosan extracts added (two loaves of each formula were tested each day of baking).

making. Further research is necessary to study the economy and safety of the pentosans.

ACKNOWLEDGMENTS

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