Changes in Wheat Flour Pentosans as a Result of Dough Mixing and Oxidation[1]

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ABSTRACT

Purified pentosans were extracted from flour, dough, and potassium iodate-treated dough (30 ppm KIO_3), with each dough mixed for three different mixing times. Flour yielded more pentosans than did dough or iodate-treated dough. Protein content of the pentosans decreased as mixing time increased; adding KIO_3 increased protein losses. Thus, pentosans extracted from overmixed, treated dough had lower protein contents than did flour pentosans. Overmixing dough caused a loss of the high molecular weight part of the arabinoxylan fraction; overmixed KIO_3-treated dough showed the greatest loss. Absorbance at 320 nm and the Folin-Ciocalteau method were used to study the ferulic acid content in the samples. Ferulic acid was associated only with the largest molecular weight part of the arabinoxylan fraction. Increased mixing time decreased ferulic acid 30% in overmixed dough pentosans and 45% in overmixed treated dough pentosans.

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Cereal chemists and technologists have long been interested in the water-soluble pentosans of wheat flour. 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.

Water-soluble pentosans form a gel when certain oxidizing agents are added (Baker et al 1943, Durham 1925). The oxidative gelation is a unique property of water-soluble, wheat flour pentosans. The UV spectrum shows a disappearance of the peak at 320 nm when oxidizing agents are added to pentosan solutions Kuendig et al 1961b). Ferulic acid is believed to be involved in the oxidative gelation mechanism (Fausch et al 1963). Most work on oxidative gelation has been in vitro. For example, oxidizing agents were added to pentosans extracted from flour.

In this study, water-soluble pentosans were extracted from flour, dough, and treated (KIO_3, 30 ppm) dough; each dough treatment was mixed for three different mixing times. The purified extracted pentosans from those samples were characterized by fractionation with saturated ammonium sulfate and gel filration, using

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Sepharose 2 and 4B. Ferulic acid contents of the various fractions were measured by UV absorbance and by the Folin-Ciocalteau method. The study was designed to gain a better understanding of the change in pentosans caused by oxidative reactions (from mixing and flour improvers) in the dough system.

MATERIALS AND METHODS

Flour

Untreated, unbleached flour milled from a composite of many hard winter wheat varieties grown at many locations was used throughout the experiment. The flour had a protein content of 12.5% and a moisture content of 13%.

Doughs

Doughs made of flour (100 g, 14% mb) and water were mixed in air for 1 min (undermixing), 3 min (optimum mixing), and 6 min (overmixing). Doughs made of flour (100 g, 14% mb), KIO_3 (30 ppm, flour basis), and water were mixed for the same periods. After mixing, the doughs were immediately frozen, then lyophilized.

α-Amylase

The four-times crystallized bacterial α-amlase used for pentosan purification was obtained from Sigma Chemical Co., St. Louis, MO.

Isolation of Purified Pentosan

Water-soluble pentosans were isolated and purified from flour and lyophilized ground doughs according to the procedures (Fig. 1) ofCawley (1964), Kuendig et al (1961a), and Fincher and Stone (1974).

Carbohydrate Determination

The phenolsulfuric acid method of Dubois et al (1956) was used to estimate carbohydrate in the purified pentosan, pentosan fractions, and column chromatography eluates. D-Xylose was used as a standard. Absorbance was measured at 480 nm.

Protein Estimation

Protein concentrations in the purified pentosan, pentosan fractions, and column chromatography eluates were determined by the method of Lowry et al (1951), with crystalline bovine serum albumin as a standard.

Gel Filtration Chromatography on Sepharose 4B

To study the molecular size distribution of pentosans and their fractions, Sepharose 4B column chromatography was used (Fincher and Stone 1974). Purified pentosans (15 mg) or arabinoxylan (10 mg) or arabinogalactan (4 mg) was dissolved in 1 ml of 0.01 *M* phosphate buffer, pH 6.8, and loaded on a column (54 × 2.5 cm) of Sepharose 4B (Pharmacia Fine Chemicals). The samples were eluted at a flow rate of approximately 11 ml/hr with a solution of 0.3% NaCl containing 0.05% sodium azide; 7–8-ml fractions were collected. Each effluent fraction was analyzed for carbohydrate by the phenolsulfuric acid procedure, for protein concentration by the Lowry procedure, and for ferulic acid content by absorption at 320 nm.

The void volume and total bed volume of the column were determined by applying a mixture of 10 mg of blue dextran-2,000 and 2 mg of D-xylose in 1 ml of 0.01 *M* phosphate buffer (pH 6.8) on the column. The blue dextran-2,000 was used to determine the void volume (V_o) of the column. The total bed volume (V_t) was determined by measuring the elution volume of D-xylose, assayed for by phenolsulfuric acid.

Fractionation of Purified Pentosan by Saturated Ammonium Sulfate

Purified pentosans were divided into soluble and insoluble fractions according to Fincher and Stone's procedure (1974). Purified pentosans extracted from flour or doughs (2 mg/ml) were dissolved in 0.1 *M* phosphate buffer (pH 7.0), and $(NH_4)_2SO_4$ was slowly added to satutation. The solutions were allowed to stand overnight at room temperature. The precipitated polysaccharide was collected by filtration on glass fiber paper (Whatman GF/A). The precipitate was then dissolved in water and dialyzed against water to remove the $(NH_4)_2SO_4$. The supernatant was also dialyzed to remove the $(NH_4)_2SO_4$. Both the insoluble and the soluble fractions were recovered by freeze-drying.

Ferulic Acid Content Determination

Liberation of Ferulic Acid. To determine the total ferulic acid content in the soluble pentosans, the bound ferulic acid was liberated from the pentosan molecule by an alkaline saponification procedure (Fausch et al 1963) Fifteen milligrams of 0.5 *N* KOH solution was added to 100 mg of purified pentosans or their fractions and the solution kept under nitrogen gas at 60°C for 90 min. After acidification with HCl to pH 3.0, three extractions were made with ethyl acetate. The ethyl acetate extract was dried with

TABLE I
Yield, Total Carbohydrate, and Protein of Purified Pentosans from Flour and Doughs

Samples	Purified Pentosan (%)	Total Carbohydrate (%)	Protein (%)
Flour	0.44	72.5	9.20
Dough			
Undermixed	0.38	73.4	9.48
Optimum mixed	0.42	71.6	8.42
Overmixed	0.35	72.5	8.55
KIO_3-treated dough			
Undermixed	0.39	74.4	8.80
Optimum mixed	0.42	72.7	7.44
Overmixed	0.38	73.4	7.04

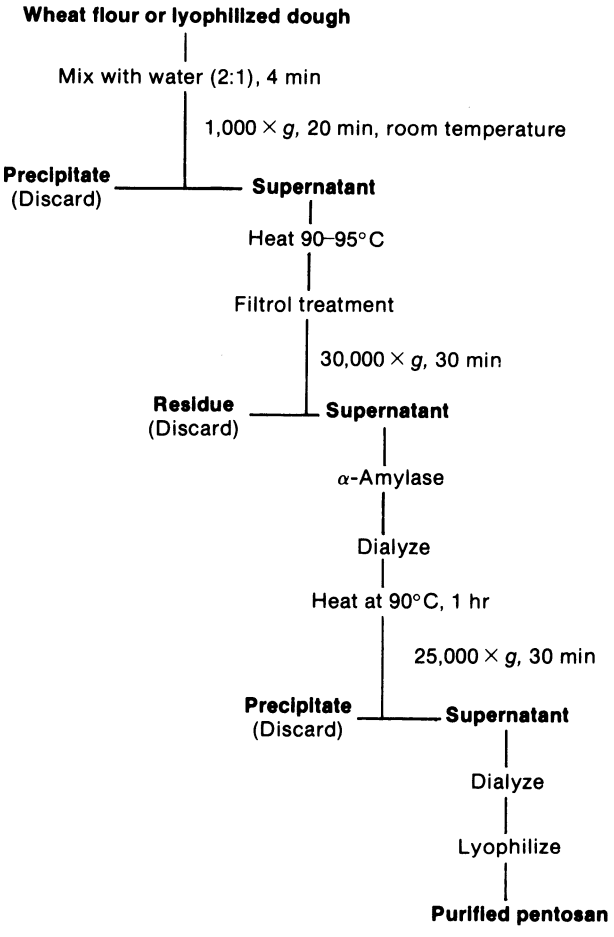

Fig. 1. Procedure for isolating purified pentosans from flour and doughs.

Na_2SO_4 and concentrated under vacuum.

Qualitative Determination. The concentrated ethyl acetate extracts were evaporated to dryness and the residue was taken up in a small amount of methanol. The aqueous fractions were also concentrated under vacuum.

Silica gel 60 F-254 precoated TLC plates were obtained from Brinkmann Company.

Solvent systems were A) benzene/dioxane/acetic acid (90:25:4) and B) chloroform/ethyl acetate/formic acid (5:4:1).

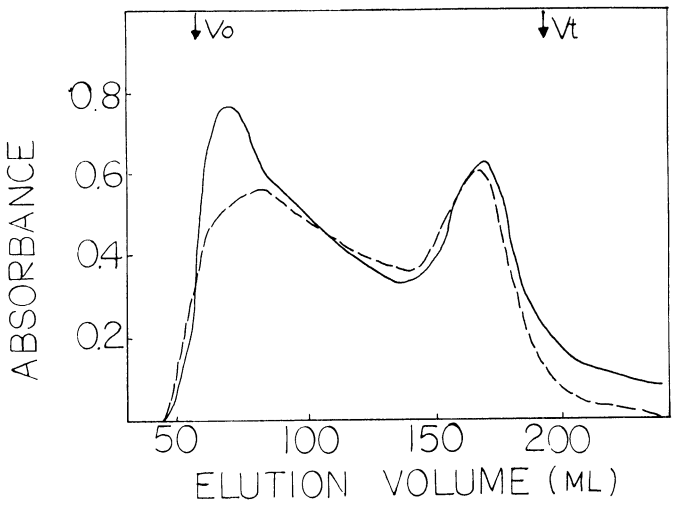

Fig. 2 Elution profile of purified pentosans extracted from flour (—) and overmixed, KIO_3-treated dough (--) on Sepharose 4B.

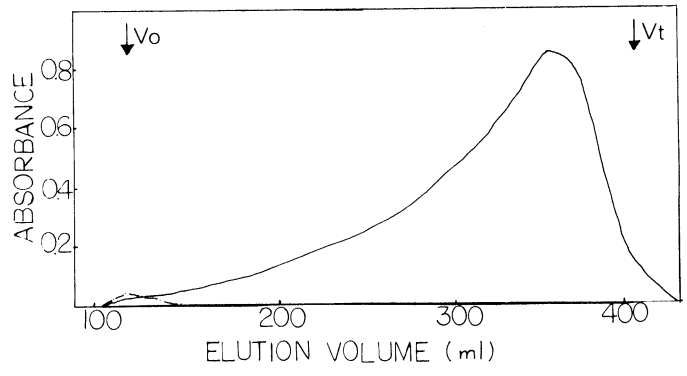

Fig. 3. Elution profile of purified pentosans extracted from flour on Sepharose 2B: (——) = carbohydrate, (—·—·—) = ferulic acid, 320nm.

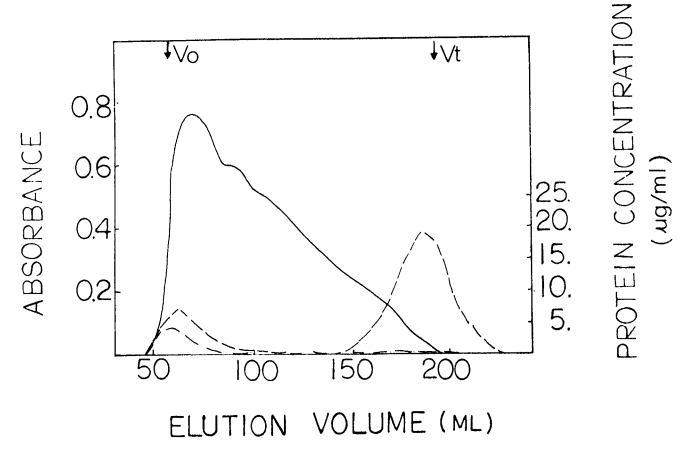

Fig. 4. Elution profile of the arabinoxylan extracted from flour on Sepharose 4B: (—) = carbohydrate, (--) = protein, (—·—·—)=ferulic acid, 320 nm.

The developed plates were examined under a UV lamp before and after exposure to NH_3 vapor and were then photographed under UV light.

Quantitative Determination of Polyphenols

Ultraviolet Method. The concentrated ethyl acetate solution was measured at 320 nm with a double beam spectrophotometer, using ferulic acid as the standard.

Folin-Ciocalteau Method. The concentrated ethyl acetate solutions were evaporated to dryness and the residue taken up in methanol. A 0.5-ml sample of phenol-containing methanol solution, pipetted into a test tube, and 2.5 ml of Folin-Ciocalteau reagent (Hoff and Singleton 1977) was added. The solution was then mixed and allowed to stand for 10 min. Saturated Na_2CO_3 solution (2 ml) was added; the solution was heated at 45° C for 15 min and then cooled. After filtering, the absorption was read at 720 nm, with ferulic acid used as a standard.

RESULTS AND DISCUSSION

Extraction of Purified Pentosans

The yield, the total carbohydrates, and the protein contents of purified pentosans extracted from flour and from ground lyophilized doughs are shown in Table I. The protein content of the purified pentosans ranged from 7 to 9.5%. Literature values range from 8 to 22% protein for the water-soluble pentosan fraction (Lin and Pomeranz 1968, Lineback et al 1977, Patil et al 1975). The major difference in our procedure was heating to remove α-amylase, rather than precipitating with trichloroacetic acid. Perhaps heating removes part of the associated protein from the pentosans.

The total carbohydrate was determined by the phenolsulfuric acid procedure (Dubois et al 1956) with D-xylose as a standard. Thus, the total carbohydrate is expressed as xylose. Xylose is the predominant component of pentosan, but pentosans contain significant amounts of arabinose and galactose. Because xylose gives a much higher color yield than do the other sugars, the total carbohydrate values are understated and should be considered minimal.

Molecular Size Distribution of Purified Pentosan

Fincher and Stone (1974) have shown that pentosans can be fractionated into two obvious, but not completely separated,

TABLE II
Percent Yield, Total Carbohydrate, and Protein of Arabinoxylan Fractions

Samples	Yield (%)	Total Carbohydrate (%)	Protein (%)
Flour	67.21	86.33	11.40
Dough			
Optimum mixed	66.37	84.67	8.01
Overmixed	64.80	84.33	7.14
KIO_3-treated dough			
Optimum mixed	66.57	82.12	5.22

TABLE III
Percent Yield, Total Carbohydrate, and Protein of Arabinogalactan Fractions

Samples	Yield (%)	Total Carbohydrate (%)	Protein (%)
Flour	25.98	44.67	9.62
Dough			
Optimum mixed	27.00	47.13	7.95
Overmixed	26.00	47.47	8.40
KIO_3-treated dough			
Optimum mixed	23.79	46.13	7.68

fractions by gel filtration on Sepharose 4B. They reported that the first broad peak is a high molecular weight arabinoxylan and the second, a lower molecular weight arabinogalactan associated with protein.

Samples of purified pentosans from the flour and doughs listed in Table I were fractionated on Sepharose 4B. In general, the first fraction shows a higher peak height than the second one from all samples except those from doughs that had been overmixed, which had about equal peak heights. The elution profiles for the pruified pentosan from flour and from an overmixed dough (Fig. 2) show a marked decrease in peak 1 for the overmixed dough. The elution profile of the second peak is essentially not affected, which indicates a loss of arabinoxylan from the water-soluble pentosan as a result of overmixing.

Significant UV absorption at 280 nm and 320 nm was observed in peak 1 for all the pentosan samples, which confirms the finding that ferulic acid is associated with the arabinoxylan fraction of the pentosans (Painter and Neukom 1968). The ferulic acid (absorption at 320 nm) eluted at the void volume not only from Sepharose 4B, but also from Sepharose 2B (Fig. 3), indicating that ferulic acid is associated with the largest arabinoxylan fraction in the pentosans.

Fractionation of Pentosans with Ammonium Sulfate

Four pentosan samples, (from flour, optimum mixed dough, optimum mixed dough containing KIO_3, and overmixed dough) were fractionated into soluble and insoluble fractions by saturated $(NH_4)_2SO_4$. The yields, total carbohydrates, and protein contents are given in Tables II and III. As Fincher and Stone (1974) had shown, the insoluble fraction is essentially an arabinoxylan, containing protein not associated with the carbohydrate (Fig. 4). The soluble fraction (Fig. 5) is an arabinogalactan, apparently associated with protein (Fincher et al 1974). About two-thirds of the purified pentosan is in the arabinoxylan fraction.

Protein contents of pentosan fractions extracted from doughs were lower that those extracted from flours. The same trend was found for both fractions, but the difference was more evident with the arabinoxylan fraction. Increased mixing decreased the protein content of the arabinoxylan fraction, and the presence of KIO_3 during mixing decreased it further. A UV spectrum showed absorption at 280 and 320 nm in the arabinoxylan fraction but only at 280 nm in the arabinogalactan fraction, which indicates that ferulic acid is present only in the arabinoxylan.

UV Spectra of Purified Pentosans and Their Fractions

Kuendig et al (1961b) and Neukom et al (1962) have shown that the absorbance maximum at 320 nm disappeared after a gel was formed by oxidation of the pentosans. The peak's disappearance suggested that ferulic acid was involved in the mechanism of oxidative gelation.

The UV absorption spectra of the arabinoxylan fractions extracted from flour, optimum mixed dough, overmixed dough, and optimum mixed dough containing KIO_3 are shown in Fig. 6. The absorption peak at 320 nm is less pronounced as a result of dough mixing and oxidation, apparently the result of increased absorption at about 280 nm. The arabinogalactan fractions do not absorb at 320 nm and show no change in absorption spectra from dough mixing or oxidation.

Quanitative Determination of Phenolics

The pentosan fractions were saponified with dilute base and the released phenolics extracted with ethyl acetate. Thin-layer chromatography confirmed that ferulic acid was the main phenolic extracted from the water-soluble pentosans. Several other weakly

TABLE IV
Ferulic Acid Content[a] of Purified Pentosans from Flour and Doughs

Samples	Method	
	UV	Folin-Ciocalteau
Flour	0.56	0.62
Dough		
Undermixed	0.54	0.50
Optimum mixed	0.57	0.56
Overmixed	0.41	0.45
KIO_3-treated dough		
Undermixed	0.56	0.53
Optimum mixed	0.52	0.47
Overmixed	0.31	0.34
Standard deviation	0.043	0.055

[a]Milligrams of ferulic acid per gram of pentosan.

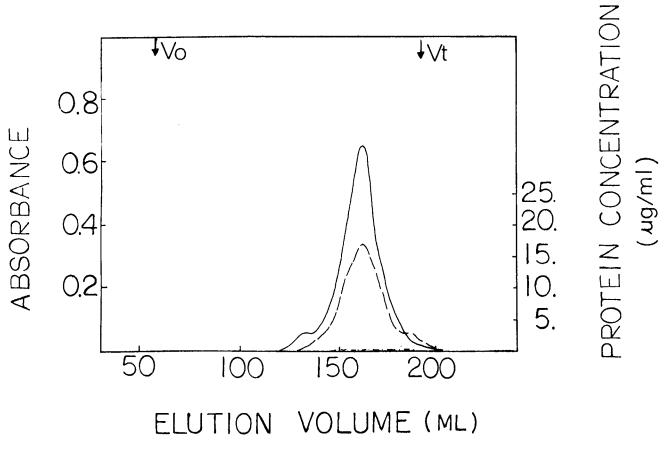

Fig. 5. Elution profile of the arabinogalactan extracted from flour on Sepharose 4B: (—) = carbohydrate, (--) = protein, (—·—·—) = ferulic acid, 320 nm.

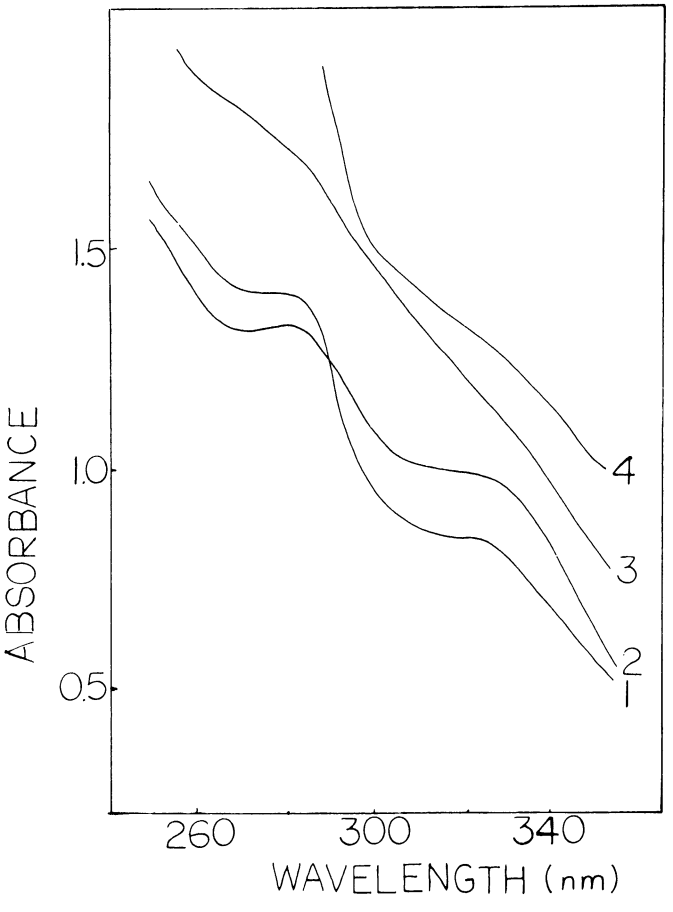

Fig. 6. Ultraviolet spectra of the arabinoxylan fraction extracted from **1**, optimum mixed dough; **2**, flour; **3**, overmixed dough; and **4**, dough overmixed with KIO_3.

fluorescent compounds also were present. The ethyl acetate extracts were analyzed by two methods, UV absorption at 320 nm and the Folin-Ciocalteau procedure for phenolics. Ferulic acid was used as a standard.

The ferulic acid contents of the various purified pentosans, as determined by the two methods, are shown in Table IV. Overmixing dough in air decreased the ferulic acid in the purified pentosan by 30%. A similar loss was found by mixing the dough to optimum with KIO_3. Doughs overmixed with KIO_3 gave a 45% loss of ferulic acid in the purified pentosans. Similar reesults were obtained with ferulic acid extracted from arabinoxylan fractions. No significant amount of ferulic acid was found in any of the arabinogalactan fractions.

Both methods of determining ferulic acid content gave similar results. The loss of ferulic acid from overmixing and/or oxidation indicates that the water-soluble pentosans have undergone oxidative gelation in the dough system. The oxidized pentosan gels are insoluble and thus would not be isolated by our procedure. This would account for the reported loss of ferulic acid.

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