# Cereal Pentosans: Their Estimation and Significance. I. Pentosans in Wheat and Milled Wheat Products[1]

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**ABSTRACT**

Water-soluble, enzyme-extractable, and total pentosans were estimated in whole wheat and laboratory-milled wheat products by the orcinol-HCl method. The enzyme-extractable pentosans were estimated after treatment with a multi-component system of *Trichoderma viride*. Total pentosans were estimated after hydrolysis with 2*N* HCl at 100° C, neutralization, and fermentation with fresh, compressed baker's yeast. Whole wheat contained 0.90, 2.45, and 8.95%; wheat flours 0.70–0.83%, 1.66–1.86%, and 1.50–2.12%; and milling by-products 1.29–1.68%, 2.14–4.45%, and 9.90–28.39%, soluble, enzyme-extractable, and total pentosans, respectively. The least significant differences in wheat and milled wheat products at the 0.05 level of significance were 0.192, 0.363, and 1.28 for the soluble, extractable, and total pentosans, respectively.

Estimation of pentosan contents has assumed increasing significance as they have been known to affect rheological properties of wheat flour doughs (Hoseney 1984), breadmaking characteristics of wheat and rye flours (Casier et al 1973), and the classification of dietary fiber (Southgate et al 1978, James and Theander 1981, Prosky et al 1984, Halvarson and Alstin, 1984, and Wisker et al 1985).

The development of methodology for the determination of dietary fiber has been hampered by the fact that fiber is a complex mixture of many, loosely defined substances. As pentosans are one of the main components of dietary fiber in cereals, the estimation of pentosans is of particular interest. Ford (1981) developed a procedure for determining the ratio of hexoses to pentoses in solution. The method uses a combination of the phenol-sulfuric acid reaction and an ultraviolet absorption band in 312–325 nm for the reacted solutions. The absorbance maximum of that band increases with, and is linearly related to, the ratio of hexose to total sugars in the mixture. Preliminary investigations in our laboratories have not yielded satisfactory results in the determination of pentoses in aqueous extracts of wheats and milled wheat products and mixtures of pure hexoses and pentoses. The colorimetric phloroglucinol method of Cracknell and Moyce (1970) and Douglas (1981) eliminates the interference of hexoses in pentose estimation by determining the difference between absorbances at two wavelengths. The method was modified by Bell (1985) to eliminate interference of particulate matter in analysis of high-fiber products. The modified method showed excellent agreement with dietary fiber in wheat products (including bread) but not in barley, oats, cooked maize products, or legumes. Bell (1985) also showed that the correlation between dietary fiber and the orcinol method for wheat product samples was 0.998 and that

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pentose values estimated in white, brown, or whole wheat bread by the orcinol (after correction) and phloroglucinol methods were essentially identical.

We describe here the results of estimating water-soluble, enzyme-extractable, and total pentoses by the orcinol-hydrochloric acid method. The interference of high concentrations of glucose is eliminated by fermentation with fresh, compressed bakers' yeast.

## MATERIALS AND METHODS

Centurk 78, a hard red winter wheat, harvested in 1984 in Colby, KS, was milled on an experimental mill (Allis-Chalmers Mfg. Co., Milwaukee, WI) (Finney and Bolte 1985). The following milled products and combinations were analyzed for water-soluble, enzyme-extractable, and total pentosan: first and second middlings; sizings and third middlings; first, second, and third breaks; fourth break; first and second low grade; fourth and fifth middlings; coarse bran; fine bran; shorts; and red dog of the hard winter wheat. The combinations were selected to produce flours varying widely in ash contents (Table I).

Two hard red winter wheat varieties, TAM-101, composited from two locations in Texas (Vernon and Bushland) in 1983, and an experimental wheat selection composited from three locations in Nebraska (Meade, Clay Center, and N. Platte) in 1983, were selected on the basis of their high and low total pentosan contents, respectively, and were used to establish laboratory conditions for estimating water-soluble and enzyme-extractable pentosans from flour. The wheats were milled experimentally. Flour ash and protein contents of TAM-101 were 0.45 and 12.3%, respectively, and 0.44 and 12.3%, respectively, of the Nebraska experimental selection (14% mb).

Wheat bran, certified food grade, was purchased from the American Association of Cereal Chemists, St. Paul, MN, and used as received. A crude preparation of water-soluble pentosans from rye flour was obtained from B. Fretzdorff, Detmold, West Germany, and of water-insoluble pentosans from oat hulls was purchased from Sigma Chemical Co., St. Louis, MO.

Meicellase, a multicomponent enzyme system of *Trichoderma viride* origin, included cellulases (I and II), β-glucosidase, xylanases (A and B), β-xylosidase, and α-L-arabinosidase (Hashimoto et al 1971, Hashimoto 1982). Veron HE, a commercial pentosanase, was supplied by Rohm Tech., Inc. (New York, NY). All chemicals were reagent grade or better.

Moisture, ash, and protein were determined by AACC methods 44-15A, 08-01, and 46-11, respectively (AACC 1984).

The orcinol-hydrochloric acid method of Albaum and Umbreit (1947) for pentose determination was used. It consisted of heating in boiling water for 30 min a solution containing 3 ml of pentose, 3 ml of 0.1% ferric chloride in concentrated hydrochloric acid, and 0.3 ml of 1.0% orcinol in 100% ethanol, cooling, and determining absorbance ($A$) at 670 nm.

Methods were developed to estimate water-soluble, enzyme-extractable, and total pentosan contents of cereal grains. They are described in detail under Results and Discussion.

**TABLE I**
**Mill Fractions, Their Yields, Protein, and Ash Contents from a Hard Red Winter Wheat[a]**

| Mill Fraction[b] | Yield[a] (%) | Protein[a] (%) | Ash[a] (%) |
|---|---|---|---|
| Whole wheat | 100.0 | 11.8 | 1.49 |
| 1, 2 MDS | 20.4 | 10.0 | 0.38 |
| SIZ, 3 MDS | 25.4 | 10.4 | 0.41 |
| 1, 2, 3 BRK | 11.3 | 11.2 | 0.58 |
| 4 BRK, 1, 2 LG, 4, 5 MDS | 15.8 | 11.2 | 0.73 |
| Coarse bran | 16.9 | 13.5 | 5.55 |
| Fine bran | 5.7 | 15.1 | 4.30 |
| Shorts | 3.4 | 16.4 | 3.32 |
| Red dog | 1.1 | 15.7 | 2.35 |

[a] Variety Centurk, harvested at Colby (Fallow), KS. All values on a 14% mb.
[b] Abbreviations: MDS = middlings, SIZ = sizings, BRK = break flour, LG = low-grade flour.

## RESULTS AND DISCUSSION

Absorbances over the range from 520 to 730 nm of solutions of xylose, glucose, xylose plus glucose, glucose plus yeast, and xylose plus glucose plus yeast are shown in Figure 1. Maximum absorbance for xylose (curve II) occurs at 670 nm. Curves III and IV demonstrate the interference caused by glucose, and curves I and V show the effectiveness of yeast in removing glucose.

Solutions of xylose in water were made at concentrations of 40, 80, 120, 160, and 200 μg/3 ml and were measured spectrophotometrically after applying the orcinol-HCl method, which gave a standard curve (Fig. 2) from which concentrations of pentose or pentosan could be estimated. As shown in Figure 2, the determination of pentoses by the orcinol method was affected by very high concentrations of glucose. If the concentration of glucose is more than about five times the concentration of xylose, it may be necessary to remove glucose by fermentation.

For a 1-cm cell and for a 10-mg sample in 3 ml, $A_{670} \times m \div 100$ and $A_{670} \times m \times 0.88 \div 100$ estimated pentose and pentosan content in percent (w/v), respectively.

### Water-Soluble Pentosan

The two flour samples (100 mg each) of low and high pentosan contents were shaken in 10 ml of distilled water at 30° C for 0, 0.5, 1, 2, 3, and 20 hr and centrifuged. Aliquots of the supernatants were hydrolyzed with 4$N$ HCl for 2 hr, and pentosan contents were estimated by the orcinol-HCl method.

As extraction time of water-soluble pentosan increased from 0 to 20 hr, the amount of pentosan extracted increased for both the high

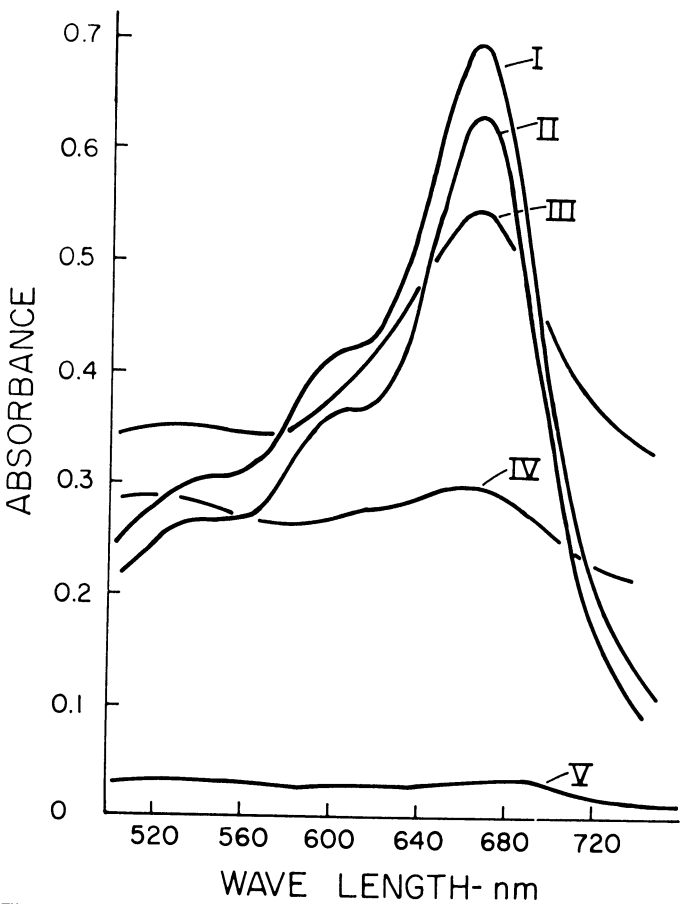


**Fig. 1.** Absorbance at 520–730 nm for solutions of: **I,** 0.9 mg of xylose, 24 mg of glucose, and yeast; **II,** 0.9 mg of xylose; **III,** 0.45 mg of xylose and 12 mg of glucose; **IV,** 12 mg of glucose; and **V,** 24 mg of glucose plus yeast.

and low pentosan containing flours (Fig. 3). In choosing an extraction time of 2 hr, three considerations were made. For the determination to be utile, 2 hr was more advantageous than 10–20 hr. Too long an extraction time could involve the action of flour pentosanases (Hashimoto, *unpublished data*). Although zero-time extraction was most advantageous for routine assay, being on the steepest part of the curve made time more critical than at 2 hr. For 0-, 0.5-, 1-, 2-, and 3-hr extraction times, pentosan extracted from the high-pentosan flour was 56, 68, 72, 78, and 81%, respectively, of the 20-hr total, and from the low-pentosan flour it was 52, 67, 70, 75, and 78% of the 20-hr total. A scheme for estimation of soluble pentosans is given in Figure 4.

**Enzyme-Extractable Pentosan**

The two flour samples (100 mg each) of high and low pentosan content were suspended in 10 ml of 0.1*M* Na-acetate buffer (pH 4.5–4.6), and 200 $\mu$l of 0.5% meicellase or 200 $\mu$l of 1% Veron HE (in 0.1*M* Na-acetate buffer, pH 4.5–4.6) were added. The mixture was shaken for 1, 2, 5, 21, and 26 hr and centrifuged. Aliquots of the supernatants were hydrolyzed with 4*N* HCl for 2 hr, and pentosan contents were determined by the orcinol-HCl method. Zero-hour extraction was carried out by extracting without enzyme for 1 hr.

As incubation time of meicellase increased, pentosan extracted from high- and low-pentosan flours increased to a maximum at about 21 hr (Fig. 5). At 0, 1, 2, 5, and 26 hr, respectively, amounts extracted from the high-pentosan flour were 42, 81, 89, 91, and 99% of the 21-hr total, and from the low-pentosan flour they were 42, 86, 89, 94, and 101% of the 21-hr total. Addition of more enzyme after 21 hr of incubation did not increase the amount of extracted pentosan. Optimum was considered to be 20 hr. About twice the amount of Veron HE as meicellase was required for equivalent activity. A scheme for estimation of enzyme-extractable pentosan is given in Figure 6.

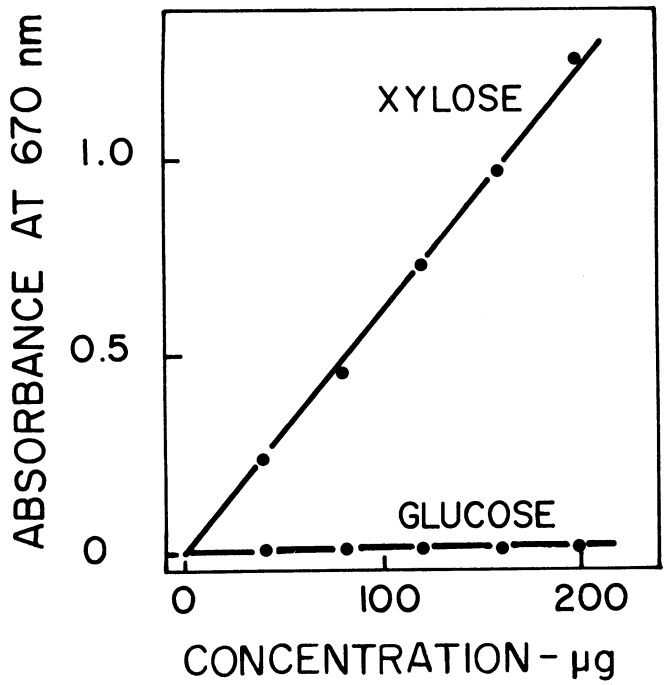


**Fig. 2.** Standard curve for absorbance vs. xylose and glucose concentrations.

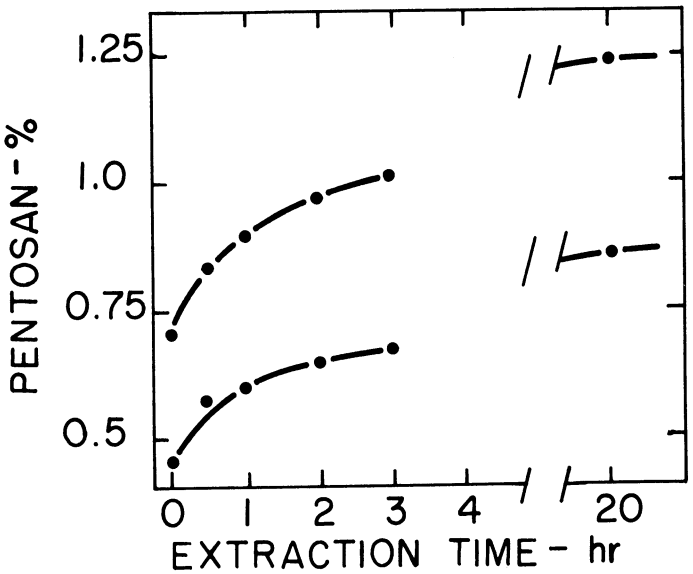


**Fig. 3.** Effect of time on extraction of water-soluble pentosan from wheat flours of low- and high-pentosan contents.

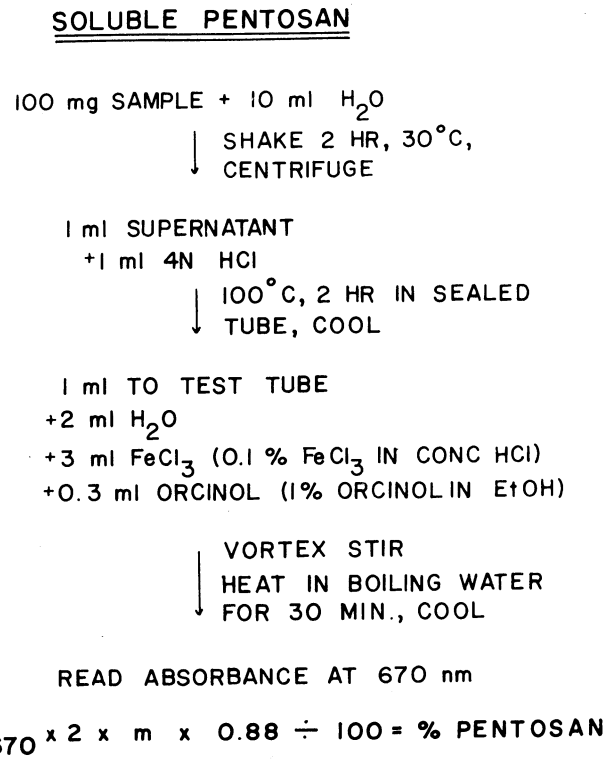


**Fig. 4.** Scheme for estimation of soluble pentosan.

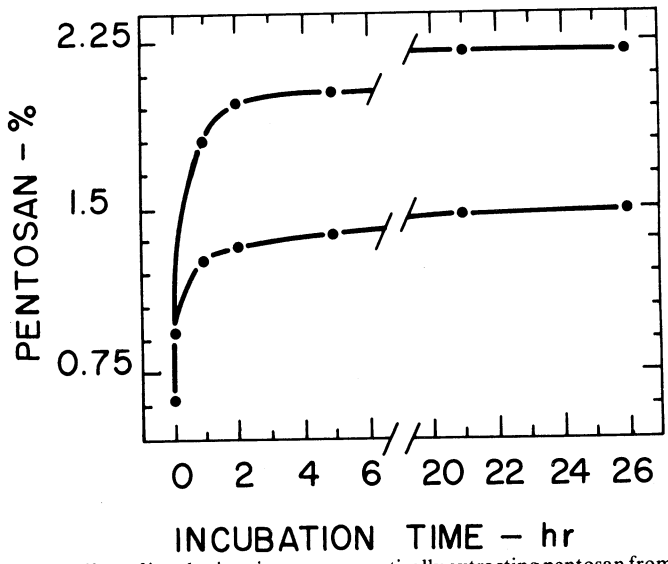


**Fig. 5.** Effect of incubation time on enzymatically extracting pentosan from wheat flours of low- and high-pentosan contents.

**Total Pentosan**

Flour (10 mg) was weighed into a 15-ml hydrolysis tube; 2 ml of 2*N* HCl was added, and the mixture was hydrolyzed at 100° C for 2.5 hr. After cooling, neutralization was effected by the addition of 2 ml of 2*N* sodium carbonate. Fermentable sugars were removed by fermentation; 2 ml of a 25 mg/ml of 0.2*M* Na phosphate buffer (pH 7.0) was added in a suspension of fresh compressed yeast (*Saccharomyces cerevisiae*), incubated, and mixed on a Vortex-Genie mixer every 20–30 min for 1.5 hr at 30° C or until fermentation was complete. Increasing the fermentation temperature to 37° C shortened the required time to a maximum of 1 hr. The mixture was centrifuged at 1,000 rcf for 10 min, and an aliquot of the supernatant was analyzed by the orcinol-HCl method.

The hydrolysis time and acid concentration we used generally are recognized as adequate for glycoproteins, whereas higher concentrations such as 6*N* would destroy the sugar. In hydrolyzing polymers of pentoses, glucose polymers were also hydrolyzed. As shown in Figures 1 and 7, fresh compressed bakers' yeast was effective in removing glucose before applying the orcinol-HCl test. The presence of yeast caused a slight increase in absorbance, probably due to the presence of nucleic acids, and therefore would limit the use of the orcinol-HCl method in baked goods containing yeast. As compressed yeast storage time increased, $A_{260}$ of a centrifuged supernatant increased. In similarly prepared samples, $A_{260}$ was much greater for dry yeasts. Nucleic acids are known to absorb in the $A_{260}$ region. Therefore, fresh yeast interfered least with total pentosan estimations. A yeast blank would be advisable. A scheme for estimation of total pentosan is given in Figure 8.

The total pentosan contents (as-is basis) were 60.4% in the water-soluble preparation from rye flour, 73.0% in the water-insoluble preparation from oat hulls, and 19.5% in the AACC wheat bran. According to the collaborative study of Prosky et al (1984), the AACC wheat bran contained 36.54–47.22% (average 42.55%) dietary fiber. Water-soluble, enzyme-extractable, and total pentosan for the whole wheat and the milled wheat products are given in Table II. The total dietary fiber contents in single samples of white and whole wheat flours in the collaborative study of Prosky et al (1984) were 3.07% (0.07–12.25%) and 12.92% (5.33–16.17%), respectively.

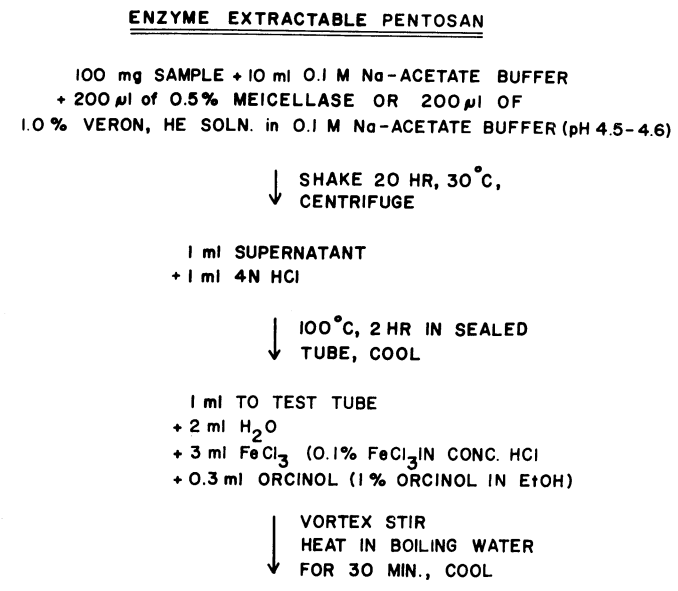


**Fig. 6.** Scheme for estimation of enzyme-extractable pentosan.

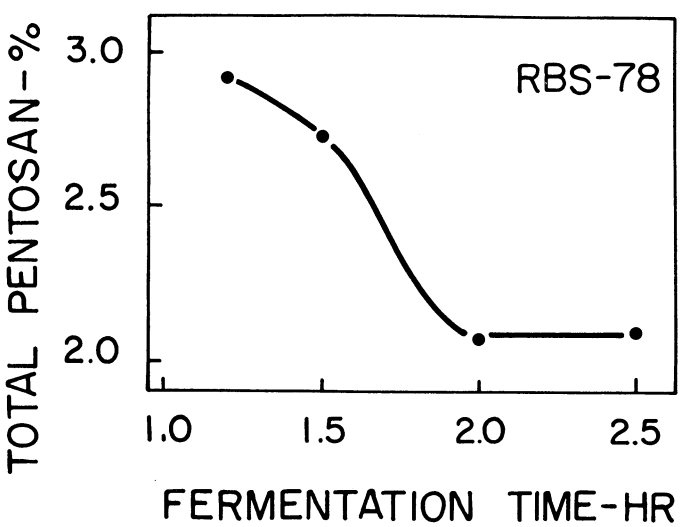


**Fig. 7.** Effect of yeast fermentation time on total pentosan determination.

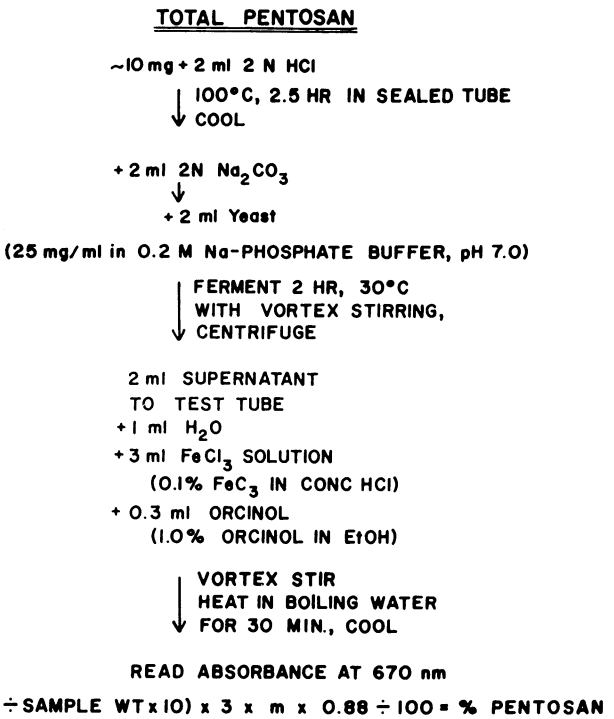


**Fig. 8.** Scheme for estimation of total pentosan.

**TABLE II**
**Mill Fractions and Their Water-Soluble, Enzyme-Extractable, and Total Pentosan Contents from a Hard Red Winter Wheat[a]**

| Mill Fraction[b] | Pentosan | | |
|---|---|---|---|
| | Soluble (%) | Enzyme-Extractable (%) | Total (%) |
| Whole wheat | 0.68 | 1.84 | 6.71 |
| 1, 2 MDS | 0.53 | 1.25 | 1.13 |
| SIZ, 3 MDS | 0.56 | 1.33 | 1.15 |
| 1, 2, 3 BRK | 0.62 | 1.34 | 1.29 |
| 4 BRK, 1, 2 LG, 4, 5, MDS | 0.59 | 1.40 | 1.59 |
| Coarse bran | 0.97 | 1.61 | 21.29 |
| Fine bran | 1.15 | 2.60 | 17.37 |
| Shorts | 1.22 | 3.34 | 10.35 |
| Red dog | 1.26 | 3.29 | 7.43 |
| Least significant difference at 0.05 | 0.192 | 0.363 | 1.28 |
| Least significant difference at 0.01 | 0.275 | 0.521 | 1.84 |

[a] Variety Centurk, harvested at Colby (Fallow), KS. All values on a 14% mb.
[b] Abbreviations: MDS = middlings, SIZ = sizings, BRK = break flour, LG = low-grade flour.

According to Bell (1985), the relation between Southgate dietary fiber (polysaccharides plus lignin [DFs]) and total pentose estimated by the orcinol method (Po) were DFs = 2.18 + 1.65 Po ($r$ = 0.998). This corresponds to about 17.0% dietary fiber in wheat containing 8.95% pentosans and to 45.1% dietary fiber in bran containing about 26% pentosans (this study). The values of Theander and Westerlund (1986) for dietary fiber were 10.6% in wheat and 39.0% in bran.

Weighted sums of pentosan values derived by multiplication of each mill fraction (yield in Table I) by its pentosan value (Table II) were compared to values determined in the whole wheat. The weighted values were 83.3% for the soluble, 104.5% for the enzyme-extractable, and 88.4% for the total pentosan. The total pentosan estimates may be affected by the fact that some carbohydrates (primarily dextrins) freed by the hot acid treatment may not be sufficiently broken down for complete removal by yeast. Additional studies should be conducted to determine the amounts and identity of those compounds.

There were significant differences in the three pentosan forms between the wheat, the flour, and the milling by-products. The least significant differences listed in Table II are for the wheat and all milling products. The determinations of wheat and milling by-products composition are associated with substantial errors resulting from sampling, grinding, and inherent heterogeneity of various particles (even in the ground form). Such errors (and associated least significant differences) are much smaller in flours of relatively uniform particle size and composition, as shown in a study on the relation between pentosan contents and breadmaking characteristics (Shogren et al 1986). In that study, multiple correlations (including protein and various forms of pentosan) predicted water absorption and loaf volume potential quite well. Estimations of pentosans in pearled cereal grains and commercial by-products of milled cereals are reported elsewhere (Hashimoto et al 1986).


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