

NOTE ON A PROCEDURE FOR THE ISOLATION OF THE WATER-INSOLUBLE PENTOSANS OF WHEAT FLOUR¹

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A procedure is described for the isolation of the pentosans associated with the "sludge," "tailings," or "squeegee" fraction of wheat flour.

Various techniques are reported in the literature on the isolation of pentosans associated with the above-mentioned fraction of wheat flour.

Yamazaki (1) purified the tailings fraction of wheat flour by passing it as a slurry through a 325-mesh sieve. Chemical analyses of the purified tailings indicated that they were low in starch and nitrogen and rich in pentosans. With a finer mesh screen (400-mesh) more of the impurities were removed. Jelaca and Hlynka (2) utilized a similar technique. After thorough washing of the tailings with distilled water over a 400-mesh stainless-steel sieve, these workers reacted the material remaining on the sieve with amyloglucosidase.

Various means of enzymatic treatment have been employed to purify the tailings fraction including that of Upton and Hester (3). Montgomery and Smith (4) found that treatment of the water-insoluble squeegee fraction of flour with pancreatin, followed by acetylation and fractionation of the acetylated polysaccharide by extraction with acetone, gave a pentosan which was soluble in water. Likewise Simpson (5) has utilized pancreatin for purification of pentosans.

A different method was employed by Cole (6) to isolate the pentosans of the squeegee component. This worker used sodium hydroxide to extract the pentosans followed by ethanol precipitation.

This study describes a method to fractionate the water-insoluble pentosans associated with the sludge fraction of wheat flour into four components.

MATERIAL AND METHODS

Flour Samples

The flour samples used for isolation of the pentosans from the sludge fraction of wheat flour included eight hard red spring wheat varieties: three conventional height samples, Chris, Waldron, and Justin, and five semidwarf samples, World Seeds 1809, Fletcher, Era, World Seeds 1812, and Red River 68.

Water-Insoluble Pentosan Isolation

Crude sludge from each flour was obtained by thorough washing of a dough ball in distilled water, followed by centrifugation of the washings. The sludge was located as a distinct layer above the prime starch in the centrifuge cup.

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Figure 1 shows the scheme used for the isolation of the pentosans from the sludge fraction. Four pentosan-containing fractions were obtained.

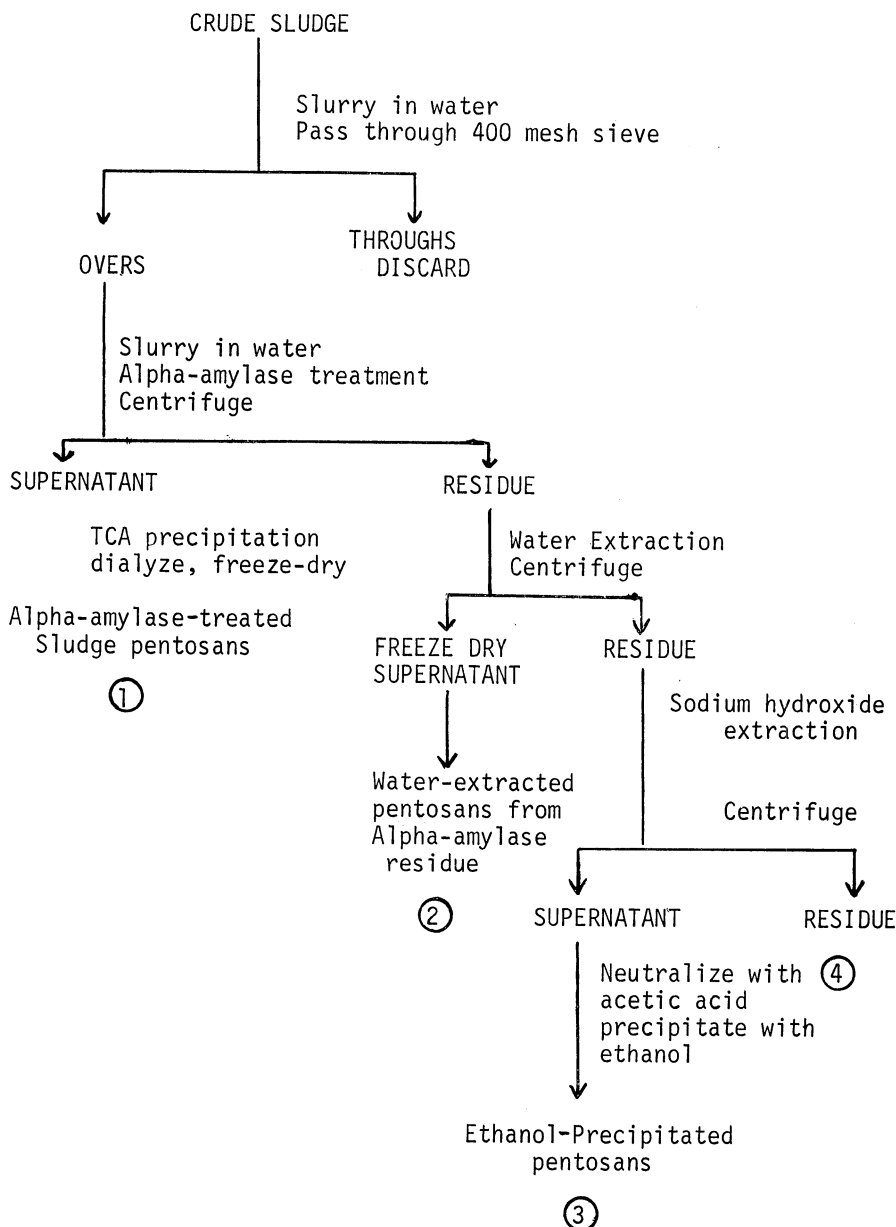


Fig. 1. Schematic diagram for the isolation of pentosans associated with the sludge fraction of wheat flour.

A thin slurry was made with water and a certain amount of sludge from each flour sample. The slurry was passed through a 400-mesh stainless-steel sieve, with the residue remaining on top of the sieve being washed thoroughly with distilled water, removed, and freeze-dried.

Six hundred milligrams of the freeze-dried residue (purified sludge) remaining on top of the 400-mesh stainless-steel sieve was subjected to α -amylase treatment with crystalline α -amylase from hog pancreas (Nutritional Biochemical Corp., Cleveland, Ohio). The purified sludge was slurried in 30 ml distilled water and 30 ml 0.02*M* phosphate buffer, pH 7.2, containing 0.04*N* sodium chloride. Enzyme (8.5 mg, which represented 5,000 units where 1 unit frees 1 μ mole of reducing groups from soluble starch at 25°C) was added and the sludge suspension dialyzed against a 1:1 dilution of the buffer for 3 days. A few drops of chloroform was added to inhibit microbial growth. The dialyzing buffer was changed twice daily. After 3 days the material in the dialysis bags was removed and centrifuged. The residue obtained was saved for further pentosan isolation. To the supernatant 15 ml 30% trichloroacetic acid was added. This was dialyzed against distilled water for 2 days followed by centrifugation, filtration of the supernatant through Whatman No. 4 filter paper, and freeze drying. The freeze-dried product represented the α -amylase-treated sludge pentosans ① shown in Fig. 1.

The residue was reextracted by stirring with distilled water, followed by centrifugation. The residue obtained in this instance likewise was saved for further pentosan isolation. The supernatant was freeze-dried and represented the second sludge pentosan fraction designated as water-extracted pentosans from α -amylase residue ②.

The residue obtained after water extraction was then extracted with 50 ml 0.5*N* sodium hydroxide with stirring under nitrogen for 1 hr. Centrifugation followed, with the residue once again being retained. The supernatant was adjusted to pH 7 with glacial acetic acid followed by precipitation with absolute ethanol supernatant:ethanol (1:4). The precipitate obtained was collected, dissolved in distilled water, dialyzed, and freeze-dried. This represented the ethanol-precipitated pentosan fraction ③ shown in Fig. 1.

The residue recovered after the sodium hydroxide extraction was dialyzed for 2 days against distilled water and then freeze-dried, and is indicated as residue ④ in Fig. 1.

Analysis of Sludge Pentosan Fractions

The four sludge pentosan fractions were analyzed for component sugars by hydrolyzing a small portion of each in 1*N* H₂SO₄ followed by paper chromatographic examination (7).

The α -amylase-treated sludge pentosan fraction ① and the ethanol-precipitated pentosan fraction ③ from the different flours were investigated further by DEAE-cellulose column chromatography (8). The ratio of component sugars in DEAE-cellulose pentosan fraction I was determined by gas chromatography (9) and the intrinsic viscosity in this fraction was determined in 0.05 *N* sodium hydroxide solution at 25°C with a Ubbelohde viscometer.

TABLE I
Recovery of Pentosan Components from Sludge Fraction after
Purification Through 400-Mesh Sieve^a

Sample	α - Amylase- Treated Pentosans	Water-Extracted Pentosans of α - Amylase Residue	Ethanol- Precipitated after NaOH Extraction of Residue	Residue after Sodium Hydroxide Extraction
	%	%	%	%
Chris	24.5	13.2	19.2	9.1
Justin	24.3	5.8	19.7	6.3
Waldron	28.0	6.5	17.7	8.2
World Seeds 1809	34.8	5.0	15.3	10.4
Fletcher	32.5	5.0	12.5	8.1
Era	30.3	8.3	17.5	5.8
World Seeds 1812	21.7	7.7	9.5	7.7
Red River 68	19.5	7.3	17.8	9.3

^aExpressed on a dry basis.

RESULTS AND DISCUSSION

A procedure has been described to fractionate the pentosans associated with the "sludge," "tailings," or "squeegee" fraction of wheat flour into four fractions.

Table I shows the recovery of pentosan components from the sludge fraction after purification through a 400-mesh sieve. The values given are based on the material remaining on top of the 400-mesh sieve. The fraction recovered in highest yield was that obtained after α -amylase treatment. The second highest-yielding fraction was that obtained by ethanol precipitation after sodium hydroxide extraction of the residue. Two of the semidwarf wheat varieties, World Seeds 1812 and Red River 68, showed the lowest recoverable yield of the α -amylase-treated pentosans.

The amylase-treated pentosans revealed the presence of arabinose and xylose as principal component sugars whereas the ethanol-precipitated pentosan fraction revealed the presence of glucose as well as arabinose and xylose.

Pentosan fractions ② and ④ (Fig. 1) revealed only arabinose and xylose as component sugars.

The protein content for pentosan fractions ①, ②, and ③, as illustrated in Fig. 1, for all of the flours investigated ranged between 4.8 and 9.6%.

DEAE-cellulose column chromatography of the α -amylase-treated sludge pentosan fraction and the ethanol-precipitated pentosan fraction revealed the highest yields for fractions ① and ② combined.

Table II shows the ratio of component sugars and intrinsic viscosity values for DEAE-cellulose pentosan fraction I, for the amylase-treated sludge pentosan fraction ①, and the ethanol-precipitated sludge pentosan fraction ③ for four of the flour samples. Fraction I was essentially a pure arabinoxylan. As noted in this table the ratio of component sugars was less for the ethanol-precipitated pentosans. A lower ratio would indicate a higher degree of branching. With the exception of Red River 68, the intrinsic viscosity values for the ethanol-

TABLE II
Data on DEAE-Cellulose Pentosan Fraction I

Sample	Amylase-Treated Sludge Pentosans		Ethanol-Precipitated Pentosans	
	Ratio ARAB:XYL	$[\eta]$	Ratio ARAB:XYL	$[\eta]$
Chris	1:1.53	2.2	1:1.38	3.5
Waldron	1:1.70	1.9	1:1.50	3.9
World Seeds 1812	1:1.80	1.9	1:1.44	4.2
Red River 68	1:1.61	3.6	1:1.31	3.0

precipitated pentosans were higher. Complete results on the comparison of starch, pentosans, and sugars of the flours used in this study have been reported elsewhere (7).

This study has indicated that different pentosan fractions could be obtained from crude sludge using various methods of treatment.

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