Note on the Effect of Purification Treatment on Water-Soluble Pentosans[1]

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This paper reports on the chemical composition and properties of water-soluble pentosans isolated from durum wheat semolina and purified according to the techniques described by Simpson (1), Kündig et al. (2), and D'Appolonia and Gilles (3).

Simpson (1) proposed the use of pancreatin to remove soluble starch from water-soluble extracts. This enzyme was chosen because it contained both amylolytic and proteolytic activities. Although pancreatin has been used mainly in the characterization of water-insoluble pentosans (4–7), it has also been used by

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Medcalf et al. (8) in their study to compare water-soluble pentosans from durum and hard red spring wheats.

Kündig et al. (2) suggested the use of crystalline α-amylase to remove soluble starch. Purification of pentosans using crystalline α-amylase has been performed by Kündig et al. (2), Lin and Pomeranz (9), Medcalf et al. (8), and D'Appolonia et al. (10) in extensive studies on the characterization of water-soluble pentosans.

More recently, D'Appolonia and Gilles (3) have used a modification of Simpson's pancreatin method to study flour pentosans associated with the gluten fraction of wheat flour.

MATERIALS AND METHODS

Semolina Sample

The semolina used for the present investigation was milled on a Buhler mill from a pure cultivar (Leeds) of durum wheat grown during the 1970 crop year.

Pentosan Isolation, Purification, and Fractionation

The water-soluble pentosans were isolated as suggested by Medcalf et al. (8), and purified by three different procedures. These included Simpson's (1) pancreatin treatment, Kündig's (2) α-amylase method, and the modified-pancreatin treatment of D'Appolonia and Gilles (3). Simpson's pancreatin treatment differs from the modification made by D'Appolonia and Gilles in that the former treatment requires acidification of the ethanolic extract with concentrated HCl, whereas the latter does not. To obtain additional information regarding the effect of both pancreatin treatments on pentosan purification, the supernatant, obtained after ethanol precipitation of the pentosans, was evaporated to near dryness, dissolved in distilled water, and freeze-dried. This material, called "ethanol supernatant", was further analyzed for constituent sugars.

The purified water-soluble pentosans obtained by the three different procedures were fractionated into five fractions by stepwise elution from a column of DEAE-cellulose (2). The carbohydrate content of each tube was evaluated by the phenol-sulfuric acid procedure (11). Tubes corresponding to each carbohydrate peak were combined, dialyzed, and freeze-dried. The protein content of each fraction was estimated by the Lowry method (12).

Paper chromatography and the ratio of component sugars were performed according to D'Appolonia and Gilles (3). The quantitative determination of the alditol acetate derivatives of the monosaccharides was performed according to the method of Sawardeker et al. (13).

RESULTS AND DISCUSSION

Figure 1 shows a comparison of the chromatographic separation of hydrolyzed water-soluble pentosans purified by the modified-pancreatin, pancreatin, and α-amylase treatments, respectively. In the pentosans purified with pancreatin, galactose was not present. The modified-pancreatin treated pentosans showed a pattern analogous to that of the α-amylase purified pentosans. The absence of galactose in the pancreatin-treated material was confirmed by gas-chromatographic analysis of the alditol acetate derivatives of the unfractionated material.

To investigate further the effect of the enzymatic treatment, the water-soluble pentosans were fractionated on a DEAE-cellulose column. Table I shows the yield, protein content, and ratio of component sugars for the pentosans obtained by the

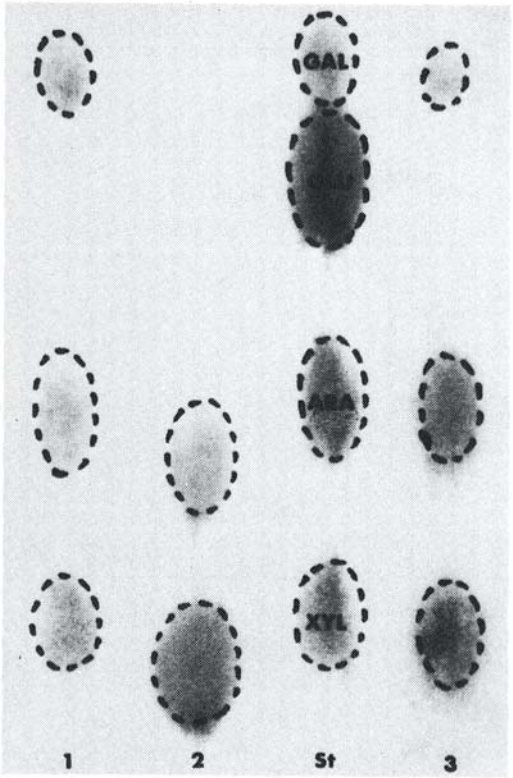

Fig. 1. Paper chromatogram showing sugars present in the hydrolyzed unfractionated water-soluble pentosans purified with 1) α-amylase; 2) pancreatin; and 3) modified-pancreatin.

three purification procedures. As can be noted, the pancreatin treatment was the most effective in removing protein. The absence of galactose is particularly noticeable in fraction III and only small amounts in fraction IV of the pancreatin-treated sample. These fractions, it should be noted, are principally arabinoxylans.

The ratios of component sugars are similar for the pentosans purified with α-amylase and the modified-pancreatin methods. In the unfractionated material, the pancreatin-treated pentosans showed a reduction in the amount of arabinose present, besides the already mentioned loss of galactose. These differences in the ratios of the component sugars may be responsible for the differences in yield of the different DEAE-cellulose fractions, and in particular of fraction IV. The low yield of fraction IV for the pancreatin-treated material can be explained as due to the loss of galactose, since this sugar is present in high amounts in the same fraction for the pentosans treated by the other two methods.

With respect to the galactose and arabinose losses, Perlin (14) has indicated the acid-labile nature of arabinofuranose branches in the pentosan molecule. Also, Kündig et al. (2) found that mild acid hydrolysis (1N HCl, 20°C., 72 hr.) affected the composition of their DEAE-cellulose fractions I and II. They showed that

TABLE I. EFFECT OF PURIFICATION TREATMENT ON WATER-SOLUBLE PENTOSANS[a]

Enzymatic Treatment	Fraction	Yield %	Protein %	Ratio Arabinose:Xylose: Galactose
α-Amylase (Kundig) (2)	Unfract.	...	17.4	1:1.05:0.53
	I	43.9	2.7	1:1.60: ...
	II	14.1	2.2	1:1.31: ...
	III	6.0	8.6	1:0.29:1.05
	IV	26.7	24.0	1:0.13:1.52
	V	9.3	33.7	1:1.78:0.04
Pancreatin (Simpson) (1)	Unfract.	...	1.5	1:1.63:trace
	I	50.9	0.7	1:1.67: ...
	II	22.3	1.3	1:1.56: ...
	III	8.8	2.2	1:1.42: ...
	IV	8.8	2.6	1:1.41:0.21
	V	9.1	1.7	1:1.80: ...
Modified-pancreatin (D'Appolonia) (3)	Unfract.	...	9.6	1:1.04:0.45
	I	45.7	0.9	1:1.62: ...
	II	14.9	2.1	1:1.32:0.15
	III + IV[b]	17.8	7.3	1:0.30:1.09
	IV	14.0	13.6	1:0.16:1.08

[a]Fractionation of pentosans performed on DEAE-cellulose 23.

[b]Fraction III contains a portion of fraction IV due to an error in removal of the fraction from the dialysis bags.

TABLE II. GAS-CHROMATOGRAPHIC QUANTITATIVE DETERMINATION OF ALDITOL ACETATES IN ETHANOL SUPERNATANT FROM PANCREATIN TREATMENT

Alditol	Unhydrolyzed mg./100 g.	Hydrolyzed mg./100 g.
Arabitol	3.44	4.62
Xylitol	0.71	0.73
Galactitol	3.18	4.30
Glucitol	1.93	2.55

during hydrolysis arabinose was cleaved from fraction I, and arabinose and galactose from fraction II (fraction I was a pure arabinoxylan).

The acidification step in Simpson's pancreatin treatment may result in the hydrolytic cleavage of arabinogalactan chains or cleavage of arabinose from the main xylan chain which results in reduction in the arabinose and galactose contents of the pentosans. Hydrolytic cleavage does not occur in the D'Appolonia and Gilles modification, and consequently no loss of either sugar can be observed in the purified sample.

Figure 2 shows a paper chromatogram of the hydrolyzed ethanol supernatant (1N H_2SO_4, 100°C., 5 hr.) (3) from the pancreatin and modified-pancreatin treatments. It can be observed from this figure that the supernatant of the pancreatin-treated material contains galactose, arabinose, and glucose, whereas the

supernatant of the modified treatment contains only glucose. This experiment provides further evidence of the fact that the modified-pancreatin treatment does remove starch from the water-soluble pentosan extracts without affecting the integrity of the pentosan molecule.

Quantitative gas-chromatography was used to investigate the products of the acid hydrolysis following Simpson's pancreatin treatment. The quantitative composition of the ethanol supernatant from the pancreatin-treated sample before and after hydrolysis (3) is shown in Table II. From these values and from the quantitative differences arising following hydrolysis, it can be concluded that the products of the hydrolytic cleavage following Simpson's method consist of free sugars as well as arabinogalactans and starch. The amount of xylose present was not changed following sulfuric acid hydrolysis.

The results of this investigation indicate that the modified-pancreatin method preserves the molecular structure of the purified pentosan, whereas the Simpson method causes considerable hydrolysis of arabinose and galactose from the pentosan chain.

The Simpson method was the most effective in removing protein material, as is shown by the data in Table I for the unfractionated pentosan preparations;

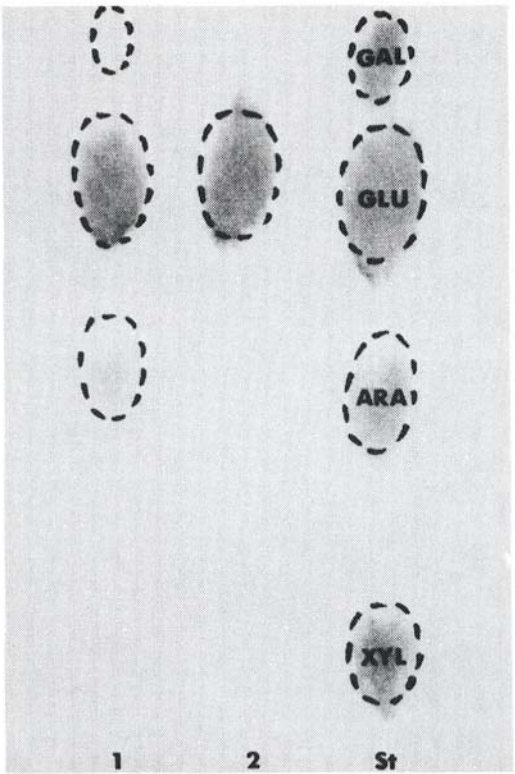

Fig. 2. Paper chromatogram showing the sugars present in the hydrolyzed ethanol supernatant from 1) pancreatin; and 2) modified-pancreatin treatments.

however, some destruction of the pentosan moiety resulted. The other two procedures gave unfractionated pentosan preparations of higher protein content without disrupting the pentosan structure.

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