

# Effects of Low-Level Gamma Radiation on Water-Soluble Nonstarchy Polysaccharides Isolated from Hard Red Spring Wheat Flour and Bran<sup>1</sup>

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Water-soluble pentosans, a small percentage of wheat carbohydrates, are involved in the gas-retaining properties of dough and have been shown to have a beneficial effect on loaf volume (D'Appolonia 1971). Milner (1961) and MacArthur and D'Appolonia (1983) reported detrimental effects on baking quality of bread as radiation levels increased. A closer look at the water-soluble nonstarchy polysaccharides of gamma-irradiated wheat was in order, since low levels of irradiation (100–500 Krad) have been used to free wheat and other grains from insect infestation (Hassett and Jenkins 1952, Cornwell 1966).

## MATERIALS AND METHODS

### Samples

Three cultivars of hard red spring wheat (Olaf, Waldron, and an experimental line) were used. The cultivars were chosen according to their strong (A), intermediate (B), and weak (C) mixing characteristics. Irradiation was accomplished using gamma rays emitted from a cobalt-60 source at a rate of 9,891 rads/min. The time of exposure was calculated to equal a radiation level of 300 Krads. After irradiation, the wheat was stored at 5°C for 1 week. The samples were milled on a Buhler experimental mill to a 72.0% extraction.

### Isolation and Purification of Water-Soluble Nonstarchy Polysaccharides

Crude pentosans were isolated from flour according to the procedure of D'Appolonia (1973); pentosans were isolated from the bran samples using the dilute alkaline extraction method of D'Appolonia and MacArthur (1976). The crude pentosans were treated with hog pancreas  $\alpha$ -amylase, 2 x crystallized (Nutritional Biochemical Corp., Cleveland, OH) to remove soluble starch according to Kündig et al (1961). Crude pentosan (2 g) was treated with  $\alpha$ -amylase, (1,260 units/mg) and dialyzed against phosphate buffer (pH 7.2) for three days at ambient temperature. The buffer was changed twice daily. After treatment, the enzyme was inactivated by the procedure of Fincher and Stone (1974), with the following modifications. The solution was heated to 94°C, held at this temperature for 4 min, cooled, and centrifuged at 10,000  $\times$  g for 5 min. The supernatant, free from enzyme, was dialyzed at 5°C against distilled water for three days. The water was changed twice daily. Following dialysis, the supernatant was shell-frozen and freeze-dried.

### Diethylaminoethyl-Cellulose Column Chromatography

The purified pentosans were fractionated by diethylaminoethyl (DEAE)-cellulose chromatography in the borate form. A column

(2.4  $\times$  30 cm) was prepared according to the procedure of Neukom and Kündig (1965). The DEAE-cellulose (Whatman DE23) had an exchange capacity of 1.0 meq/g. The sample (300 mg) was dissolved in a small amount of distilled water and applied to the top of the column. After the sample penetrated into the DEAE-cellulose, elution was accomplished with the following solvents: 1) distilled water, 2) 0.0025M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 3) 0.025M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 4) 0.250M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, and 5) 0.4N NaOH.

### Sugar Composition of DEAE-Cellulose Fractions

A portion (3–5 mg) of each DEAE-cellulose pentosan fraction obtained from the control and the irradiated flour and bran samples was hydrolyzed with 5 ml of 1N H<sub>2</sub>SO<sub>4</sub> for 5 hr at 100°C, followed by neutralization with barium carbonate. The component sugars were identified by paper chromatography, and the relative percentage of each was determined by gas-liquid chromatography (Medcalf et al 1968).

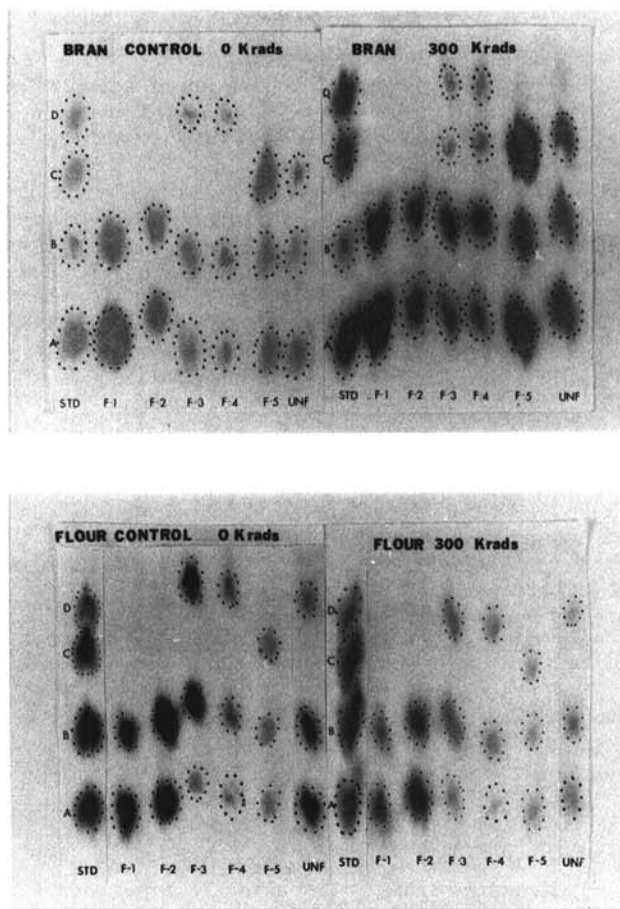


Fig. 1. Paper chromatograms of component sugars in hydrolyzed unfractionated and Diethylaminoethyl-cellulose fractionated (F1–5) water-soluble nonstarchy polysaccharides of control and irradiated flour and bran of sample C. A = arabinose, B = xylose, C = glucose, D = galactose.

<sup>1</sup>Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or products not mentioned.

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**TABLE I**  
**Intrinsic Viscosity of Diethylaminoethyl-Cellulose**  
**Chromatography Fractions 1 and 2 from Control**  
**and Irradiated Flours and Brans<sup>a</sup>**

Sample	Radiation Level (Krad)	Fraction	
		1 ( $\eta$ )	2 ( $\eta$ )
Flour			
A	0 (control)	5.33	5.05
	300	5.70	5.53
B	0 (control)	3.12	1.51
	300	3.35	2.97
C	0 (control)	2.62	3.10
	300	3.37	3.75
Bran			
A	0 (control)	2.85	1.94
	300	3.64	2.60
B	0 (control)	1.48	1.15
	300	3.73	1.33
C	0 (control)	2.97	1.80
	300	3.66	2.38

<sup>a</sup> Average of two determinations.

### Intrinsic Viscosity

The intrinsic viscosity of the DEAE-cellulose chromatography fractions 1 and 2 from each sample was measured after dissolving a portion (0.4–0.6 g) in 2 ml 0.5N NaOH and determined using a constant temperature oil bath at 25°C. An Ubbelohde (Cannon Fenske) viscometer, capillary size 75, equipped with an automatic viscosity timer (Jupiter Instrument Co., Jupiter, FL) was used.

## RESULTS AND DISCUSSION

The amounts of crude pentosans recovered from the flour were 0.95–1.21% (mean, 1.06%) and 1.03–1.39% (mean, 1.18%), respectively, for the control and irradiated samples. The corresponding amounts recovered from bran were 9.7–10.4% (mean, 10.0%) and 12.2–13.0% (mean, 12.6%). After treatment with amylase, the average amounts of pentosans were 0.63 and 0.71% for the flour and 5.0 and 6.5% for the bran, from control and irradiated samples, respectively. Finney et al (1960) also reported an increase in water-soluble pentosans in samples subjected to high radiation levels.

Paper chromatograms showing the four component sugars in the DEAE-cellulose chromatography fractions of sample C (flour and bran) are shown in Figure 1. The other two samples, A and B, were essentially the same as sample C. Fractions 1 and 2 of both the flour and bran were essentially pure arabinoxylans. No glucose was found in the unfractionated material from the flour samples; therefore, the glucose in DEAE fraction 5 probably was from the DEAE-cellulose as a result of elution with 0.4N NaOH (D'Appolonia 1973). The high levels of glucose in bran fraction 5 represented the glucose eluted from the DEAE-cellulose and that already present in the bran.

For the flour samples, the amounts of arabinose and xylose increased, whereas the amount of galactose in each sample

decreased. No consistent trends were evident for the bran samples.

Table I shows the intrinsic viscosities of DEAE-cellulose chromatography fractions 1 and 2 from the control and irradiated flour and bran samples, respectively. For all samples, intrinsic viscosity increased with radiation. One of the unique properties of water-soluble pentosans is their ability to form a viscous gel, largely because they are highly branched polymers. Higher intrinsic viscosities, such as those obtained for the irradiated samples, generally indicate a higher degree of branching, where more L-arabinose units are present on the D-xylose chain. Gamma rays, like X-rays, can hydrolyze chemical bonds, thereby cleaving molecules into smaller fragments. Therefore, one would expect a lower intrinsic viscosity as a result of irradiation. However, the effect of radiation in this study apparently changed the number or sequence of branching within the pentosan molecule, creating a more viscous gel. Perhaps the level of irradiation was not high enough to break the chemical bonds leading to reduced viscosity but was high enough to alter the structure. The bran pentosans appeared to be more linear, with less arabinose side chains present.

In summary, the results of this study suggest that low-dosage irradiation (300 Krad) does not impair the water-soluble pentosans.

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