

# Effect of Gamma-Radiation on a Water-Insoluble Arabinoxylan

ANNA EBRINGEROVÁ, JOZEF PRUŽINEC,\* MARTA KAČURÁKOVÁ, and ZDENKA HROMÁDKOVÁ, *Institute of Chemistry of the Centre of Chemical Investigation, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia*

## Synopsis

The effect of gamma radiation on a water-insoluble L-arabino-D-xylan has been studied by functional group and structural analysis using chemical methods and IR spectroscopy, by sugar composition analysis, and by viscosity measurements. The gamma irradiation has been shown to modify both sugar components of the arabinoxylan, accompanied by cleavage of the glycosidic linkages, depolymerization, and sugar decomposition, depending on the applied radiation dose, thus increasing the molecular and chemical heterogeneity of the polysaccharide.

## INTRODUCTION

Arabinoxylans are the main type of hemicelluloses of all species of grasses.<sup>1</sup> Due to their relatively easy and simple isolation from the plant material using alkaline extraction, they represent a potential source of biopolymers for industrial application. In order to characterize the molecular properties of the arabinoxylans, a series of chemically well-defined arabinoxylan fractions with a narrow range of DP is needed, which requires a suitable degradation method for depolymerizing a high-molecular arabinoxylan. However, the commonly used acidic hydrolytic treatments are not favorable, because of the preferential cleavage of the arabinosidic linkages. High-energy treatment, which was found to be very effective not only in cellulose and starch depolymerisation,<sup>2-6</sup> but in hemicellulose degradation, too,<sup>7</sup> may be an alternative method for depolymerizing the arabinoxylan. So far, no references concerning the radiolysis of isolated arabinoxylans had appeared.

The objective of the present work was to study the molecular and chemical changes of a water-insoluble arabinoxylan after its gamma irradiation in solid state.

## EXPERIMENTAL

**Materials.** The starting arabinoxylan was isolated from rye bran holocellulose by an alkaline fractional extraction procedure<sup>8</sup> and used in the freeze-dried form. The polysaccharide with the intrinsic viscosity  $[\eta] = 197 \text{ cm}^3 \text{ g}^{-1}$ , contained in dry matter D-xylose (69.4%), L-arabinose (20.2%), D-glucose (6.8%), D-galactose (1.6%), and D-glucuronic acid (< 1%).

\*Slovak Technical University, Faculty of Chemical Technology, 812 37 Bratislava, Czechoslovakia.

*Procedure:* Irradiation was performed on laboratory equipment RCH gama 30 (USSR);  $^{60}\text{Co}$  was used as the source and its activity was measured by Fricke dosimetry.<sup>8</sup> Air-dry samples (1 g) in glass vials (Unihost, CSSR) were irradiated at room temperature in air atmosphere.<sup>8</sup>

Hydrolysis of the irradiated samples and their fractions were performed in 2M trifluoroacetic acid at 100°C for 2 h and the sugar composition of the hydrolysates was determined by gas-liquid chromatography of the alditol trifluoro acetates.<sup>9</sup> The carboxyl groups were determined alkalimetrically by potentiometric titration; the "apparent aldehyde group" content was estimated by hypiodite oxidation.<sup>10</sup> Endiol groups were estimated by iodine consumption in acidic medium, the hydroperoxide groups iodometrically using ammonium molybdate as catalyst.<sup>11</sup> Reduction of the irradiated samples was performed with 0.1M sodium borohydride at room temperature for 16 h. After acidification with acetic acid the insoluble part was filtered and washed with water, methanol, and acetone.

The IR spectra were measured in KBr pellets and in Fluorolube mull on KBr discs and recorded with a Perkin-Elmer G983 spectrophotometer using DS 3700. The intrinsic viscosity  $[\eta]$  was measured in dry DMSO at  $21 \pm 0.1^\circ\text{C}$  using an modified Ubbelohde viscosimeter. The UV spectra were recorded with an Unicam-SP 1700 ultraviolet spectrophotometer.

**Fractionation of the Irradiated Arabinoxylan.** The sample (0.5 g) was stirred in 50 mL of redistilled water at ambient temperature for 2 h. The insoluble part (WISP) was separated by centrifugation and, after washing with ethanol and acetone, dried over  $\text{P}_2\text{O}_5$ . The water-soluble polysaccharide fraction (WSP) was obtained by precipitation of the supernatant with ethanol (1:3, v/v) and after filtration dried as mentioned above. The filtrate yielded a low-molecular fraction (LMP) by evaporation in vacuum.

## RESULTS AND DISCUSSION

A water-insoluble L-arabino-D-xylan with a high  $\text{DP}^{9,12}$  was the object of the radiation-effect study. The backbone of the polysaccharide is built up by (1  $\rightarrow$  4)- $\beta$ -linked D-xylopyranosyl residues and  $\alpha$ -L-arabinofuranosyl units are attached as single side chains on 0-3 positions of the xylose residues. The polysaccharide was contaminated with a small amount of hexosans. As shown in Table I, the main effects of the gamma-radiation treatment of the arabinoxylan are depolymerization and degradation. This follows from the deca-

TABLE I  
Solubility and Viscosity Changes of the Arabinoxylan  
Depending on the Gamma-Radiation Dose

Dose (kGy)	Water extract (% of irradiated arabinoxylan)	Intrinsic viscosity $[\eta]$ ( $\text{cm}^3 \text{g}^{-1}$ )
0	0	197
90	27.4	77
180	35.2	64
270	45.7	51

TABLE II  
Chemical Analysis of Functional Groups of the Irradiated Arabinoxylan

Dose (kGy)	Carboxyl <sup>a,c</sup> group (%)	Hydroperoxide group (mmol g <sup>-1</sup> × 10 <sup>2</sup> )		"Apparent aldehyde group" <sup>a</sup> (%)	Endiol group <sup>b</sup> (mmol g <sup>-1</sup> × 10 <sup>2</sup> )
		a	b		
0	0.12	0	0	0.05	0
90	0.46	3.2	2.0	0.84	1.3
180	0.65	4.2	2.8	1.13	1.6
270	0.95	15.9	4.4	1.28	2.6

<sup>a</sup> Before drying.

<sup>b</sup> After drying at 105°C.

<sup>c</sup> Determined in aqueous suspension (0.5 g in 50 cm<sup>3</sup>).

ing intrinsic viscosity  $[\eta]$ , which is a measure of the relative molecular mass of the polysaccharide, and so from the increasing water solubility. The course of the viscosity decrease was similar to that observed with cellulose,<sup>3</sup> starch,<sup>6</sup> and glucuronoxyylan.<sup>7</sup> After an initial high viscosity drop a leveling-off effect was noted at higher radiation doses.

The various functional groups, which were found in the irradiated arabinoxylan, are presented in Table II. As expected with regard to the limited amount of oxygen and moisture in the sample under radiation treatment, hydroperoxide groups were formed with increasing radiation dose, but not in a direct proportion. Their content is lowered by subsequent thermal treatment of the irradiated sample. The titratable acidity of the aqueous suspensions of the irradiated arabinoxylan originates mainly from acidic groups, formed by various reactions of the carboxy or carboxyalkoxy radicals<sup>2</sup> and arising with increasing radiation dose. The "apparent aldehyded group" content was higher than both hydroperoxide and carboxyl group contents. Due to the progressive degradation reaction and acidic group formation, at the highest applied radiation dose the water solubility of the arabinoxylan reached a value near 50%. The WSP fraction was isolated from the water extract by ethanol precipitation, in amounts of 12.5–27%, related to the irradiated sample (Table III).  $[\eta]$  of the WSP fractions was about twice as low as that of the appropriate WISP fraction. Comparing both carboxyl group and "apparent aldehyde group" content of the irradiated arabinoxylan with those of both isolated polymeric fractions WSP and WISP (Tables II and III), it is evident that a substantial amount of these groups is present in the LMP fraction. Apart from a mixture of unidentified neutral and acidic compounds xylo-oligosaccharides were detected in LMP by means of p.c.<sup>9</sup> The UV spectrum of the aqueous solution of LMP (Fig. 1) displays an absorption band with  $\lambda_{\max} = 234$  nm and one with  $\lambda_{\max} = 270$  nm. After addition of alkali the second band was shifted to 280 nm. It is known that unsaturated compounds with extended conjugation systems (endiol, dienol, carbonyl groups) absorb<sup>2</sup> in the region of 230–300 nm and they were found in irradiated aqueous solutions of carbohydrates.<sup>2,13,14</sup> The presence of endiol structures in the irradiated

TABLE III  
Chemical and Molecular Properties of the WISP and WSP Fractions  
of the Irradiated Arabinoxylan

Dose (kGy)	Yield %	Intrinsic viscosity $[\eta]$ ( $\text{cm}^3 \text{g}^{-1}$ )	Carboxyl group (%)	"Apparent aldehyde group" (%)	Endiol group ( $\text{mmol g}^{-1} \times 10^2$ )
<i>WISP fraction</i>					
90	72.6	80	0.19	0.69	1.3
180	64.8	67	0.23	0.61	1.9
270	54.3	55	0.27	0.95	2.5
<i>WSP fraction</i>					
90	12.5	41	0.11	0.51	—
180	18.2	35	0.15	0.65	1.1
270	27.0	31	0.19	0.67	1.9

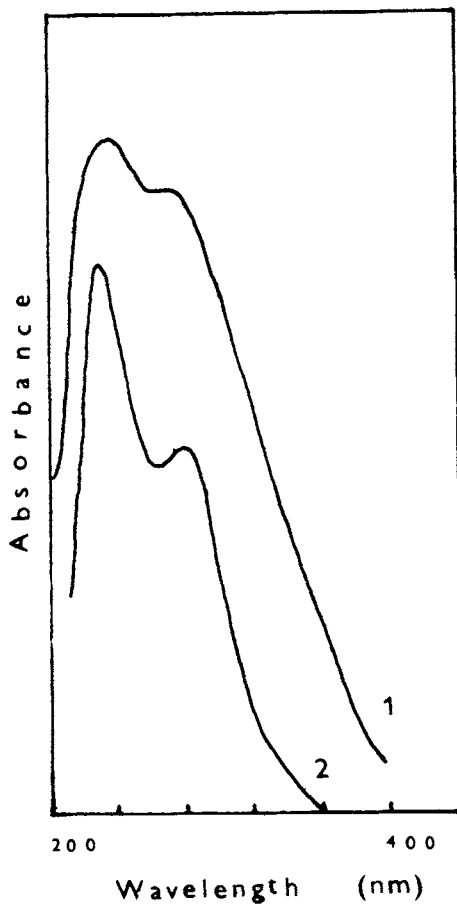


Fig. 1. UV spectra of the LMP obtained from arabinoxylan irradiated at (1) 90 and (2) 270 kGy.

arabinoxylan was supported by their ability to reduce iodine in acidic medium (Table II).

In addition to chemical analysis also IR spectral investigations were made to characterize the structural changes in the irradiated arabinoxylan. The IR spectra (in KBr) of the arabinoxylan irradiated at different radiation doses were similar and exhibit main changes in the region of 1500–1800  $\text{cm}^{-1}$ , where carbonyl group vibrations are located<sup>15</sup> (Fig. 2). The intensity of the characteristic peak of carbonyl and carboxyl groups  $\nu(\text{C}=\text{O}) \sim 1725 \text{ cm}^{-1}$  increased after irradiation. Furthermore, a less intense broad band appeared in the water absorption region at 1638  $\text{cm}^{-1}$  and a small one at 1540  $\text{cm}^{-1}$ . The enolic form of  $\text{C}=\text{O}$  and  $\text{C}=\text{C}$  conjugations shows a band between 1500 and 1600  $\text{cm}^{-1}$ , which includes the absorption of both functional groups.<sup>16</sup> More pronounced differences in the mentioned region were noted in the IR spectra of WSP and WISP in KBr (Fig. 3). The intensity of the absorption band at 1725  $\text{cm}^{-1}$  was lowered in WSP and in the insoluble residue after  $\text{NaBH}_4$  reduction, in which the band at 1540  $\text{cm}^{-1}$  disappeared. In the spectrum measured in Fluorolube (Fig. 4) the carbonyl peak appears as an unresolved doublet (1744 and 1723  $\text{cm}^{-1}$ ), belonging to carboxyl and carbonyl stretching vibrations  $\nu \text{C}=\text{O}$ , respectively. In addition, a band at 1695  $\text{cm}^{-1}$ , corresponding to  $\nu \text{C}=\text{O}$  of these groups in conjugation with  $\text{C}=\text{C}$  bonds appeared. Further noted absorption bands at 1669 and 1618  $\text{cm}^{-1}$  were unresolved in KBr (Fig. 3) and may originate from other types of unsaturated bonds.<sup>16</sup> After  $\text{NaBH}_4$  reduction the bands overlapped in the Fluorolube spectrum in an intense broad absorption peak in the range of 1675–1560  $\text{cm}^{-1}$  in the both polymeric fractions. The  $\nu \text{C}=\text{O}$  absorption in the WSP and WISP fractions is shifted to lower frequency (1714  $\text{cm}^{-1}$ ) in Fluorolube. The aldehyde and carboxyl group contents (Table II and III) of the polymeric fractions are lower than those of the original sample. The IR spectra showed a similar distribution pattern for the carbonyl group vibrations, what is demonstrated in Table IV by the intensity ratio of the peaks at 1725 and 2920  $\text{cm}^{-1}$  (from Figs. 2 and 3). The “apparent aldehyde group” content of the polymeric fraction (Table III) exceeds several times the theoretical value expected from the estimated  $[\eta]$ , if the Mark–Houwink constants for the 4-O-methylglucuronoxylan<sup>17</sup> are used in the  $\text{DP}_v$  calculation. The obtained results show that gamma radiation produces carbonyl groups of various types and localization sites in the arabinoxylan chain. According to the accepted, although not fully elucidated, degradation mechanism of cellulose and starch radiolysis,<sup>2,4,6,18</sup> it is possible to assume that also during gamma irradiation of arabinoxylan stable radicals are formed on the C1 and C4 positions of the glycosidic linkages, thus resulting in cleavage of the xylan chain and formation of new functional groups. Moreover, radicals are probably formed also on the glycosidic linkages of the arabinosyl side chains. However, other reactions like hydrolytic and oxidative cleavage of the glycosidic linkages are not excluded due to the present acidic and hydroperoxide groups.

Radical decomposition reactions comprising fragmentation, disproportionation, rearrangement, dehydration, etc.<sup>2,3</sup> produce carbonyl groups depending on the radiation conditions, which may be neighbors with deoxy or hydroxyl groups, thus forming enol, endiol, or unsaturated structures as the result of

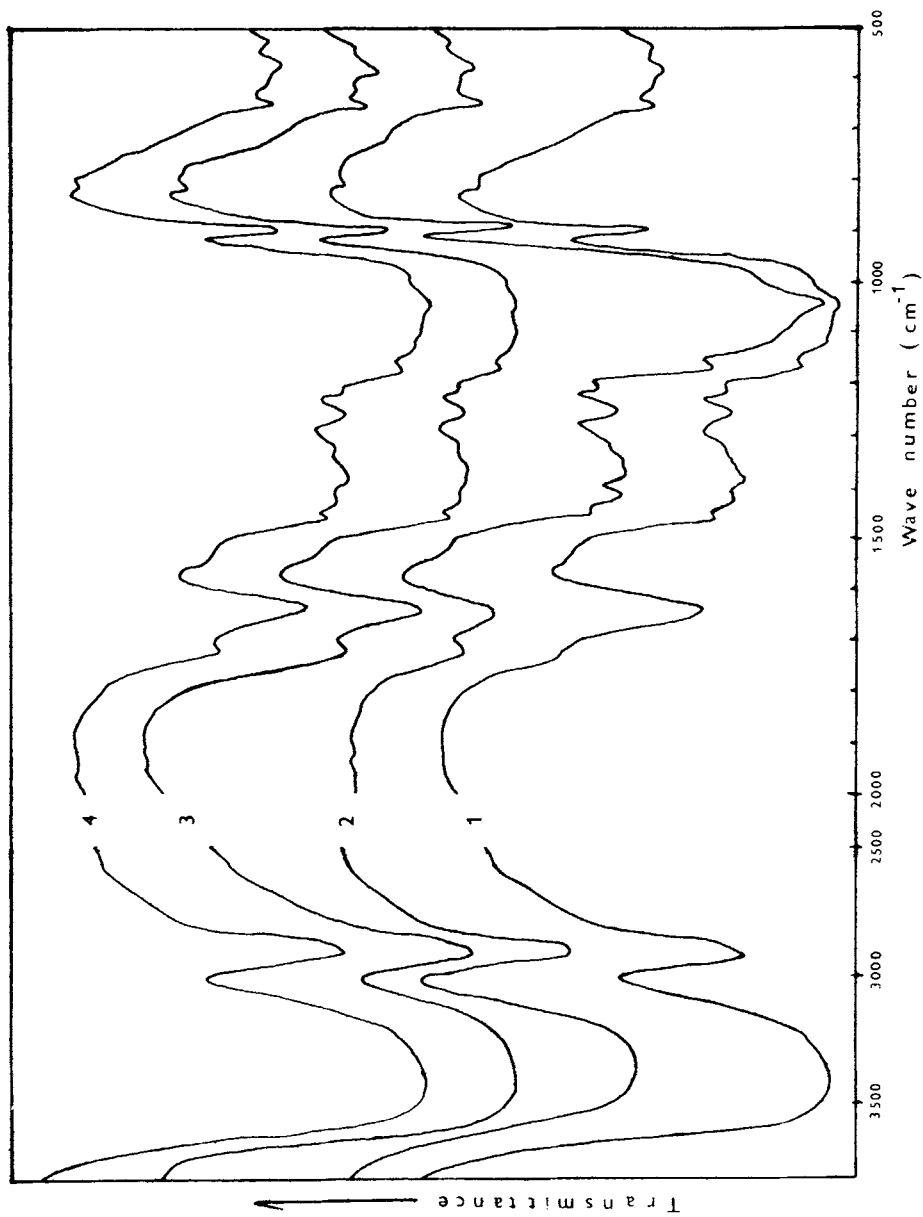


Fig. 2. IR spectra of the arabinoxylan irradiated at: (1) 0 kGy; (2) 90 kGy; (3) 180 kGy; (4) 270 kGy.

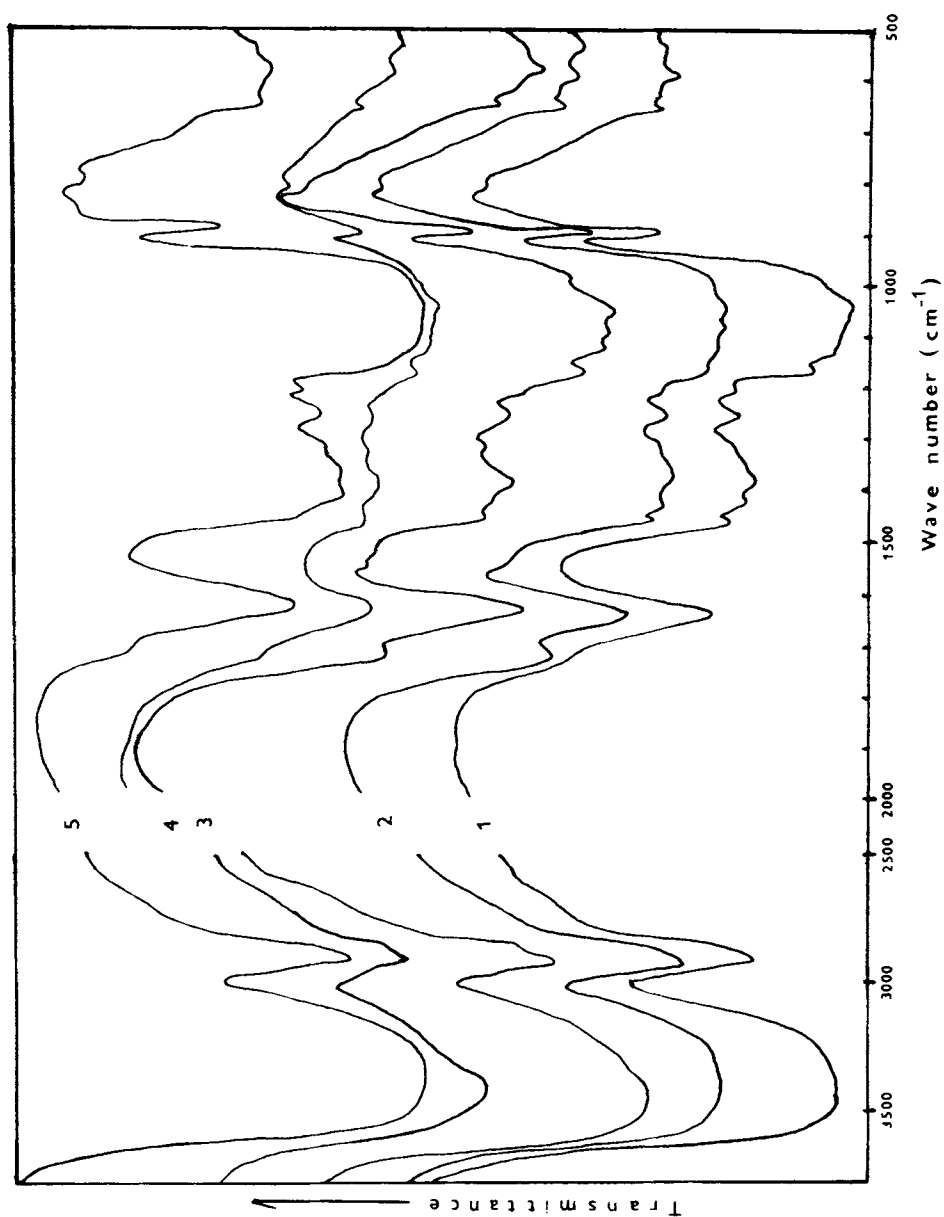


Fig. 3. IR spectra of the irradiated arabinoxylan and its polymeric fractions (in KBr): (1) arabinoxylan before irradiation; (2) arabinoxylan irradiated at 180 kGy (sample 2); (3) WISP of sample 2; (4) sample 2 after  $\text{NaBH}_4$  reduction; (5) WSP of sample 2.

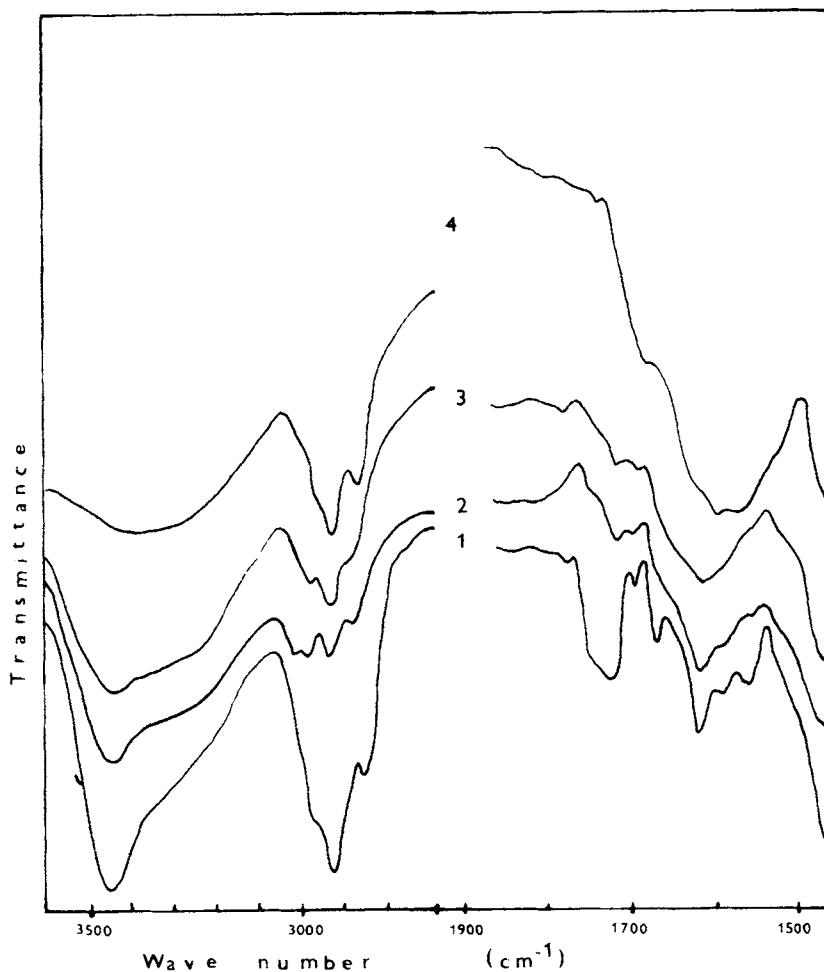


Fig. 4. IR spectra of the irradiated arabinoxylan (sample 2) and its polymeric fractions (in Fluorolube): (1) sample 2; (2) WISP of sample 2; (3) WSP of sample 2; (4) sample 2 after  $\text{NaBH}_4$  reduction.

TABLE IV  
Distribution of the Carboxyl Groups in Irradiated Arabinoxylan  
and Its WISP and WSP Fractions, Expressed as  $I_{1725}/I_{2920}$

Dose (kGy)	Arabinoxylan	WISP fraction		WSP fraction
		a	b	
90	0.52	0.42	0.38	0.15
180	0.78	0.73	0.58	0.38
270	0.80	0.68	0.29	0.30

<sup>a</sup> Determined before  $\text{NaBH}_4$  reduction.

<sup>b</sup> Determined after  $\text{NaBH}_4$  reduction.



TABLE V  
Molar Proportion of Neutral Sugar Components in the Hydrolysate  
of the Irradiated Arabinoxylan and Its WISP and WSP Fractions

Dose (kGy)	AR <sup>a</sup> (%)	Arabinose	Xylose	Glucose	Galactose
<i>Arabinoxylan</i>					
0	0	29	100	8	2
90	2.6	26	100	6	2
180	4.8	23	100	5	1
270	6.1	21	100	5	1
<i>WISP fraction</i>					
90	1.5	19	100	9	1
180	3.8	12	100	9	1
270	4.3	15	100	7	Trace
<i>WSP fraction</i>					
90	0.4	30	100	7	2
180	0.8	36	100	6	1
270	0.7	31	100	6	1

<sup>a</sup>AR = Acid resistant matter.

various consecutive reactions.<sup>19,20</sup> The absorption at 1691 and 1618  $\text{cm}^{-1}$ , detected in both polymeric fractions of the irradiated arabinoxylan (Fig. 4), can be ascribed to  $\nu$  C=O and  $\nu$  C=C vibrations of conjugated systems, which were found also after thermal treatment of xylans.<sup>21,22</sup> The highest intensity of this absorption band was observed in the WISP fraction and corresponds to the shape of the multiplet in the area of  $\nu$  CH vibrations in Fluorolube. The irradiated arabinoxylan, its insoluble residue after  $\text{NaBH}_4$  reduction, and the WSP fraction show absorption maxima at  $\nu_{as}$   $\text{CH}_3$  (2964  $\text{cm}^{-1}$ ),  $\nu_{as}$   $\text{CH}_2$  at 2926  $\text{cm}^{-1}$ , and  $\nu_s$   $\text{CH}_2$  at 2855  $\text{cm}^{-1}$  (Fig. 4). The main absorption peak at 2926  $\text{cm}^{-1}$  is less intense in WISP and a new band appears near 3010  $\text{cm}^{-1}$ , due to  $\nu$  =CH vibrations.<sup>16</sup>

In addition to the above-presented structural changes determined by functional group analysis, a decrease of the arabinosyl content of the unfractionated irradiated arabinoxylan with increasing radiation dose and, in particular, of that of its WISP fraction was observed (Table V). The WSP fractions exhibit, on the contrary, a somewhat higher arabinose to xylose ratio. The lowering of the arabinose content can be explained by a higher reactivity of the arabinosidic linkage in radiolytic scission in comparison to that of the xylosidic ones and/or by radiochemical modification of the arabinosyl unit, which is not cleaved off, but is destroyed under the acid hydrolytic treatment of sugar analysis. Furthermore, the formation of an acid-resistant part during polysaccharide hydrolysis with a tendency to higher values at higher radiation doses (Table V) was observed. This finding is in accordance with the increasing carbonyl and endiol group content of the polymeric fractions (Tables III and IV) as insoluble polymeric products had been found after acidic treatment of sugar lactones and endiolic structures,<sup>2</sup> too.

In view of the obtained results we assume that the formation of WISP and WSP fractions with a different arabinose to xylose ratio after irradiation of the water-insoluble arabinoxylan is due not only to the observed radiochemi-

cal modification of both sugar components, but may be also traced back to a nonuniform distribution of the arabinosyl side chains along the xylan backbone of the unirradiated polysaccharide.

### CONCLUSIONS

Gamma irradiation of the solid arabinoxylan in the presence of a limited amount of moisture and air produces a complex of various reactions resulting in changes of the sugar composition, formation of new functional groups, cleavage of glycosidic linkages and decomposition of the sugar units, decrease of the DP, and increase of the water solubility of the polysaccharide. The results of the investigations indicate a higher reactivity of the arabinosyl moieties in comparison with the xylosyl ones and, furthermore, a nonuniform distribution of the arabinosyl side chains on the xylan core of the nonirradiated polymer. It is possible to conclude that, in contrast to cellulose, both molecular and chemical heterogeneity of the arabinoxylan arose during the applied radiation conditions, the heteropolymeric character of the hemicellulose and the different glycosidic linkage types being the main reasons for the noted differences.

### References

1. K. C. B. Wilkie, *Adv. Carbohydr. Chem. Biochem.*, **36**, 215 (1979).
2. G. O. Phillips, *Adv. Carbohydr. Chem.*, **16**, 13 (1961).
3. B. G. Jerchow and A. S. Klimentow, *Usp. Khim.*, **53**, 2056 (1984).
4. Y. W. Han and A. Ciegler, *Annual Reports on Fermentation Processes*, Academic, New York, 1983, Vol. 6, p. 299.
5. J. Pružinec, Š. Varga, and J. Kadlečík, Research report, CHTF SVŠT, Bratislava, 1983.
6. J. Pružinec, O. Holá, and J. Kadlečík, Research report, CHTF SVŠT, Bratislava, 1986.
7. I. I. Beinart, T. N. Tochilovskaya, and E. L. Rajavets, *Khim. Drev.*, **6**, 39 (1978).
8. J. Pružinec, J. Kadlečík, Š. Varga, and F. Piovarníček, *Chem. Vlákna*, **32**, 267 (1982).
9. Z. Hromádková and A. Ebringerová, *Nahrung*, **31**, 149 (1987).
10. S. K. Chanda, E. L. Hirst, J. K. N. Jones, and E. G. V. Percival, *J. Chem. Soc.*, **1950**, 1289.
11. O. Tomiček, *Quantitative Analysis* (in Czech), SZN, Prague, 1954, p. 209.
12. Z. Hromádková, A. Ebringerová, E. Petráková, and J. Schraml, *Carbohydr. Res.*, **164**, 73 (1987).
13. T. Kudo and H. Heusinger, *Carbohydr. Res.*, **123**, 41 (1983).
14. H. Scherz, *Radiation Res.*, **43**, 12 (1970).
15. R. G. Zbankow, *Infrared Spectra of Cellulose and Its Derivatives* (in Russian), Nauka i tehnika, Minsk, p. 19.
16. M. Avram and G. Mattescu, *Infrared Spectroscopy*, Wiley-Interscience, New York, 1966, p. 380.
17. R. G. Lebel and D. A. I. Goring, *J. Polym. Sci. C*, **21**, 29 (1963).
18. B. G. Jerchow, *Radiation Technology and Feedstuff Production* (in Russian), Moscow, 1986, p. 19.
19. Y. Nakamura, Y. Ogiwara, and G. O. Phillips, *Polym. Photochem.*, **6**, 135 (1985).
20. C. von Sonntag, *Adv. Carbohydr. Chem. Biochem.*, **37**, 7 (1980).
21. K. Shimizu, F. Teratani, and K. Miyazaki, *J. Jpn. Wood Res. Soc.*, **15**, 114 (1969).
22. A. Ebringerová, *Cellulose Chem. Technol.*, **10**, 121 (1976).

Received March 15, 1989

Accepted April 10, 1989