Pakistan J. Sci. Ind. Res., Vol. 15, No. 3, June, 1972

POLYSACCHARIDE COMPONENTS OF SUNFLOWER HEADS

Part I. The Pectins

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(Received May 31, 1971; revised November 18, 1971)

Abstract. Samples of polysaccharides were extracted from three varieties of sunflowers, in a stepwise manner, with water, ammonium oxalate and disodium ethylenediamine tetracetate (EDTA). The polysaccharide samples had similar uronic acid anhydride (u.a.a.) contents and specific rotations and were found to contain D-galacturonic acid as major sugar component with small amounts of L-arabinose, D-galactose, L-rhamnose, D-xylose and 2-*O*-methyl-L-fucose. Samples of pectins were fractionated by diethylaminoethylcellulose chromatography and graded precipitation with sodium acetate. The results of this fractionation show that the polysaccharides are homogeneous and the neutral sugars are the integral constituents of the pectin extracted from these varieties of sunflower, under mildest possible conditions.

Seeds of sunflowers contain appreciable quantities of edible oil and different varieties of the plant are being experimented for cultivation in Pakistan. The sunflower heads left after removal of seeds contain another commercially important substance—the pectin. Bishop[1] has extracted pectin from a variety of sunflower, with ammonium oxalate–oxalic acid solution and examined its structure. The polysaccharide was found to contain D-galacturonic acid as major sugar constituent with small amounts of D-galactose and L-arabionse. Extraction of the residual plant material with alkali furnished a mixture of polysaccharides which was thought to contain three polysaccharides including a xylan. In later investigations.[2] the pectin sample extracted with ammonium oxalate–oxalic acid solution was fractionated by graded precipitation with sodium acetate, into four fractions of pectic acid containing varying amounts of galacturonic acid and neutral sugars, including a pure galacturonan whose structure has been investigated.[3]

In the present investigations, samples of polysaccharides were extracted from three different varieties of sunflower. Since pectins are very susceptible to degradation and extraction with acidic solution (may cause the degradation of polysaccharide), the plant material was extracted with mild solvents like water, ammonium oxalate and disodium EDTA (pH 6). The dried plant material (each sample of the available three varieties of sunflower heads) was first extracted with ethanol–water (4:1) to inactivate the enzymes and to remove the colouring matter, soluble sugars and proteins. The dried and powdered residue was then extracted, in a stepwise manner, with water to extract the water-soluble polysaccharide(s), with ammonium oxalate to extract pectins as ammonium pectate, and with disodium EDTA to extract the residual pectic material still remained in the plant as calcium salt.

It was observed that the pectin content (on dry-weight basis) of Russian variety and American variety Su-264 is about 23% and American variety OH-I of sunflowers is 17%. The results of analysis of various pectin samples are given in Table 1.

The polysaccharides are similar with slight variations in the relative amounts of acidic and neutral sugars. (Table 1). They represent a typical pectin sample and have more or less the same neutral sugars, which have been found to be present in other pectin samples obtained from a number of fruits and vegetables.[4–6] D-Galactose and L-arabinose have been reported previously to be present in the pectin[1,2] obtained from a variety of sunflower. In addition to these neutral sugars, D-xylose, L-rhamnose and 2-*O*-methylfucose have been identified chromatographically, in various pectin samples obtained from three varieties of sunflower heads. These neutral sugars alongwith other sugars, such as L-fucose and 2-*O*-methylxylose, have been reported to be present in pectins of sisal flash,[5] lucerne,[6] soyabeans[7,8] and Amabilis fur.[9]

Fractionation of Pectins

(a) *Diethylaminoethylcellulose Chromatography.* Samples of pectins, extracted with water from three varieties of sunflower heads, were chromatographed on diethylaminoethylcellulose by Neukom's method.[10] All the three samples gave similar elution pattern. Most of the polysaccharide was eluted from the column of this ion exchanger in a single broad band with sodium hydroxide. Traces of polysaccharide, eluted with 0.5M sodium dihydrogen phosphate buffer, resembled the original polysaccharide in the presence of constituent sugars. No pure galacturonan, or any neutral polysaccharide could be fractionated by means of diethylaminoethylcellulose chromatography. Similar results have been obtained by diethylaminoethylcellulose chromatography of pectins of lucerne,[11] lemon peels,[12,13] soyabeans,[7,8] orange peels and turnips.[14] The pectin samples of sunflower heads seem to be homogeneous by criterion of Neukom's chromatographic[10] method.

TABLE 1. ANALYSIS OF PECTIN SAMPLES.

Variety	Pectin sample	Wt (g)	Sp. rot.	u.a.a. (%)	Constituent sugars
(A) Russian variety (10 g)	Extracted with water	0·47	+305	77	Galacturonic acid, galactose, arabinose, xylose (tr)
	Extracted with ammonium oxalate	1·36	+280	66	Galacturonic acid, galactose, arabinose, xylose (tr), rhamnose (tr)
	Extracted with EDTA	0·54	+225	70	Galacturonic acid, galactose, arabinose, xylose (tr), rhamnose (tr)
(B) American variety OH-I (54 g)	Extracted with water	4·5	+225	80	Galacturonic acid, galactose, arabinose, xylose (tr), rhamnose (tr), 2-*O*-methylfucose
	Extracted with ammonium oxalate	4·0	+225	75	Galacturonic acid, galactose, arabinose, rhamnose (tr), 2-*O*-methylfucose
	No pectin was extracted with EDTA				
(C) American variety Su-264 (34 g)	Extracted with water	3·0	+225	78	Galacturonic acid, galactose, arabinose, rhamnose (tr), xylose (tr), 2-*O*-methylfucose (tr)
	Extracted with ammonium oxalate	4·8	+270	80	Galacturonic acid, galactose, arabinose, xylose (tr), rhamnose (tr)

tr, traces.

(b) *Fractionation of Pectic Acids by Graded Precipitation with Sodium Acetate.* Samples of pectins obtained from three varieties of sunflower heads were also fractionated by graded precipitation with sodium acetate.[2] Since pectins are readily degraded in alkaline solution,[15] the sunflower head pectins were deesterified to give pectic acids, under mildest possible conditions.[16] Free pectic acids were then fractionated by Bishop's method.[2] It was observed that each pectic acid sample gave only one major polysaccharide fraction. Traces of pectic acid were obtained at various concentrations of sodium acetate, and it is remarkable that all the pectic acid samples contained all constituent monosaccharide present in the original pectins. No pure galacturonan was obtained as a result of this fractionation, whereas in some instances, pure galacturonans have been fractionated by graded precipitation with electrolytes.[2,3,9] These pectic acid samples have been isolated, as well as deesterified, under mildest possible conditions and may represent the original undegraded polysaccharide of the plant material.

The combined results of diethylaminoethylcellulose chromatography and sodium acetate fractionation of pectic acid samples point to the presence of a single homogeneous polysaccharide component in the samples of pectins, extracted from three varieties of sunflowers, under very mild conditions. These results also show that various neutral sugars are the integral constituents of this polysaccharide. The pectin of sunflower heads as well as various fractions of pectic acid have been found to contain D-galacturonic acid as major sugar constituent with small amounts of D-galactose, L-arabinose, L-rhamnose, D-xylose and 2-*O*-methyl-L-fucose. These neutral sugars have been found to be present in the pectic substances of lucerne,[6] citrus fruits,[12,13] soyabeans[7,8] and turnips.[14]

Experimental

General Methods of Investigation. Paper partition chromatography was carried out on Whatman filter paper No. 1, using the following solvent systems:

(a) Ethyl acetate–pyridine–water (10:4:3)

(b) Ethyl acetate–acetic acid–formic acid–water (18:3:1:4).

Chromatograms were developed by spraying with aniline oxalate or aniline phthalate.

Column chromatography was carried out on diethylaminoethylcellulose (phosphate form) column,[10] which was eluted in a stepwise manner with 0.05M, 0.1M, 0.25M, and 0.5M, sodium dihydrogen phosphate buffer (pH 6) and finally with 0.3N NaOH. Cations were removed by Amberlit resin, IR–120(H), and anions by IR–45 (OH). Samples of polysaccharides were hydrolysed in sealed tubes with 1N H_2SO_4 at 100°C for 18 hr. The hydrolysates were neutralized with $Ba(OH)_2$ and $BaCO_3$. Insoluble inorganic salts were removed by centrifugation. The supernatant was deionized with IR–120 (H) resin, concentrated and examined by paper chromatography. Uronic acid anhydride (u.a.a.) contents of samples of polysaccharides were determined by Anderson's decarboxylation method.[17] Specific rotations of aqueous solutions of pectins (0.2%) were measured at 20°C.

Extraction and Purification of Pectins

(a) *Sunflower Head (Russian Variety) Pectin.* Dried and crushed sunflower heads (10 g) were extracted twice with ethanol–water (4:1; 250 ml). The residue (7.5 g) was then dried and extracted, in a stepwise manner, with water (250 ml), ammonium oxalate solution (0.5%, 2×250 ml; at 90°C for 2 hr) and

EDTA (2%, pH 6, 2×250 ml, at 90°C for 2 hr). The polysaccharide from each extract was precipitated with ethanol (1:1, v/v) and dried by solvent exchange method. In this way three samples of sunflower head pectins were obtained. The analysis of the polysaccharide samples is given in Table 1. The plant material left after extracting the pectin was 4.5 g.

(b) *Pectins of Sunflower Heads (American Variety OH-I).* Dried, powdered sunflower heads (54 g) were extracted twice with ethanol–water (4:1). The residual plant material (50 g) was then extracted in a stepwise manner with water (2×1 litre) and ammonium oxalate (0.5%, 2×1 litre at 90° for 2 hr). The polysaccharide from each extract was recovered as described previously. The analysis of the samples of pectins are given in Table 1. The residue (14.1 g) left after extraction of the pectins with water and ammonium oxalate on further extraction with EDTA gave no polysaccharide.

(c) *Pectins of Sunflower Heads (American Variety Su-264).* Dried, crushed sunflower heads (34 g) were first extracted with ethanol–water (4:1) and the residue (28 g) was extracted, in a stepwise manner, with water (2×1 litre) and ammonium oxalate (0.5%, 3×500 ml, at 90°C for 2 hr), and the polysaccharides from each extract were recovered as described above. The analyses of the samples of the pectins are given in Table 1. The residual plant material was 9.13 g.

Fractionation

Fractionation of each variety of sunflower head pectin was carried out by two methods:

(a) *Chromatography on DEAE-Cellulose Column.*[10] DEAE-cellulose (phosphate form) column was prepared and the samples of polysaccharides were chromatographed as follows:

(i) Sunflower head (Russian variety): Pectin (0.1 g) extracted with water was adsorbed on DEAE-cellulose column (5 g, 2×6 cm) which was then eluted in a stepwise manner with 0.05M, 0.1M, 0.25M, and 0.5M, sodium dihydrogen phosphate buffer (pH 6) and 0.3M NaOH. The eluted polysaccharides were precipitated with ethanol (1:1, v/v). Most of the polysaccharide was eluted with sodium hydroxide solution and only traces of it were eluted with sodium dihydrogen phosphate (0.5M) buffer. Hydrolysis of these polysaccharides and paper chromatographic examination of hydrolysates in solvent systems A and B showed the presence of D-galacturonic acid, D-galactose, L-arabinose, and traces of D-xylose.

(ii) Sunflower head (American variety OH-I): Pectin (0.1 g) extracted with water was adsorbed on the above-mentioned column and then eluted in a stepwise manner with sodium dihydrogen phosphate buffers and finally with 0.3M NaOH. The polysaccharides were precipitated from the eluant with ethanol (1:1, v/v) and dried by solvent exchange method.

Most of the polysaccharide was eluted with sodium hydroxide solution and very small quantity of it with 0.5M sodium dihydrogen phosphate buffer. Paper chromatographic examination of hydrolysates, obtained from both samples of polysaccharides in solvent systems A and B, showed the presence of D-galacturonic acid, D-galactose, L-arabinose, and traces of D-xylose and L-rhamnose.

(iii) Sunflower head (American variety Su-264): Pectins (0.1 g) extracted with water was chromatographed on DEAE-cellulose column as described above. Most of the polysaccharide was eluted with NaOH (0.3M) and only traces of it were eluted with sodium dihydrogen phosphate (0.5M) buffer. Both the samples of polysaccharide on hydrolysis and paper chromatographic examination of hydrolysates, in solvent systems A and B, showed the presence of D-galacturonic acid, D-galactose, L-arabinose and traces of D-xylose and L-rhamnose.

(b) *Fractionation by Graded Precipitation with Electrolyte.* (i) Sodium acetate fractionation[2] of sunflower (American variety OH-1) pectic acid: The solution (1%) of sunflower head pectin (1 g) was converted to pectic acid[16] by dropwise addition of NaOH (2M), so that the pH of solution became 12. The solution was kept at 0°C for 2 hr, the pH being maintained at 12. The pectic acid was then precipitated from aqueous solution by addition of 18% hydrochloric acid. Pectic acid (0.91 g) was filtered, washed with acidified ethanol-water (3:2, litre solution containing 5 ml HCl) and dried by solvent exchange method.

Pectic acid (0.9 g) was dissolved in water (90 ml) at pH 6.5 by dropwise addition of dilute NaOH. Sodium acetate (2M, 5.4 ml) was added to this solution, so that the molarity of sodium acetate in the solution was 0.12. The solution was kept at 0°C for 18 hr. The precipitated polysaccharide (0.55 g) was removed at the centrifuge, washed with acidified ethanol–water (3:2, 1 litre containing 5 ml HCl) and dried by solvent exchange method. A sample of this polysaccharide was hydrolysed and the hydrolysate was examined by paper chromatography in solvent systems A and B. Galacturonic acid, galactose, arabinose, rhamnose and xylose were identified.

More amount of sodium acetate (2M, 1.2 ml) was added to the solution so that the molarity of sodium acetate in the solution became 0.14. The solution was kept at 0°C for 18 hr. The precipitated polysaccharide (0.04 g) was removed at centrifuge, washed with acidified ethanol–water and dried by solvent exchange method. Hydrolysis of this sample of polysaccharide and paper chromatographic examination of hydrolysate in solvent systems A and B showed the presence of galacturonic acid, galactose, arabinose and traces and rhamnose and xylose.

Another portion of sodium acetate (2M, 2 ml) was added to the mother liquor from the second fractionation so that the molarity of sodium acetate in the solution became 0.18. The solution was kept at 0°C for 18 hr, the precipitated polysaccharide (0.01 g) removed by centrifugation and dried by solvent exchange.

Hydrolysis of the sample of pectic acid and paper chromatographic examination of hydrolysate, in solvent systems A and B, showed the presence of the sugars present in the original polysaccharide.

More sodium acetate (2M, 1.3 ml) was added to the solution so that the molarity of sodium acetate in the solution became 0.2M. Solution was kept at 0°C for 18 hr, the precipitated polysaccharide (only

TABLE 2

Fraction No.	Sodium acetate	Wt of pectic acid (≠g)
1.	0.12M	0.14
2.	0.14M	0.02
3.	0.18M	0.01
4.	0.20M	0.03
5.	Pectic acid precipitated with ethanol	0.04

TABLE 3

Fraction No.	Sodium acetate	Wt of pectic acid (≠g)
1.	0.12M	0.18
2.	0.14M	0.02
3.	0.18M	0.01
4.	0.20M	0.03
5.	Pectic acid precipitated with ethanol	0.06

traces) removed at centrifuge and dried by solvent exchange. Hydrolysis of this sample and paper chromatographic examination (in solvent systems A and B) of hydrolysate showed the presence of sugars present in the original polysaccharide.

The residual polysaccharide (0.5 g) was precipitated from mother liquor by alcohol (1:1, v/v) and dried by solvent exchange method. Hydrolysis of a sample of this polysaccharide and paper chromatographic examination of hydrolysate in solvent systems A and B, showed the presence of galacturonic acid, galactose, arabinose and traces of rhamnose and xylose.

(ii) Sodium acetate fractionation of sunflower head (American variety Su-264) pectic acid: Pectin (0.4 g) extracted with water from this variety of sunflower heads was converted to pectic acid[16] (0.32 g) as described above.

The pectic acid (0.3 g) was fractionated by portionwise addition of sodium acetate (2M), in such a way that the molarity of sodium acetate in solution was 0.12M, 0.14M, 0.18M, and 0.2M, respectively, for first, second, third and fourth fractionations. The weight of pectic acid fractions obtained at different concentrations of sodium acetate are given in Table 2.

The hydrolysis of each fraction and paper chromatographic examination of hydrolysates, in solvent systems A and B, showed the presence of galacturonic acid, galactose, arabinose and traces of xylose, and rhamnose.

(iii) Sodium acetate fractionation of sunflower head (Russian variety) pectic acid: Pectic acid (0.40 g) obtained by deesterification of pectin (0.50 g), extracted with water, was fractionated by portionwise addition of sodium acetate (2M) as described previously. The weights of pectic acid fractions obtained at different concentrations of sodium acetate are as given in Table 3.

The hydrolysis of all the samples of pectic acid and paper chromatographic examination of hydrolysates in solvent systems A and B, showed the presence of galacturonic acid, galactose, and arabinose and traces of xylose and rhamnose.

Acknowledgement. The authers thank the University of Karachi, for the provision of Laboratory facilities to carry out this research work and the Agriculture Research Institute, Tandojam, for the supply of samples of sunflower heads.

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