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EXTRACTION OF PECTIC SUBSTANCES FROM SUNFLOWER HEADS

M. QUDRAT-E-KHUDA, IKRAM-UR-REHMAN SIDDIQI and S.M. AMIR*

Biochemical Research Division, PCSIR Laboratories, Karachi 39

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In 1948, Sloikoft[1] studied the carbohydrate content of sunflower heads and suggested that the extraction of pectin in commercial quantities from this source was feasible. Subsequent investigations[2–4] revealed that extraction with a dilute solution of ammonium oxalate–oxalic acid yielded the most satisfactory product, which possessed a relatively high degree of homogeneity.

We have now examined the pectin content of several varieties of sunflower heads grown in Pakistan with a view to their evaluation as a commercial source of pectin.

Material and Methods

Sunflower heads, derived from the varieties designated as AI, SU-264, HO-I and Kra-I, were dried in the sun for several days and then ground to coarse powder. The dried powder (5 g) was extracted with a mixture of ethanol and benzene (150 ml, 1:2 v/v) at room temperature for 48 hr. The supernatant was decanted off, and the residue extracted with a solution of ammonium oxalate–oxalic acid (0.5%, 110 ml) at 75°C for 1.5 hr with constant stirring. The pectin extract was removed, cooled and pectin precipitated by addition of ethanol (2 vol) acidified with 0.5% HCl. Thereafter, the suspension was cooled at 4–10°C for 24 hr, centrifuged and the residue successively washed with 95% ethanol (10 ml) and acetone (10 ml). The powdered pectin was dried *in vacuo* ($CaCl_2$). The residual crude powder was reextracted twice, as above.

In a second series of experiments, the extraction of pectin was affected as follows. The powdered sunflower heads (5 g) were initially defatted by treatment with the ethanol–benzene mixture, as described earlier. The residue, so obtained, was dried and extracted with water (110 ml) at 75°C for 1.5 hr. The extraction mixture was cooled, filtered and pectin precipitated by addition of two volumes of ethanol to the filtrate. The suspension was centrifuged and the residue obtained successively washed with ethanol and ether. The product was dried *in vacuo* and the extraction method repeated twice. The yields of pectic substances, as obtained by the use of the two methods, are shown in Tables 1 and 2.

The pectin was hydrolyzed by heating either with 1.5N or 3N HCl for 2 and 4 hr. Descending paper chromatography was performed for detection of sugars, using Whatman No. 1 filter paper and the following solvent systems: (a) n-butanol–ethanol–water (4:1:5, v/v); (b) n-butanol–acetic acid–water (4:1:5, v/v).

The sugars were detected by silver nitrate spray method of Trevelyan *et al.*[5] Uronic acid was determined by the carbazole reaction, as described by Dische,[6] using D-glucuronic acid as standard. Total carbohydrates were determined either by Dische's cysteine–H_2SO_4 method[7] or Anthrone reaction,[8] using a mixture of glucose and galactose in equal amounts as standard. Whenever the amounts of pectin permitted it, the specific rotations were recorded in aqueous solution (0.1 or 0.5%) in a 0.5-decimeter rotation tube.

Results and Discussion

As can be noted from Tables 1 and 2, considerably higher percentages of the crude pectin can be obtained by the use of ammonium oxalate–oxalic acid extraction method as compared to the aqueous extraction. The residues extracted by the former method showed only poor solubility in water.

The percentages of the crude pectin in the dried powders, derived from four different varieties of sunflower, ranged from 9.06 to 26.2. Bishop[3] found that the average pectin content of sunflower heads grown in Southern Manitoba was 27.5%, which is close to the maximum figure we obtained for one of our varieties. The wide variation noted in the pectin content may reflect, besides species differences, the degree of maturity of the flowers at the time of collection.

TABLE 1. PECTIN CONTENT OF SUNFLOWER HEADS.

Sunflower varieties	Ammonium oxalate–Oxalic acid extraction		Yield (% of the dried powder)
	1st extract (g)	2nd extract(g)	
AI	1.1658	0.1490	26.296
SU-264	0.6022	0.2520	17.084
HO-I	0.4452	0.3852	16.608
Kra-I	0.3080	0.1453	9.067

TABLE 2. PECTIN CONTENT OF SUNFLOWER HEADS.

Sunflower varieties	Aqueous extraction		Yield (% of the dried powder)
	1st extract (g)	2nd extract (g)	
AI	0.1580	0.0450	4.062
SU-264	0.0533	0.0026	1.118
HO-I	0.1182	0.0114	2.592
Kra-I	0.0472	—	0.944

*Now at the National Institutes of Health, U.S Department of Health, Education and Welfare, Bethesda, Md. 20014, U.S.A.

TABLE 3. COMPOSITION OF PECTIN PREPARATIONS EXAMINED.

Sunflower varieties	Total uronic acids(%)		Total carbohydrates(%)		Specific rotation		Sugars detected*
	1st extr.	2nd extr.	1st extr.	2nd extr.	1st extr.	2nd extr.	
Ammonium oxalate–oxalic acid extract							
SU-264	35.93	51.00	8.59	12.50	+33[3a]	+180[b]	Gal. Arab.
HO-I	56.00	15.00	11.40	13.50	+80[b]	+120[b]	Gal. Arab.
Kra-I	78.00	32.00	13.00	10.50	+266[b]	+112[b]	Glu. Arab. Gal.
Aqueous extract							
SU-264	46.00	—	22.00	—	+108[c]	—	Glu. Arab.
HO-I	53.00	—	14.50	—	+86[c]	—	Gal. Arab.
AI	69.00	53.50	22.50	21.50	+172[c]	+108[c]	Glu. Arab.
Kra-I	25.00	—	64.00	—	+63.2[c]	—	Gal. Arab.

*Other than D–galacturonic acid.

[a] c, 0·06, [b] c, 0·1, [c] c, 0·5.

The aqueous extraction method yielded relatively much lower quantities of pectin, the maximum being 4.06%. Somewhat similar results were reported earlier for lemon peel pectin when it was found that no more than a third of the total quantity present could be removed by aqueous extraction.[9] It is likely that the water-soluble pectin represents a distinct species, differing in the degree of polymerization and/or containing a higher methyl ester content.

Hydrolysis and paper chromatography of the pectin preparations revealed, besides D-galacturonic acid, the presence of D-galactose, D-arabinose and, occasionally, D-glucose (Table 3). No evidence, however, could be found of the presence of L-rhamnose in our hydrolysates, although it has frequently been reported to be a constituent of pectic substances.

It is noteworthy that Bishop[3] isolated a polyuronide by extracting the sunflower head powder with ammonium oxalate–oxalic acid solution, which yielded only D-galacturonic acid residues on hydrolysis. On following the same procedure, we obtained a product which, in addition to uronic acid, contained neutral sugars as well. It is not clear whether the latter were present because of contamination by neutral polysaccharides or because they constitute an integral part of the pectin structure.

These preliminary studies indicate that the varieties of sunflower grown in West Pakistan may be suitable as a commercial source of pectin. However, further studies, on a larger scale, are required before optimum conditions for extraction can be delineated.

Acknowledgement. The sunflower varieties named AI, Su-264, HO-I were grown and developed at the Agricultural Research Institute, Tandojam, Sind, and we would like to thank Mr. Altaf Hussain Chaudry, of this Institute, for kindly supplying us with the sunflower heads. Kra-I was collected locally from around the PCSIR Laboratories, Karachi.

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POLYSACCHARIDE COMPONENTS OF SUNFLOWER HEADS

Part II. The Hemicelluloses

SOFIA BAQAI, SOHELA RIAZ and MAHBOOB UDDIN

Department of Chemistry, University of Karachi, Karachi 32

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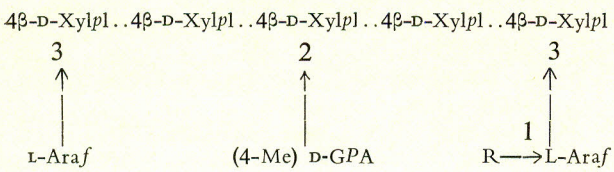
Hemicelluloses constitute an important group of cell-wall polysaccharides.[1] Among various hemicelluloses xylans and related polysaccharides are very common in plant kingdom. Xylan group of hemicelluloses include[2,3] xylans, 4-*O*-methyl glucaronoxylans, *O*-acetyl-4-*O*-methyl-glucuronoxylans, arabinoxylans, 4-*O*-methylglucurono-arabinoxylans and similar type of polysaccharides of complex structure containing a

number of neutral sugars in addition to xylose and acidic sugar, 4-*O*-methylglucuronic acid. In a few instances pure xylans have been isolated[4] whereas in most of the cases the polysaccharide of this group of hemicelluloses isolated from various plants, has been found to contain a number of monosaccharides. In cases[5,6] where hemicelluloses are extracted directly with alkali from plant material, without prior removal of other polysaccharides like pectins, the complex nature of the polysaccharide may be due to the presence of other accompanying polysaccharides. In contrast, the purified, homogeneous polysaccharide of this group[7] has been found to contain L-arabinose, L-rhamnose and traces of D-galactose together with D-xylose and 4-*O*-methylglucuronic acid.

The main chain of this group of polysaccharides consists of 1—→4 linked β-D-xylopyranose units.[1-3] In some cases the main chain is interrupted occassionally by L-rhamnose residues,[7] the linkage being 1—→3. 4-*O*-Methylglucuronic is present as single unit side-chain linked at No. 2 carbon atom of xylose units and arabinofuranose is present either as single unit or multiple unit side-chain. The main chains of 1—→4 linked D-xylose units have also some branching in hard wood xylans.[8]

The following general structural pattern is present in xylan group of hemicelluloses:



In a previous investigation on polysaccharides of sunflower heads,[9] a sample of polysaccharide was extracted with dilute alkali from residual plant material left after removal of pectins. The polysaccharide was thought to be a mixture of three polysaccharides including a xylan. In the present investigations the plant material was extracted[11] successively, with ethanol–water (4:1) to remove much of the proteins, colouring matter and soluble sugars, with water to remove water-soluble polysaccharides, with ammonium oxalate to remove pectins and finally with dilute sodium hydroxide to extract hemicelluloses. Samples of hemicelluloses were extracted from three varieties of sunflower heads, and purified via precipitation as insoluble copper complex. Hydrolysis of the samples of hemicelluloses gave D-xylose, as major sugar component, 4-*O*-methyl-D-glucuronic acid, and traces of L-arabinose and D-galactose. The percentage of 4-*O*-methyl-D-glucuronic acid in the polysaccharide samples is slightly higher than the percentage of this sugar in other xylans.[1-3] However, the hemicelluloses of sunflower heads resemble the typical xylans present in different plants.[1-3]

Experimental

General Methods. Paper partition chromatography was carried out on Whatman filter paper No. 1, using the solvent systems (a) ethyl acetate–pyridine–water (10:4:3); (b) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (c) butanol–ethanol–water (4:1:5; upper layer); (d) butanol–acetic acid–water (4:1:5; upper layer).

The chromatograms were developed by spraying with *p*-anisidine hydrochloride, aniline oxalate or aniline phthalate.

Samples of polysaccharide (5–10 mg) were hydrolysed in normal sulphuric acid and hydrolysates were neutralized with barium hydroxide and barium carbonate. Insoluble inorganic salts were removed by filteration, filterates were deionized, concentrated and examined by paper partition chromatography. Cations were removed from sugar solutions with Amberlit resin I.R.-120(H) and anions with I.R.-45(OH). All concentrations were done at or below 50°C. Specific rotations of polysaccharides were measu red in normal sodium hydroxide solution, at 20°C.

TABLE 1. ANALYSES OF XYLANS OF SUNFLOWER HEADS.

Variety of sunflower heads	Wt of plant material* (g)	Wt of hemicelluloses (g)	Specific rotation	u.a.a. (%)	Constituent sugars
American variety Su-264	8.28	0.78	—32°	12.4	Xylose, 4-*O*-methyl-glucuronic acid galactose (tr) arabinose (tr)
American variety OH-I	5.00	0.40	—35	12.2	Xylose, 4-*O*-methyl-glucuronic acid arabinose (tr) galactose (tr)
Russian variety	2.6	0.23	—38	11.8	Xylose, 4-*O*-methyl-glucuronic acid arabinose (tr) galactose (tr)

*After extraction with ethanol–water (4:1), water, ammonium oxalate, and EDTA. tr., traces.

Uronic acid anhydride (u.a.a.) contents of the samples of hemicelluloses were determined by Anderson's decarboxylation method.[10]

Extraction and Purification of Hemicelluloses. The residual plant material[11] obtained after successive extraction with ethanolnwater (4:1), water, ammonium oxalate (0.5%) and ethylenediaminetetraacetate disodium salt (2%), was extracted with aquous sodium hydroxide (2%; 3×200 ml) for 2 hr. The residue was removed at the centrifuge and the polysaccharide was precipitated from the supernatant solution with alcohol (1:1; v/v), the pH of the mixture being maintained between 4 and 5 with acetic acid. The precipitated polysaccharide was removed at the centrifuge, washed with acidified ethanol–water (1:1, v/v, 1 litre of the solution containing 4 ml glacial acetic acid), and dried by solvent exchange method. The residual plant material, on further extraction with aqueous sodium hydroxide (5%) gave no polysaccharide. The polysaccharide was purified by precipitation (three times) as insoluble copper complex formed on addition of Fehling's solution to alkaline solution of polysaccharide. In this way, samples of polysaccharides were isolated from three varieties of sunflower heads.

Analyses of the Polysaccharides. Samples of hemicelluloses were analysed for (a) uronic acid anhydride contents by Anderson's decarboxylation method (b) specific rotations, and (c) constituent sugars. The results are given in Table 1.

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SHREDDED-LEAF DISEASE OF THE PAPAYA TREE

MOHSIN AZIZ FARUQI and M. RAFI-UZ-ZAMAN

PCSIR Laboratories, Karachi 39

S. MAHDIHASSAN

S.D. 34, Block A, North Nazimabad, Karachi 33

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In the North Nazimabad suburb of Karachi, some papaya trees, growing in a house garden, were found suffering from a virus infection named here, shredded-leaf disease. In such a garden belonging to one of us a few years previously two lemon trees were infected by a roll-leaf virus disease. On account of the severity of the symptoms they had to be coppiced. No papaya tree at the time was subject to the disease under consideration. But later on a healthy leaf would show gradual development of a withering disease. The leaf would gradually deteriorate so that each rib would be carrying a 'rag' of leaf mass and finally left bare as 'shred', hence the name given to it here. The disease begins earliest when the tree is some eight months old and about four feet high. Usually, if not invariably, the top-most leaves begin to reveal evidence of infection. The leaf margin as a whole begins to curl upwards from all sides. On the contrary the normal leaf remains flat and droops a little. The leaf surface would later reveal blisters as dark green spots on the surface which usually becomes paler than the normal. The under-surface would correspondingly show pits or concavities. The blisters increase and the leaf surface gets paler and reduced in area. Thus the blisters are darker than the normal leaf colour but the main surface of the diseased leaf abnormally pale. As the disease increases the blisters multiply but the leaf mass gets 'dissolved out' leaving each rib with less of leaf mass. This seems to be reduced further when a rib seems to carry a 'rag' of leaf mass finally denuded completely and left as bare shreds.

Figure 1 shows an easily recognizable or sufficiently advanced stage of the disease. The most vertical rib in the picture best reveals blisters on the surface. Another leaf showing a more developed form of the disease, than in Fig. 1, carries four ribs and a leaf mass hanging like pieces of rags (Fig. 2). The portion to our left is lighter in colour, being pale green or chlorotic. Here the blisters are few and by contrast darker. A similar portion is obviously darker because of the intense occurrence of blisters. Thus the two ribs represent the contrast between chlorotic colouration and the intensified deep green colour due to blisters; the ribs being close together were equally illuminated. As the disease progresses the leaf mass correspondingly gets reduced. Thus in Fig. 3 there are again only four ribs with 'rags' of leaf hanging at their tips, the worst, to our extreme left, with hardly more than a residue of it. Besides the

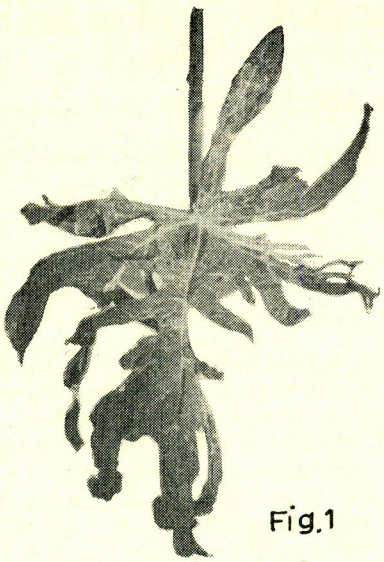

Fig.1

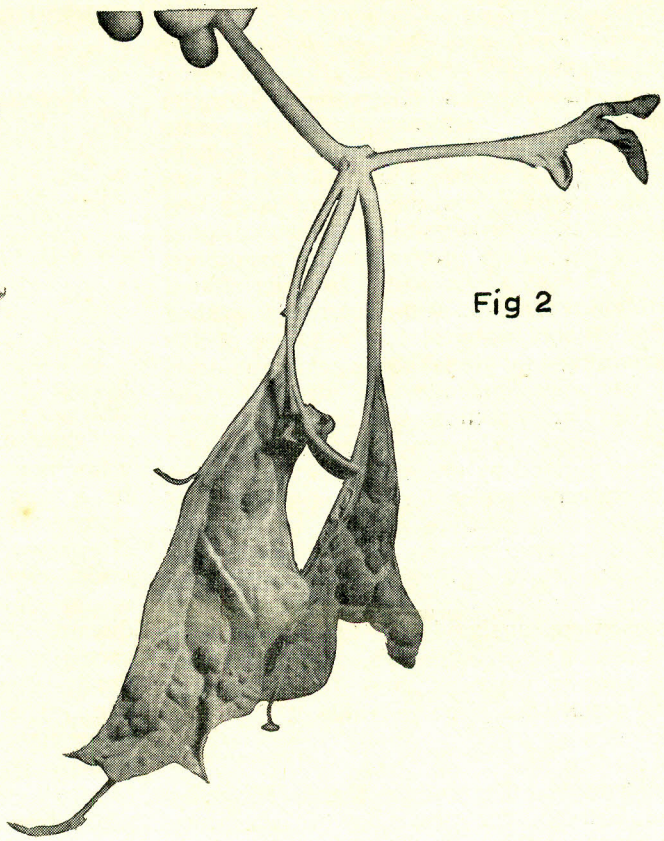

Fig 2

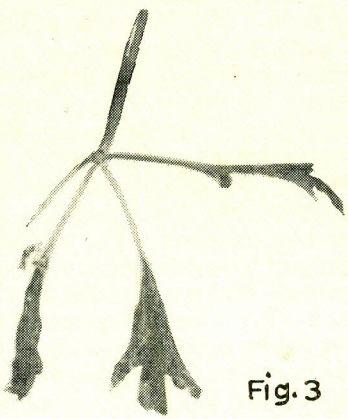

Fig.3

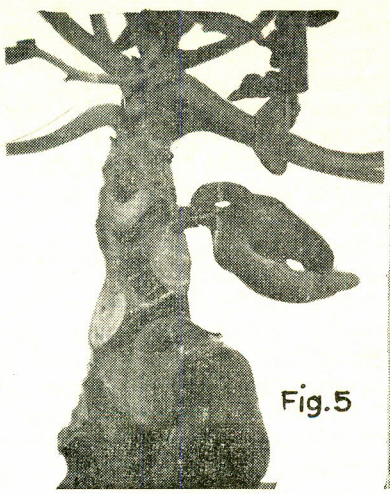

Fig.5

Fig.4

Fig. 1. A papaya leaf in advanced stage of disease. Its seven ribs are visible bearing ragged leaf mass. The top-most portion shows dark blisters on a pale chlorotic leaf. Fig. 2. A leaf with four ribs, one on the left with pale chlorotic leaf mass, with few dark blisters. Next, a rib with leaf mass full of dark blisters. Fig. 3. A further advanced stage of the disease, showing four ribs of the leaf and with ends carrying leaf mass reduced to rags. Fig. 4. A form of disease showing more obvious blisters but mis-shaped, as also a part lacking in strength resulting in shrivelling on account of it. Fig. 5. When the disease is intense fruits which grow reveal monstrosity as shown here.

shredded form another malformation is illustrated in Fig. 4. Here the leaf surface does not shows blisters but it is misshaped, and part of it lacks in strength so that it droops. While being photographed the larger portion had to be lifted and placed properly like a piece of cloth which is not starched and tends to shrivel. Figs. 1–3 show leaves removed from their plants, but Fig. 4 had to be photographed on the tree itself, not by choice but on account of the soft nature of the part of the leaf. When infection, starting from leaves already full grown, permeates into the system of the plant the young leaves that emerge are chlorotic and highly misshaped. Even fruits show malformation in a tree with a long duration of the disease. Fig. 5 represents a monstrosity as a fruit. There are about a dozen papaya trees between 5 to 1 years old, but all were found suffering from the disease. Its infective nature seems to be self-evident. In the neighbourhood some half a mile away a few gardens of papaya trees were also found suffering from the same infection.

For over a year a daily inspection of the papaya trees showed that hardly any insect visits them, at least during the day. A few species of flies and the black camponotus ant were occasionally seen but none could be suspected as the probable transmitter of infection. The problem of biochemical interest is the dissolution of a normal leaf into a whithered condition leaving only the ribs. Hyperactivity of diastase and phosphatase is suspected as induced by the virus. In human pathology leprosy is such a disease where even the bone gets dissolved by the enzymes of the germ. The virus disease begins with leaves nearly full grown which gradually whither into shredded leaves.