Carbohydrates of Field Bean (*Dolichos lablab*)

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ABSTRACT

Field bean endosperm sugars soluble in 70% ethanol were D-glucose, D-galactose, D-fructose, sucrose, raffinose, stachyose, and verbascose. The endosperm was composed principally of starch, whereas the hulls were rich in dietary fiber components. In vitro digestion of the native starch granule by glucoamylase and salivary α-amylase revealed characteristic enzyme degradation patterns in scanning electron microscopy. Glucoamylase digestion of a granule resulted in typical onion-type layering, unlike α-amylase digestion, which resulted in depressions and selective opening of the granule. Hot water soluble gum from the endosperm was a noncellulosic β-D-glucan. A cold water soluble fraction from the hulls was identified as a mixture of arabinogalactans; a hot water soluble fraction was found to be a composite aggregate of water-soluble gums and pectic fractions. Pectic fractions from the hulls had gelling characteristics that were superior to those from the endosperm. Hemicellulose A from the hulls was an amyloid-type polysaccharide, and hemicellulose B was either a glucomannan-type polysaccharide or a mixture of a glucomannan and a cellulosic β-D-glucan. Glucose was the predominant sugar in hemicelluloses A and B of the endosperm. Alkali-insoluble residue from the endosperm was a macromolecular aggregate of pentosans and cellulose, whereas that from the hulls was composed of cellulose.

Carbohydrates are the principal constituents of most diets normally consumed in India. In addition to providing energy, food carbohydrates that constitute dietary fiber are important in terms of food texture, nutrition, and physiology (Southgate 1976). In the last two decades, the role of dietary fiber in human nutrition has been of great interest to researchers (Reiser 1976, Southgate 1978). Starchy and nonstarchy food carbohydrate fractions may be better understood if their physical and physiological characteristics are investigated in detail.

Field bean (*Dolichos lablab*) is one of the legumes consumed by the people of south India. In addition to large proportions of protein (26%) and starch (48%), field bean contains various other carbohydrates, collectively referred to as dietary fiber or unavailable carbohydrate. Information on the chemical nature of these useful carbohydrate fractions is limited. A study was accordingly initiated to investigate the chemical and structural nature of starchy and nonstarchy carbohydrates of this legume.

Subba Rao (1966) found 6.5% soluble sugars, consisting of sucrose, raffinose, stachyose, and verbascose; however, no monosaccharides were identified. Studies by Hasegawa et al (1951) and Nigam and Giri (1961) using paper chromatography (PC) showed that varying amounts of glucose, fructose, sucrose, raffinose, stachyose, and verbascose were present in the endosperm. Comparative studies of the starch properties of certain leguminosae, including field bean, also have been done (Rosenthal et al 1971).

MATERIALS AND METHODS

Materials

Field bean (*Dolichos lablab*, cultivar lignosus) was purchased in the local market during December through January of 1978.

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Freeze-dried seeds were dehulled manually, either dry or after preliminary soaking in water for 12 hr. Hulls amounted to about 18% of the dry weight of the seeds. The hulls and the endosperm were powdered to size 60 mesh in a Waring Blendor.

Methods

All rotary evaporations were done at a bath temperature of 40° C. Protein was removed either by proteolysis with pronase (100 mg/100 g of material activity: 45,000 proteolytic units per gram, Calbiochem, CA) in 0.2*M* phosphate buffer (pH 7.5 at 25° C for 24 hr) or by treatment with aqueous phenol (60%) at 60° C for 20 min, followed by cooling in an ice-water bath and siphoning out the aqueous layer (Westphal and Jann 1965). The protein was then dialyzed and lyophilized. Total sugar, uronic acid, glucose, and reducing sugar were estimated by phenol-H_2SO_4 (McKelvy and Lee 1969), carbazole (Knutson and Jeanes 1968), glucose-oxidase (Dahlqvist 1964), and Nelson-somogyi (Nelson 1944) methods, respectively. Glucose, glucuronic acid, and maltose served as the calibration standards for total sugar/glucose, uronic acid, and reducing sugar determinations. Protein was estimated either by Lowry's method (Lowry et al 1951) or by the micro-Kjeldahl method (N × 6.25). Specific rotations of monosaccharides were determined in a Carl-Zeiss triple-beam polarimeter on a 1% aqueous solution of the material at room temperature. Lignin was determined by subjecting the residue, left after 72% H_2SO_4 hydrolysis at 26° C for 3 hr, to ashing at 550° C for 3 hr. The weight loss was measured as crude lignin.

Isolation of Carbohydrate Fractions

Free sugars from the defatted, depigmented (hexane-$CHCl_3$, 1:2 extraction; 10 ml/g; three times at 80° C for 2 hr each) material were extracted with 70% ethanol (10 ml/g, three times) at room temperature. The combined alcoholic extracts were purified by passage through Dowex 1 × 8 (H^+) and Dowex 50 W (OH^-) resins, concentrated and analyzed chromatographically. The quantitation of ethanol-soluble sugars was done by gas-liquid chromatography (GLC) as trimethylsilyl ethers (Brobst and Lott 1966).

The alcohol-insoluble residue (240 g) from the endosperm was then gelatinized by cooking in water followed by amylolysis of the starch with a 50:1 mixture of glucoamylase (from *Aspergillus niger*, Anil Starch Co., Hyderabad, India) and α-amylase (Sigma Chemical Co.) in acetate buffer (pH 4.8, 0.05*M*, 60° C). Dialysis and centrifugation of the hydrolyzed suspension gave a supernatant from which the hot water soluble gum was recovered by precipitation with alcohol (0.37 g).

Three successive extractions with aqueous ammonium oxalate (0.5% at 85° C for 2 hr each) of the starch-free residue, followed by precipitation of the extract with alcohol (three volumes) gave pectic

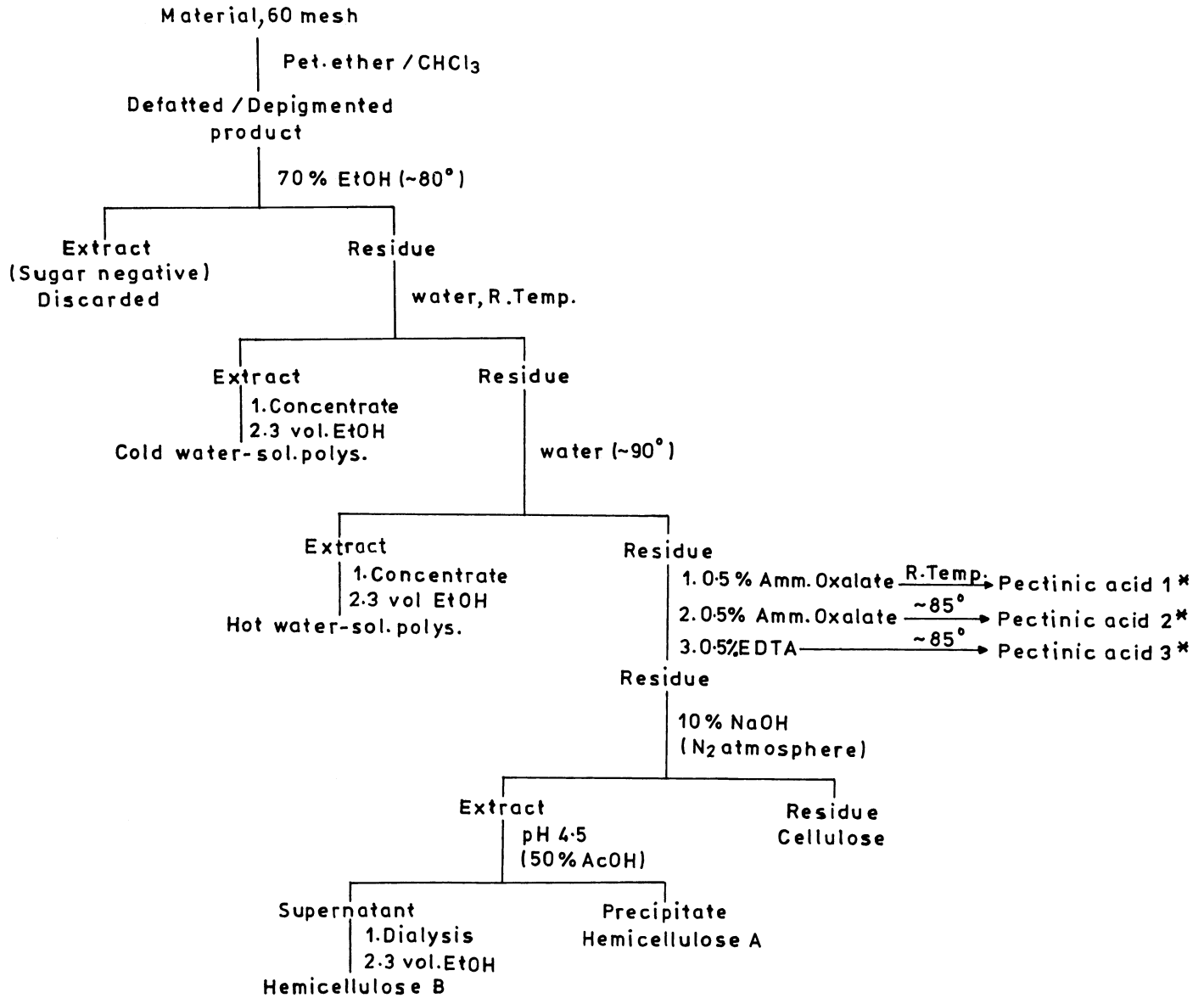

Fig. 1. Scheme of extraction of polysaccharides from field bean hull.

substances (1.1 g). The resulting pectin-free residue was suspended in 10% carbonate-free NaOH, and hemicelluloses were extracted over a period of 5 hr in an N_2 atmosphere by the method of Whistler and Feather (1965). Centrifugation and acidification (to pH 5) of the extract at ice-cold temperature with 50% acetic acid precipitated hemicellulose A which was collected by centrifugation. Addition of acetone (three volumes) to the clear centrifugate yielded hemicellulose B. The alkali-insoluble residue left after alkali extraction represented the cellulosic fraction.

Starch from the endosperm was isolated and purified by the method described by Paramahans et al (1980).

The alcohol-insoluble residue from the hulls was subjected to sequential extraction as outlined in Fig. 1.

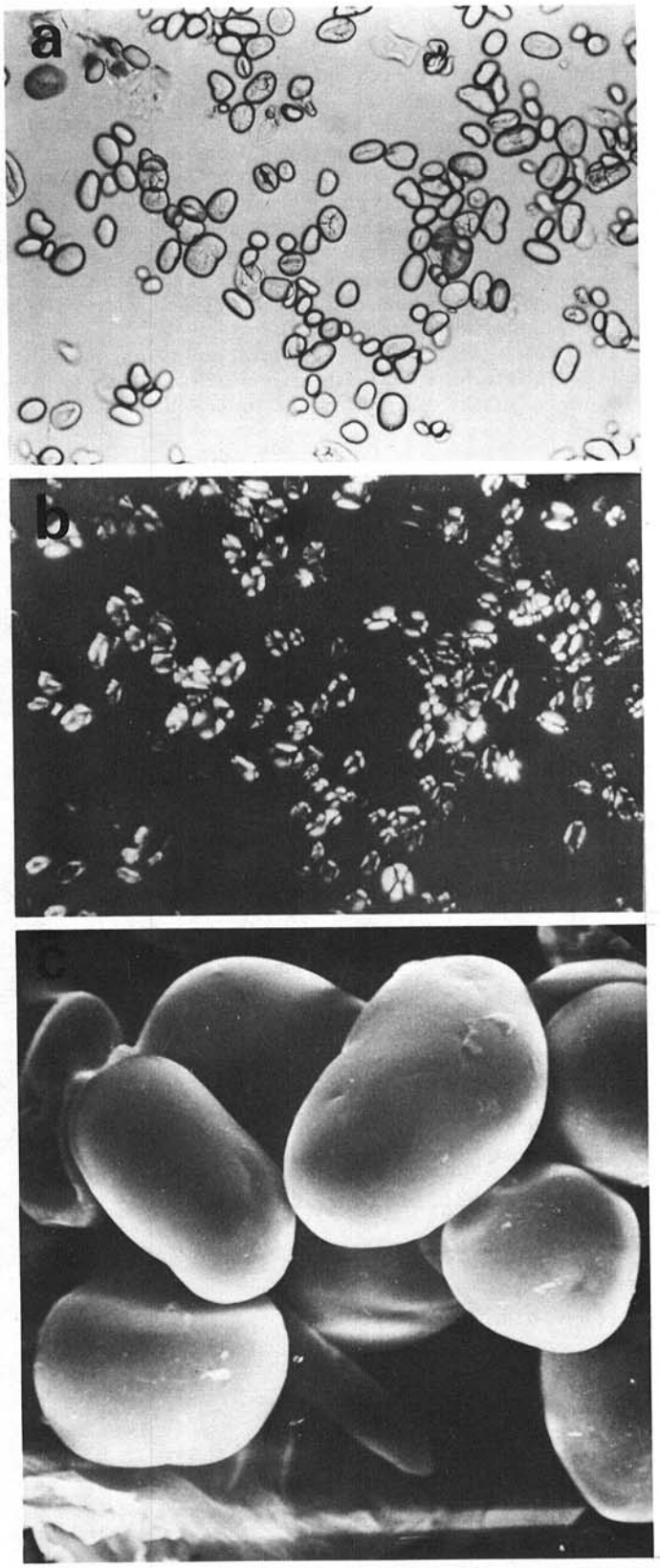

Fig. 2. Photomicrographs of field bean starch granules: **a,** under ordinary light (×100); **b,** under polarized light (×100); **c,** scanning electron micrograph (×2,200).

Hydrolytic Conditions

Water-insoluble fractions were hydrolyzed by 72% H_2SO_4 solubilization as follows. The fraction (10 mg) was suspended in water (0.5 ml) for 2 hr, followed by dropwise addition of concentrated H_2SO_4 (AR,BDH) to 72% concentration at ice-bath temperature. After approximately 1 hr, it was diluted to 8% acid, and hydrolysis was continued by heating at 95° C for 8–10 hr. Water-soluble fractions were hydrolyzed with $2N$ H_2SO_4 for 8 hr at 95° C. The hydrolysates were neutralized (solid $BaCO_3$), deionized Amberlite IR 120, H^+, concentrated, and chromatographed by PC, thin-layer chromatography (TLC), or GLC as described by Paramahans and Tharanathan (1980). Quantitation of the sugars was done by GLC as alditol acetates (Paramahans and Tharanathan 1980).

In Vitro Digestibility of Raw Starch Granules

Starch granules (100 mg) were incubated with 740 units of glucoamylase (Sigma Chemical Co.) in acetate buffer (100 ml, pH 4.8, 0.05M at 55° C) or with salivary α-amylase (3,900 units, 100-ml of freshly drawn human saliva diluted with an equal volume of acetate buffer at 37° C). Aliquots (5 ml) from the reaction mixture and control were withdrawn after 0, 30, and 120 min of incubation, added to ethanol (10 ml), and centrifuged. The centrifugates for glucoamylase were assayed for D-glucose by the glucose oxidase method (Dahlqvist 1964), and centrifugates for α-amylase were assayed for reducing sugar as maltose by the Somogyi method (Nelson 1944). The sediments were washed with aqueous ethanol (70%) and ethanol and then were lyophilized. The dried materials were used for scanning electron microscopy (SEM).

For SEM, the samples were mounted on brass stubs containing double-sided adhesive tape and vacuum-coated with gold-palladium alloy (400 Å). They were viewed at 25 kV in a JEOL-35 scanning electron microscope.

RESULTS AND DISCUSSION

Mono- and oligosaccharides (7.3%) from the endosperm were identified as fructose, galactose, glucose, sucrose, raffinose, stachyose, and verbascose by a combination of chromatographic methods (Table I). Specific rotation values of glucose (+55°), fructose (−91°), and galactose (+83°) indicated that the monosaccharides have a D-configuration. Stachyose was the major sugar, representing about 29% of the alcoholic extract (2.1% of the dry weight of the endosperm). This corroborates well with the large amounts of stachyose in legume carbohydrate fractions found by Jeanes and Hodge (1975). Soaking the beans in water for 12 hr resulted in the decrease of galactose and galactose-containing oligosaccharides, perhaps through α-galactosidase activity. The decrease in sucrose content (Table I) may have been a result of invertase activity, as the concentrations of fructose and glucose increased concomitantly.

A negative phenol-H_2SO_4 assay of the extract from the hulls indicated the absence of free sugars.

Starch, 76% of which was recovered, was the major constituent of the endosperm. The purified starch had a very low protein content (0.2%). The granules were 10–30 μ and of various shapes, ranging from round to oval to irregular. This irregularity in shape has been reported in many legume starch granules (Greenwood 1976). Light-microscopic observations (Fig. 2) showed hilum and lamellae in the starch granules. Field bean hilum was of varying shapes and lengths, as are those of lima bean (Salunkhe and Pollard 1955), Great Northern bean (Sathe and Salunkhe 1981), and starch grains of *Phaseolus* species. As can be seen from SEM analysis, the

starch granules have a smooth surface with fewer dents, due to developing starch granules and protein bodies.

The in vitro digestibility of raw (uncooked) starch granule was followed by chemical and microscopic analyses. Follow-up of amylolysis at specified intervals revealed considerable hydrolysis attack by glucoamylase; 96% glucose was released after 20 min of incubation. The characteristic onion-type layering of the granule is clearly discernible in Fig. 3a. Marshall and Whelan (1970) noted that complete hydrolysis of starch (and glycogen) is possible only when the enzyme preparation is contaminated with α-amylase. Similar action patterns were observed by Fuwa et al (1978a, 1978b) on the mutant and normal starch granules of maize. Hydrolysis of the field bean starch granule was fast and quantitative, unlike hydrolysis of starch granules of groundnut (*Arachis hypogea*), ragi (*Eleusine coracana*) (Tharanathan et al 1980), and varagu (*Paspalum scrobiculatum*) (Paramahans and Tharanathan 1982), wherein enzyme action was sluggish, probably because the studies were done at 37° C. In the present study, the incubation temperature also was higher (55° C), and some preliminary swelling and partial gelatinization are likely to have occurred.

TABLE I
Chemical Composition[a] (%) of 70% Alcohol-soluble Materials from Field Bean Endosperm

Manual Dehulling Method	Yield	Total Sugar	Protein	Moisture	Ash	Sugars Detected (g)
Dry	7.32	87.57	1.05	10.25	1.20	Fructose, 0.84 Galactose, 0.82 Glucose, 0.22 Sucrose, 1.80 Raffinose, 0.59 Stachyose, 2.07 Unidentified component, 0.04 Verbascose[b]
After soaking	6.33	87.20	0.99	10.15	1.25	Fructose, 1.19 Galactose, 0.65 Glucose, 0.24 Sucrose, 1.31 Raffinose, 0.49 Stachyose, 1.60 Unidentified component, 0.03 Verbascose[b]

[a] Each value represents the mean of two determinations.
[b] The concentration of verbascose could not be determined.

Salivary α-amylase also showed some hydrolyic ability, as evidenced in Fig. 3b; 58% reducing sugar was released after incubation for 120 min. The attack is localized; selective digestion is followed by opening of the granule.

The hulls are rich in nonstarchy polysaccharides, but the endosperm has very little such material. The hot water soluble fraction from the latter was predominantly a polysaccharide of D-glucose (Table II).

The cold and hot water soluble fractions from the hulls (Fig. 1), on the other hand, were entirely different from the endospermic fractions and contained a highly complicated sugar profile. Proteolysis of the cold water soluble fraction with pronase gave a carbohydrate-rich fraction containing galactose and arabinose (1:2) and having a uronic acid content of 6.0% (Table III). Diethylaminoethyl-cellulose chromatography of the fraction afforded two homogeneous arabinogalactins. Sugar analysis (Table III) showed that the hot water soluble fraction was a macromolecular aggregate consisting of hexosan, pentosan, and pectic fractions.

The pectic fractions from the hulls were chemically and functionally much more interesting than the endosperm fractions. The hull pectic fractions, obtained in a higher yield (7.7% of the hulls, db, Table III), contained significant amounts of uronic acid and exhibited characteristic viscogenic properties, unlike the endosperm pectic fractions, which did not possess any viscosity. This may be ascribed to the lower percentage of esterified carboxyls in the latter (10.2%), than in the former (Table III). The endosperm pectic fraction (Table II) exhibited a ratio of 1:1.8:1.9 for deoxyhexose to pentose to hexose, suggesting that the isolated fraction is probably a heteroglycan consisting of a mixture of

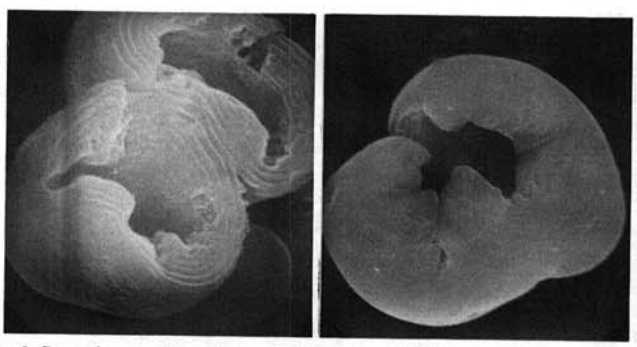

Fig. 3. Scanning electron photomicrographs of field bean starch granules: **a,** attacked by glucoamylase (×4,200); **b,** attacked by salivary α-amylase (×3,200).

TABLE II
Chemical Composition[a] (%) of Field Bean Endosperm and Its Fractions

	Flour	Water-Soluble	Ammonium Oxalate-Soluble	Alkali-Soluble Hemicellulose A	Alkali-Soluble Hemicellulose B	Alkali-Insoluble
Yield	(100)	0.15	0.44	6.09	0.55	2.23
Moisture	9.65	ND[b]	ND	ND	ND	8.00
Ash	3.10	ND	ND	3.02	ND	4.00
Methoxyl	–[c]	–	1.67	–	–	–
Esterification	–	–	10.23	–	–	–
Protein	21.95	10.80	5.30	6.05	2.60	0.28
Total sugar	65.00	75.40	69.40	75.00	81.44	80.70
Uronic acid	12.34	15.00	44.13	1.60	8.40	17.46
Sugars detected						
Rhamnose/Fucose	+[d]	–	5.33	1.08	–	0.85
Arabinose	0.13	3.30	6.72	2.42	3.72	18.84
Xylose	+	+	3.01	1.21	1.86	2.29
Mannose	+	–	–	6.83	–	–
Galactose	1.04	3.30	4.17	5.62	13.03	13.32
Glucose	51.50	53.38	6.03	57.80	54.32	27.90

[a] Each value represents the mean of two determinations.
[b] ND = not determined.
[c] – = detectable but not measurable.
[d] + = present in trace amounts.

TABLE III
Chemical Composition[a] (%) of Field Bean Hull and Its Fractions

	Flour	Cold Water Soluble (Arabino-galactan)	Hot Water Soluble	Cold Ammonium Oxalate (Pectinic acid 1)	Hot Ammonium Oxalate (Pectinic acid 2)	Hot EDTA Soluble (Pectinic acid 3)	Alkali Hemicellulose A	Alkali Hemicellulose B	Alkali-Insoluble
Yield	(100)	0.83	1.08	3.00	4.20	0.50	7.50	2.66	42.00
Moisture	7.40	ND[b]	ND	ND	ND	ND	ND	ND	8.50
OMe	–[c]	ND	ND	2.60	2.20	1.90	–	–	–
Esterification	–	ND	ND	15.80	13.20	11.80	–	–	–
Protein	9.20	6.50	11.40	3.90	2.64	5.60	8.20	10.40	5.95
Total sugar	81.50	90.00	78.20	80.10	84.50	78.00	80.00	79.60	80.40
Uronic acid	20.00	6.00	32.50	70.00	65.00	37.00	7.30	0.20	3.92
Sugars detected									
Rhamnose	+[d]	–	1.19	1.20	–	–	–	–	–
Arabinose	+	25.42	6.56	6.00	12.50	13.10	–	3.17	–
Xylose	4.96	–	3.00	2.40	7.00	14.30	30.40	+	–
Mannose	+	–	1.31	–	–	–	–	20.39	–
Galactose	+	54.20	7.06	–	–	6.60	–	+	–
Glucose	56.51	–	26.37	–	–	6.90	42.66	56.04	80.00

[a]Each value represents the mean of two determinations.
[b]ND = not determined.
[c]– = detectable but not measurable.
[d]+ = present in trace amounts.

polysaccharides of varied sugar composition.

The pectinic acids of the hulls varied in their degree of esterification, extent of heterogeneity, and neutral sugar profile (Table III).

The hemicellulosic fractions from the endosperm and hulls had grossly dissimilar sugar composition. The endosperm hemicellulose A, mainly a proteinaceous material (60% protein), was subjected to proteolysis by pronase. The resulting carbohydrate-rich fraction (75%) had the sugar composition shown in Table II. Glucose was the major sugar and probably originated from the alkali-soluble cellulosic fraction. The presence of both arabinose and galactose is interesting because arabinogalactans are unusual in hemicellulose fractions (Cook and Stoddart 1973). The presence of mannose in hemicellulose A is noteworthy; it may be present as mannan or glucomannan-type polysaccharides or both or as a constituent of lectin, a sugar-binding glycoprotein (Narayana Rao et al 1976).

Hemicellulose B from the endosperm, obtained in 0.55% yield, was soluble in water and could be easily hydrolyzed. The sugar composition (Table II) revealed predominantly glucose followed by galactose, arabinose, and xylose. Thus, this may also represent a glucan-type polysaccharide.

In contrast, hemicellulose A from the hulls, obtained in 7.5% yield, was a brown product rich in carbohydrates (Table III). The fraction was insoluble in water but soluble in dilute alkaline solutions. Examination of the acid hydrolysate showed xylose and glucose in the proportion of 1:1.4 (Table III). The fraction gave a positive starch-blue color when treated with I_2-KI solution, suggesting that the fraction is an amyloid-type polysaccharide, such as those identified in rapeseed (Siddiqui and Wood 1977), tamarind (Srivastava and Singh 1967), and jojoba (Watanabe et al 1980). However, unlike the former, the latter amyloids contain residues of fucose, rhamnose, and galactose, in addition to xylose and glucose.

Hemicellulose B from the hulls, a cream-colored, light and fluffy material, was easily soluble in water and contained considerable protein contamination (10.4%) (Table III). Sugar analysis showed that the fraction was a glucomannan with a 3:1 glucose-to-mannose ratio. The fraction also contained small amounts of arabinose, xylose, and galactose and two unidentified components.

The endosperm alkali-insoluble residue obtained after successive extractions of hemicelluloses with 10% NaOH constituted 2.2%. It was protein-free and consequently rich in carbohydrates (Table II). The fraction was very resistant to hydrolysis with 72% H_2SO_4. Even drastic conditions such as 80% H_2SO_4-solubilization followed by dilute acid (8%) hydrolysis at elevated temperatures left about 20% of the insoluble residue unhydrolyzed. The nature of this acid-insoluble residue was not identified further, but it might represent a macromolecular aggregate of cellulose and lignin. Its estimated lignin content was 1%. The neutral sugars identified in the hydrolysate were rhamnose/fucose, arabinose, xylose, glucose, and galactose (Table II). The pentose-hexose ratio of 1:1.9 suggests that the extraction of the hemicellulose components is still far from complete. Thus, despite two successive extractions with 10% NaOH, a pentose-free cellulose component could not be isolated from the endosperm. Presumably the fiber fraction is involved in more stable macromolecular interactions with other cell wall polymers. Alkali-insoluble residues invariably represent the cellulosic (fiber) fraction and exclusively consist of glucose with few if any of other sugars (Tharanathan et al 1975, Wankhede and Tharanathan 1976).

Recovery of the alkali-insoluble residue from the hulls was 42%. The fraction was resistant to hydrolysis, probably for the reasons mentioned. Drastic hydrolytic conditions (80% H_2SO_4, etc) showed predominantly glucose. The lignin content of the hulls was 3.2%

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