NOTE

Carbohydrate Profile of Black Gram (*Phaseolus mungo*)

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Black gram (*Phaseolus mungo*), a legume grown widely in India, is used traditionally for the preparation of fermented and steamed puddings (*idlis*) and a variety of other leavened foods with special textural qualities. A few preliminary reports on the chemistry and some functional characteristics of black gram mucilage are available (Kadkol et al 1971, Susheelamma and Rao 1978, 1979). In addition to protein (~24%), the bulk of the grain composition is carbohydrates (~60%), both starchy and nonstarchy (Reddy et al 1982). Although starch has been isolated and partially characterized (Sathe et al 1982), no systematic investigation on the unavailable carbohydrates of black gram is reported so far. We considered, therefore, that a detailed, systematic analysis of the pulse was necessary.

MATERIALS AND METHODS

Materials

Locally grown black gram was obtained in a single harvest; the botanical and agricultural history were unknown. The sun-dried seeds were smeared lightly with 0.1% castor oil to facilitate dehusking and again sun-dried. Later, the seeds were dehusked in a modified disk sheller at low peripheral speed (900 ft/min), and the dehusked seeds were powdered to 60-mesh in a Strong-Scott pearling machine.

Methods

All rotary evaporations were done at a bath temperature of 40°C. Total sugar, uronic acid, glucose, and reducing sugar were estimated by phenol-sulfuric acid (McKelvy and Lee 1969), carbazole (Knutson and Jeanes 1968), glucose oxidase (Dahlquist 1964), and Nelson-Somogyi (Nelson 1944) methods, respectively. Nitrogen was estimated by the micro-Kjeldahl method using a Buchi Kjeldahl unit (322/342/430) with on-line titration and calculation systems. Protein content was calculated as N × 6.25. The amylose content in starch was estimated by iodometry (McCready and Hassid 1943), and the X-ray powder diffraction pattern was obtained by exposure for 5 hr at 10 Å/30 kV to Co-K_α filtered radiation. The ionic character of the starch was determined by treating it with an aqueous solution of cationic (safranine and methylene green) and anionic (orange G and fuchsin) dyes. After approximately 10 min the suspension was centrifuged, and the sediment was washed with water and viewed under the microscope (Schoch and Maywald 1956). The solubility of starch in Me_2SO was determined as described earlier (Ramadas Bhat and Tharanathan 1983).

Isolation of Carbohydrate Fractions

The various polysaccharides, i.e., starch, pectic substances, hemicelluloses, and alkali-insoluble fractions were extracted sequentially by a method reported earlier (Paramahans and Tharanathan 1982a).

Hydrolytic Conditions

Polysaccharides were hydrolyzed either by 2*M* trifluoroacetic acid at 100°C for 2 hr, or by 1*N* sulfuric acid at 100°C for 6 hr followed by precipitation with 3 vol of alcohol of the degraded polysaccharide, which was further completely hydrolyzed by 72%, followed by 8% sulfuric acid (Paramahans and Tharanathan 1982a). Identification of the sugars was done by chromatographic techniques: 1) descending paper chromatography was on Whatman No. 1 filter paper sheets using a solution of *n*-propanol, ethanol, and water (7:1:2, v/v/v) and *n*-butanol, ethanol, and water (10:1:2, v/v/v) as irrigating systems; and 2) gas-liquid chromatography of the alditol acetates (Sawardeker et al 1965) was performed in a Packard model 437 gas-liquid chromatograph fitted with a flame ionization detector and using OV-225 (3% on Gas Chrom Q, 100–120 mesh) column (6 ft × 1/8 in.) and operating at 190°C (isothermal). The injector and detector port temperatures were 250°C, and nitrogen was the carrier gas used (15 ml/min). The quantitation of the components was achieved by the Packard model 604 recording data processor.

RESULTS AND DISCUSSION

Polysaccharides were separately isolated from black gram husk and endosperm. The husk, which accounts for approximately 15% of total black gram, was removed by milling. The yield and chemical composition of the various fractions derived from the husk and endosperm are given in Table I.

From the compositional data it is apparent that the cold water-soluble fraction of the husk is predominantly a hexosan-type polysaccharide, whereas the hot water-soluble fraction is of pentosan type. The hot water-soluble fraction of the endosperm was essentially a glucan-type polysaccharide. The pectic fractions (cold oxalate, hot oxalate, and hot ethylenediaminetetraacetic acid soluble fractions in Table I) from both the husk and the endosperm contained arabinose as the major constituent. All the pectic fractions, in addition, contained considerable amounts of uronic acid, which was identified as galacturonic acid. The carbohydrate profile of the pectic fractions, however, reveals that they are composed of a range of (pectic) polysaccharides. Linear 1,4-linked galactan, 3,6-linked arabinogalactan, and a highly branched arabinan are the commonly associated polymers of pectic complexes (Aspinall and Molloy 1968, Talmadge et al 1973). Conspicuously, the endosperm cold oxalate-soluble pectic fraction was rich in glucose. The possibility of any starch contamination in this fraction was ruled out, as it was negative to I_2-KI. The available evidence, however, does not rule out the presence of a contaminating glucan that does not stain with I_2.

The hemicellulosic fractions of the endosperm and husk showed grossly dissimilar sugar composition. Hemicellulose A from the husk had rhamnose and xylose as the major sugars, whereas the fraction from the endosperm was rich in arabinose and was totally devoid of xylose. Hemicellulose B from the husk and the endosperm contained arabinose and galactose, but the husk fraction also had a high xylose content. Conspicuously, xylose was totally absent in all the fractions of the endosperm. Xylans (and arabinoxylans) are the major polysaccharides in the endospermic hemicellulose A fractions of leguminous plants (Gaillard 1965), but this was not the case in the present study. In field bean (*Dolichos lablab*), husk xyloglucans possessing unusual structures have been identified (Paramahans and Tharanathan 1982b). Glucose, probably originating from the cellulosic material, was the major sugar in the alkali-insoluble fractions of both the husk and the endosperm. However, other sugars were also present in the

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TABLE I
Chemical Composition (%) of Black Gram Husk and Endosperm Fractions[a]

	Cold Water-Soluble	Hot Water-Soluble	Cold Oxalate-Soluble	Hot Oxalate-Soluble	Hot EDTA-Soluble[b]	Alkali-Soluble		Alkali-Insoluble
						Hemicellulose A	Hemicellulose B	
Husk								
Yield	1.2	1.6	3.3	4.6	4.2	10.9	2.6	53.4
Total protein (N × 6.25)	13.4	17.5	8.9	6.6	7.0	6.3	4.8	2.7
Total sugar	78.5	72.9	82.3	82.9	74.6	82.8	85.3	88.5
Uronic acid	10.5	15.0	25.3	43.8	40.5	Tr	16.2	···
Sugars detected								
Rhamnose	16.8	Tr	7.4	5.2	2.6	49.2	12.7	7.6
Arabinose	8.7	35.5	26.8	17.4	15.2	5.9	7.8	5.1
Xylose	3.1	···	2.2	1.6	1.0	20.2	19.2	10.2
Mannose	3.1	7.8	···	···	Tr	···	···	···
Galactose	20.6	7.1	7.9	5.6	3.9	2.2	29.4	1.1
Glucose	16.1	7.5	12.7	9.1	11.3	4.8	···	64.5
Endosperm								
Yield	···	2.7	3.3	4.8	3.5	5.7	2.6	2.4
Total protein (N × 6.25)	···	2.4	1.7	2.9	4.1	4.9	2.4	1.1
Total sugar	···	92.5	91.3	88.0	84.8	82.3	87.7	81.8
Uronic acid	···	10.2	9.3	15.6	23.0	4.8	7.5	2.1
Sugars detected								
Rhamnose	···	2.6	2.8	4.0	3.9	12.9	8.1	13.3
Arabinose	···	6.1	21.5	49.2	41.1	51.7	54.8	···
Mannose	···	···	···	···	···	Tr	···	···
Galactose	···	9.8	17.6	19.2	11.9	12.9	17.3	···
Glucose	···	63.9	40.1	···	4.8	···	···	66.4

[a] Values represented on dry weight basis.
[b] EDTA, ethylenediaminetetraacetic acid.

husk fraction. This may have resulted from incomplete extraction of the hemicellulose(s) or from a close macromolecular association. Such polymer-polymer interactions, mainly through hydrogen bonding, are known to render cellulose more soluble in aqueous medium (Tharanathan 1977).

Black gram starch granules, obtained in approximately 38% yield, ranged from 10–12.5 μm in size and had characteristic oval shapes. The granules appeared smooth without fissures and exhibited birefringence. The purified starch had very little protein (0.9%) and was non-ionic. The amylose content was high (31.5%) as in other legume starches (Naivikul and D'Appolonia 1979, Lineback and Ke 1975, El Faki et al 1983). Black gram starch was highly soluble in Me_2SO; over 95% solubility was achieved in less than 50 hr. This probably indicated easy penetration of the solvent into the granular matrix. In accord with this observation, the X-ray powder diffraction pattern of the isolated starch showed diffraction angles characteristic of the A-type starches, which is typical of the legume starches in general (Goering and Brelsford 1966).

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