Seed Polysaccharides of Some Winged Bean Varieties

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The seed polysaccharide composition of five winged bean (*Psophocarpus tetragonolobus* L. DC) varieties was investigated. Varieties SLS 3, SLS 6, SLS 44, and TPT 1 contained little starch, while no starch was detected in SLS 1. Nonstarch polysaccharide yields represented 38.8, 40.4, 41.2, 37.3, and 41.6% of the defatted flour of SLS 1, SLS 3, SLS 6, SLS 44, and TPT 1, respectively. The nonstarch polysaccharide fractions contained 63.2–67.5% carbohydrates, 12.8–15.2% protein, 10.4–12.1% lignin, and 3.2–4.8% ash. Galactose (44.0–54.9%) was the major sugar in all five varieties. Nonstarch polysaccharides also contained 5.1–6.5% arabinose; 4.3–6.5% xylose; small amounts of glucose, rhamnose, and fucose; 12.8–14.3% uronic acid; and 17.7–23.8% cellulose. The results suggest that winged bean polysaccharides are essentially nonstarch polysaccharide and are heterogeneous and insoluble.

The winged bean (*Psophocarpus tetragonolobus* L. DC), an underutilized tropical legume with high protein (35%) and oil (17%), is envisioned as a replacement for soybeans. While high-protein winged bean seeds could play a significant role in improving the nutritional status of tropical population, consumption of seeds is not as widespread as that of pods with a protein content of only 2–3% or even soybeans. The lack of tradition in consuming the seeds is evidently related to the hard nature, bitter taste, harsh nutty flavor, and prolonged cooking time required to tenderize the seed even after soaking for considerable time.

Winged bean seeds also contain a high content (34%) of carbohydrates (Garcia and Palmer, 1980b). Though the constituent carbohydrates as determined by various researchers (Garcia and Palmer, 1980b; Sajjan and Wankhede, 1981; Kute et al., 1984; Ravindran and Palmer, 1984) have been presented as conflicting results, our earlier study on variety TPT-2 (Ravindran and Palmer, 1984) and that of Garcia and Palmer (1980b) has revealed the presence of 25–28% polysaccharides in the mature seeds with little or no starch as is the case with mature soybeans reported by Altschul (1958). The detergent fiber analysis of the winged bean seeds indicates a relatively low content (2–4%) of hemicelluloses and an unusually high content (12–14%) of cellulose or cellulose-like polysaccharides (Garcia and Palmer, 1980a). The available literature suggests a unique polysaccharide composition for mature winged bean seeds that probably plays an important role in determining the wider acceptability of winged bean seeds via its effects on cooking, digestion, and texture. Hence, the present investigation was undertaken to study the composition of polysaccharide in mature winged bean seeds of five varieties.

MATERIALS AND METHODS

Sample Preparation. Mature winged bean seeds of five varieties, SLS 1, SLS 3, SLS 6, SLS 44, and TPT 1, from the International Winged Bean Institute, Pallekelle, Sri Lanka, were ground in a Wiley Laboratory mill to pass through a 60-mesh sieve. The samples were defatted with a solvent mixture of chloroform–methanol (2:1) for 12 h and oven-dried at 60 °C to a final moisture content of about 6%.

Analytical Methods. Starch in the defatted winged bean flour was assayed by two methods, the enzymatic method (Thivend et al., 1972) and the perchloric acid method (Pucher et al., 1948).

The nonstarch polysaccharide (NSP) fraction was isolated from defatted flour by the procedure of Selvendran et al. (1981). This method involved removal of intracellular compounds with sodium deoxycholate, soluble sugars with 85% ethanol, protein with phenol–acetic acid–water (2:1:1), and starch with dimethyl sulfoxide (DMSO). The resulting insoluble residue was the crude nonstarch polysaccharide.

To determine the neutral sugars, the polysaccharide fractions (10 mg) were hydrolyzed with 3.0 mL of 1 N H_2SO_4 at 100 °C for 4 h, and 0.2 mL of the hydrolyzed samples was derivatized into aldononitrile acetates, as described by McGinnis (1982) and quantified by gas–liquid chromatography (Gow-Mac Instrument Co., Bridgewater, NJ). A reference sample with a known weight and composition of pure monosaccharides was subjected to the hydrolysis procedure together with the sample. The derivatized sugars in 2-μL aliquots of hydrolysates or standard solutions were eluted isothermally at 195 °C (helium, 25 mL/min) from a nickel alloy column (3.05 m × 3.2 mm) packed with 1% diethylene glycol adipate coated on 100–1200-mesh Chromosorb WHP (Supelco, Inc., Bellefonte, PA), using methyl α-D-*gluco*-pyranoside as internal standard.

The sugars in hydrolysates were quantified from peak areas relative to appropriate standard solutions prepared from high-purity sugars (Sigma Chemical Co., St. Louis, MO). Sugars were identified from GLC retention times relative to standards and the identities confirmed by thin-layer chromatography (Gauch et al., 1979). An independent analysis by high-performance liquid chromatography (HPLC), as described earlier (Ravindran and Palmer, 1984), was carried out to confirm the content of galactose, the major sugar of winged bean NSP.

Uronic acid in the polysaccharide fractions was determined by the method of Scott (1979). This method involved dissolution of 5-mg samples in 2 mL of concentrated H_2SO_4 at 4 °C, dilution to 10 mL, and reaction of the uronic acids with 125 μL of 2% NaCl, 2 mL of H_2SO_4 at 70 °C for 10 min, and then 0.1 ml of dimethylphenol in glacial acetic acid, followed by measurement of absorbance at 450 and 400 nm versus galacturonic acid standards. The uronic acid in winged bean polysaccharides has been earlier identified as mainly consisting of galacturonic acid (Ravindran and Palmer, 1984).

Cellulose was determined by the procedure of Updegraff (1969), which involved extraction of cellulose with acetic acid–nitric acid reagent and dissolution in 67% H_2SO_4. Sugars in the hydrolysate were determined by the phenol–sulfuric acid method (Dubois et al., 1956).

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Table I. Percent Composition of Polysaccharides in Defatted Winged Bean Seed Flour, Dry Weight Basis[a]

variety	% starch: perchloric acid	% starch: enzymatic	% starch: av	crude NSP	total polysacch
SLS 1	0.0	0.6	0.3	38.8	39.1
SLS 3	3.2	2.4	2.8	40.4	43.2
SLS 6	3.4	3.9	3.6	41.2	44.8
SLS 44	4.7	4.5	4.6	37.3	41.9
TPT 1	1.7	2.1	1.9	41.6	43.5

[a] Each value represents the mean of three determinations.

Nitrogen was determined by a micro-Kjeldhal procedure (Robinson, 1956) and lignin by permanganate oxidation (Goering and Van Soest, 1970).

To determine the solubility of the polysaccharide, the isolated fractions (5 mg) were boiled in 250 mL of water for 1 h, with constant stirring, filtered, dried, and weighed, and weight loss was determined. Supernatant was analyzed for carbohydrates by the phenol–sulfuric acid method versus a glucose standard.

RESULTS AND DISCUSSION

The proximate compositon of the seeds of the five winged bean varieties has been reported previously (Gajameragedera and Ravindran, 1988). The total carbohydrate content (carbohydrate content plus the fiber value) of the five cultivars ranged from 34.3–43.1%.

No starch was detected in cultivar SLS 1, while the other four cultivars contained 1.9–4.6% starch in defatted seed meal (Table I). These values are consistent with those reported by Kute et al. (1984) but differ from the value of 36.5% in defatted flour obtained by Sajjan and Wankhede (1981) and starch values reported by other workers (Garcia and Palmer, 180b; Ravindran and Palmer, 1984). Similarly Gajameragedara and Ravindran (1986) found no starch in the seeds of winged bean varieties SLS 1, SLS 3, and SLS 6, which were harvested at full maturity, but detected small amounts in the early stages of seed development. Hence, the small amounts of starch detected in the current study may have been due to the presence of immature seeds in the sample.

Nonstarch polysaccharide in the defatted flour of the five seeds ranged from 37.3 to 41.6% (Table I). The NSP values when added to their corresponding starch contents yielded total polysaccharide, which ranged from 39.1% in SLS 1 to 44.8% in SLS 6. These values are higher than the total carbohydrate contents obtained in the proximate analysis (Gajameragedera and Ravindran, 1988). The values from the latter included soluble sugars as well. This suggests that the NSP fractions may also contain some noncarbohydrate components.

Analysis of the NSP for liignin, protein, and ash confirmed the presence of 27.4–32.4% noncarbohydrate components (Table II). The relatively high percentage of associated protein (12.8–15.2%) in the NSP indicates a high degree of impurity. The ineffectiveness of phenol, a strong dissociating agent, suggests that this protein is an integral part of the polysaccharide. Due to its close association with the cell wall structures, this type of protein is reported to be extremely resistant to digestion (Theander, 1976). Whether this indigestible protein should be considered part of the polysaccharide complex is debated. Lignin is generally considered, but not clearly proven, as being partly linked to polysaccharide cell wall components, rendering extra rigidity and hydrophobicity to the cell wall.

Carbohydrate components in the crude NSP fractions account for 63.2–67.5%, with neutral sugars accounting for 39.6–46.0%, while cellulose and uronic acid represent 11.8–15.8% and 8.5–9.4%, respectively.

The composition of polysaccharides (Table III) was obtained by correcting the crude NSP fractions for the noncarbohydrate components. The composition of neutral sugar constituents indicates the presence of galactose, arabinose, xylose, glucose, rhamnose, and fucose and a clear preponderance of galactan (44–55%) in all the five winged bean varieties. Predominance of galactose was further confirmed by HPLC determination, which revealed the presence of 61, 62, 50, and 43% respectively, in SLS 1, SLS 3, SLS 6, and SLS 44 varieties. Our earlier work on winged bean, variety TPT-2, also showed galactan to be the major polysaccharide (Ravindran and Palmer, 1984).

Presence of 5.1–6.5% arabinose, 4.3–6.5% xylose, and small amounts of glucose, rhamnose, and fucose indicate heterogeneity in the polysaccharide. Glucose, arabinose, and xylose could also have arisen from fragments split from cellulose. Traces of arabinose and xylose are known to be intimately associated with the glucose molecules of the cellulose. Hydrolysis with 1 M H_2SO_4 for $2^1/_2$ h at 100 °C have been reported to hydrolyze about 5–10% of the cellulose in cereal products (Selvendran and Du Pont, 1980). Substantial amounts of uronic acid (12.8–14.3%) and the presence of rhamnose could suggest an independent pectic substances fraction, as well.

In most legumes, galactan, arabinan, and arabinogalactan are the main hemicellulosic NSP, though glucans and xylans are also reported to be present. Galactose is the major sugar in soybean and lupin, whereas arabinose is the major one in black gram, great northern bean, and ground nut (Brillouet, 1982). Winged bean NSP resembles those of soy and lupin but contains lower amounts of ar-

Table II. Percent Composition of Crude Polysaccharide Fractions of Defatted Winged Bean Seed Meal, Dry Weight Basis[a]

	SLS 1	SLS 3	SLS 6	SLS 44	TPT 1
galactose[b]	36.7 ± 1.3	36.1 ± 1.8	29.2 ± 1.3	30.6 ± 1.3	28.9 ± 1.7
arabinose[b]	3.6 ± 0.4	3.8 ± 0.3	4.3 ± 0.5	3.4 ± 0.3	3.2 ± 0.7
xylose[b]	2.9 ± 0.2	3.1 ± 0.5	4.3 ± 0.1	3.6 ± 0.2	3.2 ± 0.3
glucose[b]	0.8 ± 0.3	1.4 ± 0.1	2.1 ± 0.2	1.0 ± 0.1	2.5 ± 0.4
rhamnose[b]	0.7 ± 0.3	0.8 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.1
fucose[b]	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.3	0.9 ± 0.3	0.7 ± 0.2
total neutral sugars[b]	(45.6 ± 2.6)	(46.0 ± 3.0)	(42.0 ± 2.6)	(40.5 ± 2.4)	(39.6 ± 3.4)
cellulose	11.8 ± 1.9	12.2 ± 2.2	15.8 ± 1.6	13.9 ± 1.3	14.8 ± 1.1
uronic acid	9.4 ± 1.0	9.3 ± 0.4	8.5 ± 0.8	9.1 ± 1.3	8.8 ± 0.5
protein	13.6 ± 0.3	13.1 ± 0.2	12.8 ± 0.9	15.2 ± 0.2	14.7 ± 1.0
ash	4.8 ± 0.4	3.9 ± 0.7	4.3 ± 0.3	4.5 ± 0.3	3.2 ± 0.3
lignin	13.0 ± 1.4	10.4 ± 1.8	12.1 ± 1.2	12.6 ± 1.1	14.5 ± 1.9
recovery[c]	98.2 ± 7.6	94.9 ± 8.3	95.5 ± 7.4	95.8 ± 7.5	95.6 ± 8.1

[a] Each value represents the mean of three determinations ± standard deviation. [b] Neutral sugars are expressed as polysaccharides. [c] Sum of neutral sugars, cellulose, uronic acid, protein, ash, and lignin.

Table III. Percent Yield and Distribution of Sugars as Percent of Total Polysaccharide, Dry Weight Basis[a]

	SLS 1	SLS 3	SLS 6	SLS 44	TPT 1
yield	25.9	27.3	27.3	23.7	26.3
galactose	54.9	53.5	44.0	48.2	45.7
arabinose	5.4	5.6	6.5	5.3	5.1
xylose	4.3	4.6	6.5	5.7	5.1
glucose	1.2	2.1	3.2	1.6	3.9
rhamnose	1.0	1.2	1.6	1.6	1.7
fucose	1.3	1.2	1.5	1.4	1.1
cellulose	17.7	18.1	23.8	21.9	23.4
uronic acid	14.1	13.8	12.8	14.3	13.9
galactose to arabinose ratio	10:1	9:1	7:1	9:1	9:1

[a] Calculated from mean values of Table II.

abinose relative to galactose (Table III). Galactose to arabinose ratios of 2:1 and 2.5:1 have been reported in soy and lupin, respectively (Brillouet, 1982).

Yields of NSP from SLS 1, SLS 3, SLS 6, SLS 44, and TPT 1, after correction for noncarbohydrate components, were 25.9, 27.3, 27.3, 23.7, and 26.3%, respectively (Table III). These figures are lower than the value of 31% obtained for the variety TPT 2 (Ravindran and Palmer, 1984). Other than varietal differences, it is possible that the DMSO, which was used in the current study during the NSP extraction procedure to remove starch, may have solubilized some NSP (Selvendran and Du Pont, 1979).

In the current study, the NSP was isolated under mild conditions involving no heat. The extracted NSP was therefore cooked to determine their extent of solubility. The gravimetrical determination revealed that only 11.2, 12.4, 14.6, 9.2, and 12.7% of SLS 1, SLS 3, SLS 6, SLS 44, and TPT 1, respectively, were soluble at 100 °C for 1 h. These figures are slightly higher than the values obtained by total sugar analysis. The corresponding values obtained by sugar analysis are 10.1, 10.8, 12.3, 8.7, and 10.9%, respectively. The results suggest that winged bean polysaccharides are basically insoluble.

The overall results indicate that winged bean polysaccharide is virtually all NSP, which is a heterogeneous and insoluble component, incorporating protein, minerals, and lignin. The basic consistency of the data with regard to yields and the relative proportions of the sugars in the five varieties would indicate very little varietal effect on the polysaccharide composition of winged bean.

Registry No. Starch, 9005-25-8; lignin, 9005-53-2; cellulose, 9004-34-6; galactan, 9037-55-2.

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