Nonstarchy Polysaccharide Analysis of Cotyledon and Hull of *Lupinus albus*[1]

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ABSTRACT

The cotyledon and hull of a lupin cultivar sample (*Lupinus albus* L.2043N) were analyzed for polysaccharide composition. Nonstarchy polysaccharides (NSP) were obtained from the whole grain, hull, and cotyledon fractions after defatting and pronase/α-amylase hydrolysis. Cotyledon polysaccharides of the water-insoluble fraction (11.7%) contained galactose > arabinose > mannose. Cotyledon's water-soluble fraction (88.3%) contained galactose > mannose > arabinose. The presence of galactose and mannose suggested the presence of galactomannan. As expected, a high ratio of galactose to arabinose (3.3) in *L. albus* L2043N cotyledon indicated a high content of cell wall material. Fractions of cellulose, hemicellulose A and B, and oxalate-soluble material were separated by solubility properties. Arabinose was the dominant sugar in the hull and galactose in the cotyledon. The hull contained 56% NSP, most of which was hemicellulose, which represented 65% of the total NSP in the whole grain. The oxalate-soluble and cellulose fractions were not completely separated from hemicellulose, as indicated by the presence of arabinose and xylose in the former and arabinose in the latter.

Since the 1960s, interest in lupin as a protein and oil source for humans and animals has increased. The development of a low alkaloid species enhanced lupin utilization for human and feedstock (Gladstones 1970).

Cell wall material varies among lupin species ranging from 8 to 35% (Brillouet and Riochet 1983). However, high amounts of cell wall material in the cotyledon may lower its nutritional value (Carre et al 1985). The cell wall material (CWM) of lupin cotyledon can be fermented in the hindgut of ruminants and produce available energy (Rerat 1978, Nyman and Asp 1982).

Tomata and Kitamura (1967) found that two major polysaccharides from yellow lupin (*L. luteus*) contained mainly galactose and arabinose residues associated with galacturonic acid. Hirst et al (1947) reported that the cotyledon cell wall polysaccharides from white lupin were linear β(1→4) galactans. Carre et al (1985) suggested that galactose in lupin is a linear galactan linked to rhamnogalacturonan backbones. The galactan in the cell wall has been reported as a storage carbohydrate that is utilized during germination (Crawshaw and Reid 1984, Salimath and Tharanathan 1982). During germination of *Lupin luteus* cotyledons, the arabinogalactan side chains of the pectic polysaccharide fraction were selectively hydrolyzed leaving a primary wall with high uronic acid content (Matheson and Saini 1977).

Raymond et al (1974) used a mixture of cellulase and hemicellulase to hydrolyze a depectinated hull fraction of *L. angustifolius*. After a 48-hr digestion, the glucose oxidase method showed that cellulose was higher than hemicellulose. The oxalate-soluble fraction contains galactose, arabinose, xylose, and uronic acids (McNeil et al 1980, Evans et al 1993). Xylose has been reported as the main sugar in the hemicellulose A fraction and arabinose in the hemicellulose B fraction of *L. angustifolious* (Brillouet and Riochet 1983). According to Evans et al (1993), the hull of a *L. angustifolius* contained 88% fiber on dry basis as measured by the AOAC gravimetric method.

Lupin hull forms 25% of the whole seed (Raymond et al 1974). The hull from different lupin cultivars is poorly lignified (Evans et al 1993). Lignin causes growth depression, and a lack of lignin in lupin seeds is advantageous for its use as feed.

Limitation of lupin seed adapted to United States environments is reflected in a few literature reports and a little utilization of lupin from this area of the world. The breeding program directed by Gene Aksland (Resource Seeds Inc., Gilroy, CA) has improved several production aspects of lupin cultivation in the Northern Plains region of the United States.

The objective of this study was to investigate the starch and nonstarchy polysaccharide fractions of cotyledons and hull from a lupin cultivar adapted to the Northern Plains environment.

MATERIALS AND METHODS

Chemicals

Pronase from *Stryptomyces bacillus* (6 units/mg), α-amylase (E.C. 3.2.1.1) from *Bacillus subtilis* (1,350 units/mg), trifluoracetic acid, sodium borohydride (20 mg of $NaBH_4$+ 1 ml of dimethylsulfoxide), 1-methylimidazole, glacial acetic acid, and ammonium hydroxide were from Sigma Chemical Co. (St Louis, MO).

Lupin Sample

L. albus L.2043N seed, a low alkaloid (0.009%) line, was donated by Gene Aksland, Resource Seeds Inc., Gilroy, CA. The seeds were grown in Gilroy, CA, in 1992 and stored at –7°C until analysis.

Sample Preparation

Lupin seeds were hand-dissected, and the germ was discarded. Hull and cotyledon were analyzed separately.

Cotyledon Polysaccharides

Cotyledon samples were ground to pass through a 40 U.S. mesh sieve (425 μm). Lipids and pigments were extracted in a Soxhlet apparatus, by successive treatment with chloroform-methanol (2:1, v/v) and methanol-water (80:20, v/v). The samples were extracted overnight, and solvent was allowed to evaporate at room temperature inside a hood. Five grams of defatted samples were suspended in 150 ml of 0.1*M* sodium phosphate dibasic buffer, pH 7.5, containing 0.02% sodium azide. The suspension was homogenized for 10 min at medium speed, using an Ultra Turrax homogenizer (Janke & Kunkel KG, Germany).

Proteolysis was accomplished with pronase (27 mg in 5 ml of 0.1*M* phosphate buffer, pH 7.5) for 9 hr at 30°C, followed by

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centrifugation at 2,060 × *g* for 15 min. The residue was resuspended in phosphate buffer (0.1*M*, pH 7.5) containing 15 mg of pronase and incubated at 30°C for 6 hr, followed by centrifugation at 2,060 × *g* for 15 min. The supernatant was saved, and the residue was heated in 70 ml of water to 95°C with constant stirring. The suspension was cooled to 50°C and treated with α-amylase (0.0833 mg in 7 ml of 0.2*M* sodium acetate buffer, pH 5.6) for 3 hr. The suspension was centrifuged at 2,060 × *g* for 15 min and the supernatant saved. The residue was washed five times with excess water and dried with excess ethanol, acetone, and diethyl ether. The resulting white powder was considered water-insoluble polysaccharides (WIP).

Supernatants from pronase and α-amylase treatments were pooled and filtered in an Amicon ultrafiltration device (W. R. Grace & Co. Beverly, MA) using YM1 1,000 MW cutoff Diaflo membrane and 20 psi of N_2 to separate the peptides and sugars produced by the enzymatic action. The water-soluble polysaccharides (WSP) with MW > 1,000 retained in the residue were recovered, and five volumes of ethanol were added. The resulting precipitate was recovered by centrifugation, washed with water, and dried with solvents.

Compositional Analysis of Cotyledon Polysaccharide.

The WIP and WSP were hydrolyzed with 2*M* TFA for 2 hr at 121°C, as described by Albersheim et al (1967). Liberated neutral sugars were analyzed as their alditol acetate derivatives with gas chromatography, as described by Blakeney et al (1983). A gas chromatograph Hewlett Packard HP 5890 Series 2 (Hewlett Packard, San Fernando, CA) with a mass selective detector (MSD) HP5971 and a SP2380 (Supelco, Bellefonte, PA) column (30 m × 0.25 mm) with 0.25 μm film thickness was used. A two-stage program was utilized; the initial temperature of 100°C was increased to 230°C at 30°C/min rate. The second stage increased the temperature to 250°C at 4°C/min. Myo-inositol (Sigma) was used as an internal standard in the lupin fractions and standard samples. Results were expressed on a dry weight basis.

Hull Polysaccharides

The dry hull was milled to pass through a 40 U.S. mesh sieve (425 μm). Four-gram samples were boiled in 400 ml of water for 10 min and filtered through glass microfiber filter paper (GF/G, Whatman Int., Maidstone, England) The filtrate was considered WSP. The total carbohydrate content in this fraction was determined with the phenol-sulfuric acid method (Dubois et al 1956) using anhydroglucose as a standard.

The residue was refluxed for 2 hr in 400 ml of 0.5% ammonium oxalate and filtered (Whatman paper #1). This filtrate was considered mostly pectin material associated with hemicellulose. The filtrate was acidified with 0.1*N* HCl to pH 6.5, and five volumes of ethanol were added to precipitate pectins. The residue was recovered by centrifugation and dried with excess ethanol, acetone, and diethyl ether.

The residue from the oxalate was treated with 0.5% KOH for 6 hr under N_2 and constant mixing. The solution was filtered, and the residue was treated with 24% KOH overnight (16 hr) under N_2 and filtered. The filtrates from the two treatments were combined and acidified to pH 5.8 to precipitate the hemicellulose A which was recovered by centrifugation (2,060 × *g*, 15 min). The residue was dried with solvents.

The supernatant was added to five volumes of 95% ethanol to precipitate the hemicellulose B, which was recovered by centrifugation (2,060 × *g*, 15 min). The residue after the KOH treatments was washed five times with water and dried with solvents. The washed residue was considered the cellulose fraction. A residue from the oxalate treatment received an extended treatment with 0.5% KOH and 24% KOH for 24 hr and 26 hr, respectively, to improve the separation of cellulose and hemicellulose.

Compositional Analyses of Hull Carbohydrates

For the carbohydrate analyses, all the fractions were hydrolyzed according to the method of Albersheim et al (1967) with the following modifications: 1) 250 μl of 3*M* TFA were added to 2 mg of sample and hydrolyzed at 135°C for 4 hr; 2) liberated neutral sugars were dried under N_2 and reduced with 0.5 ml of sodium borohydride for 90 min at 40°C, and nine drops of glacial acetic acid were added; 3) sugars were acetylated with 100 μl of 1-methylimidazole and 0.5 ml of acetic anhydride for 25 min at room temperature; and 4) 5 ml of water and 1 ml of dichloromethane were added to extract the derivatives. The bottom layer containing the derivatives was removed, placed into new tubes, and dried under N_2. The dried derivatives were redissolved in 1 ml of acetone and aliquots injected into the GC-MS.

The fractions from the procedure that used 24 and 26 hr KOH treatments were hydrolyzed, according to the method of Fengel and Wegener (1979), but modified by using 100% TFA and 90°C temperature in the hydrolysis. The liberated sugars were derivatized to alditol acetates according to the method of Blakeney et al (1983). The derivatives (3 μl) were injected in a gas chromatograph HP5890A (Hewlett Packard, San Fernando, CA) equipped with a flame ionization detector (FID). An SP2330 (Supelco, Bellefonte, PA) column (30 m × 0.25 mm) with 0.20 μ film thickness of nonbonded 90% biscyanopropyl and 10% phenyl polysiloxane was used. The oven and detector temperatures were 250°C and the injector temperature was 230°C. Helium was used as a carrier gas at a column flow rate of 1 ml/min.

Proximate Analysis

Protein (N × 6.25) was determined using AACC method 46-10 Moisture was determined using method 44-19 (AACC 1983).

RESULTS AND DISCUSSION

Whole Grain Composition

Lupin whole grain polysaccharides are reported in Table I. The starch content (3%) was comparable to the reports in soybeans and, as expected, all was contained in the cotyledon. The total NSP were 36.9% from which the majority were water-insoluble (79.9%).

TABLE I
Percentage of Polysaccharides in Whole Grain, Cotyledon, and Hull of *Lupinus albus* L.2043N[a]

Fraction	Water-Soluble NSP %	Water-Insoluble NSP %	Cellulose	Hemicellulose
Cotyledon	3.2	24.1	...	...
Hull	0.9	55.8	19.7	70.8

[a] Average of two determinations expressed on a dry weight basis.

TABLE II
Neutral Sugar Composition of the Cotyledon Nonstarch Polysaccharides of *Lupinus albus* L.2043N Analyzed by Gas Chromatography Mass Spectroscopy[a]

	Sugar, %						
Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
Water-soluble	Tr[b]	0.1	12.5	2.8	18.1	55.6	10.9
Water-insoluble	2.2	1.8	22.1	9.9	ND[c]	59.7	4.5

[a] Average of two determinations and expressed on dry weight basis.
[b] Trace.
[c] Not detected.

Composition of the WSP in *L. albus* Cotyledon

The cotyledons water-soluble NSP (Table I) represented about a two-thirds yield of the total water-soluble NSP polysaccharides. The water-soluble NSP fraction contained 3% protein that resisted pronase hydrolysis; the fraction may contain glycoproteins, or protein physically entrapped, linked to polysaccharides or phenolic compounds (Carre et al 1985).

The water-soluble NSP from *L. albus* cotyledon comprised 11.7% by weight (db) of the total polysaccharide (Table I) content in the cotyledon. The water-soluble NSP were mainly made up of galactose, followed by mannose and arabinose (Table II). This sugar composition may indicate the presence of galactomannan similar to that of other legumes (Bailey 1967, Bailey et al 1974) but further investigations are needed to confirm this. Evans et al (1993) reported galactose (67.3%) and arabinose (13%) are the main monosaccharides in cotyledon from *L. angustifolius* grown in Australia. In contrast, 4*M* KOH-extractable pectic polysaccharides from mung bean cotyledon cell wall had highly branched arabinorhamnogalacturonans with significant amounts of terminal xylopyranosyl residues (Gooneratne et al 1994). The cell wall material and the soluble fraction from lupin cotyledons behave like soluble fiber, i.e., they are fermentable, lower the hindgut pH, and reduce the plasma cholesterol in ruminants and rats (Carre et al 1985, Evans and Cheung 1991).

Composition of the WIP in the Cotyledon

The first pronase treatment hydrolyzed most of the proteins, probably storage proteins. However, 2.3% protein (db) still remained associated to the WIP fraction and resisted pronase action. The enzymatic determination of starch content yielded 3% and was comparable to other reports of low (2.5%) starch content in lupin seeds (Evans et al 1993) and <1% in soybeans (Snyder and Kwon 1987). The lupin cotyledon sample (Table I) showed high water-insoluble NSP yield (24.1%) compared to that of other legumes, e.g., broad beans (7.1%), soybeans (9%), peas (7.1%), and *L. luteus* (12.3%) (Salimath and Tharanathan 1982, Brillouet and Carre 1983).

Lupin cotyledon water-insoluble fraction was made up mainly of galactose (59.7%), followed by arabinose (22.1%) (Table II). The low glucose content (4.5%) could indicate the presence of cellulose. The galactose composition agreed with results reported on lupin by Brillouet and Riochet (1983), but had lower rhamnose and higher arabinose contents than those reported by Evans et al (1993).

The sugar composition of lupin cotyledon nonstarchy polysaccharides (Table II) showed lower amounts of rhamnose than in other legumes (Brillouet and Riochet 1983). Variation of galactose content in lupin seeds was reported by Brillouet and Riochet (1983). Galactose is also found in other crops, such as potatoes which contain mainly galactose in the cell walls (Ring and Selvendran 1979). The galactose to arabinose (gal:ara) ratio seems to be related to the amount of cell wall material in lupin cotyledon (Brillouet and Carre 1983) and in this study there was a 3.3 gal/ara ratio. This value is higher than that reported in pea and broad bean (≈0.1) and soybeans (≈2.4) (Brillouet and Carre 1983).

Composition of Lupin Hull Fraction

Five fractions were obtained from lupin hulls. The alkaline extraction was accomplished without delignification, yielding two fractions. The hemicellulose was fractionated into hemicellulose A and B. The second fraction was insoluble in 24% KOH. The results suggested that cellulose remained associated with hemicellulose (Table III). The cellulose fraction contained 37.8% arabinose, 28.8% glucose, and 13.9% galactose, indicating a contamination with hemicellulose and pectic substances. The fractions of the extended treatment with KOH 24 and 26 hr slightly improved the separation (Table IV). The cellulose fraction contained less arabinose (35.9%), xylose (15.4%), and galactose (12.7%), and higher glucose content (33.9%) than the procedure using 6 and 16 hr KOH treatment.

The occurrence of both galactose and mannose could indicate the presence of galactomannan in soybean hull (Aspinall and Whyte 1964), but further confirmation is required. The hemicellulose A fraction contained xylose as dominant sugar, followed by arabinose (Table IV). A large portion of this xylose might have originated from the secondary cell wall, as reported by Bailey et al (1974). The hulls from North American lupin cultivars can contain low cellulose (Brillouet and Riochet 1983).

Bailey et al (1974) reported that hemicellulose B of *L. angustifolius* hulls contained arabinose as the dominant sugar. However, xylose was the major sugar in the *L. albus* used in this study (Tables III and IV). The hemicellulose B fraction contained higher glucose content than that reported in the literature, indicating the presence of cellulose. Small amounts of rhamnose were detected in the hemicellulose B fraction, and no mannose was found in hemicellulose A (Tables III and IV).

The hull contained 56.7% total NSP, and most of it was hemicellulose (70.8%). The neutral sugar content of the oxalate-soluble fraction was identified as arabinose, xylose, and galactose (Tables III and IV). The hull of *L. angustifolius*, grown in Australia, contained 85% NSP and 13.6% oxalate-soluble substances (Evans et al 1993); both values higher than *L. albus* used in this study (65.7 and 7.9%, total NSP and oxalate soluble substances in the whole grain, respectively; Table I). These results confirm the higher content of pectic substances in lupins than in soybean hulls (4.4%) (Bailey et al 1974).

Bailey et al (1974) reported that none of the sugars (arabinose, xylose, and galactose) interfered with the 3,5-dimethylphenol during the determination of uronic acids. The sugar composition of the oxalate-soluble fraction showed an association between

TABLE III
Neutral Sugar Composition of the Hull Nonstarch Polysaccharides of *Lupinus albus* L.2043N Analyzed by Gas Chromatography Mass Spectroscopy[a]

	Sugar, %						
Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
Cellulose	ND[b]	0.3	37.8	17.3	1.9	13.9	28.8
Oxalate soluble	ND	0.7	58.7	16.7	3.7	17.0	3.2
Hemicellulose-A	ND	ND	10.0	84.2	ND	1.4	4.4
Hemicellulose-B	0.2	1.5	12.0	62.7	1.0	6.3	16.4

[a] Average of two determinations expressed on a dry weight basis.
[b] Not detected.

TABLE IV
Neutral Sugar Composition of the Hull Nonstarch Polysaccharides[a] of *Lupinus albus* L.2043N Extracted 50 hr with KOH[b] and Analyzed by Gas Chromatography Flame Ionization Detection

	Sugar, %						
Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
Cellulose	ND[c]	0.2	35.9	15.4	1.9	12.7	33.9
Oxalate-soluble[d]	ND	0.7	58.7	16.7	3.7	17.0	3.2
Hemicellulose-A	ND	ND	12.2	82.1	ND	1.7	4.1
Hemicellulose-B	0.2	1.6	12.6	65.6	0.9	6.6	12.5

[a] Average of two determinations expressed on a dry weight basis.
[b] 0.5% KOH and 24% KOH for 24 and 26 hr, respectively.
[c] Not detected.
[d] Analyzed by gas chromatography mass spectroscopy.

pectic substances and arabinogalactan (Tables III and IV) as reported by Blanche and Gailard (1958) in other legumes. Rhamnose was not detected in the oxalate-soluble fraction (Tables III and IV).

Legume hulls contain undigestible material, and a dehulling step may be needed before processing for oil and meal extraction, i.e., during soybean processing. Lupin hull material has been reported 89% digested by pigs (Gladstones 1971). All lupin cultivars reported in the literature have low lignin content (Bailey et al 1974, Evans et al 1993). There are no reports of growth depression in animals fed with lupin, probably due to the acceptable levels of polysaccharide digestibility and low lignin content (Reichert 1981). Unlike other legumes, dehulling is not necessary when lupin is used as animal feed.

CONCLUSIONS

Cotyledon NSP of *L. albus* L.2043N showed a water-soluble fraction was mostly made of galactose and mannose. The cotyledon contained 27.3% total NSP, of which 3.2% was water-soluble, 24.1% was water-insoluble, and 3% was starch. The water-insoluble fraction was mainly a mixture of galactose and arabinose.

The hull contained 63% of the seed's polysaccharides, of which 70.8% was hemicellulose and 19.7% was cellulose, and 1.6% was water-soluble material. Extended time (50 hr) treatment of the oxalate-residue with KOH resulted in slightly better separation of the cellulose fraction with less hemicellulose contamination. Xylose was the most abundant sugar in the hull followed by arabinose. The oxalate-soluble substances in lupin whole grain (7.9%), were higher than those in soybeans (4.4%).

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