Cell Wall Polysaccharides of Navy Beans (*Phaseolus vulgaris*)

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Cell wall (CW) materials of selected navy bean cultivars/line (C-20, Seafarer, Fleetwood, and experimental line 84004) were isolated and fractionated into six fractions: hot water soluble polymer (HWSP), ammonium oxalate soluble polymer (AOSP), hemicelluloses A and B, cellulose, and lignin. Seed coat CW was rich in cellulosic structural polysaccharides (range 58.7–65.0%) and lignin (range 1.4–1.9%), whereas cotyledon CW was composed principally of matrix polysaccharides and was especially rich in HWSP (range 25.7–32.5%) and H_B (14.6–19.2%). Cotyledon CW contained 11–14% protein which was resistant to proteolysis and/or inaccessible to proteolytic enzymes. Fractionation of CW materials should provide a better basis for understanding the diverse physiological responses encountered when dietary fiber is consumed than if only dietary fiber, soluble fiber, or nonsoluble fiber is known.

INTRODUCTION

Starch and protein components of dry beans (*Phaseolus vulgaris*) have been extensively studied, whereas cell wall polysaccharides (fiber) have received little attention until recent years. Because of increased consumer awareness in health benefits of dietary fiber, several studies have been conducted to develop a standard methodology for measuring total dietary fiber (Prosky et al., 1984; Mongeau and Brassard, 1986; Asp and Johansson, 1981). In addition, Anderson and Bridges (1988) suggested that not only total dietary fiber but also soluble polysaccharides, noncellulose polysaccharides, cellulose, and lignin, and constituent sugars of the soluble and noncellulose polysaccharides be considered in clinical research. These data are essential for accurate prediction and explanation of the physiologic and therapeutic effects of fiber since different types of fiber elicit different physiological responses such as decreasing gastrointestinal transit time, lowering blood cholesterol, slowing nutrient absorption, reducing hyperglycemia, decreasing diverticulosis, and perhaps aiding maintenance of appropriate weight [see references cited in Leeds (1990)]. For food research, detailed qualitative and quantitative information on plant cell wall components and their chemical composition and structural organization is vital for a better understanding of the interrelationships between their functional properties and product performance under various processing conditions.

Methods for isolation and characterization of legume polysaccharides have not been well characterized and established. The polysaccharide content of various legume seeds has been studied with a variety of isolation and fractionation procedures. These include pinto bean (Monte and Maga, 1980), field bean (Salimath and Tharanathan, 1982), smooth field pea, broad bean, and soya (Brillouet and Carre, 1983), and kidney bean, lentil, and chickpea (Champ et al., 1986). Only one group, Salimath and Tharanathan (1982), has classified cell wall components according to solubility, which is regulated by bond types and bonding strength in differential extraction solvents.

The composition of polysaccharides varies qualitatively and quantitatively according to type of plant tissue studied. The purposes of this research were to prepare cell wall materials from navy bean seed coats and cotyledons and then to characterize the isolated cell walls by using sequential solvent extraction. The hot water soluble polysaccharides of cotyledon tissue were further analyzed for their neutral sugar composition and uronic acid content. Navy bean cultivars C-20, Seafarer, and Fleetwood and experimental line 84004 were selected due to their inherent diversity in general processing quality and acceptability as previously reported by Ruengsakulrach (1990).

MATERIALS AND METHODS

Materials. Navy bean samples (C-20, Seafarer, Fleetwood, and experimental line 84004) were obtained locally. Enzymes used to prepare cotyledon cell walls were components of a total dietary fiber assay kit (TDFAB-1) from Sigma Chemical Co. which included α-amylase, amyloglucosidase, and protease. Galacturonic acid monohydrate, sodium tetraborate, *m*-hydroxybiphenyl, and standard sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) were purchased from Aldrich Chemical Co.

Methods. *Sample Preparation.* Whole dry bean seeds were soaked at 4 °C in deionized distilled water for 45 min prior to manual decortication. This soaking treatment, as shown by analysis of whole seeds and soaking water, did not result in a significant loss of material into soaking water. The seed coats and cotyledons were subsequently freeze-dried and ground with a Udy cyclone mill through 20-mesh screen to yield seed coat and cotyledon flours.

Cell Wall Preparation. All flour samples were first defatted with petroleum ether in a Goldfisch extractor for 4 h. Since seed coat tissues contain a small amount of proteins and no starch, their cell walls were prepared by removing small, relatively polar molecules (such as sugars and phenolic acids) from defatted seed coat flours with 80% alcohol. The defatted seed coat flour was extracted (3×) with 80% ethanol at 80 °C for 30 min to solubilize free sugars and phenolic compounds. The insoluble residue, termed alcohol-insoluble cell wall (AICW), was collected by centrifugation (17300*g* for 20 min), dried under vacuum, and kept for cell wall fractionation.

Isolation of cotyledon cell wall required additional steps to remove the large quantities of starch and protein found in bean cotyledon. Enzymatic hydrolyses used for total dietary fiber determination (AOAC, 1985) were applied with extended incubation periods as follows: amylase, 2 h; protease, 8 h; and amyloglucosidase, 16 h. Four volumes of ethanol were used to precipitate soluble polysaccharides. The precipitates recovered by centrifugation (17300*g* for 20 min) were designated as purified

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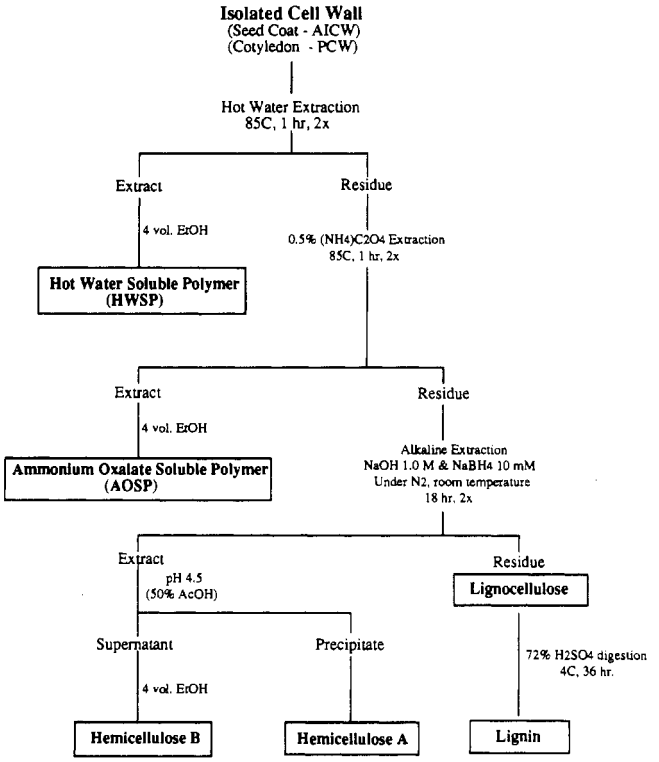

Figure 1. Procedure for fractionation of cell walls.

cell wall (PCW), which were further washed with 80% aqueous ethanol (1×) and absolute ethanol (2×) and then dried under vacuum. A reagent blank was run along with the samples and used to correct for precipitates contributed by reagents. Parts of PCW were analyzed for ash (AACC Method 08-01) and protein (AOAC Method 1424.038).

Cell Wall Fractionation. Cell wall materials (AICW and PCW) were fractionated as outlined in Figure 1. This fractionation scheme is from the methods of Ring and Selvendran (1980) and Salimath and Tharanathan (1982). On the basis of these methods, six fractions were obtained: hot water soluble polymer (HWSP), ammonium oxalate soluble polymer (AOSP), hemicellulose A (H_A), hemicellulose B (H_B), and lignocellulose (cellulose plus lignin). Cellulose content was obtained by subtracting lignin content from the lignocellulose content. In this scheme, cellulose, H_A, and H_B are based on gravimetric determinations after selective, sequential extraction of AICW and PCW rather than on sugar analyses.

Uronic Acid Determination. The uronic acid content of the HWSP was analyzed by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hanson, 1973) using galacturonic acid as a standard.

Neutral Sugar Analysis. The methodology of York et al. (1985) was utilized for neutral sugar determination. To obtain free glycoses, cell wall samples (15–20 mg) were hydrolyzed for 1 h at 121 °C with 1 mL of 2 M trifluoroacetic acid (TFA). After hydrolysis, the TFA was evaporated at 40 °C under a stream of N_2. Isopropyl alcohol (0.5 mL) was added to the apparently dry hydrolysate and then evaporated to remove remaining traces of TFA. The glycoses were then reduced to the corresponding alditols by adding 1 mL of 1 M ammonia containing 10 mg/mL sodium borohydride. The reaction was allowed to continue for 1 h at room temperature, after which time the excess sodium borohydride was destroyed by dropwise addition of glacial acetic acid. Glacial acetic acid–methanol (1:9 v/v, 0.5 mL) was added, and the resulting solution was evaporated to dryness at 40 °C. The drying process with glacial acetic acid–methanol was repeated two more times. Three evaporations with 0.5 mL of methanol were then performed. The alditols were then acetylated with acetic anhydride by adding 1 mL of a 1:1 mixture of acetic anhydride and pyridine. Samples were heated at 121 °C for 1 h to complete acetylation. After acetylation, toluene (0.5 mL) was added to the resulting solution and the solution was evaporated at room temperature under a N_2 stream. The dried sample was mixed with 0.5 mL of toluene and evaporated a second time. Finally, the acetylated alditols were partitioned between dichloromethane (1 mL) and deionized distilled water (1 mL). The dichloromethane layer was transferred to a clean tube and evaporated at room temperature under a stream of N_2. The alditols were then redissolved in 0.5 mL of dichloromethane and kept at −20 °C until they were analyzed by gas–liquid chromatography (GLC). General procedures for GLC analysis of alditol acetates are described in York et al. (1985) and in Bulletin 774B from Supelco, Inc.

The acetylated alditols were analyzed with a Varian 3700 gas chromatograph equipped with a flame ionization detector. The alditol acetates were separated on a 30 m × 0.53 mm i.d., wide bore, capillary column with a 0.2 μm thick film of SP-2380 (Supelco, Inc.). Column temperature was programmed from 160 to 240 °C at 5 °C/min. Injector and detector temperatures were set at 260 °C. Carrier gas (He) flow was 5 mL/min, and make-up gas (N_2) flow was 30 mL/min. Relative weight percentages of the alditol acetates were quantitated with a Varian CDS-111 integrator. Peaks were identified by their retention times compared to standard alditol acetate mixtures purchased from Supelco, Inc.

RESULTS AND DISCUSSION

Cell Wall Preparation. Seed coat and cotyledon tissues require different methods for cell wall preparations due to the differences in chemical composition. The alcohol-insoluble cell wall fraction (AICW) in navy bean seed coats ranged from 91.96% for Seafarer to 95.33% for C-20 (Table I).

Cotyledon tissues require extensive enzymatic hydrolysis treatments to remove starch and protein. The cotyledon flours contained 16.32–18.02% PCW and PCW contained 6.94–8.47% ash and 11.36–14.24% protein as shown in Table I. With an 8-h proteolysis treatment, 90–93% of the protein in cotyledon flours was successfully removed as determined by micro-Kjeldalh analysis. Negative I_2 test indicated the absence of starch in PCW. Only slight variations in PCW residual protein and residual ash were found among navy bean cultivars/line.

Cell Wall Fractionation. The isolated cell wall fractions are classified according to their solubility, which is determined by types of bonds and bonding strength. As shown in Table II, 93.3–96.1% of the seed coat AICW was recovered in the various cell wall fractions. For the cotyledon, 75.21–89.84% of the PCW was recovered in the various cell wall fractions. The lower recoveries for PCW may be related to residual protein and ash in the PCW. If residual protein or ash in PCW is solubilized during the fractionation procedure, this would lead to apparent low recoveries of PCW.

Cellulose was the major carbohydrate in seed coats. Significant differences ($P < 0.05$) in cellulose content were found among cultivars. This implies differences in seed coat cell wall strength since the crystalline nature of cellulose provides rigid structural characteristics to the cell wall. Hemicellulose was the second largest constituent of seed coat cell walls. Seed coat cell wall hemicellulose had equal or more H_A than H_B. The contents of H_A and H_B varied among cultivars, especially H_B. The ratios of H_A to H_B ranked from 1.97 in experimental line 84004 to 1.06 in Seafarer. The relationship of H_A and H_B to cell wall strength is not clear from the literature.

The cation-bound pectin (AOSP) present in bean seed coats ranged from 9.54 to 12.71%. This fraction possessed gellike characteristics in 80% ethanol. Precipitation of this material by centrifugation at 23000*g* for 15 min produced a transparent, hard, gellike material, indicating good liquid-holding capability. Seed coat cell walls contain very small amounts of HWSP (2.59–3.98%); C-20 con-

Table I. Data (%, Dry Basis) from the Cell Wall Preparations of Seed Coat and Cotyledon Tissues[a]

		cotyledon			
bean cultivar	sead coat AICW[b]	total protein[c]	PCW[b]	residual protein[d]	residual ash[d]
C-20	95.33 ± 0.10c	30.02 ± 0.07c	16.62 ± 0.47a	14.24 ± 0.32b	8.47 ± 0.91a
Seafarer	91.96 ± 0.27a	25.77 ± 0.12a	17.08 ± 0.60ab	11.36 ± 1.25a	7.07 ± 0.02a
Fleetwood	92.24 ± 0.03a	28.19 ± 0.04b	18.02 ± 0.55b	14.27 ± 0.19b	6.94 ± 1.46a
84004	93.52 ± 0.09b	30.45 ± 0.16d	16.32 ± 0.93a	13.55 ± 0.88ab	8.09 ± 0.57a

[a] Means in a column followed by different letters are significantly different ($P < 0.05$). [b] The yields of alcohol-insoluble cell wall (AICW) and purified cell wall (PCW) were reported as percent (dry basis) of seed coat ($n = 3$) and cotyledon tissues ($n = 7$), respectively. [c] Total protein expressed as percent (dry basis) in cotyledon flour. [d] Residues are expressed as percent (dry basis) in purified cell wall (PCW) ($n = 2$).

Table II. Percentage Distribution of Cell Wall Fractions[a] in Seed Coats and Cotyledons[b]

bean component	bean cultivar	HWSP	AOSP	hemicellulose (H) total	H_A	H_B	cellulose	lignin	sum of fractions
seed coat	C-20	2.42 ± 0.38a	9.85 ± 0.24ab	18.68 ± 0.52a	10.53 ± 0.55a	8.14 ± 0.03b	60.70 ± 0.83c	1.68 ± 0.25bc	93.32 ± 1.01a
	Seafarer	3.79 ± 0.09b	12.13 ± 0.77c	19.66 ± 0.33ab	10.11 ± 0.27a	9.55 ± 0.14c	58.43 ± 0.58b	1.37 ± 0.05ab	95.38 ± 0.98b
	Fleetwood	3.57 ± 0.16b	8.90 ± 0.07a	20.80 ± 0.98b	13.48 ± 1.23b	7.32 ± 0.25a	58.65 ± 1.15b	1.38 ± 0.06ab	93.29 ± 0.31a
	84004	3.55 ± 0.10b	9.87 ± 0.62b	24.40 ± 0.55c	16.17 ± 0.32c	8.23 ± 0.31b	56.42 ± 0.51a	1.86 ± 0.22c	96.10 ± 0.85b
cotyledon	C-20	23.70 ± 1.86b	9.05 ± 0.34a	22.01 ± 1.15c	4.50 ± 0.51d	17.52 ± 0.87b	27.12 ± 0.91a	0.39 ± 0.10a	82.15 ± 1.91b
	Seafarer	25.23 ± 1.79b	12.81 ± 0.30c	20.52 ± 0.79b	1.30 ± 0.14a	19.22 ± 0.74c	30.91 ± 0.57b	0.56 ± 0.02a	89.84 ± 1.91c
	Fleetwood	19.35 ± 0.49a	12.40 ± 0.25c	17.18 ± 0.43a	2.58 ± 0.05b	14.60 ± 0.38a	25.98 ± 0.29a	0.45 ± 0.03a	75.21 ± 1.06a
	84004	28.41 ± 1.25c	11.42 ± 0.37b	20.64 ± 0.21bc	3.78 ± 0.29c	16.86 ± 0.37b	26.80 ± 1.73a	0.39 ± 0.09a	87.53 ± 0.30c

[a] All cell wall fractions: hot water soluble polymer (HWSP), ammonium oxalate soluble polymer (AOSP), hemicellulose (H), cellulose, and lignin, were reported as percent of AICW and PCW in seed coat and cotyledon, respectively. [b] $n = 3$. Means in a column followed by different letters are significantly different ($P < 0.05$).

tained a lower amount ($P < 0.05$) of HWSP than the other three cultivars/line. The lignin content of seed coat cell walls (1.44–1.94%) is comparable to earlier findings (Champ et al., 1986), with small, but significant ($P < 0.05$) differences among the four cultivars/line.

In contrast to the seed coat cell walls, cotyledon cell walls contained much more HWSP and H_B with much less cellulose and lignin. Cellulose made up approximately one-third (30.61–34.55%) of the cotyledon cell walls, whereas cellulose constitutes nearly two-thirds of the seed coat cell walls (58.71–65.04%). Although the total amounts of hemicellulose in cell walls of seed coats and cotyledons are comparable, the relative amounts of H_A and H_B were very different in cotyledon compared to seed coats. H_B is the predominant form in the cotyledon cell wall, while H_A is equal to or greater than H_B in the seed coat cell wall. AOSP contents are comparable in cotyledon cell wall and in seed coat cell wall. The HWSP fractions of cotyledon cell walls (25.73–32.47%) were dramatically higher than those observed in the seed coat (2.59–3.82%). Further, 2.5–3.0 times less lignin was found in cotyledon cell walls (0.44–0.63%) than in seed coats. Cell wall composition indicates that cotyledon cell walls are more flexible (less rigid) than seed coats. These findings are consistent with the concept that bean seed coats limit seed expansion and water absorption.

Among the four bean samples, the cotyledon cell wall of 84004 had the highest HWSP content (32.47%) and the lowest cellulose content (30.61%). Conversely, the cotyledon cell wall of Fleetwood was lowest in HWSP content (25.73%) and highest in cellulose content (34.55%). H_B contents of C-20 and Seafarer were significantly higher than those in Fleetwood and 84004. Significant differences ($P < 0.05$) in H_A and AOSP contents were observed among the four bean cultivars/line, suggesting genetically dependent effects. There was no significant difference in lignin content among dry bean samples. The cell wall composition patterns reported here (both seed coat and cotyledon) are in general agreement with earlier reports when all data are expressed per gram of starting tissue (Monte and Maga, 1980; Champ et al., 1986; Anderson and Bridges, 1988). Differences among these four papers

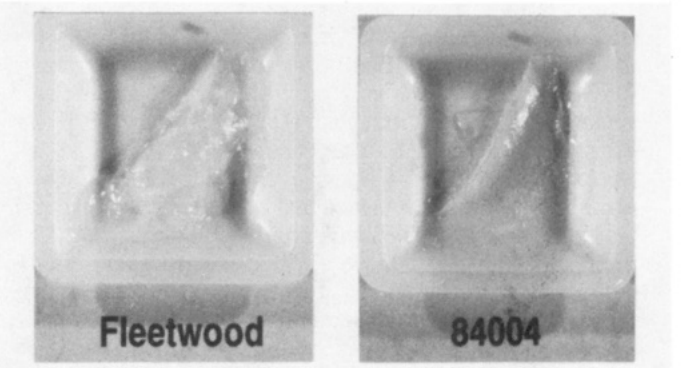

Figure 2. Ethanol precipitates of hot water soluble polysaccharides extracted from cotyledon cell walls after centrifugation at 23000*g* for 15 min. Note the transparent gellike appearance for Fleetwood compared to 84004.

are likely due to differences in methodology for cell wall preparation and/or cell wall fractionation.

During the recovery of extracted cotyledon HWSP by 80% ethanol, the Fleetwood HWSP fraction exhibited a fibrous, gellike characteristic. This material aggregated and stayed in suspension in 80% ethanol, whereas the HWSP from other beans completely precipitated. Precipitation of cell wall polymers by making the sample 80% ethanol is based on competition for water molecules between ethanol and the cell wall polymer. Normally when the sample is 80% ethanol, there is insufficient free water available to keep the solubilized polymers fully hydrated, which results in precipitation of the polymer. Upon centrifugation (23000*g*, 15 min), the Fleetwood HWSP precipitate retained its gellike, transparent structure which is characteristic of materials with a high capacity for liquid entrapment (Figure 2). However, the HWSP precipitates of C-20, Seafarer, and 84004 did not have transparent gellike appearances. Entrapment of liquid in a polysaccharide matrix is thought to occur during formation of "junction zones" (Graessley, 1974) which probably occurs through noncovalent bonding between linear polymer molecules and/or linear sections of branched molecules.

The HWSP fraction contained pectin and associated neutral sugars. Pectin is estimated by uronic acid content even though pectins frequently contain neutral sugars as

Table III. Sugar Composition of HWSP from Studied Bean Cotyledons[a]

bean	uronic acid, %	rhamnose, %	neutral sugars (expressed as weight ratio to rhamnose)						
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc
C-20	10.05b	0.68	1	1.38	20.51	3.49	0.73	3.38	2.41
Seafarer	12.67c	0.88	1	1.26	16.11	3.46	0.48	3.35	1.97
Fleetwood	12.99c	0.98	1	0.66	16.15	2.46	0.42	2.24	1.99
84004	6.74a	0.50	1	1.40	22.66	4.33	0.52	4.06	2.86

[a] Means in a column followed by different letters are signficantly different ($P < 0.05$).

integral constituents (Aspinall, 1981). Neutral sugars are usually associated with pectin as polymers bound to rhamnose. Rhamnose is the only neutral sugar interspersed in the (1,4) D-galacturonic acid or galacturonic ester chain of pectin (Jarvis, 1984). Rhamnose serves as a branch point in the pectin molecule and binds neutral sugar polymers as side chains. Occasionally neutral sugar polymers are isolated as discrete polymers that are not bound to rhamnose. However, since most neutral sugars exist as side chains of rhamnose, the neutral sugar associated with pectin is often expressed relative to rhamnose. The neutral sugar patterns of HWSP, therefore, were reported as a weight ratio to rhamnose (Table III). The uronic acid content varied from 6.74% in experimental line 84004 to 12.99% in Fleetwood. The uronic acid content of cotyledon HWSP in navy beans is comparable to the uronic acid content of HWSP in field bean (15%) (Salimath and Tharanathan, 1982) and to kidney bean (9.6%) (Champ et al., 1986). Sugar analysis showed that the HWSP was a mixture of pentosans, hexosans, and pectin (Table III). Arabinose was the predominant neutral sugar in the HWSP followed by xylose, galactose, glucose, fucose, rhamnose, and mannose, respectively. Fleetwood had much less fucose, xylose, and galactose compared to the other bean cultivars. These data suggest that pectin in the Fleetwood cultivar has more side chains with a shorter average chain length than the other cultivars/line. Additional, quantitative analyses of the neutral sugars of the HWSP are required before the unusual gelling characteristic of the Fleetwood HWSP can be related specifically to uronic acid and neutral sugar composition.

SUMMARY AND CONCLUSION

Cell wall composition of four navy bean cultivars/line varied according to tissue and genetic background. Seed coat cell wall is rich in cellulose and lignin, indicating its strong physical property, thus supporting its biological function as a protective tissue. The more flexible cell wall structure of bean cotyledon, a storage tissue, is shown by its high content of matrix polysaccharides (HWSP and H_B) with much less cellulose, H_A, and lignin. As a result, the cell wall polysaccharides of the seed coat and cotyledon are expected to produce different physiological effects in man. Distinct gelling characteristics of Fleetwood cotyledon HWSP in 80% ethanol may be related to the number of side chains and the average length of side chains in the pectin molecule.

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LITERATURE CITED

AACC. *American Association of Cereal Chemists Approved Methods*, 8th ed.; American Association of Cereal Chemists: St. Paul, MN, 1983.

Anderson, J. W.; Bridges, S. R. Dietary fiber content of selected foods. *Am. J. Clin. Nutr.* **1988**, *47*, (3); 440–447.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

AOAC. Method 43, A14-A20. Total dietary fiber in foods. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 399.

Asp, N.-G.; Johansson, C.-G. Techniques for measuring dietary fiber; principal aims of methods and a comparison of results obtained by different techniques. In *The analysis of dietary fiber in food*; James, W. P. T., Theander, O., Eds.; Dekker: New York, 1981; pp 173–189.

Aspinall, G. O. Constitution of plant cell wall polysaccharides. In *Plant Carbohydrates II. Extracellular Carbohydrates*; Springer-Verlag: Berlin, 1981; Vol. 13B, pp 3–8.

Blumenkrantz, N.; Asboe-Hanson, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.

Brillouet, J. M.; Carre, B. Composition of cell walls from cotyledons of Pisum savitum, Viva faba and Glycine max. *Phytochemistry* **1983**, *22*, 841–847.

Champ, M.; Brillouet, J. M.; Rouau, X. Non-starchy polysaccharides of Phaseolus vulgaris, Lens esculenta, and Cicer arietinum seeds. *J. Agric. Food Chem.* **1986**, *34*, 326–329.

Graessley, W. W. The entanglement concept in polymer rheology. *Adv. Polym. Sci.* **1974**, *16*, 1–179.

Jarvis, M. C. Structure and properties of pectin gels in plant cell walls. *Plant Cell Environ.* **1984**, *7*, 153–64.

Leeds, A. R. *Dietary fibre perspectives reviews and bibliography 2*; Leeds, A. R., Ed.; Libby: London, 1990.

Mongeau, R.; Brassard, R. A rapid method for the determination of soluble and insoluble dietary fiber: comparison with AOAC total dietary fiber procedure and Englyst's method. *J. Food Sci.* **1986**, *51*, 1333–1336.

Monte, W. C.; Maga, J. A. Extraction and isolation of soluble and insoluble fiber fractions from the pinto bean (Phaseolus vulgaris). *J. Agric. Food Chem.* **1980**, *28*, 1169–1174.

Prosky, L.; Asp, N. G.; Furda, I.; Devries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of total dietary fiber in foods, food products and total diets: Interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 1044–1052.

Ring, S. G.; Selvendran, R. R. Isolation and analysis of cell wall material from beeswing wheat bran (*Triticum aestivum*). *Phytochemistry* **1980**, *19*, 1723–1730.

Ruengsakulrach, S. Navy bean physico-chemical characteristics and canned product quality. Ph.D. Dissertation, Michigan State University, E. Lansing, 1990.

Salimath, P. V.; Tharanathan, R. N. Carbohydrates of field bean (*Dolichos lablab*). *Cereal Chem.* **1982**, *59*, 430–435.

York, W. S.; Darvill, A. G.; McNeil, M.; Stevenson, T. T.; Albersheim, P. Isolation and characterization of plant cell walls and cell wall components. *Methods Enzymol.* **1985**, *118*, 3–40.

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Registry No. H_A, 63100-39-0; H_B, 63100-40-3; AOSP, 9000-69-5; lignocellulose, 11132-73-3; cellulose, 9004-34-6; lignin, 9005-53-2.