CELL WALL POLYSACCHARIDES OF COMMON BEANS (*Phaseolus vulgaris* L.)[1]

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SUMMARY

The soluble and insoluble cotyledon (SPF-Co and IPF-Co) and tegument (SPF-Te and IPF-Te) cell wall polymer fractions of common beans (*Phaseolus vulgaris*) were isolated using a chemical-enzymatic method. The sugar composition showed that SPF-Co was constituted of 38.6% arabinose, 23.4% uronic acids, 12.7% galactose, 11.2% xylose, 6.4% mannose and 6.1% glucose, probably derived from slightly branched and weakly bound polymers. The IPF-Co was fractionated with chelating agent (CDTA) and with increasing concentrations of NaOH. The bulk of the cell wall polymers (29.4%) were extracted with 4.0M NaOH and this fraction contained mainly arabinose (55.0%), uronic acid (18.9%), glucose (10.7%), xylose (10.3%) and galactose (3.4%). About 8.7% and 10.6% of the polymers were solubilised with CDTA and 0.01M NaOH respectively and were constituted of arabinose (52.0 and 45.9%), uronic acids (25.8 and 29.8%), xylose (9.6 and 10.2%), galactose (6.1 and 3.9%) and glucose (6.5 and 3.8%). The cell wall polymers were also constituted of small amounts (5.6 and 7.2%) of cellulose (CEL) and of non-extractable cell wall polymers (NECW). About 16.8% and 17.2% of the polymers were solubilised with 0.5 and 1.0M NaOH and contained, respectively, 92.1 and 90.7% of glucose derived from starch (IST). The neutral sugar and polymers solubilization profiles showed that weakly bound pectins are present mainly in SPF-Co (water-soluble), CDTA and 0.01-0.1M NaOH soluble fractions. Less soluble, highly cross-linked pectins were solubilised with 4.0M NaOH. This pectin is arabinose-rich, probably highly branched and has a higher molecular weight than the pectin present in SPF-Co, CDTA and 0.01-0.1M NaOH fractions.
Keywords: legume; pectin; carbohydrate; gas chromatography.

RESUMO

POLISSACARÍDEOS DE PAREDE CELULAR DE FEIJÕES (*Phasealus vulgaris* L.). Foram isoladas por método enzimático-químico as frações da parede celular de feijão (*Phaseolus vulgaris* L.) contendo polímeros solúveis e insolúveis obtidos do cotilédone (SPF-Co e IPF-Co) e tegumento (SPF-Te e IPF-Te). A análise da composição de açúcares mostrou que a SPF-Co era composta por 38,6% de arabinose, 23,4% de ácidos urônicos, 12,7% de galactose, 11,2% de xilose, 6,4% de manose e 6,1% de glicose, provavelmente, provenientes de polímeros fracamente ligados e contendo baixo grau de ramificação. A IPF-Co foi fracionada com agente quelante (CDTA) e com concentrações crescentes de NaOH. A maior parte dos polímeros da parede celular (29%) foram extraídos com NaOH 4,0M e continham, principalmente, arabinose (55,0%), ácido urônico (18,9%), glicose (10,7%), xilose (10,3%) e galactose (3,4%). Cerca de 8,7% e 10,6% de polímeros foram solubilizados com CDTA e NaOH 0,01M apresentando em sua composição, arabinose (52,0 e 45,9%), ácidos urônicos (25,8 e 29,8%), xilose (9,6 e 10,2%), galactose (6,1 e 3,9%) e glicose (6,5 e 3,8%) respectivamente. Os polímeros da parede celular também eram constituídos por pequenas quantidades (5,6 e 7,2%) de celulose (CEL) e polissacarídeos não-extraíveis (NECW). Cerca de 16,8% e 17,2% dos polímeros foram solubilizados com NaOH 0,5 e 1,0M e estes continham, respectivamente, 92,1 e 90,7% de glicose proveniente de amido (IST). O perfil de solubilização dos polímeros e de açúcares neutros mostrou que as pectinas fracamente ligadas estão presentes principalmente nas frações SPF-Co (solúvel em água), CDTA e NaOH 0,01-0,1M. Pectinas menos solúveis e contendo grande quantidade de ligações cruzadas foram solubilizadas com NaOH 4,0M. Estas pectinas ricas em arabinose, provavelmente, são altamente ramificadas e apresentam maior peso molecular do que as pectinas presentes nas frações SPF-Co, CDTA e NaOH 0,01-0,1M.
Palavras-chave: leguminosa; pectina; carboidrato, cromatografia de gás.

1– INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a member of the Leguminosae, family Phaseoleae, subfamily Papilionoideae, and originated as a crop in the New World and spread to all the continents. Bean seeds serve as an important source of food energy and nutrients, especially for the populations of developing countries [6, 31].

The cell wall of different leguminous plants revealed a pectin rich structure, primarily containing arabinose [4, 11, 15, 19, 20, 22, 25, 33, 37]. In leguminous plants the bulk of the cell wall polysaccharides is only extracted with high hydroxide concentrations [19, 20], showing a strong association between the polymers. REICHERT [30], working with the cotyledon cell wall of peas, observed that it was composed to a large extent of pectic substances and hemicellulose, while its tegument consisted primarily of cellulose.

Numerous reviews have depicted plant cell walls as dynamic structures, composed of complex polysaccharides (cellulose, hemicellulose and pectins), small amounts of phenolic compounds (lignins and hydroxycinnamic acids esterified to the wall polysaccharides) and proteins (extensin and enzymes), with ionic and covalent linkages stabilizing its components [3, 9, 12, 32]. Its polymeric structure dictates the textural quality of the plant based foods [7]. The de-polymerization of these polysaccharides during the cooking process leads to bean tissue softening.

Texture is one of the most important quality attributes of legume seeds, because it affects palatability and consequently consumer acceptability. Plant food texture is associated with the cell wall characteristics. Amongst these characteristics, the degree of polysaccharide solubility and the depolymerization during cooking process, is a determinant factor of texture quality.

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Many studies have associated changes in the cell wall and middle lamella polysaccharides with the development of the hard-to-cook defect, which renders legume seeds resistant to cooking [1, 18, 23, 34, 35, 40].

A better understanding of the cell wall polysaccharide composition and solubilization pattern could be useful to the comprehension of how these polymers are interconnected and behave during tissues heating. The polysaccharide solubilization profile is a good indication of the nature of the polymer linkages influencing food texture. Enzymatic treatments of bean tissues give us a notion of the digestion of the cell contents and of the cell wall polysaccharide behavior under enzymatic attack.

The detailed composition of the cell wall and middle lamella polysaccharides is also relevant when associating the physical and physiological properties (nutritional effects) as related to the effect of fiber in human health [26]. Moreover, a better knowledge of polymer structure help us to understand the cell wall polysaccharides disassembling mechanisms. So, the aim of this work was to suggest a new method of cell wall polymer extraction, under mild conditions, and study the polysaccharide composition and its solubilization patterns.

2 – MATERIALS AND METHODS

2.1 – Plant Material

Common beans *(Phaseolus vulgaris* L. cv. IAC – Carioca) seed, (9.9% moisture), harvested in July, were kindly provided by the Instituto Agronômico de Campinas (Campinas, SP – Brazil).

2.2 – Reagents

Heat-stable α-amylase (A-3306), protease (P-3910), amyloglucosidase (A-9913) and D(+) fucose standard sugar (F-2127) were purchased from Sigma Chemical Co. (St. Louis, MO). The others standard sugars, L-rhamnose (17,198-0), L-arabinose (A9, 190-6), D-xylose (X107-5), a-D-glucose (25,307-3), D-galactose (11,2589-3), D-mannose (11,258-5) and inositol (I-665-2) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Molecular porous dialysis membrane utilized was Spectra/Por® Membrane, MWCO 12-14,000 Da from Spectrum® (Houston, TE); BCA-Protein Assay Reagent kit (Pearce®).

2.3 – Moisture determination

Moisture content was taken as weight loss after heating whole bean flour at 105°C for 12h.

2.4 – Beans cell wall isolation and fractionation

The soluble (SPF-Co) and insoluble (IPF-Co) cotyledon and tegument (SPF-Te and IPF-Te) materials were isolated according to the chemical-enzymatic method, adapted from the procedures of PROSKY *et al.* [29], CARPITA [8] and Mc LAUGHLING and GAY [27]. The seeds were manually de-hulled and freed from germ. The tegument and the cotyledon were ground separately in blade mills. The flours were passed through a 0.5mm sieve. About 500mg of cotyledon and tegument flour were incubated with 0.1mL of α-amylase in 25mL of 0.08M sodium phosphate buffer at pH 6.0/30min in a boiling water bath with manual stirring every 5min. After cooling, the pH was adjusted to 7.5 and treated with 0.1mL of protease (5mg/mL) at 60°C and with 0.3mL of amyloglucosidase at pH 4.3 with stirring (100 r.p.m.) for a period of 1h for each enzyme added (*Figure 1*).

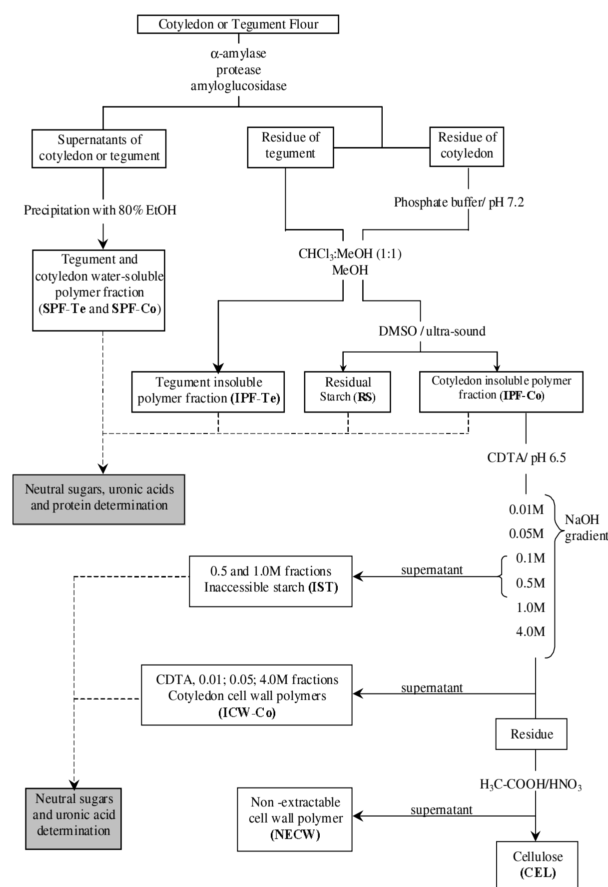

FIGURE 1. General flow chart of isolation, fractionation and analysis of the cell wall polymers of common beans (*Phaseolus vulgaris* L.)

The suspensions were cooled and centrifuged at 8000g/10min and the residues washed (3x) with 10mL of distilled water. The supernatants and the washing waters obtained from the cotyledon and tegument, were passed through fiberglass filters (GF/F Whatmann) when necessary. The extract was brought to 80% (v/v) ethanol and the mixture allowed to precipitate for 24h at -20°C. The alcohol insoluble precipitates obtained were washed (3x) with 10mL of ice-cold 80% ethanol, suspended in distilled water, freeze dried and weighed, corresponding to the tegument and cotyledon soluble polymer fractions (SPF-Te, SPF-Co) (*Figure 1*). The residues of the tegument and cotyledon resulting from the enzymatic treatment, were submitted to distinct chemical treatments.

2.4.1 – Cotyledon chemical treatment

The residue from the enzymatic hydrolysis of the cotyledon was treated (2x) with 10mL of 0.5M sodium phosphate buffer, pH 7.2 and with 10mL of chloroform: methanol (1:1; v:v)/45°C/30min and with 10mL of methanol (2x). The remaining residue was treated with 10mL (2x) of 90% dimethyl sulfoxide (DMSO) for 20min in an ultrasonic bath, washed (2x) with 10mL of 90% DMSO and rinsed (5x) with distilled water. The DMSO supernatants were dialyzed 48h against distilled water, freeze dried and weighed, corresponding to the residual starch fraction (RS) (*Figure 1*). The final residues were suspended in water, freeze dried and weighed, corresponding to the insoluble polymer fractions of the cotyledon (IPF-Co). At each stage of extraction the suspensions were pelleted by centrifugation at 8000g/15min and the residues washed with water (*Figure 1*).

2.4.2 – Tegument chemical treatment

The residues obtained from the enzymatic hydrolysis of the tegument were treated (2x) with 10mL of chloroform: methanol (1:1 v:v)/45°C/30min and with 10mL of methanol (2x) and washed with 10mL (3x) distilled water. The suspensions were centrifuged at 8000g/20min at each step of extraction. The residues obtained were suspended in distilled water, freeze dried and weighed, corresponding to the insoluble polymer fraction of the tegument (IPF-Te) (*Figure 1*).

2.4.3 – Fractionation of insoluble cotyledon polymers

The IPF-Co was fractionated (*Figure 1*) following the methodology described by CARPITA [8]. Thirty milligrams of IPF-Co were extracted with 10mL/0.05M trans-1, 2-diaminocyclohexane-N, N, N', N', -tetraacetic acid (CDTA)/pH 6.5/16h/22°C by stirring with a few drops of toluene. The suspension was centrifuged at 1500g/10min and the supernatants filtered through a GF/F filter. The filtrate was dialyzed against distilled water at 22°C/72h, freeze dried and weighed, resulting in the CDTA soluble fraction. The residue was sequentially treated with 10mL of 0.01, 0.05, 0.1, 0.5 and 1.0M NaOH containing 3mg/mL $NaBH_4$ for 1h/22°C under an atmosphere of N_2 with constant stirring and with 4.0M NaOH/16h under the same conditions. The NaOH extracts were chilled in an ice bath and neutralized with glacial acetic acid and dialyzed for 48h against distilled water, then freeze dried and weighed, resulting in the 0.01, 0.05, 0.1, 0.5, 1.0 and 4.0M soluble fractions.

The insoluble material (residue) remaining after IPF-Co fractionation was washed, suspended in distilled water, freeze dried and weighed. Crystalline cellulose (CEL) was obtained after digestion of the non-cellulosic polymers with a mixture of water: glacial acetic acid: nitric acid (2:8:1;v: v: v). About 30mg of residue was treated with 10mL of nitric acid reagent [39] and incubated for 90min in a boiling water bath. The highly cross-linked material solubilised corresponded to the non-extractable cell wall polymer (NECW).

2.5 – Protein composition

The protein content was determined by the bicinchoninic acid (BCA) method [36, 41], using the BCA kit (Pearce).

2.6 – Carbohydrate composition

The IPF-Co, IPF-Te, SPF-Co, SPF-Te, RS were characterized for protein, neutral sugars and uronic acid contents. The sugar compositions of the IPF-Co fractions were also analyzed.

2.7 – Uronic acids determination

Samples were suspended or dissolved in distilled water (0.5mg/mL) and whenever necessary, homogenized in a tissue homogenizer with a Teflon® pestle. The uronic acids were determined according to FILISETTI-COZZI and CARPITA [16]. To a 400µL sample (0.5mg/mL) in a test tube kept on ice, 40µL of a 4.0M sulfamic acid-potassium sulfamate (pH 1.6) solution were added and mixed thoroughly. Analytical grade (96.4%) H_2SO_4 containing 75mM sodium tetraborate (2.4mL) was added, and the solution stirred vigorously by vortex mixing. The solution was incubated for 20min in a boiling water bath. After cooling, 40µL of 0.15% (w/v) m-hydroxydiphenyl in NaOH 0.5% (w/v) was added and the mixture stirred vigorously by vortex mixing. The pink color develops to completion in about 5 to 10min, and is stable for about 1h. Absorbance was read at 525nm using a standard curve containing 50, 100, 150 and 200nmoles of galaturonic acid.

2.8 – Neutral sugars determination

The neutral sugars rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal) and glucose (Glc), excluding the glucose derived from the cellulose, released by acid hydrolysis of the polysaccharides [2], were quantitatively determined by gas-liquid chromatography after reduction and acetylation [5, 10, 17]. About 1mg of freeze dried product was hydrolyzed in a screw-cap vial with 1mL of 2M trifluoroacetic acid (TFA) containing 1µmol of *myo*-inositol (internal standard)/90min/120°C in a heating block and submitted to stirring at 30min intervals towards the end of incubation, then cooled and centrifuged at 800g/5min. The supernatant was poured into a dry vial and 1mL of *tert*-butyl alcohol (TBA) added. The mixture obtained was dried under N_2 flow at 45°C. In parallel to each group of analyses, 1mL of the standard mixture (3µmoles from each standard sugar per 1mL of 2M TFA, containing 1µmol of *myo*-inositol) was submitted to hydrolysis. The sugars were dissolved in 0.5mL of 20mg/mL of sodium borohydrate ($NaBH_4$) in DMSO (w/v), 100µL of 1.0M NH_4OH was added and the mixture incubated at 40-45°C/90min. The solution was neutralized with 100µL of glacial acetic acid, and acetylated with 100µL of 1-methylmidazol and 0.5mL of acetic anhydride. The mixture was incubated in a water bath at 40-45°C/30min. About 1.5mL of distilled water was added to the final product containing the alditol acetate. The aqueous

mixture was extracted (2x) with 0.5mL of dichloromethane (CH_2Cl_2) with vigorous stirring and separated by centrifuging at 800g/5min. The CH_2Cl_2 extracts were washed (2x) with 1mL of distilled water. The washed CH_2Cl_2 extracts were poured into clean flasks and dried under N_2 flow at 40-45°C. The derivatives were dissolved in ethyl acetate and 1-3µL samples injected into a gas chromatograph equipped with a flame ionization detector (FID). The alditol acetates were separated using a 30m x 0.25mm i.d. SP-2330 fused-silica capillary column with a 0.20µmm film thickness (Supelco, Inc., Bellefonte, PA). The column temperature was programmed from 170 to 240°C at 10°C/min with a 20min hold at the highest temperature. Injector and detector temperatures were set at 250°C. Nitrogen was used as the carrier gas at a flow rate of 30mL/min.

3 – RESULTS AND DISCUSSION

The bean seeds (*Phaseolus vulgaris* L., cv Carioca) were constituted of 11.2% tegument, 87.5% cotyledon and 1.3% embryo by weight.

After the enzymatic-chemical treatment, the bean cotyledon rendered 23.1% of undigested cotyledon material (CM) composed of IPF-Co, SPF-Co and RS (*Table 1*).

TABLE 1. The sugar and protein compositions of bean cell wall polymers.

FRACTION		%	NEUTRAL SUGAR (µg/mg)							NS	UA	Protein	Total	NS/UA
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	µg/mg				
TM (77.9%)	SPF-Te	5.5	0	0	50.4	20.8	34.8	36.8	21.0	163.9	143.7	184.0	491.6	1.1
	IPF-Te	72.4	8.6	0	82.2	143.7	0	15.8	22.0	272.3	167.0	252.8	692.0	1.6
CM (23.1%)	SPF-Co	9.0	7.7	0	193.6	56.3	32.2	63.7	30.5	383.9	117.2	213.8	714.9	3.3
	IPF-Co	12.3	9.4	0	249.9	50.2	0	33.9	295.5	638.9	91.2	145.3	875.4	
	RS	1.8	0	0	104.4	17.9	54.6	54.0	212.7	443.6	17.1	244.0	704.7	
					Fractionation of IPF-Co									
ICW-Co (66%)	CDTA	8.7	0	0	454.2	83.9	0	53.4	57.0	648.5	225.2		873.7	2.9
	0.01M	10.6	5.2	0	158.9	35.3	16.7	13.6	13.2	242.9	103.2		346.1	2.4
	0.05M	2.5												
	0.1M	1.9												
	4.0M	29.4	15.1	0	499.9	93.4	0	30.7	97.2	736.3	172.0		908.3	4.3
	CEL	5.6												
	NECW	7.2												
IST (34%)	0.5M	16.8	0	0	10.5	0	0	18.8	645.5	674.8	26.0		700.8	
	1.0M	17.2	0	0	16.4	0	0	28.6	627.8	672.7	19.5		692.2	
	Total	100.0												

SPF-Te, IPF-Te, tegument soluble and insoluble polymers fraction; SPF-Co, IPF-Co, cotyledon soluble and insoluble polymer fractions; ICW-Co, cotyledon cell wall polysaccharide; CM and TM, cotyledon and tegument material; CDTA and 0.01-4.0M, CDTA soluble fraction and 0.01-4.0M hydroxide soluble fractions; CEL cellulose; NECW, non extractable cell wall polymers; RS, residual starch undigested by enzyme treatment and extracted by DMSO treatment; IST, inaccessible starch; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; NS, total neutral sugars; UA, uronic acids.

The bean cotyledon cell wall polymers were constituted of about 9% of weakly bound material extracted with water (SPF-Co). The mannose content in the SPF-Co and SPF-Te originated from the polymers present in the enzyme used for cell wall extraction. In a recent study in our laboratory, NEVES [28] also extracted bean cell wall using the same enzyme kit to hydrolyze starch and protein. NEVES [28] verified that high amounts of protein and mannose-rich polymers were present in the bean cell wall material obtained. The analysis of the composition of the enzyme kit revealed that it contained a polymer that was composed mainly

of mannose. Hence, the presence of mannose-rich polymers in the SPF was probably due to enzyme contamination. The enzyme polymers contained high amounts of mannose (62.3%), glucose (14.1%), uronic acid (12.4%), galactose (4.9%), rhamnose (3.2%), xylose (1.3%), arabinose (1.1%) and fucose (0.9%), as can be seen in NEVES [28].

The high protein content was also attributed to the presence of enzymes in the SPF. In a recent work, a good separation of enzyme contamination was achieved using anion exchange chromatography (unpublished data).

About 1.8% of the starch (RS) was not extracted by the enzymatic treatment and was solubilised with DMSO. The bulk (12.3%) of the polymers was insoluble in hot-water and DMSO and constituted the IPF-Co fraction. About 34% of the IPF-Co contained high amounts of inaccessible starch (IST), that remained even after DMSO washing, being extracted by 0.5-1.0M NaOH (*Table 1*). The starch (RS plus IST) found in the CM is extremely hard to remove, and is well established in the literature as resistant starch (RS[1]) by ENGLYST, KINGMAN and CUMMINGS [13]. However, this starch has beneficial effects, contributing in the same way as ICW-Co as an insoluble dietary fiber as can be seen in the review by HARALAMPU [21].

About 66% of the IPF-Co was constituted of wall polymers, composed of CDTA and mildly alkali soluble pectic polymers, 4.0M soluble polymer, crystalline cellulose (CEL) and non-extractable cell wall polymers (NECW). Protein was also found in the IPF-Co fraction (*Table 1*).

The weakly bound CDTA (8.7%) and mildly alkali (0.01-0.1M) soluble polymers were composed of arabinose-rich pectic material. The low sugar recovery in the 0.01M fraction was due to the presence of large amounts of hydroxyproline in this fraction, as can be seen in NEVES [28]. The mass of highly cross-linked pectic polymers (29.4%) was solubilised with 4.0M NaOH. This polymer is branched (NS/AU = 4.28), with a high molecular weight and its structure is entangled with cellulose and hemicellulose microfibrils. The beans cotyledon cell wall was also composed of small amounts (5.6 and 7.2%) of crystalline cellulose (CEL) and non-extractable cell wall polymers (NECW) (*Table 1*).

The sugar analysis of the 4.0M NaOH soluble polymer fractions showed that they were composed of arabinose-rich polysaccharides (55.0%) containing uronic acids (18.9%), glucose (10.7%) and xylose (10.3 %) (*Table 2*).

Probably the high levels of glucose found in the 0.5 and 1.0M fractions were derived from starch, because xylose was not found in these fractions (*Table 2*). The high amounts of glucose and the absence of xylose confirm that both fractions were mainly composed of inaccessible starch (IST). These fractions also contained small amounts of wall polymer (about 9%) composed of uronic acids (3-4%), arabinose (1.5-2.4%) and galactose (3-4%), probably derived from pectins.

The CDTA and 0.01M NaOH solubilised materials were mainly composed of arabinose (52.0 and 45.9%), uronic acids (25.8 and 29.8%) and xylose (9.6-10.2%) (*Table 2*). The cell wall polymers extracted from each fraction showed little difference in sugar composition between them, but the differences in extractability were perceptible when the amounts of material extracted by the different solvents were observed (*Tables 1* and *2*). The difference in extractability probably resulted from the quantity and quality of the cross-links stabilizing the wall components. The SPF-Co pectic polysaccharides contained less arabinose than the 4.0M fraction polymers (*Tables 2* and *3*) and were probably less branched and bound.

TABLE 2. Sugar composition of the cotyledon insoluble cell wall polymer (IPF-Co) fractions.

FRACTIONS	%	NEUTRAL SUGARS (%)							SUGARS (%)		
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	NS	UA	Total
CDTA	8.7	0	0	52.0	9.6	0	6.1	6.5	74.2	25.8	100
0.01M	10.6	1.5	0	45.9	10.2	4.8	3.9	3.8	70.2	29.8	100
0.5M	16.8	0	0	1.5	0	0	2.7	92.1	96.3	3.7	100
1.0M	17.2	0	0	2.4	0	0	4.1	90.7	97.2	2.8	100
4.0M	29.4	1.7	0	55.0	10.3	0	3.4	10.7	81.1	18.9	100
CEL	5.6										
NECW	7.2										

CDTA and 0.01-4.0M, CDTA soluble polymer and 0.01-4.0M hydroxide soluble polymers. Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; NS, total neutral sugars; UA, uronic acids; CEL, cellulose, NECW, non extractable cell wall polymers. The shaded area shows the fractions containing large amounts of inaccessible starch (IST).

TABLE 3. Sugar composition of soluble and insoluble polymer fractions from the tegument (SPF-Te and IPF-Te), cotyledon (SPF-Co and IPF-Co) and residual starch (RS).

RACTIONS	%	NEUTRAL SUGARS (%)							SUGARS (%)		
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	NS	U.A.	Total
SPF-Te	5.5	0	0	16.4	6.8	11.3	12.0	6.8	53.3	46.7	100
IPF-Te	72.4	2.0	0	18.7	32.7	0	3.6	5.0	62.0	38.0	100
SPF-Co	9.0	1.5	0	38.6	11.2	6.4	12.7	6.1	76.6	23.4	100
IPF-Co	12.3	1.3	0	34.2	6.9	0	4.6	40.5	87.5	12.5	100
RS	1.8	0	0	22.7	3.9	11.8	11.7	46.2	96.3	3.7	100

SPF-Te and IPF-Te, soluble and insoluble polymer fractions of tegument; SPF-Co and IPF-Co, soluble and insoluble polymer fractions of cotyledon; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; N.S., total neutral sugars; U.A., uronic acids; R.S., residual starch.

The monosaccharide composition found, suggests that the bean ICW-Co was basically composed of arabinose-rich pectic polysaccharides (*Table 1*), probably derived from arabinan or arabinogalactans and medium amounts of hemicellulose (xyloglucan). These results are very coherent with the legume seed sugar compositions reported in the literature [11, 15, 19, 20, 25, 27, 33, 37]. BHATTY [4] inferred that the lentil cell wall was probably composed of arabinan and arabinogalactan polysaccharides, because such polysaccharides are commonly distributed in the primary cell wall of cotyledonous plants. The common beans showed a composition similar to that of *Vigna radiata* [19, 20] containing polysaccharides less readily extractable by

CDTA and slightly alkaline solutions, the majority of the polysaccharide being solubilised by 4.0M base.

In a work carried out with beans (*Phaseolus vulgaris* L. cv Pérola), the starch was not totally eliminated by the enzymatic treatment (heat-stable α-amylase and amyloglucosidase) and by extensive washing with DMSO (unpublished data). DMSO was shown to be a bad solvent when an accurate cell wall composition is required. The material extracted by this solvent (RS) was rich in glucose (46.2%), but also contained arabinose (22.7%), mannose (11.8%), galactose (11.7%), xylose (3.9%) and uronic acids (3.7%), derived from the cell wall polysaccharides (*Table 3*), also containing protein in its composition (*Table 1*). The cell walls obtained from beans (cv Pérola) after efficient cell disruption, showed low levels of 0.5 and 1.0M NaOH solubilised polymers; 6.1 and 3.4% respectively (unpublished data), instead of the 16.8 and 17.2% previously obtained (*Table 2*). NEVES [28] also found high amounts of glucose derived from inaccessible starch in the 0.5-1.0M NaOH soluble fractions.

Hence, 0.5 and 1.0M alkali extracted mainly starch instead of hemicellulose and we concluded that bean cell wall xyloglucan cannot be separated by solubilisation in these alkali concentrations. Also, hemicellulose was extracted together with arabinose-rich pectins and was found in all cell wall polymer fractions obtained, as can be seen by the xylose and glucose content (*Table 2*). This shows that almost all cell wall polymers are composed of arabinose-rich pectic polysaccharides extracted by hot-water, CDTA and strong alkali solutions and are difficult to separate from hemicellulose.

DMSO washing is inefficient in removing inaccessible starch (IST) without an efficient cell rupture, especially for legume seeds because the starch seems to be protected against enzymatic and chemical attack by the cell wall structure. Moreover, DMSO also extracts wall polymers. Enzymatic-chemical extraction must be carefully evaluated, according to the purpose of the analysis. To characterize cell wall components, more effective sample homogenization is necessary before the enzymatic treatment, to obtain cell wall polysaccharides, and the use of DMSO to extract starch must be reevaluated.

The tegument was composed of 77.9% of undigested tegument material (TM), 72.4% of insoluble polysaccharide fraction (IPF-Te) and 5.5% of water-soluble polysaccharide fraction (SPF-Te) (*Table 1*). So, the bean cell wall furnishes large amounts of insoluble dietary fiber derived from the tegument (IPF-Te) and cotyledon (IPF-Co).

The IPF-Te polysaccharide was primarily constituted of xylose (32.7%) with a medium level of arabinose (18.7%) (*Table 3*) The high levels of xylose in IPF-Te could mean the presence of xylans and the glucose content (5%) suggests the presence of small amounts of xyloglucans. The high quantities of uronic acids and protein (*Table 1*) in SPF-Te (46.7%) and in IPF-Te (38.0%) may be attributed, in part, to the interference of phenolic compounds in the colorimetric methods adopted. A better alternative to overcome phenolic interference in the uronic acid determination could be the use of gas chromatography [24, 38] or high performance liquid chromatography [14].

A careful observation of the bean cell wall composition, shows that the SPF-Te of beans was constituted of arabinose-rich polysaccharides (16.4%), also containing galactose (12.0%), and mannose (11.3%), with small amounts of xylose (6.8%) and glucose (6.8%)(*Table 3*). The xylose and glucose were found in a 1:1 proportion, and probably these sugars were derived from hemicelluloses.

4 – CONCLUSIONS

The cotyledon water-soluble polymer fraction (SPF-Co) of the beans was constituted of 38.6% arabinose, 23.4% uronic acids, 12.7% galactose, 11.2% xylose, 6.4% mannose and 6.1% glucose.

The cotyledon insoluble polymer fraction (IPF-Co) was mainly constituted of polysaccharides solubilised with CDTA (8.7%), 0.01M (10.6%) and 4.0M NaOH (29.4%) and by inaccessible starch (IST), solubilised with 0.5 and 1.0M NaOH. The polysaccharides solubilised with 4.0M NaOH contained mainly arabinose (55.0%), uronic acid (18.9%), glucose (10.7%), xylose (10.3%) and galactose (3.4%). Polysaccharides solubilised with CDTA and 0.01M NaOH were constituted of arabinose (52.0 and 45.9%), uronic acids (25.8 and 29.8%), xylose (9.6 and 10.2%), galactose (6.1 and 3.9%) and glucose (6.5 and 3.8%). The polysaccharide solubilization profile showed that the bean cell wall was composed mainly of less cross-linked polysaccharides, present in the SPF-Co, CDTA and 0.01M NaOH fractions and by highly cross-linked pectins solubilised by 4.0M NaOH. These pectins are rich in arabinose and, to a lesser degree, in galactose. Medium amounts of hemicellulose, composed of glucose and xylose, were present too.

The 0.5M (16.8%) and 1.0M (17.2%) NaOH extracted material contained respectively, 92.1 and 90.7% of glucose, which was mainly constituted of inaccessible starch (IST) and negligible pectic material. The residual starch (RS) that remained after enzymatic treatment and the IST represent together an insoluble dietary fiber in the human diet. It is very hard to obtain starch free cell wall material from beans, due to the presence of resistant starch. This starch, constituted of retrograded starch protected by the cell wall structure, is not attacked by enzymes and is little extracted by DMSO.

The polysaccharide solubilization profile showed the nature of the polymer linkages. These linkages influence food texture and could contribute to an understanding of the development of the hard-to-cook defect where the nature and number of inter and intra-molecular cross-links could contribute to alter the rheological properties and render the beans hard.

5 – REFERENCES

[1] AGUILERA, J.M.; RIVERA, R. Hard-to-cook defect in black beans: hardening rates, water imbibition and multiple

mechanism hypothesis. **Food Res. Int.**, v. 25, n.2, p. 101-108, 1992.

[2] ALBERSHEIM, P.; NEVINS, D.J.; ENGLISH, P.D.; KARR, A. A method for analysis of sugars in plant cell wall polysaccharides by gas chromatography. **Carbohydr. Res.**, v. 5, p. 340-345, 1967.

[3] BENNET, A.B.; ROSE, J.K.C. Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening. **Trends Plant Sci.**, v. 4, n.5, p. 176-183, 1999.

[4] BHATTY, R.S. Cooking quality of lentils: the role of structure and composition of cell walls. **J. Agric. Food Chem.,** v. 38, n. 2, p. 376-383, 1990.

[5] BLAKENEY, A.B.; HARRIS, P.J.; HENRY, R.J.; STONE, B.A. A simple and rapid preparation of alditol acetates for monosaccharides analysis. **Carbohydr. Res**., v. 113, n. 2, p. 291-299, 1983.

[6] BRESSANI, R. Grain quality of common beans. **Food Rev. Int.,** v. 9, n. 2, p. 237-297, 1993.

[7] BRETT, C.; WALDRON, K. **Physiology and biochemistry of plant cell walls**, 2nd ed.; Chapman & Hall, London, p. 173-186, p. 222-224, 1996.

[8] CARPITA, N.C. Hemicellulosic polymers of cell walls of Zea coleoptiles. **Plant Physiol.,** v. 72, n.2, p. 515-521, 1983.

[9] CARPITA, N.C.; GIBEAUT, D.M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. **Plant J.**, v. 3, n. 1, p. 1-30, 1993.

[10] CARPITA, N.C.; WHITTERN, D. A highly substituted glucuronoarabinoxylan from developing maize coleoptile. **Carbohydr. Res.,** v.146, n.1, p.129-140, 1986.

[11] CHAMP, M.; BRILLOUET, J.M.; ROUAU, X. Non-starchy polysaccharides of *Phaseolus vulgaris, Lens esculenta*, and *Ciccer arietinum* seeds. **J. Agric. Food Chem.,** v. 34, n.2, p. 326-329, 1986.

[12] COSGROVE, D.J. Assembly and enlargement of the primary cell wall in plants. **Annu. Rev. Cell Dev. Biol**, v.13, p.171-201, 1997.

[13] ENGLYST H.N.; KINGMAN S.M.; CUMMINGS J.H. Classification and measurement of nutritionally important starch fraction. **Eur. J. Clin Nutr** (suppl 2), v. 46, p. 33S-50S, 1992.

[14] ENGLYST, H.N.; QUIGLEY, M.E. Determination of the uronic acid constituents of non-starch polysaccharides by High-performance liquid chromatography with pulse amperometric detection. **Analyst**, v. 119, n. 7, p. 1511-1518, 1994.

[15] EVANS, A.J.; CHEUNG, P.C.K.; CHEETHAM, N.W.H. The carbohydrate composition of cotyledons and hulls of cultivars of *Lupinus angustifolius* from Western Australia. **J. Sci. Food Agri.,** v.61, n. 2, p.189-194, 1993.

[16] FILISETTI-COZZI; T.M.C.C.; CARPITA, N.C. Measurement of uronic acid without interference from neutral sugars. **Anal. Biochem**., v. 197, n.1, p. 57-162, 1991.

[17] FOX, A.; MORGAN, S.L.; GILBART, J. Preparation of alditol acetate and their analysis by gas chromatography (CG) and mass spectrometry (MS). In Biermann, C.J., McGinnis, G.D. (Ed.) **Analysis of carbohydrates by GLC and MS**. Boca Raton: CRC Press, 1989. p 87-117.

[18] GARCIA, E; LAJOLO, F.; SWANSON, B.G. A comparative study of normal and hard-to-cook brazilian common bean (*Phaseolus vulgaris*): Ultrastructural and histochemical aspects. **Food Struct.,** v. 12, p. n.2, 147-154, 1993.

[19] GOONERATNE, J.; MAJSAK-NEWMAN, G.; ROBERTSON, J.A.; SELVENDRAN, R.R. Investigation of factors that affect the solubility of dietary fiber, as nonstarch polysaccharides, in seed tissues of mung bean (*Vigna radiata*) and black gram (*Vigna mungo*). **J. Agric Food Chem.,** v. 42, n.3, p. 605-611, 1994a.

[20] GOONERATNE, J.; NEEDS P.W.; RYDEN, P.; SELVENDRAN, R.R. Structural features of cell wall polysaccharides from the cotyledons of mung bean *Vigna radiata*. **Carbohydr. Res.,** v. 265, n.1, p. 61-77, 1994b.

[21] HARALAMPU, S.G. Resistant starch – a review of the physical properties and biological impact of RS_3. **Carbohydr. Polym**., v. 41, n. 1, p. 285-292, 2000.

[22] HUISMAN, M.M.H.; SCHOLS, H.A.;VORAGEN A.G.J. Enzymatic degradation of cell wall polysaccharides from soybean meal. **Carbohydr. Polym.,** v. 38, n. 4, p. 299-307, 1999.

[23] JONES, P.M.B., BOULTER, D. The cause of reduced cooking rate in *Phaseolus vulgaris* following adverse storage conditions. **J. Food Sci.,** v. 48, n. 2, p. 623-649, 1983.

[24] KIM, J.B., CARPITA, N.C. Changes in esterfication of uronic acid groups of cell wall polysaccharides during elongation of maize coleoptiles. **Plant. Physiol**, v.98, n.2, p. 646-653, 1992.

[25] LOZOVAYA, V.V.; ZABOTINA, O.A.; WIDHOLM, J.M. Synthesis and turnover of cell wall polysaccharides and starch in photosynthetic soybean suspension cultures. **Plant Physiol.,** v. 111, n. 2, p. 921-929, 1996.

[26] MC DOUGALL, G. J.; MORRISON, I.M.; STEWART, D.; HILLMAN, J.R. Plant cell wall as dietary fibre: range, structure, processing and function. **J. Sci. Food. Agric.,** v. 70, n.2, p. 133-150, 1996.

[27] Mc LAUGHLIN, M.A.; GAY, M.L. Differentiation of dietary fiber sources by chemical characterization. In FURDA, I., BRINE, C.J.(Ed.), **New developments in dietary fiber**; New York: Plenum, 1990; p. 295-310.

[28] NEVES T.R.M. Endurecimento de feijão (*Phaseolus vulgaris* L.): proteínas da parede celular ricas em hidroxiprolina. São Paulo, 2000. Tese de doutorado (Doutora em Ciência dos Alimentos) - Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (USP).

[29] PROSKY, L.; ASP, N.G. SCHWEIZER,T.F.; DEVRIES, J.W.; FURDA, I. Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. **J. Assoc. Off. Anal. Chem.,** v.71, n. 5, p.1017-1023, 1988.

[30] REICHERT, R.D. Quantitative isolation and estimation of cell wall material from dehulled pea (*Pisum sativum*) flours and concentrates. **Cereal Chem.,** v. 58, n. 4, p. 266-27, 1981.

[31] REYES-MORENO, C.; PAREDES-LÓPEZ, O. Hard-to-cook phenomenon in common beans - A review. **Crit. Rev. Food Sci. Nutr.,** v. 33, n. 3, p. 227-286, 1993.

[32] RODIONOVA, N.A.; BEZBORODOV, A.M. Localization of enzyme systems that degrade cell wall polysaccharides in higher plants: pectinases (review). **Appl. Biochem. Microbiol.,** v. 33, n. 5, p. 467-487, 1997.

[33] RYDEN, P.; SELVENDRAN, R. Cell-wall polysaccharides and glycoproteins of parenchymatous tissues of runner bean (*Phaseolus coccineus*). **Biochem. J.,** v. 269,n. 2, p. 393-402, 1990.

[34] SELVENDRAN, R.R.; O'NEILL, M. Isolation and analysis of cell walls from plant material. **Methods Biochem. Anal.,** v.32, p. 25-153, 1987.

[35] SHOMER, I.; PASTER, N.; LINDNER, P.; VASILIVER, R. The role of cell wall structure in the hard-to-cook phenomenon in beans (*Phaseolus vulgaris* L.). **Food Struct.,** v. 9, n. 2, p. 139-149, 1990.

[36] SMITH, P.K.; KROHN R.I.; HERMANSON G.T.; MALLIA A.K., GARTNER F.H.; PROVENZANO M.D.; FUJIMOTO E.K.; GOEKE N.M.; OLSON B.J.; KLENK D.C. Measurement of protein using bicinchoninic acid. **Anal. Biochem.**, v. 150, n. 1, p. 76-85, 1985.

[37] TALBOTT, L.D.; RAY, P.M. Molecular size and separability features of pea cell wall polysaccharides. **Plant Physiol.**, v.98, n. 1, p.357-368, 1992.

[38] TAYLOR, R.L., CONRAD, H.E. Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. **Biochemistry**, v.11, n.8, p. 1383-1388, 1972.

[39] UPDEGRAFF, D.M. Semimicro determination of cellulose in biological materials. **Anal. Biochem** v.32, n. 3, p. 420-424, 1969.

[40] VARRIANO-MARTSON, E.; JACKSON, G.M. Hard-to-cook phenomenon in beans. Structural changes during storage and inhibition. **J. Food Sci.**, v.46, n. 3, p.1379-1385, 1981.

[41]WIECHELMAN, K.J.; BRAUN, R.D.; FITZPATRICK. Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation. **Anal. Biochem**., v. 175, n.1, p. 231-237, 1988.

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