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Cell wall polysaccharides of common beans (*Phaseolus vulgaris* L.)—composition and structure

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Abstract

A better knowledge of cell wall structure and composition is useful to understand the physiological properties of dietary fibre and the textural defects that affect legume seeds. Hence, cell wall polymers from cotyledon and tegument of common beans were fractionated, chemically and enzymatically degraded and analysed using GC-FID, GC-EIMS and HPLC. Results revealed that bean cotyledon is composed of 16.6% of cell wall, mostly constituted of 4 M NaOH and hot-water soluble polymers (WSP). Middle amounts of polymers were also solubilised by CDTA (1.2%) and 0.01 M NaOH (0.7%). Beans cell wall polymers are made up of arabinose-rich pectins, β-glucans and galacturonans. Endopolygalacturonase treatment in conjunction with methylation analysis revealed that galacturonans in beans cell wall are composed of xylogalacturonans and rhamnogalacturonans rather than homogalacturonans. The bulk of xyloglucan, the main hemicellulose found in cotyledon, was extracted with 4 M NaOH and showed to be fucogalactoxyloglucan. All fractions contained large ramified arabinans, highly substituted at *O-3* and *O-2* positions with short oligomers and terminal arabinoses. Galactans were found in low amounts probably as a linear 1,4-linked structures. Bean hulls contained high amounts of 1,4-linked xyloses and 1,5-linked arabinose, probably derived from xylans and large unbranched linear arabinans.

Keywords: Legume; Pectin; Carbohydrate; Gas chromatography

1. Introduction

The cooking quality of leguminous seeds is related to the thermal degradation and solubilization of cell wall polymers and, therefore, to the structure and chemical composition of their pectic polysaccharides. The cell wall of leguminous plants is generally depicted as a pectin-rich structure, rich in arabinan (Bhatty, 1990; Gooneratne, Needs, Ryden, & Selvendran, 1994b, Talbott and Ray, 1992). The bulk of the wall polysaccharides can be extracted with high hydroxide concentrations, which indicates a strong association between them (Gooneratne, Needs, Ryden, & Selvendran, 1994b; Ryden and Selvendran, 1990). Studies carried out by Reichert (1981), showed that the cell wall of pea cotyledon was composed to a large extent of pectic Ara-rich polysaccharides and hemicellulose, whilst its tegument consisted primarily of cellulose. Preliminary evidence suggested the presence of a different class of acidic polysaccharide in mung bean cell wall, probably xylogalacturonans (XGA) (Gooneratne, Needs, Ryden, & Selvendran, 1994b). This class of pectin is composed of a backbone of galacturonan containing terminal xylopyranosil (*t*-xyl*p*) substituted to the 3-position of some galacturonosyl residues. Huisman et al. (2001)

Abbreviations used CEL, cellulose; PMAA, partially methylated alditol acetate; TFA, trifluoroacetic acid; RG, rhamnogalacturonan; PG, polygalacturonan; HG, homogalacturonan; AG, arabinogalactan; UA, uronic acid; XGA, xylogalacturonan; XG, xyloglucan; WSP, water soluble polymers; WBP, weak-base soluble polymers; P1–P3, pools obtained from anion exchange chromatography peaks; EPG, endopolygalacturonase. Linkages were deduced from PMAA; *t*-ara*f*, terminal-arabinofuranose; *t*-xyl*p*, terminal-xylopyranose; 5-ara*f*, a arabinosylfuranoside residue containing a *O*-5 linkage; 2-xyl*p*, 2-xylopyranose; 3,5-ara*f*, a arabinosyl residue containing *O*-3 and *O*-5 linkages; The *p* and *f* notation means pyranose and furanose.

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confirmed the presence of XGA in the CDTA-soluble pectic substance from soy bean. Their studies also revealed the presence of rhamnogalacturonan (RG), but not of homogalacturonan (HG). It is quite possible that beans cell wall also contain these polysaccharides in its composition.

Phaseolus vulgaris seed cell wall polysaccharides are not extensively described in the literature and only few structural studies have been reported. O'Neill and Selvendran (1980) studied XG of beans and depicted its structure as a branched polymer containing also domains with low branching degree. The structure proposed has some similarities to the XG of soybean studied by Huisman, Schols, Voragen (2000). Literature also provides some descriptions of the cell wall structure of *Phaseolus coccineus, Vigna mungo* and *Vigna radiata* cell walls (Gooneratne, Majsak-Newman, Robertson, & Selvendran, 1994a; Gooneratne, Needs, Ryden, & Selvendran, 1994b; Ryden and Selvendran, 1990). Nevertheless, data refers to the tissue of bean pods or seed hulls and not to cotyledons. The cell wall of *P. vulgaris* and *P. coccineus* pods was minutely studied by O'Neill and Selvendran (1983, 1985).Nevertheless, to the better understanding of the legume textural defect and the nutritional properties of dietary fibre, further studies involving the structure of the cell wall of cotyledon are necessary.

Bean seeds (*P. vulgaris* L.) are important and nourishing food sources, especially for the populations of developing countries (Bressani, 1993). They furnish significant amounts of dietary fibre (DF), starch, vitamins, minerals and protein. Because of the increased awareness of the different properties of dietary fibres, accurate knowledge of the cell wall polysaccharide structure and composition is also important to have a better understanding of how fibres elicit physiological responses (McDougall, Morrison, Stewart, & Hillman, 1996). Furthermore, the cell wall determinates the texture of plant-based foods; this is an important quality attribute of legume seeds. The arrangement and interactions of plant cell wall polymers, both physically and chemically, largely determine the mechanical properties of the food (Waldron et al., 2003). The degree of polysaccharide solubility and of depolymerization during the cooking process, are determinants to quality. Some 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 (Garcia, Lajolo, & Swanson, 1993; Jones and Boulter, 1983; Liu, 1995; Shomer, Paster, Lindner, & Vasiliver, 1990).

In a previous paper, we obtained preliminary results on the cell wall polysaccharide sugar composition (Shiga, Lajolo, & Filisetti, 2004). In this study, we further investigate the cell wall polysaccharide structure and composition.

2. Materials and methods

2.1. Plant material

Common beans (Family Fabaceae; Genus, Phaseolus L.; Specie, *Phaseolus vulgaris* L; c.v. Carioca-Pérola) seeds were kindly provided by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)-GO-Brazil).

2.2. Moisture determination

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

2.3. Beans cell wall isolation

The cotyledon and tegument water-soluble (WSP) and water-insoluble (WIP) cell wall polymers were isolated according to the chemical-enzymatic method, adapted from the procedures of Prosky, Asp, Schweizer, Devries, and Furda (1988), Carpita (1983) and McLaughling and Gay (1990). 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 0.5 mm sieve. About 1 g of cotyledon or tegument flour were incubated with 15 mL $CHCl_3$:methanol (1:1, v/v) at 45 °C for 30 min and centrifuged at 9000*g* for 15 min (2×). The residue was washed with 15 mL methanol (2×) and 15 mL acetone and dried. The de-fatted flour was homogenised with 40 mL of 0.08 M phosphate buffer using a tissue homogenizer with a Teflon® pestle. The suspension pH was adjusted to 6.0 and 0.1 mL of α-amylase (Sigma-Aldrich Co., USA) was added and incubated for 30 min in boiling water. After cooling, the pH was adjusted to 7.5 and treatment with 0.1 mL with a protease (5 mg/mL solution; Sigma-Aldrich Co., USA) for 1 h at 60 °C followed. The pH was readjusted to 4.3 and then, 0.3 mL of amyloglucosidase (Sigma-Aldrich Co., USA) was added and incubated for 1 h at 60 °C. The suspension was allowed to cool and was then centrifuged at 8000*g* for 15 min. The supernatants were dialysed for 48 h against distilled water, freeze dried and weighed. This is the water-soluble cell wall polymers (WSP). The residue was washed exhaustively with distilled water and treated with 15 mL of 0.5 M sodium phosphate buffer, pH 7.2 (2×). The remaining residue was treated with 15 mL (2×) of 90% dimethyl sulfoxide (DMSO) for 20 min in an ultrasonic bath, washed (2×) with 15 mL of 90% DMSO and rinsed (5×) with distilled water. The final residues were suspended in water, freeze dried and weighed. This fraction is the water-insoluble polymers (WIP). At each stage of extraction the suspensions were centrifuged at 9000*g* for 15 min and the residues washed with water.

2.3.1. Chemical fractionation of WIP

The WIP was fractionated following the methodology described by Carpita (1983) with modifications. Sixty

milligrams of WIP were extracted with 15 mL of 0.05 M trans-1,2-diaminocyclohexane-N, N, N′, N′,-tetraacetic acid (CDTA), pH 6.5, for 16 h at 22 °C by stirring with a few drops of toluene. The suspension was centrifuged at 1500*g* for 10 min and the supernatant filtered through a GF/F filter. The filtrate was dialysed against distilled water at 22 °C for 72 h, freeze dried and weighed, resulting in the CDTA soluble fraction. The residue was sequentially treated with 15 mL of 0.01, 0.5 and 1 M NaOH containing 3 mg/mL of $NaBH_4$ for 1 h at 22 °C under an atmosphere of N_2 with constant stirring and with 4 M NaOH for 16 h under the same conditions. The NaOH extracts were chilled in an ice bath and neutralised with glacial acetic acid and dialysed for 48 h against distilled water, then freeze dried and weighed, resulting in the 0.01, 0.5, 1 and 4 M soluble fractions (0.01–4 M). The remaining cellulose-rich material (Residue) that resulted from WIP fractionation was washed, suspended in distilled water, freeze dried and weighed.

2.3.2. Enzymatic degradation

The cell wall polymers were treated with endo-1,4-βglucanase (Megazyme International Ireland Ltd, Ireland). Solutions (1 mg/mL) of cell wall polymers in 40 mM ammonium acetate buffer (pH 5.0) containing 5 U/mL of endo-1,4β-glucanase were incubated at 30 °C during 24 h. At the end, the enzymes were inactivated by heating at 100 °C for 10 min. The digests were precipitated with 4 vols. of ethanol (95%), warmed in a water bath at 60 °C for 15 min, chilled in ice and centrifuged at 10,000*g* for 10 min. The residue was re-suspended in water and freeze-dried. This was termed the alcohol-insoluble fraction; this contained mainly pectic polymers. Both fractions were analysed using a GC-EIMS system after derivatization.

2.3.3. Anion-exchange chromatography of WSP

Approximately 25 mg of WSP was fractionated on a XK 26/20 column (26 mm × 20 cm) containing Q-Sepharose FastFlow (Amersham Pharmacia Biotech, Uppsala, Sweden), which was initially equilibrated in 0.02 M phosphate buffer; pH 6.8 containing 5 mM sodium azide and 20 mM NaCl. The sample was eluted with the equilibrating buffer for 3 h 15 min at an elution rate of 5 mL/min. After that, a liner gradient (20 mM to 1 M NaCl) was applied. Fractions (5 mL) were collected and assayed for total sugar (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) and uronic acid content (Filisetti-Cozzi & Carpita, 1991). The appropriate fractions were pooled, concentrated, dialysed, freeze dried and analysed for neutral sugar composition and uronic acid content.

2.3.4. Molecular weigh determination

Size exclusion chromatography was performed on a C16/70 column, 16 mm × 70 cm (Pharmacia, Sweden), packed with Sepharose CL4B (Pharmacia). The column was equilibrated with 0.2 M NaOH for the elution of WSP and CDTA fractions and with 0.5 M NaOH for the elution of 0.05 to 4 M fractions. The void and included volumes of the columns were determined with blue dextran and NaCl. The molecular weight standards used were dextran polymers (fractionation range 50–3500 kDa, Fluka, Switzerland). The flow was fixed in 0.2 mL/min and 2 mL fractions were collected.

2.4. Carbohydrate composition

2.4.1. Uronic acids determination

Samples were suspended or dissolved in distilled water (0.5 mg/mL) and whenever necessary, homogenised in a tissue homogenizer with a Teflon® pestle. The uronic acids were determined according to Filisetti-Cozzi and Carpita (1991). Absorbance was read at 525 nm using a standard curve containing 50, 100, 150 and 200 nmoles of galacturonic acid. (retirei a descrição)

2.4.2. Neutral sugars determination

The neutral sugars rhamnose (Rhm), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal) and glucose (Glc), excluding the glucose derived from the cellulose (CEL), released by acid hydrolysis of the polysaccharides, were quantitatively determined by gas–liquid chromatography after reduction with $NaBH_4$ and acetylation (Blakeney, Harris, Henry, & Stone, 1983; Carpita and Whittern, 1986; Fox, Morgan, & Gilbart, 1989). The derivatives were dissolved in ethyl acetate and 1–3 μL samples injected into HP 6890 gas chromatograph (GC) equipped with a flame ionization detector (FID) (Hewlett Packard, USA). The alditol acetates were separated using a 0.25 mm × 30 m SP-2330 fused-silica capillary column with a 0.20 μm film thickness (Supelco, Inc., Bellefonte, PA). The column temperature was programmed from 170 to 240 °C at 10 °C for minute with a 20 min hold at the highest temperature. Injector and detector temperatures were set a 250 °C. Helium was used as the carrier gas at a flow rate of 30 mL/min. (retirei descrição do preparo)

2.4.3. Cellulose determination

Glucose derived from cellulose (CEL) was obtained after hydrolysis of the non-cellulosic polymers with Trifluoro-acetic acid (TFA). About 1 mg of cellulose-rich material (Residue) was hydrolysed with 1 mL of 2M TFA at 120 °C for 60 min in a screw-capped conical vial. At the end, the hydrolysate was centrifuged at 2000*g* for 5 min and the supernatant was discharged. Exact 1 mL of 2 M H_2SO_4 was added to the remaining residue and the mixture was hydrolysed at 120 °C for 90 min. At the end, the hydrolysate was neutralised with NaOH 50% (w/w) and the sugar content was analysed on a CarboPac PA10 (Dionex Corp., Sunnyvale, USA) pellicular anion-exchange analytical column (250 × 4 mm) with the correspondent guard column. The HPLC system used for this work was a DX 500 (Dionex, USA) with a gradient pump GP40, equipped with a autosampler AS50. The carbohydrates were detected by

a PAD system ED40 in the integrated amperometry mode, using a gold working electrode and an Ag/AgCl reference electrode. The running was performed isothermally and isocratically (25 °C; 2 mM NaOH; at 1.0 mL/min flow rate) for 38 min followed by a cleaning sequence with 300 mM NaOH for 10 min and more 10 min of re-equilibration.

2.5. Preparation of partially methylated alditol acetate derivatives (PMAA)

The PMAA preparation was performed according to Gibeaut and Carpita (1991).

The partially methylated derivatives were hydrolysed, reduced with $NaBD_4$ and acetylated according to 2.4 item. The PMAA was cleaned-up according to Gibeaut and Carpita (1991), using a stock solution prepared by adding equal volumes of CCl_4 (Carlo Erba Reagenti, Italy) and methanol–water solution (40:60, v/v) in a reagent bottle. After clean-up procedures, sugar derivatives were suspended in CH_2Cl_2 and analysed by GC-EIMS using a GC-MS system composed of HP 6890 coupled to a quadrupole electron impact ionization analyser HP 5973, equipped with a automated injection system HP 7683 (Hewlett Packard, USA). The source temperature was fitted at 250 °C and 70 eV. The derivatives were separated using a SP-2330 glass WCOT capillary column (Supelco, USA) in a temperature programmed of 160–210 °C at 2 °C/min and to 240 °C at 5 °C/min and Helium was used as the carrier gas. (Retirei a descrição do preparo)

2.6. Data treatment

The tables showing sugar and polymer composition was expressed as the percentage of total recovery (μg/100 mg of bean flour). A substrate blank was performed concurrently to each wall extraction. The water-soluble material that resulted from the substrate blank had its neutral sugars levels analysed, revealing high amounts of Man (62%). Methylation analysis showed the presence of *t*-man*p*, *2*-man*p* and *2,6*-man*p* residues in this material and, consequently, in the WSP. Hence, the Man content was not computed as being part of wall polymers.

3. Results

The bean seeds (*P. vulgaris* L., c.v. Carioca-Pérola) were constituted of 9.9% hull, 89% cotyledon and 2% embryo by weight. After the enzymatic-chemical treatment, the bean cotyledon rendered about 9.6% of WIP and 7% of WSP; hulls contained 7% of WIP and 0.3% of WSP. Thus, beans contained about 16.6% of cell wall polymers derived from cotyledon and 5.9% derived from hulls.

3.1. Cotyledon cell wall polysaccharides: carbohydrate composition

The WSP corresponded to 6.2% of whole cotyledon and sugar analysis showed that Ara is the most abundant monosaccharide (42%), followed by UA (13%), Man (13%), Gal (11%), Xyl (11%), Glc (7%), Rhm (2%) and Fuc (0.6%) (Table 1). The WIP corresponded to about 10.9% of the cotyledon and was composed of 56% Ara, 7% UA, 6% Gal, 13% Xyl, 0.5% Man, 3% Glc, 2% Rhm and 0.8% Fuc. About 11.5% of the total Glc derived from cellulose (Table 1).

All WIP fractions, except the cellulose-rich residue, showed a similar sugar profile. With the increasing strength of the extraction reagent a decrease in the acidic polymers is observed (Table 1). The major part of bean cell wall is composed of polymers that are released by 4 M NaOH. Table 1 shows that polymers solubilised with 4 M NaOH corresponded to 5% of the whole sample and are mainly constituted of Ara (58%), Xyl (12%), Glc (10%) and UA (10%). Approximately 1.2% of the polymers were extracted with the CDTA solution. The WSP and CDTA polymers were composed of Ara-rich (42 and 64%) and UA-rich (13 and 15%) pectic material, also containing Gal (11 and 6%), Rhm (3 and 2%), Fuc (0.8 and 0.6%), Xyl (12 and 11%) and Glc (10 and 7%) (Table 1). Polysaccharides released by

Table 1
Bean cell wall polymer and sugar composition

Fractions	(w/w%)[a]	Neutral sugars (w/w%)[a]							U.A. (w/w%)[a]
		Rhm	Fuc	Ara	Xyl	Man	Gal	Glc	
WSP	6.2±0.97	1.8±0.04	0.6±0.09	42.1±2.36	11.2±0.29	13.3±0.75	11.1±0.57	7.1±0.17	12.7±0.00
CDTA	1.2±0.40	3.4±0.15	0.4±0.03	63.8±4.31	9.1±0.93	nd	5.9±0.00	2.0±0.04	15.2±0.86
WIP=10.9%									
0.01 M	0.7±0.26	4.1±0.55	0.3±0.06	52.7±1.11	13.5±0.32	3.7±0.48	3.0±2.20	1.1±0.07	21.6±2.17
0.5 M	0.7±0.11	3.6±0.38	0.6±0.16	54.3±8.08	13.8±1.58	1.4±1.85	5.7±0.28	3.9±0.63	16.7±1.17
1 M	0.6±0.19	3.1±0.14	1.1±0.14	54.2±4.85	14.4±2.12	0.4±0.05	6.6±0.49	8.6±0.62	11.6±0.09
4 M	5.3±0.04	2.9±0.47	0.8±0.55	57.9±5.74	11.9±2.14	1.0±1.30	6.1±1.23	9.8±2.17	9.5±0.25
Residue	2.3±0.55	nd	nd	11.3±1.50	0.4±0.23	17.7±2.64	nd	56.2±3.22	14.4±0.68

WSP, water-soluble polymers; WIP, water insoluble polymers; CDTA and 0.01–4.0 M, CDTA soluble fraction and 0.01–4.0 M hydroxide soluble fractions; Residue, cellulose-rich residue. Rhm, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids. nd, not determined; (n=3).

[a] Sugar and polymer composition expressed as the dry weight percentage of total recovery.

saponification with 0.01, 0.5 and 1 M NaOH solutions constitute a modest part of whole cell wall polymers (0.7, 0.7 and 0.6%, respectively). Polymers extracted with 0.01 to 1 M NaOH contained, respectively, Glc (1–9%), Gal (3–7%), UA (12–22%), Rhm (3–4%), Fuc (0.3–1%), Xyl (14%) and Ara (53–54%) (Table 1).

The cellulose-rich residue corresponded to 2.3% of bean cotyledon (Table 1). This fraction was composed of about 56% Glc, mostly derived from cellulose. This fraction also contained 18% of Man, 14% of UA, 11% of Ara and 0.4% of Xyl (Table 1).

3.2. Water soluble polysaccharides (WSP) fractions: carbohydrate composition

The WSP of bean cotyledons was separated by anion exchange chromatography, producing three distinct peaks (Fig. 1). The elution profile revealed polymers with neutral and charged (acidic) domains similar to those obtained from pea pectin by Talbott and Ray (1992).

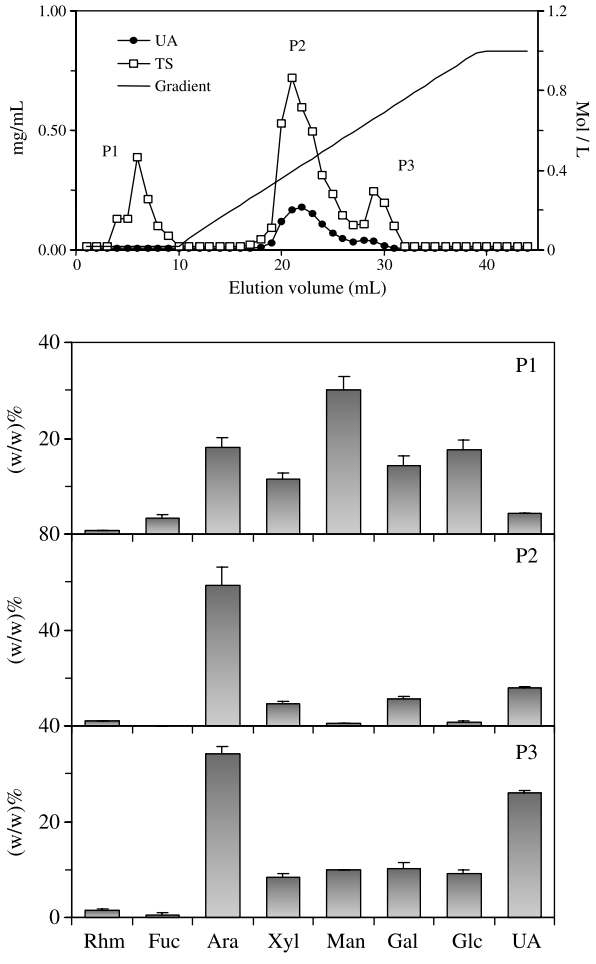

Fig. 1. Elution profile and carbohydrate compositions of the bean water-soluble polymers (WSP) on anion-exchange chromatography. P1, polymers of peak 1; P2, polymers of peak 2, and P3, polymers of peak 3. Column fractions were assayed for total sugar by Dubois et al. (1956) and uronic acids by Filisetti-Cozzi and Carpita (1991). Rhm, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid; TS, total sugar.

The peak 1 (P1) corresponded to 13% of the total polymers by weight (Fig. 1). These fractions contained mostly neutral sugars, especially Glc (18%), Xyl (12%), Fuc (3.4%), Ara (18%) and Gal (14%). Just 4% of UA were present in this fraction.

According to the results, P1 contains high amounts of Xyl and Glc, which strongly suggest the presence of XGs. This fraction also exhibits the highest Fuc content that may be associated to the presence of the trisaccharide Fuc*p*-(1-2)-Gal*p*-(1-2)-Xyl*p* in the structure of the XGs.

The polymers from peak 2 (P2) and peak 3 (P3) corresponded to 34% and 21% of the total recovery, respectively (Fig. 1). These fractions contained medium amounts of Gal (11 and 10%) and Xyl (9 and 8%) and small amounts of Fuc (0.2 and 0.5%) and Rhm (1.9 and 1.5%). The UA accounted for (16 and 26%) of the dry mass, respectively. The Ara residues were particularly high, accounting for 59 and 34% of the dry mass.

The Ara-rich polymers in P2 suggest the presence of arabinans, which may be associated to acidic RGs, since coeluted with UA rich-polymers. About 9% of the Xyl residues could be associated to pectic material instead of XGs, since just low amount of Glc (2%) were found.

3.3. Molecular weight profile (mol wt) of the cell wall polymers

The apparent mol wt of the cell wall polysaccharides obtained by gel filtration showed that bean cell wall were constituted by high mol wt polymers (mol wt range between 1576 and 3505 kDa). Polymers in CDTA fraction have the highest mol wt and the polysaccharides extracted with 4 M NaOH the lowest one.

Polymers elution produced just one peak for each running, suggesting strong interactions between wall pectic polysaccharides. These interactions were stable enough to resist to the action of strong alkali concentrations (4 M NaOH) used to solubilise the material. According to Talbott and Ray (1992), when pea pectin solutions are concentrated by evaporation, some aggregation occurs, leading to a modest upshift of the main mol wt profile. Since there are similarities between beans and peas cell walls, freeze-drying process may have produce aggregates.

3.4. Hull cell wall polysaccharide: carbohydrate composition

The WSP of hulls was composed by 38% Ara, 21% UA, 11% Gal, 11% Xyl, 10% Man, 5% Glc, 3% Rhm and 0.1% Fuc (Fig. 2). Uronic acid and Rhm contents was respectively, 1.6 and 2 times higher than in WSP of cotyledon. The WIP contained 22% Ara, 14% UA, 12% Gal, 35% Xyl, 13% Man, 0.2% Glc, 2% Rhm and 0.4% Fuc. About 12% of hulls Glc derived from cellulose (Fig. 2).

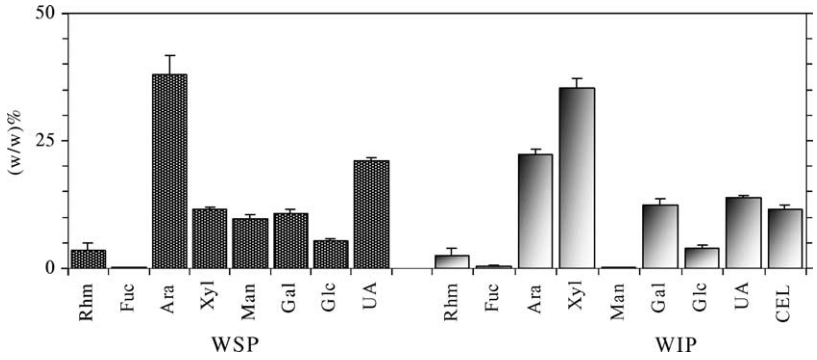

Fig. 2. Hull cell wall sugar composition of soluble and insoluble polymer fractions from the tegument (WSP and WIP). Rhm, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; CEL, glucose derived from cellulose; U.A., uronic acids.

The sugar composition of bean hulls were similar to that obtained by Weightman, Renard, Gallant, and Thibaut (1995) with pea. Both revealed predominance of Xyl, Ara and cellulose in its composition. Compared to WIP of cotyledon, tegument contained 2.5 times less Ara and 2.1, 2.3 and 2.7 times more UA, Gal and Xyl contents.

3.5. Structural aspects of the cell wall polysaccharides

3.5.1. Cotyledon cell wall polysaccharides

Methylation analysis of selected fractions revealed the main glycosidic linkages. Although, the results were not quantitative and UA content was not determined. For practical reasons, polymers released by 0.01–1 M NaOH were pooled and named weak-base-soluble polymers (WBP).

All polymers fraction of Carioca beans contain high amounts of *5*-ara*f* (26–34%) which could suggests the presence of a long 1,5-linked Ara backbone (Table 2). Methylation analysis also showed the presence of *3,5*-ara*f* and *2,5*-ara*f* residues (about 5–7% and 3–7%, respectively), accusing the presence of branching points at *O-3* and *O-2* position of arabinan backbone. Middle amounts of $Ara(OAc)_5$ (6–10%) revealed monomers of Ara completely substituted. The values of *t*-ara*f* (24–30%) and *5*-ara*f* (24–34%) are close from one another. This composition could indicate arabinan with long main backbone, with sidechains composed of short oligomers or just one residue of Ara

Table 2
Partially methylated alditol acetate from selected cotyledon cell wall fractions

Related polymer	Monomers	WSP	P2	CDTA	WBP	4 M
		Mole %				
Arabinan	t-araf	24.8	29.6	28.8	23.5	28.4
	5-araf	26.0	34.4	32.4	29.9	23.6
	3,5-araf	6.4	7.3	6.8	6.5	4.8
	2,5-araf	2.7	5.5	6.8	6.5	4.9
	$Ara(OAc)_5$	7.4	8.9	9.1	9.6	6.4
XGA Xylan XG	t-glcp	1.9	–	1.6	1.5	2.5
	4-glcp	1.0	1.0	0.6	1.6	2.0
	4,6-glcp	2.1	–	1.1	2.2	4.8
	t-xylp	9.4	6.5	6.7	11.5	13.3
	2-xylp+4-xylp	1.3	0.8	0.7	1.7	2.9
	t-fuc	0.5	–	0.2	0.2	0.8
	t-galp	2.0	1.3	1.6	1.7	2.8
	2-galp	1.1	–	0.2	0.7	1.0
Galactan AGII RGI	3-galp	0.6	0.5	0.5	0.04	–
	4-galp	0.1	0.5	0.1	0.1	–
	6-galp	0.4	0.8	0.4	0.4	0.1
	3,6-galp	1.4	–	–	–	–
	4,6-galp	0.9	1.2	0.9	0.8	0.7
	2-rhm	0.8	1.0	0.9	1.2	0.8
	2,4-rhm	0.6	0.7	0.7	0.5	0.3
-	t-manp	5.8	–	–	–	–
	2-manp+2-glcp	2.2	–	–	–	–
	2,6-manp	0.7	–	–	–	–

CDTA, polymers extracted with CDTA solution; P2, polymers of peak 2 of WSP anion exchange chromatography; WBP, weak-base soluble polymers (related to polymers solubilised by NaOH 0.01-1M); 4 M, polymers extracted with 4 M NaOH; XG, xyloglucan; XGA xylogalacturonan; AGII, arabinogalactan type II; RGI, rhamnogalacturonan type I; ($n=3$).

attached to the *O-2* and *O-3* positions (Table 2). Another hypothesis is that leguminous arabinans have short chains, having no more than 3 Ara residues (O'Neill and Selvendran, 1980). The former supposition was adopted because the molecular weights of all polymers of Carioca beans showed to be high.

The wall polymers of Carioca bean also contain low amounts of *4*-glc*p* (0.6–2%), *4,6*-glc*p* (1.1–4.8%), *t*-glc*p* (1.5–2.5%), *t*-fuc (0.2–0.8%), *2*-gal*p* (0.2–1.1%) and *t*-xyl*p* (6.5–13.3%) residues, which are characteristic of the presence XG (Table 2). The WBP and 4 M polymers contained the highest percentage of *4*-glc*p*, *4,6*-glc*p* and *t*-glc*p* residues.

Another neutral polymer, that possibly is present in bean cell wall, is a galactan. It is described in the literature as a polymer constituted of 1,4-β-linked Gal (Carpita and McCann, 2000). Low amounts of *4*-gal*p* (0.1–.5%) together with the absence of *3,4*-gal*p* residues allows the conclusion that galactans in beans have a linear structure due to the absence of branching point at *O-3* (Table 2). The absence of *3,4*-gal*p* in legume seed was also observed by Talbott and Ray (1992) who inferred that pea cell wall contains only arabinans and linear galactans rather than arabinogalactans. The absence of *3,4*-gal*p* residues may suggest the absence of arabinogalactans type I (AGI).

The *3,6*-gal*p* residues, characteristic of arabinogalactan type II (AGII), were only found in the WSP fraction (1.4%) being absent in the other fractions (Table 2). Small amounts of *4,6*-gal*p* (0.7–1.2%), *3*-gal*p* (0.04–0.6%) and *6*-gal*p* (0.1–0.8%) were also found in the wall polymers (Table 2). Galactans composed of long 1,4-linked linear regions punctuated with some 1,4,6-linked Gal containing short 1,3- and/or 1,6-linked Gal as sidechains at *O-6* position, were documented in plant material such as apple (Oechslin, Lutz, & Amadó, 2003). A galactan with similar structure could also be present in the bean cell wall, since we observed points of linkage at 3-; 6- and 4,6- in gal*p* residues. However, more studies are necessary to confirm the presumption.

Bean cell wall polymers is also characterised by high *t*-xyl*p* /*4,6*-glc*p* ratios in all polymer fractions, which suggest that *t*-xyl*p* may be associated to another group of polymer, diverse from XGs (Table 2). The separation of β-glucans and arabinans, present in the WSP, from its acidic polymers (P2) by means of anion-exchange chromatography revealed that high amounts of *t*-xyl*p* residues were associated to the uronic acid-rich polymers (Table 2). This result provides an indication of the existence of XGA in bean wall polymers. Furthermore, the amount of *t*-xyl*p* residues for WSP, CDTA, WBP and 4M fractions are 2–6 times higher than *4,6*-glc*p* residues, revealing that almost all *t*-xyl*p* residues did not arise from XG but from XGA (Table 2). This separate class of substituted HG, frequently cited as a component of legume cell wall, contains appendant ?α-D-Xyl units at the *O-3* position of about half of the galacturonic acid units (Carpita and McCann, 2000; Renard, Weightman, & Thibault, 1997). This polysaccharides is cited by Gooneratne, Needs, Ryden, & Selvendran (1994b)who found preliminary evidences of their presence in the cotyledon cell wall of *V. radiata*. The XGA was also documented in pea hulls by Le Goff, Renard, Bonnin, and Thibault (2001). According to the authors, it is a subfraction of 'hairy' regions and could be related to the protection of galacturonan backbone of microbial contamination during seed dormancy, because xylose substitution in XGA inhibited EPG degradation.

Hence, since EPG treatment did not produce appreciable reduction in the pectins mol wt (data not shown), we concluded that bean cell wall might contain more XGA than HG. The low amounts of *2*-rhm (0.8–1%) and *2,4*-rhm (0.3–0.7%) also suggest a structural composition of smooth domains of XGA interspaced with some hairy regions of RG (Table 2). The backbone of RGI is depicted as a polymer composed of alternating sequence of 1,4-linked galacturonic acid units and 1,2-linked Rhm residues, the last one carrying sidechains at *O-4*. The results are in accordance with the recent studies conducted by Huisman et al. (2001) with soybean cell wall, which reveal a structure composed of XGA and RG but not HG.

3.5.2. Oligomers released by enzymatic treatment

Cellulase (β1,4-endoglucanase) and endopolygalacturonase (EPG) was used with the intent of separate 1,4-β-linked glucans from 1,4-α-linked galacturonan. The EPG treatment was not effective to reduce pectins mol wt; therefore, only the data obtained from cellulase treatment were presented. Selected cell wall polymer fractions treated with cellulase were separated by precipitation by adding 4 vols. of 95 °GL ethanol and both, alcohol-soluble (AS) and insoluble (AI) subfractions, were dried and converted to PMAA (Tables 3–5).

The AS subfractions obtained from 4M and WBP fractions contained *2*-gal*p, t*-fuc, *4,6*-glc*p* and *4*-glc*p* residues which confirm the presence of XG oligomers (Table 3). Based on results of Table 3, a degree of branching for the XG was calculated from the amounts of *4*-glc*p* and *4,6*-glc*p*. For example, the high value of *4,6*-glc*p*/*4*-glc*p* ratios in WBP and 4 M fractions leads to the assumption that XGs are more branched in the alkali soluble fractions (Fig. 3), in opposition to observed in literature (O'Neill and Selvendran, 1983). Obviously, the values of degree of branching are just an estimation. The AI subfractions contained 4–8% of *t*-xyl*p* residues, probably derived from XGA (Table 3).

All ethanolic subfractions also exhibited *t*-ara*f*, *5*-ara*f*, *3,5*-ara*f* and *2,5*-ara*f* monomers derived from short arabinans (Table 4). These polymers possess a short 1,5-linked backbone with substitutions at *O-2* and *O-3* positions. In turn, arabinans of AI fractions have high mol wt, most probably composed of long chains of 1,5-linked Ara*f* containing high amounts of substitutions at *O-2* and *O-3* positions. These substitutions is likely to be composed of a single *t*-ara*f* residue. The approximate degree of branching

Table 3
Partially methylated alditol acetate from selected cotyledon cell wall fraction, obtained after cellulase treatment; fractions containing mainly XG oligomers

Related polymers	Monomers	Fractions			
		WSP	CDTA	WBP	4 M
	Alcohol insoluble fraction (molar %)				
XGA	*t*-glc*p*	1.5	0.6	0.9	1.0
Xylan XG	*4*-glc*p*	1.5	0.1	0.8	1.1
	4,6-glc*p*				
	t-xyl*p*	7.4	4.1	8.0	3.7
	2-xyl*p*+*4*-xyl*p*	0.5	–	0.4	0.5
	t-fuc	–	–	–	–
	t-gal*p*	1.2	1.0	1.7	3.1
	2-gal*p*	0.3	–	0.3	0.7
	Alcohol soluble fraction (molar %)				
	t-glc*p*	1.2	2.1	4.2	5.9
	4-glc*p*	24.4	3.5	5.8	4.4
	4,6-glc*p*	2.1	2.2	9.7	13.6
	t-xylp	6.4	4.7	15.0	13.4
	2-xyl*p*+*4*-xyl*p*	1.0	0.6	5.2	3.8
	t-fuc	0.0	0.6	2.0	1.5
	t-gal*p*	1.3	1.7	4.2	7.5
	2-gal*p*	6.9	2.1	8.6	11.1

CDTA, polymers extracted with CDTA solution; WBP, weak-base soluble polymers (related to polymers solubilised by NaOH 0.01–1 M); 4 M, polymers extracted with 4 M NaOH; XG, xyloglucan; XGA xylogalacturonan; ($n=3$).

was calculated from the amounts of *5*-ara*f*, *2,5*-ara*f*, *3,5*-ara*f* and Ara(OAc)$_5$ for arabinans (Fig. 3). The results show that the arabinans degree of branching increases from WSP to 4 M fraction.

Residues of *2*-rhm and *2,4*-rhm were also found in very low quantities and may reveal small domains of RG containing branches in the *O-4* position of some Rhm residues (Table 5). Large amounts of *t*-xyl*p* in the absence

Table 4
Partially methylated alditol acetate from selected cotyledon cell wall fraction, obtained after cellulase treatment; fractions containing mainly arabinans oligomers

Related polymer	Monomer	Fractions			
		WSP	CDTA	WBP	4 M
Arabinans	Alcohol insoluble fraction (molar %)				
	t-araf	28.7	28.6	33.2	23.0
	5-araf	34.1	45.3	36.7	41.7
	3,5-araf	8.9	8.7	6.5	8.3
	2,5-araf	4.2	3.2	3.8	5.6
	Ara(OAc)$_5$	6.2	5.9	4.9	7.4
	Alcohol soluble fraction (molar %)				
	t-araf	19.5	31.5	18.6	17.3
	5-araf	25.6	30.6	16.6	13.1
	3,5-araf	4.9	6.4	3.7	3.0
	2,5-araf	2.9	5.8	3.0	2.4
	Ara(OAc)$_5$	3.1	6.5	3.3	2.7

CDTA, polymers extracted with CDTA solution; WBP, weak-base soluble polymers (related to polymers solubilised by NaOH 0.01–1 M); 4 M, polymers extracted with 4 M NaOH; ($n=3$).

Table 5
Partially methylated alditol acetate from selected cotyledon cell wall fraction, obtained after cellulase treatment; fractions containing galactans and galacturonans oligomers

Related polymers	Monomer	Fractions			
		WSP	CDTA	WBP	4 M
	Alcohol insoluble fraction (molar %)				
Galactans	*3*-gal*p*	1.0		0.2	–
AGII RGI	*3,6*-gal*p*	–	–	–	–
	4-gal*p*	1.0	0.4	0.2	–
	6-gal*p*	1.7	2.2	1.3	1.8
	2-rhm	0.9	–	0.7	1.1
	2,4-rhm	0.8	–	0.5	1.0
	Alcohol soluble fraction (molar %)				
	3-gal*p*	–	–	–	–
	3,6-gal*p*	–	–	–	–
	4-gal*p*	–	0.2	–	–
	6-gal*p*	0.8	0.7	–	
	2-rhm	–	0.6	–	–
	2,4-rhm	–	0.3	–	–

WSP, water-soluble polymers; CDTA, polymers extracted with CDTA solution; WBP, weak-base soluble polymers (related to polymers solubilised by NaOH 0.01-1M); 4 M, polymers extracted with 4 M NaOH; AGII, arabinogalactan type II; RGI, rhamnogalacturonan type I; ($n=3$).

of 1,4,6-linked Glc indicate that Xyl residues derived from XGA. Galactose residues was almost absent in the AS subfractions but concentrated primarily in the AI subfractions (Table 5). Almost all Gal in the AI fractions were β1,4-linked indicating the galactan to be present as a linear oligomer (Table 5).

3.5.3. Hulls cell wall polysaccharides

Beans hulls was characterised by its high amounts of glucans. Opposing to Reichert (1981) that found high amounts of cellulose in pea hulls, beans contained just 11% glucose derived from cellulose. Almost all glucose was found as *4*-glc*p* residue, probably derived from XG (Table 6).

The presence of *4*-xyl*p* residues are usually associated to the presence of xylans and XGs. Xylans are the well-known origin of 1,4-linked xylosyl residues in dicotyledonous plant and were documented to be present at cell walls of another

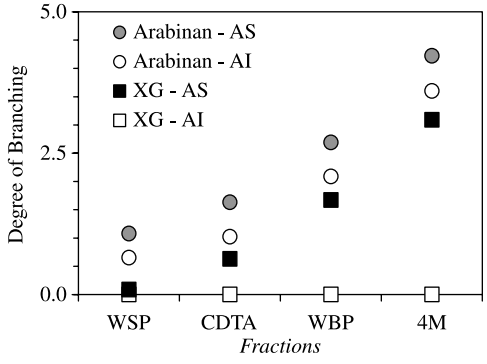

Fig. 3. Beans cell wall arabinans and xyloglucans (XG) degree of branching. AS, alcohol-soluble fraction; AI, alcohol-insoluble fraction.

Table 6
Partially methylated alditol acetate from hull cell wall fractions

Related polymers	Monomers	WIP	WSP
		Mole %	
Arabinan	t-araf	–	1.9
	5-araf	16.9	60.8
	3,5-araf	4.4	3.0
	2,5-araf	1.1	2.5
	$Ara(Ac)_5$	–	0.4
XGA Xylan XG	t-glcp	–	2.7
	4-glcp	36.9	0.6
	4,6-glcp	3.3	–
	t-xylp	–	1.4
	4-xylp+2-xylp	26.2	12.0
	t-fuc	–	1.1
	t-galp	–	2.9
	2-galp	11.2	0.2
Galactan AGII	3-galp	–	1.2
RGI	4-galp	–	0.1
	6-galp	–	1.6
	2,4-galp	–	0.2
	2-rhm	–	1.2
	2,4-rhm	–	0.7
	t-manp	–	5.5
	2-manp	–	0.1

WIP, water-insoluble polymers; WSP, water-soluble polymers; XG, xyloglucan; XGA xylogalacturonan; AGII, arabinogalactan type II; RGI, rhamnogalacturonan type I; ($n=3$).

legume seeds (Gooneratne, Needs, Ryden, & Selvendran, 1994a,b; Stolle-Smits, Beekhuizen, Van Dijk, Voragen, & Recourt, 1995). Beans hulls yielded high amounts of *4*-xyl*p* and *2*-xyl*p* in the WIP and WSP (26 and 12%), which could characterise the presence of xylans (Table 6).

Neither galactans nor RG were found in the WIP, but WSP contained both polysaccharides, as can be seen by *2*-rhm; *2,4*-rhm; *4*-gal*p* and *6*-gal*p* residues. About 11.2% of 1, 2-linked Gal residues was found in WIP, may be associated to XGs. High amounts of 1,4-linked Xyl were found in the WSP, part of them very likely to be associated with xylans. Commonly an indicative of XG, the *t*-xyl*p* residues found in WSP could be derived from XGA, since no *4,6*-glc*p* was observed (Table 6). The presence of this polymer was already reported by Renard, Weightman, & Thibault (1997) in peas.

Another similarities between both seed hulls is the presence of heteroxylans, glucans and arabinans of low degree of branching (Renard, Weightman, & Thibault, 1997). The low *4,6*-glc*p*/*4*-glc*p* ratio suggest the presence of XG with a low degree of branching (Table 6). Arabinans of beans hulls also seems to have long backbones and low degree of branching. This is observed by the high amounts of 1,5-linked Ara in WIP (17%) and WSP (61%) and small amounts of *t*-ara*f*; *3,5*-ara*f* and *2,5*-ara*f* (Table 6). These low-branched XG and arabinans may confers the water insolubility of the seed tegument, since their unbranched structures permit associations that results in crystalline regions stabilised by H bonds.

4. Discussion

Beans produce a fractionation profile similar to that obtained by Gooneratne, Needs, Ryden, & Selvendran (1994a,b)with *Vigna mungo,* despite of some differences in both extraction process. The fractionation profile shows high amount of pectins released by strong alkali solution, and middle amounts released by CDTA and mild alkali solutions. According to our results and to the literature, in contrast to another parenchymatous tissues, legume seeds have high amounts of pectic material that are released by 4 M alkali.

Loosely bound polymers solubilised by water also represent a considerable part of bean cell wall. In a review Brillouet (1982) shows the yields of water-soluble pectic material obtained from various sources. According to the work, the yields of water-soluble polymers obtained from genus *Phaseolus* varied from 13.2% in *P. glabra* to 0.2% in *P. vulgaris*. The amount of material released can also vary according to the extraction process that is carried out with cold (or most usually, hot) water or buffers. The review shows that the yields of water-soluble material is affected by temperature, as can be seen in *P. mungo* seeds that renders 1.3% with cold water and about 6% with hot water. The last data is very close to that obtained in this work.

Mild alkali solution released protein rich polymers resulting in low recovery of neutral and acidic sugars, especially polymers of 0.01 M fraction. Protein content was not determined, however, Neves (2000) documented high amounts of hydroxyproline in the polymers solubilised by weak alkali solution.

The monosaccharide composition obtained from beans also showed similarities with other legume seeds, such as groundnut, broad bean, kidney bean, lentil, chickpea and mung bean cell walls (Bhatty, 1990; Colonna, Gallant, & Mercier, 1980; Champ, Brillouet, Rouau, 1986; Gooneratne, Needs, Ryden, & Selvendran, 1994a,b; Tharanathan, Wankhede, & Raghavendra Rao, 1975). Methylation analysis and enzymatic treatment revealed that bean pectic material can be constituted of neutral and acidic domains. According to this work and evidences cited in the literature, acidic domains seems to be formed by XGA and RG substituted at the *O-4* position of Rhm (Gooneratne, Needs, Ryden, & Selvendran, 1994a,b; Huisman et al., 2001).

Size-exclusion chromatography indicates that pectins may have very large structures with a mol wt higher than 1500 kDa. Arabinans probably are the major sidechains. The size-exclusion chromatography gives an estimate of values, thus the high mol wt obtained is probably a consequence of molecular shape and aggregates formation. Structures with straight and long shape are harder to elute than structures with branched spherical shape and could lead to misleading results. Talbott and Ray (1992) working with pea seeds (*Pisum sativum*) found polymers in a range around either 100 kDa or 5–10 kDa. They suggested noncovalent

associations of polyuronides into multichain aggregates to explain the higher mol wt obtained.

O'Neill and Selvendran (1980) assumed that *P. coccineus* polymers were constituted of short arabinans containing no more than 2 or 3 units long backbone due to its high amounts of *t*-ara*f* residues. In this work, we considered that high amounts of *t*-ara*f* may be associated to a large polymers containing high amounts of single Ara residues as branching. Cooking beans cotyledons until softness, previously to extraction (about 40 min at atmospheric pressure), cause a significant reduction of Ara content in 4M fraction and increase of free fragments of arabinans (data not shown). The degradation of pectic material is normally cited in the literature as a source of errors (McDougall and Selvendran, 2001). The cooking process results in degradation of arabinose-rich pectic material in green beans (Stolle-Smits, Beekhuizen, Van Dijk, Voragen, & Recourt, 1995). Some degradations eventually may have occurred during the extraction, since free fragments of short-chain arabinans were found. Thus, the amounts of short arabinans may be the inevitable result of neutral pectins degradation that occurs during the extraction procedure. Even though, the mild extraction conditions used in this work resulted in good recovery of WSP and suggesting a low degradation of the pectic polysaccharides. Hence, in opposition of what was found by O'Neill and Selvendran (1980) for dwarf French beans and *P. coccineus*, Carioca bean arabinans seems to have a large and highly branched structure, capable to establish physical interactions that may result in aggregates formation.

Linkage analysis together with enzymatic treatment showed high amounts of *t*-xyl*p* in close association to acidic polysaccharides. The removal of XG from the sample by means of enzymatic treatment with cellulase reveal that high amounts of *t*-xyl*p* unequivocally originated from pectins, presumably from XGA. The XGA is described as a separate class of substituted galacturonan, with appendant ?α-D-Xyl units at the *O-3* position of about half of the GalA units (Carpita and McCann, 2000).

The presumption that *t*-xyl*p* could be derived from XGA is corroborated by the fact that this polymer is frequently cited in the literature as a component of legume cell wall. For example, preliminary evidences of the presence of XGA was found in mung beans (*V. radiata*) by Gooneratne, Needs, Ryden, & Selvendran (1994b). Huisman et al. (2001) also found that soy bean cell wall has a structure composed of XGA and RG but not HG.

The observation that bean pectin was not attacked by EPG and that residues of *2*-rhm and *2,4*-rhm were detected in almost all polymer fractions lead to the conclusion that beans is constituted of XGA and RG rather than HG. Furthermore, the low recovery of CDTA soluble polymers suggests that HG comprise only few domains of RG.

Bean XG is mainly extracted by 1M and 4 M NaOH, in the same way of soybean and another legume polysaccharides (Gooneratne, Needs, Ryden, & Selvendran, 1994a,b; Huisman et al., 2000). The XG of Carioca beans showed a structure similar to that found in soybean, a polymer composed of XXXG-building blocks (Huisman et al., 2000). The presence of XG is clearly observed by the presence of 1,4,6- and 1,4-linked glucopyranosyl residues and by the oligomers obtained from hydrolysis with cellulase.

The material extracted by 0.01–1 M alkali basically contained the same monosaccharide profile of the other fractions, made up of high amounts of Ara, Xyl and UA. The results suggest that there are only minor differences in the sugar composition of all cotyledon cell wall fractions. The difference in extractability may be more the result of the quantity and quality of the cross-links that stabilise the wall components.

Hull was the main source of Xyl-rich polysaccharides in bean seed, most of them derived from xylans and XGs. Xylans were also documented as being a constituent of another legume seed cell walls (Gooneratne, Needs, Ryden, & Selvendran, 1994a,b; Stolle-Smits, Beekhuizen, Van Dijk, Voragen, & Recourt, 1995). Its cell wall was also made up of acidic galacturonans, debranched and low branched arabinans and low branched XGs. Renard, Weightman, & Thibault (1997) also reported the presence of high amounts of Xyl and UA-rich polysaccharides in the cell wall of peas hull which were related to the presence of XGA. They found xylan and low branched arabinans, as well.

The presence of linear polymers in the hulls is in accord with the low water permeability showed by legume seeds. An example of this is that the alteration in the highly branched structure of cotyledon arabinans could be related to the development of beans textural defect known as hard-to-cook. Evidences that degree of branching of arabinans decreases during beans ageing were observed in this work (data not shown). The debranching of arabinans increases the strength of beet arabinans gels (McCleary, 1986). Hence, the debranching of arabinans occurred during beans hardening could also be associated to the hard-to-cook defect. The importance of this work was based on the fact that a better knowledge of cell wall polysaccharide structure and composition could help us to understand their role in the development of textural defects and on its physiological properties to human heath.

5. Conclusions

Bean cell wall polysaccharides structure and composition showed similarities with other legume seeds. Cotyledon cell wall was constituted of high amounts of pectins solubilised by strong alkali solution and hot-water. Methylation analysis of selected fractions suggests the presence of XG, large and highly substituted neutral arabinans, and short linear galactans. The arabinans

sidechains could be constituted of short oligomers or a single Ara residue. Preliminary evidences also permits to conclude that the main galacturonans in beans are XGAs, which probably are interspaced with small domains of HG and RG. The xylan was found as a minor component in the cotyledon.

Bean hulls polymer composition showed similarities with peas. The evidences indicate that xylan comprise, together with arabinans, the main polysaccharides in bean hulls. Evidences of the presence of high amounts of long-chain branchless arabinans and low-branched XG in the WIP were also found. The WSP of bean hulls was mainly constituted of acidic RG and XGA, xylan and low-branched arabinans.

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