

# Polysaccharides of green Arabica and Robusta coffee beans

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## Abstract

Two independent procedures for the quantitative determination of the polysaccharide content of Arabica Caturra (*Coffea arabica* var. *Caturra*) and Robusta ROM (*Coffea canephora* var. *ROM*) green coffee beans showed that they both contained identical amounts of polysaccharide. Cell wall material (CWM) was prepared from the beans and partial solubilisation of component polysaccharides was effected by sequential extraction with water, 1 M KOH, 0.3% NaClO<sub>2</sub>, 4 M KOH and 8 M KOH. The monosaccharide compositions of the CWMs were similar, although Arabica beans contained slightly more mannose than Robusta. In the latter, more arabinogalactan was solubilised during preparation of the CWM and the water-soluble fraction of the CWM contained higher amounts of galactomannan than in Arabica. Linkage analysis indicated that the galactomannans possessed unbranched to branched mannose ratios between 14:1 and 30:1 which is higher than previously reported. No major difference in the structural features of the galactomannans between species was found. The arabinogalactans were heterogeneous both with regard to the degree of branching and the degree of polymerisation of their arabinan side-chains. Compared to Arabica, Robusta appeared to contain greater amounts of arabinogalactans with longer side chains. It is concluded that there was no detectable difference between the Arabica and Robusta varieties of this study in their absolute polysaccharide content or in the gross structural features of their galactomannans. Differences were apparent both in the structural features and ease of solubility of the arabinogalactans but a more detailed study of several varieties of Arabica and Robusta will be required to determine whether these differences occur consistently between species. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Cell walls; Polysaccharides; Coffee beans

## 1. Introduction

Cell wall polysaccharides constitute half the dry weight of the native coffee bean but there is still much to be revealed about the detailed structure of individual polysaccharides and how they interact to give the integrated structure of the cell wall. Thaler<sup>1,2</sup> and Wolfrom<sup>3–6</sup>

detected the presence of mannans, galactomannans, arabinogalactans and cellulose as the main polysaccharides in green and roasted coffee beans. A more recent study<sup>7,8</sup> has reported detailed structural data on the two principal non-cellulosic polysaccharides. The mannan purified by Bradbury was composed of (1 → 4)-linked  $\beta$ -mannan chains substituted at O-6 with single galactose residues approximately every 100 residues.

The arabinogalactan isolated by these authors had an arabinose/galactose ratio of 0.4/1

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and consisted of a backbone of (1 → 3)-linked  $\beta$ -galactose substituted at O-6 with arabinose and/or galactose residues. The side-chains contain arabinose and galactose residues with arabinose as terminal residue. These linkages are characteristic of type-II arabinogalactans, a polymer which is usually covalently linked to protein.

Some more recent work<sup>9</sup> has focussed on the polysaccharides from hot water extracts of roasted Arabica beans. An arabinogalactan and a mannan with similar structural features to the polymers described above were isolated despite the fact that structural modifications were induced by roasting.

Differences in total polysaccharide content as well as structural differences between Arabica and Robusta species have been discussed in the literature. Reliable values are difficult to obtain as they generally have to be extrapolated and recalculated from a series of values obtained after various treatments and extractions which are not comparable.<sup>8,10–12</sup> Most of these sources report a lower polysaccharide content for Robusta compared to Arabica. Clifford<sup>11</sup> reported a total polysaccharide content between 38 and 48% for Robusta and 48–55% for Arabica, but experimental information detailing how the values were obtained is lacking. In contrast, Bradbury and Halliday<sup>8</sup> report a total polysaccharide content of 48.1% for green Robusta coffee from the Ivory Coast and ‘a lower polymeric carbohydrate content for Arabica beans’.

Clifford<sup>11</sup> also reports that Arabicas may contain more arabinogalactan (9–13%) than Robusta (6–8%) and more galactomannan (25–30% vs. 19–22%). He suggests that the galactomannan in Robusta is more highly branched and thus less crystalline but these speculations are not well substantiated. This is also used to explain why, for a same degree of roasting, Robustas generally produce more soluble solids than Arabicas.<sup>10–12</sup>

The present study compares the total polysaccharide content of a variety of Arabica and of Robusta and report data for the structural features of arabinogalactan and galactomannan isolated from each.

## 2. Materials and methods

**Raw material.**—Dry, depulped and deparched beans of Arabica Caturra (*Coffea arabica* var. *Caturra*) and Robusta ROM (*Coffea canephora* var. *ROM*) coffee which were harvested at full maturity in Ecuador were used for this investigation.

**General.**—Polysaccharide fractions and cell wall material (CWM) were hydrolysed with 2 M TFA for 1 h at 110 °C and converted to alditol acetates for GLC analysis as described in Ref. 14. Insoluble materials were hydrolysed using Saeman hydrolysis with 72% H<sub>2</sub>SO<sub>4</sub> at room temperature for 3 h then dilution to 1 M H<sub>2</sub>SO<sub>4</sub> and hydrolysed at 105 °C for 2 h. The samples were then analysed by HPAE-PED liquid chromatography.

Polysaccharides were methylated using a modification of the method of Ciucanu and Kerek.<sup>13</sup> GLC–MS of the partially methylated alditol acetates was accomplished as described previously.<sup>14</sup>

**Isolation and fractionation of cell wall material.**—Triplicate samples were taken of each species of beans. Unless specified, all operations were performed at ambient temperature.

CWM was prepared and fractionated using procedures adapted from existing methods used for fruit tissue.<sup>15</sup> Cryo-milled (IKA-Universalmühle, Staufen, Germany) coffee beans were homogenised in two volumes of cold (4 °C) (2:1:1 w/v/v) phenol–AcOH–water (PAW). The PAW-soluble fractions were combined, dialysed and the polymers recovered after freeze-drying. The residue, hereafter called CWM, was suspended in water and dialysed for 2 days at 4 °C then freeze-dried.

Before attempting to fractionate the polysaccharides, the CWM was defatted by stirring 1 g overnight in 100 mL of dichloromethane and air-drying. Defatted CWM (1 g) was sequentially extracted with 100 mL of water (2 h), 1 M KOH (2 h), NaClO<sub>2</sub> (0.3 g/100 mL + 0.12 mL AcOH, under argon, 2 h, 70 °C), 4 M KOH (2 h), 8 M KOH (2 h). All alkaline solvents contained 20 mM NaBH<sub>4</sub>. Solubilised polymers were recovered following neutralisation of the solution to pH 5.0, dialysis and freeze-drying.

Table 1

Yield of dichloromethane-soluble, PAW-soluble, PAW-precipitate and CWM fractions isolated during CWM preparation from Arabica Caturra and Robusta ROM coffee beans (mean values;  $n = 3$ )

Fraction	Amount (g per 100 g fwt)	
	Robusta	Arabica
Dichloromethane	9.4	9.7
PAW-soluble	1.9	4.8
PAW-precipitate	3.1	trace
CWM	51.7	53.7

**Size-exclusion chromatography.**—A column of Sephacryl S-300 (100 cm × 16 mm) was used at a flow rate of 25 mL/h with a sodium acetate buffer, 0.05 M, pH 6 containing 125 mM NaCl. The column was calibrated using dextran standards.

Samples corresponding to approximately 20 mg of carbohydrate material were dissolved in 1 mL of acetate buffer. Fractions were collected and analysed for total sugars using the phenol–H<sub>2</sub>SO<sub>4</sub> test. Selected fractions were pooled, freeze-dried and used for monosaccharide determination and methylation analysis.

**TCA precipitation of the PAW-soluble material.**—Water (2 mL) was added to the PAW-soluble fraction (100 mg) followed by 2 mL of trichloroacetic acid 0.4 M (TCA). The samples were left for 2 h at room temperature, then centrifuged for 10 min at 3000 rpm. The supernatant was dialysed with a membrane cut-off of 3.5 kDa. The freeze-dried supernatant was then partially methylated and acetylated for linkage analysis.

**Total polysaccharide content.**—Green beans were cryo-milled to a fine powder and 10 g were refluxed in 100 mL of 80% EtOH for 15

min. The samples were centrifuged and the residue was stirred for 2 h in 100 mL of 80% EtOH at ambient temperature. The supernatants were combined and dried down. The residues were suspended in water and dialysed for 2 days in a membrane with a molecular weight cut-off of 3.5 kDa. The entire contents (residue and supernatant) of the dialysis bag were freeze-dried to give the total polysaccharide. The carbohydrate content of the total polysaccharide fraction was then determined by two procedures. By the phenol–sulfuric assay and by GLC analysis of the alditol acetates after Saeman hydrolysis of the polysaccharide.

### 3. Results

**Preparation of CWM.**—CWM was prepared by a modification of an existing method used for the isolation of CWM from fruit tissue.<sup>15</sup> PAW was used to inactivate endogenous enzyme activity and solubilise intra-cellular proteins which have a tendency to bind to the cell wall during its isolation. The CWM (Table 1) from Arabica and Robusta beans accounted for 53 and 52% of the starting material, respectively.

**Composition of CWM.**—The monosaccharide composition of the CWM was determined following Saeman hydrolysis (Table 2) and was essentially the same for Arabica and Robusta. However, there was the suggestion that Arabica contained slightly more mannose than Robusta. Mannose was the most abundant monosaccharide followed by galactose, glucose and arabinose.

**Composition of other fractions isolated from the coffee beans.**—The PAW extract separated into a soluble and insoluble fraction during

Table 2

Monosaccharide composition of CWM following Saeman hydrolysis (mean values;  $n = 3$ )<sup>a</sup>

Sample	Monosaccharide composition (mol%)							Total (µg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
Robusta	0.3	0.4	10.8	0.6	44.8	25.5	17.6	678
Arabica	0.3	trace	9.9	0.8	47.5	24.6	16.8	695

<sup>a</sup> Protein content: 13.4 and 12.5% for Arabica and Robusta beans, respectively.

Table 3

Monosaccharide composition of PAW-soluble and PAW-precipitate fractions isolated during the purification of CWM from Arabica and Robusta coffee

	Monosaccharide (mol%)							Total (µg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
<i>Robusta</i>								
PAW-soluble	12.4	0.4	37.9	2.3	7.0	38.7	1.3	402
PAW-prec.	8.7	2.4	21.7	3.1	19.2	26.1	18.8	27
<i>Arabica</i>								
PAW-soluble	6.0	0.2	33.2	3.0	11.2	40.5	5.8	121

Table 4

Monosaccharide composition and total polysaccharide content of green Arabica and Robusta coffee (mean values  $\pm$  SE,  $n = 3$ )

Sample	Fuc (mol%)	Rha (mol%)	Ara (mol%)	Gal (mol%)	Glc (mol%)	Xyl (mol%)	Man (mol%)	Total (ug/mg)
Robusta	trace	$1.0 \pm 0.1$	$12.2 \pm 0.4$	$26.4 \pm 0.1$	$16.3 \pm 0.5$	$1.0 \pm 0.2$	$43.1 \pm 1.1$	$555 \pm 1.9$
Arabica	trace	$0.4 \pm 0.0$	$10.6 \pm 0.1$	$24.5 \pm 0.1$	$16.7 \pm 0.1$	$1.1 \pm 0.1$	$46.7 \pm 0.2$	$558 \pm 1.3$

dialysis. In Robusta, the PAW-soluble fraction (Table 3) consisted of 40% polysaccharide most of which was arabinogalactan. The material that precipitated during dialysis contained very little polysaccharide and was predominantly protein (data not shown). PAW solubilised more polysaccharides from Robusta than from Arabica.

*Total polysaccharide content.*—The total polysaccharide content of the beans was measured after removing the low molecular weight carbohydrates by ethanolic extraction. The ethanol-insoluble material (total polysaccharide) was dialysed to remove the last traces of monosaccharide and recovered by freeze-drying. Total carbohydrate as determined by the phenol–sulfuric assay gave identical yields of polysaccharide in Arabica and Robusta. This was supported by GLC analysis of the alditol acetates following Saeman hydrolysis of the total polysaccharide (Table 4). Thus, in contrast to previous reports,<sup>7,8,10–12</sup> we found no evidence for differences between Arabica and Robusta in their total polysaccharide content.

*Composition of cell wall polysaccharide fractions.*—The heterogeneous nature of the cell wall means that differences in the relative amounts and sugar compositions of individual polysaccharides may exist without any appar-

ent difference in the monosaccharide composition of the CWM as a whole. Therefore, individual polysaccharide fractions were isolated by sequential extraction of CWM with water, 1 M KOH, NaClO<sub>2</sub>, 4 M KOH and 8 M KOH and their monosaccharide composition determined (Table 5).

The major variation in the yield of an individual fraction occurred in the water-soluble fraction which had a higher yield in Robusta. The mannose content of this fraction was high. Close to 10% of the total mannose from Robusta beans was solubilised in the water fraction compared to less than 1% from Arabica.

Although there were significant differences in the comparative amounts of the NaClO<sub>2</sub>-, 4 M KOH- and 8 M KOH-soluble fractions, these fractions accounted for only a small part of the total polysaccharide.

Acidified sodium chlorite is traditionally used for delignification but has also been shown to be a good solvent for glycoproteins<sup>16</sup> and it proved to be a good solvent for arabinogalactans. Increasing concentrations of KOH solubilised a mixture of polysaccharides.

The composition of the residue which accounted for 60 and 77% of the CWM of

Table 5

Yield and monosaccharide composition of fractions of CWM isolated from Arabica and Robusta beans (mean values;  $n = 3$ )

Fraction	% CWM	Monosaccharide composition (mol%)							Total (µg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
<i>Water-soluble</i>									
Robusta	15.6	1.5	0.4	14.7	1.4	36.8	29.3	15.9	537
Arabica	3.5	3.2	0.2	25.9	1.3	16.7	49.3	3.4	273
<i>1 M KOH-soluble</i>									
Robusta	9.1	1.8	0.4	17.3	2.3	35.1	33.8	9.2	404
Arabica	12.8	2.4	0.8	20.5	2.7	35.1	35.3	3.2	149
<i>NaClO<sub>2</sub>-soluble</i>									
Robusta	4.5	1.6	0.7	32.4	0.5	5.1	57.8	1.9	362
Arabica	2.4	1.3	0.3	33.3	1.0	2.4	60.7	1.0	337
<i>4 M KOH-soluble</i>									
Robusta	4.4	0.5	0.4	14.3	4.4	33.6	34.1	12.6	485
Arabica	2.1	0.6	0.2	17.7	13.9	29.7	31.4	6.5	345
<i>8 M KOH-soluble</i>									
Robusta	5.5	0.4	0.1	23.8	1.6	17.1	53.2	3.8	312
Arabica	2.6	1.1	0.2	25.9	4.3	11.6	54.6	2.4	361
<i>Residue</i>									
Robusta	61.1	0.1	nd.	8.6	0.1	46.8	26.3	18.0	698
Arabica	76.6	0.7	0.4	7.0	3.3	51.3	19.0	18.3	701

Robusta and Arabica respectively, resembled that of the original CWM, demonstrating the difficulty of solubilising not only the galactomannan but also the arabinogalactan in coffee CWM.

**Linkage analysis of PAW-soluble arabinogalactan.**—The arabinogalactans recovered in the PAW-soluble fractions were deproteinated by TCA precipitation, methylated, converted into partially methylated alditol acetates and examined by GLC–MS (Table 6).

The arabinose/galactose ratio was close to unity for both species as was the ratio of 3-linked to 3,6-linked galactose (0.9:1 for Robusta, 1.2:1 for Arabica). Arabinogalactan contained amounts of 5-linked arabinose indicating that the side-chains consisted of more than a single arabinose unit. The presence of terminal galactose indicated that some of the side-chains contained galactose as chain ending residues.<sup>7</sup>

**Characterisation of individual polysaccharides.**—Selected CWM fractions were purified further by subjecting them to size-exclusion chromatography (SEC) on Sephacryl S-300 in preparation for structural characterisation by

linkage analysis. During SEC, the arabinogalactans generally eluted at a higher MW than the galactomannans (Fig. 1, Table 7), enabling recovery of the polysaccharides in a more purified form for methylation analysis. Pooled polysaccharide fractions recovered after SEC were methylated, converted into partially methylated alditol acetates and examined by GLC–MS.

**Structural features of coffee arabinogalactan.**—The glycosyl linkages in Table 8 are consistent with the documented structure of

Table 6

Linkage analysis of polysaccharide content of PAW-soluble fraction

Linkage	Robusta	Arabica
terminal-Rha	7.4	3.7
terminal-Araf	22.1	22.8
5-Araf	11.6	13.7
terminal-Xylp	6.5	<sup>a</sup>
terminal-Gal	2.7	3.1
3-Gal	15.3	19.5
3,6-Gal	15.8	16.8
4-Man	5.5	7.1

<sup>a</sup> Not detected.

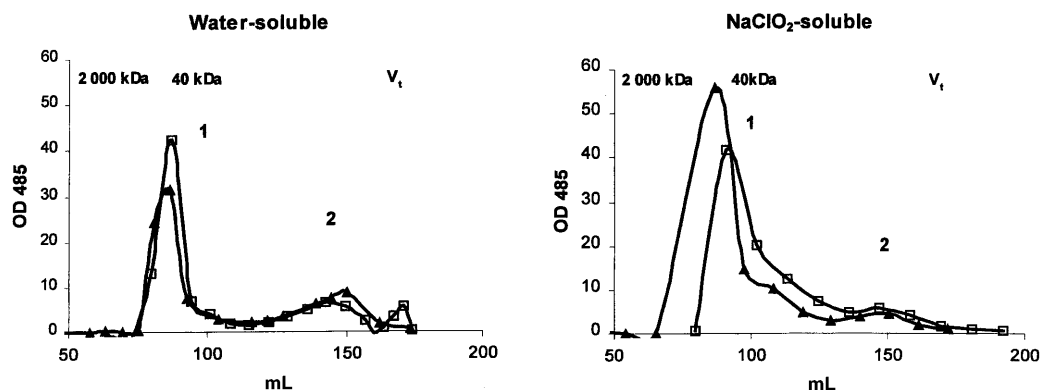


Fig. 1. Gel-permeation chromatography on Sephacryl HR-300 of water-soluble and  $\text{NaClO}_2$ -soluble fractions from Arabica (triangles) and Robusta (squares) beans.

an arabinogalactan which possesses a (1 → 3)-linked galactose backbone with a proportion of the galactose residues substituted at O-6 by side-chains of arabinose and/or galactose residues. The degree of substitution as indicated by the ratio of 3:3,6-linked galactose ranged between 0.92 (Robusta Chlorite-soluble) and 1.5 (Robusta 8 M KOH-soluble). A similar range of degree of branching was shown by the arabinogalactan in the Arabica fractions, however the pattern of distribution of the branched arabinogalactans differed between Robusta and Arabica. For example, the water-soluble arabinogalactan in Robusta was more highly branched than the equivalent fraction in Arabica and these differences were also apparent in the arabinogalactans from the sodium chlorite and 8 M KOH-soluble fractions. The PAW-soluble fraction of Robusta contained greater amounts of arabinogalactan than the same fraction from Arabica. However, linkage analysis of these arabinogalactans did not reveal any marked differences in their structural features (Table 6).

It was noticeable that the ratio of 5-linked arabinose to terminal arabinose also showed considerable variation among fractions. A higher proportion of 5-linked residues would indicate that the side chains of the arabinogalactans were more extended than those polymers with higher proportions of terminal arabinose. In general it seemed that the more extended the side-chains were the more easily the arabinogalactans were removed from the CWM. Thus, in Robusta, the ratio of terminal to 5-linked arabinose was highest in the water-soluble and 1 M KOH soluble fractions and

lowest in the 4 M and 8 M KOH-soluble fractions. Arabica exhibited a similar pattern, although the proportion of 5-linked arabinose was in general lower than in the same Robusta fractions.

*Structural features of coffee galactomannans (Table 9).*—Galactomannans were recovered in most of the CWM fractions, although in Robusta coffee a higher proportion was recovered in the water-soluble fraction. After the final extraction with 8 M KOH, the residue still contained close to 50% of mannose. The water-soluble galactomannans had ratios of unbranched to branched mannose of 19:1 for Robusta and 29:1 for Arabica. In the other cell wall fractions, the material was more branched with ratios of unbranched to branched residues close to 10:1, except in the 4 M KOH fractions where the galactomannans were less substituted (20:1).

Table 7

Distribution of the main monosaccharides (mol%) in the fractions recovered after size-exclusion chromatography of the water and  $\text{NaClO}_2$ -soluble fractions of green Arabica and Robusta CWM

		Ara	Gal	Glc	Man
<i>Water-soluble fraction</i>					
Robusta	F1	29.3	49.7	5.4	8.6
Arabica	F1	32.2	57.0	1.6	2.3
Robusta	F2	8.2	14.3	19.1	47.3
Arabica	F2	19.0	14.9	5.3	46.3
<i>NaClO<sub>2</sub>-soluble fraction</i>					
Robusta	F1	31.3	58.8	1.7	2.0
Arabica	F1	38.2	54.9	2.6	0.5
Robusta	F2	33.2	36.9	9.2	5.7
Arabica	F2	47.2	18.7	3.1	13.7

Table 8

Glycosyl-linkage analysis of the arabinogalactan-rich polysaccharide fractions from Arabica and Robusta coffee

Fraction	Water-soluble		1 M KOH-soluble		NaClO <sub>2</sub> -soluble		4 M KOH-soluble		8 M KOH-soluble	
Linkage	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica
terminal-Araf	16.0	18.1	16.0	14.8	22.8	15.1	21.5	16.3	17.8	22.9
5-Araf	12.2	9.0	14.5	10.7	7.6	10.3	7.1	8.7		7.8
2-Araf		trace	1.0	trace	1.0	2.4			trace	trace
3-Araf	0.2	trace		trace	trace	3.0			trace	trace
2,5-Araf	1.2	trace								
3,5-Araf					1.4	1.3	trace	2.5		
terminal-Xylp	trace	trace	1.2	1.4	trace		trace	3.1		
4-Xylp	2.7	trace	4.2	2.9	1.6	trace	7.0	3.8	1.2	2.0
terminal-Gal	2.9	3.9	1.9	2.7	0.7	9.9	2.7	3.8	2.2	1.8
3-Gal	28.9	32.8	26.3	22.3	27.7	30.0	29.2	24.0	40.2	27.6
4-Gal	trace	trace	1.1	trace	2.1	1.2				
6-Gal	trace	1.8	trace	1.6	trace	1.3	trace	2.2	1.3	1.4
3,6-Gal	20.9	22.5	24.9	23.3	29.8	20.6	18.9	17.8	26.6	25.7
terminal-Man	trace	trace			trace		trace		trace	
4-Man	6.2	4.5	2.4	13.1	1.9		4.3	4.3	6.8	4.4
4,6-Man	1.4	trace					1.1	0.9		
terminal-Glc	trace						1.4	3.4		
4-Glc	1.1	trace		trace	trace	trace	trace		trace	trace

An attempt was made to solubilise more galactomannan by using an 8 M KOH extraction. However, as shown in Table 5, the 8 M KOH extract contained little mannan and was predominantly arabinogalactan.

The presence of (1→4)-linked glucose moieties may be indicative of the presence of galactoglucomannans, xyloglucans or glucomannans since no starch was detected in the green beans.

*Linkage analysis of insoluble polymers.*—Structural features of the insoluble polysaccharides were investigated by repeated methylation of the residue. The main species detected were 4-linked mannose, 3- and 3,6-linked galactose and 4-linked glucose. Typical results show a ratio of unsubstituted mannose to substituted mannose of 20:1, and of 1.7:1 for unsubstituted galactose to substituted galactose for both species. The average degree of branching of the galactomannans remaining in the residue was similar to that of the material extracted with 4 M KOH.

#### 4. Discussion

The results of this study showed no difference in the total polysaccharide content or any marked difference in the structural features of the galactomannans between Robusta ROM and Arabica Caturra. However, differences in solubility, particularly in relation to the arabinogalactans, did support the idea that the polysaccharides of Robusta beans were more easily extracted than those of Arabica.

Robusta ROM contained amounts of a highly soluble arabinogalactan which possessed more branch points and more extended side-chains than arabinogalactans found in Arabica Caturra. This may be one of the reasons that the arabinogalactans of Robusta were more easily solubilised than those of Arabica. It is feasible that the accommodation within the cell wall matrix of a polysaccharide with more extended side-chains may produce a cell wall with different physicochemical characteristics (a more open matrix?) than one which does not possess such polymers. This

different architecture could effect not only the relative ease of solubility of the cell wall as a whole, but also its susceptibility to dissolution by enzymes or solvents.

Both varieties possessed galactomannans with ratios of unbranched to branched mannose between 10:1 and 30:1. While the overall structures did not differ significantly between the two varieties, higher amounts of the water-soluble galactomannan were recovered in Robusta ROM and it was more highly branched. The galactomannans which remained in the residue or were extracted with concentrated alkali had degrees of branching close to 20:1. Except in the water soluble fraction, no evidence was found to support the idea that Robusta contained more branched galactomannans than Arabica.<sup>10,11</sup> The average degree of branching observed was higher than the 100:1 value reported by Bradbury and Halliday<sup>7,8</sup> although they use harsher extraction procedures.

The main difficulty in characterising the mannans or galactomannans which are

present in the coffee bean lies in the high proportion of insoluble polymers, only about 1/3 of the total CWM was solubilised by the sequential fractionation process, despite the fact that an alkali concentration of 8 M was used.

A certain amount of arabinogalactan remained in the residue despite its intrinsic solubility, pointing to the importance of cell-wall architecture in determining the physicochemical properties of its components. This was supported by the fact that arabinogalactans with similar structural features were recovered in all the cell wall fractions, indicating that the immediate environment of the polysaccharide had more influence on its solubility than its basic structural features.

The complex heterogeneity of the arabinogalactans shown in this study justifies a more in-depth investigation of their structural features. In particular their association with protein, acidic monosaccharide content and interaction with the galactomannans are areas which need further study. Type II arabino-

Table 9

Glycosyl-linkage analysis of the galactomannan-rich polysaccharide fractions from Arabica and Robusta coffee

Fraction	Water-soluble		1 M KOH-soluble		NaClO <sub>2</sub> -soluble		4 M KOH-soluble		8 M KOH-soluble	
Linkage	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica
terminal-Araf	7.9	9.4	14.0	18.7	4.5	18.6	17.6	16.9	16.9	16.1
5-Araf	7.1	1.2	4.6	9.5	7.6	7.8	3.4	5.2		8.1
2-Araf	trace	2.0	-	4.9	4.4	9.6	1.7	2.4	trace	3.2
3-Araf		2.7	2.3	-	2.6	6.2			trace	2.3
2,5-Araf	0.5									-
3,5-Araf					1.4	2.0	trace	1.5		-
terminal-Xylp	1.2				1.9	5.6	2.2	3.0		7.3
4-Xylp	2.1	6.6	15.9		9.0	8.3	6.9	6.3	1.1	2.0
terminal-Gal	5.3	2.1	15.1	3.8	3.1	3.5	3.3	2.8	2.9	3.7
3-Gal	6.2	6.4	8.5	12.7	15.3	10.4	15.7	17.4	33.2	17.3
4-Gal			9.3	3.1	2.3	1.5				
6-Gal	trace	1.2					trace	1.2	1.5	2.8
3,6-Gal	7.1	7.9	9.8	13.8	11.4	7.8	12.9	14.4	23.4	12.7
terminal-Man	2.7	3.0	2.1	3.8	1.6	3.3	2.2	2.1	1.1	4.2
4-Man	41.0	48.7	25.0	22.8	19.6	9.6	20.0	15.4	14.5	16.2
4,6-Man	2.2	1.7	3.4	1.8	1.8	1.1	1.1	0.8	trace	trace
terminal-Glc	1.5	2.9					3.1	2.3		
4-Glc	5.9	2.9		5.0	5.8	1.9	trace	2.3	1.2	4.3



galactans such as those identified in coffee beans are often linked to hydroxyproline or serine residues, but so far no such links have been identified in coffee.<sup>17</sup>

To date there has been a general assumption that the coffee bean consists of an amalgam of arabinogalactans, galactomannans and cellulose. The presence of additional and perhaps novel polysaccharides in coffee beans is seldom discussed. The difficulty of solubilising the bulk of the cell wall polysaccharides indicates an intimate association between some of the arabinogalactans, galactomannans and cellulose. While this could be caused by non-covalent interactions between the different polysaccharides, it may also indicate the presence of covalent linkages and therefore the presence of a new class of polysaccharides possessing, as yet, undetermined structural features.

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