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Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans

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Abstract

The degree and nature of polysaccharide degradation at different roasting levels was determined for three Arabica (*Coffea arabica*) bean varieties. Between 12 and 40% of the bean polysaccharides were degraded depending on the roasting conditions. The thermal stability of the arabinogalactans, (galacto)mannans and cellulose was markedly different. The arabinogalactans and mannans were degraded up to 60 and 36%, respectively, after a dark roast, while cellulose showed negligible evidence of degradation. Roasting led to increased solubility of both the arabinogalactans and (galacto)mannans from the bean but the structural modifications, which accompanied this change in solubility, were different for each polysaccharide. Despite the moderate degradation of the (galacto)mannans, those remaining in the bean after roasting showed no evidence of change to their molecular weight even after a dark roast. In contrast, arabinogalactans were depolymerised after a light roast both by fission of the galactan backbone and loss of arabinose from the sidechains. The recently discovered covalent link between the coffee bean arabinogalactans and protein survived roasting. The glucuronic acid component of the AG was degraded markedly after a dark roast, but approximately 30% of the original content remained as part of the AG polymer. The results show that polysaccharide degradation during roasting is more marked than previously documented, and points to roasting induced changes to the polysaccharides as major factors in the changing physicochemical profile of the coffee bean during processing.

Keywords: Coffee; Arabica; Polysaccharides; Mannans; Arabinogalactans; Cellulose; Roasting; Degradation

1. Introduction

During roasting, the characteristic aroma, flavour and colour of the coffee bean is generated and the polysaccharide content is rendered more extractable.[1] The extracted polysaccharides contribute to organoleptic properties of the coffee brew such as viscosity,[2] mouth-feel[3] and foam stability in espresso coffee,[4] as well as retaining volatile substances.[5] The increase in ease of extractability is in part due to changes in the microstructure of the bean, as the beans increase in volume and large micropores appear in the cell wall.[6] Accompanying these physical changes, amounts of polysaccharide are degraded or structurally modified to a form different from that in the green bean. Much of the current knowledge of roasting-induced changes to the polysaccharides of coffee beans has been based on the data of Thaler et al., derived from studies made in the 1960–1970s.[7–9] Later reviews of the subject matter[1,3] have interpreted the data of Thaler and Arneth in different ways, making it difficult to obtain a clear idea of the actual extent of degradation of individual polysaccharides for a given set of roasting conditions. In addition, polysaccharides have been solubilised using high temperature extraction after roasting, so that the true effect of roasting on the polysaccharide structure, as opposed to extraction induced degradation, is not easily assessed. More recent studies[10] have shown that coffee arabinogalactans are particularly susceptible to degradation during roasting while the mannans are only moderately degraded. The cellulose remains largely undegraded even at longer roasting times. Other investigators have examined in

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detail the chemical structure of specific polysaccharide fractions obtained following hot-water extraction of green and roasted Arabica beans.[11,12] The fractions were depolymerised during roasting and showed an increase in the galactose/arabinose ratio as the arabinose side-chains of the arabinogalactans were preferentially degraded.

The object of the present investigation was twofold: (i) To determine, in a single study, the levels of polysaccharide degradation in Arabica beans for a defined set of roasting conditions; and (ii) to elucidate the extent of structural modification to the partially degraded arabinogalactans and galactomannans which remained in the bean at each roasting stage.

2. Experimental

Plant material.—Yellow Caturra (*Coffea arabica*, var. Yellow Caturra), Catimor CIFC (*C. arabica*, var. Catimor CIFC) and Sarchimor (*C. arabica*, var. Sarchimor) Arabica beans were obtained from Ecuador. Each variety was supplied as dried, deparched, mature green beans and at three stages of roasting as characterised by a colour test Neuhaus (CTN). The CTN values (Table 1) corresponded to a light (3–3.5 min), medium (4–5 min) and dark (7–8.5 min) roast at 240 °C.

Yariv assay for arabinogalactan proteins.—The β-glucosyl-Yariv reagent [1,3,5-tris(4-β-D-glucopyranosylphenylazo)-2,4,6-trihydroxybenzene] was purchased from Biosupplies Australia PTY. The assay was based on a protocol described by van Holst and Clarke[13] and is described in detail elsewhere.[14]

Isolation of polysaccharides.—Two methods were used to isolate the polysaccharide content of the beans. One procedure enabled the total polysaccharide content to be isolated in a single fraction with a minimum of extraction steps and was used for the determination of the level of polysaccharide degradation at each stage of roasting. Another procedure isolated the cell-wall material (CWM) free of lipids and most of the intracellular protein which co-purified with the polysaccharides in the first procedure. The CWM was used for the isolation of individual polysaccharides for structural characterisation by compositional and linkage analysis.

Total polysaccharide.—Beans were frozen in liquid N_2 and triplicate samples were cryo-milled to a powder and freeze-dried overnight. The dried coffee powders (10 g) were refluxed for 15 min in 80% EtOH (100 mL). The suspension was centrifuged (6000 rpm), the supernatant decanted and the residue stirred in 80% EtOH (100 mL) for 2 h at ambient temperature. The ethanolic supernatants were removed and the residues suspended in 50 mL of water and dialysed (MWCO, 3.5 kDa) for 3 days and the content of the dialysis bag freeze-dried to give the total polysaccharide fraction.

Cell-wall material (CWM).—Coffee powder (10 g) (prepared as in the total polysaccharide protocol) was refluxed for 15 min in 80% EtOH (100 mL). The suspension was centrifuged (6000 rpm) and the residue re-extracted in 80% EtOH (100 mL). The ethanolic extracts were decanted and the residue suspended in 1:1 $CHCl_3$–MeOH (100 mL) and stirred at ambient temperature for 1.5 h. The extraction was repeated and followed by a 10 min extraction in 100 mL of MeOH.

The final residue was suspended in 2:1:1 phenol–AcOH–water, (PAW, 100 mL) and stirred overnight at ambient temperature. The supernatant was removed by centrifugation and the residue extracted in PAW a second time for 2 h. The combined PAW extracts were dialysed. A precipitate formed and was separated from the soluble material and each recovered as separate fractions (i.e., PAW-soluble and PAW-precipitate).

The PAW-insoluble residue was dialysed for 3 days to remove traces of PAW. The contents of the bag were centrifuged and the supernatant (CWM-sol) and the residue (CWM) recovered by freeze-drying.

Solubilisation of arabinogalactans (AG) and galactomannans (GM) from CWM.—CWM (2 g) was stirred for 4 h in 8 M KOH (100 mL) containing 50 mM $NaBH_4$. The suspensions were centrifuged (6000 rpm, 10 min) and supernatants were filtered through glass fibre paper (GFA) to ensure complete removal of the CWM-residue. Both the supernatants and the residues were neutralised with AcOH and each dialysed (MWCO 3.5 kDa).

Table 1
Weight loss during roasting for three varieties of Arabica beans

Variety	CTN value	Roast bean weight (g) [a]
Catimor CIFC		
Light	112	170.7
Medium	82	165.5
Dark	50	153.0
Yellow Caturra		
Light	110	166.2
Medium	78	159.9
Dark	52	149.5
Sarchimor		
Light	106	166.3
Medium	80	161.6
Dark	50	147.5

[a] From 200 g green beans. Water content of the Catimor CIFC, Yellow Caturra and Sarchimor green beans was 10.9, 10.5 and 11.1%, respectively, and in the roasted beans between 1.0 and 1.6%.

The CWM-residue fraction was removed from the dialysis bag and separated by centrifugation into a soluble fraction and a residue. The latter was freeze-dried (CWM-res) but the supernatant was added to the dialysed 8 M KOH fraction to give the total solubilised polysaccharide fraction which was not freeze-dried, but kept as a solution, pending the next stage of purification.

Separation of AG and GM using $Ba(OH)_2$.—The procedure was based on a method described elsewhere.[15] The total solubilised polysaccharide fraction was concentrated to 40 mL and 2.4 g of NaOH added. A 6% solution of $Ba(OH)_2$ (40 mL) was added and the solutions were left for 4 h at 4 °C. The solutions were centrifuged (8000 rpm, 15 min) and the supernatants decanted. The pellet was re-suspended in 6% $Ba(OH)_2$ (5 mL), centrifuged and the pellet dispersed in 6% NaOH (5 mL). The dispersed pellet (galactomannan-enriched) and the first supernatant fraction (arabinogalactan-enriched) were adjusted to pH 5.0 with AcOH. Each was then dialysed (MWCO 3.5 kDa) and recovered after freeze-drying.

Second $Ba(OH)_2$ precipitation of AG- and GM-enriched fractions from Sarchimor and Yellow Caturra.—Amounts (100 mg) of the AG- and GM-enriched fractions from the Sarchimor and Yellow Caturra beans were subjected to a second $Ba(OH)_2$ precipitation. The samples were dissolved in 5% NaOH (5 mL) and the precipitation accomplished by the addition of satd $Ba(OH)_2$ (5 mL). The remainder of the procedure was the same as described in the previous section.

General analytical procedures.—Polysaccharide fractions and CWM were analysed for their monosaccharide composition following hydrolysis in 72% H_2SO_4 for 3 h at rt and then for 2 h at 110 °C in 1 M H_2SO_4. The hydrolysates were separated by HPAEC-PAD on Dionex DX-500 using a CarboPac PA-1 column (4 × 250 mm) equilibrated in 150 mM NaOH.

Linkage analysis was carried out following methylation of polysaccharides using a modification of the method of Ciucanu and Kerek.[16] GLC–MS of the partially methylated alditol acetates was accomplished using a SP-2380 column (30 m × 0.2 mm i.d.) maintained at 70 °C for 4 min, raised to 150 °C at 25 °C/min and then to 220 °C at 4 °C/min. The molar-response factors reported by Sweet et al. were used.[17]

Size-exclusion chromatography.—Sephacryl S-300 (column size, 100 × 1.6 cm) was equilibrated in 0.05 M sodium acetate pH 6.0. Samples of polysaccharide (20 mg) were dissolved in 1.0 mL of buffer and eluted through the column at 25 mL/h. Fractions were assayed for total sugar using the phenol–H_2SO_4 procedure.[18]

3. Results and discussion

Total polysaccharide degradation during roasting.—Table 2 shows the percentage of polysaccharide lost or degraded in each variety of bean at three roasting times. Degraded polysaccharide is defined as the difference between the polysaccharide content of the green and roasted bean as measured by summing the monosaccharide constituents of the total polysaccharide fraction following acid hydrolysis. Degradation implied that the monosaccharide constituents had been destroyed or chemically modified by roasting, since the missing carbohydrate could not be accounted for as either mono- or oligosaccharides.

At the dark roasting level, approximately 40% of the polysaccharide had been degraded in all varieties. After the light roast stage, less polysaccharide has been degraded but there was more variation in the degree of degradation among the three varieties after a light roast. Thus, Yellow Caturra showed a 25% loss of polysaccharide after a light roast compared to 11 and 15% losses in Catimor CIFC and Sarchimor, respectively.

Degradation of individual polysaccharides.—Table 3 demonstrates the relative degree of degradation of each monosaccharide constituent of the coffee polysaccharides. Arabinose, galactose mannose and glucose are the building blocks for the three main types of polysaccharide found in the coffee bean cell wall: arabinogalactan (AG), mannan and cellulose. The degradation of each is therefore indicative of the destruction of the polymers from which they were derived.

Table 2
Polysaccharide degradation during roasting

Variety and roasting stage	Polysaccharide (g/10 g)	% degradation
Catimor CIFC		
Green	4.90 ± 0.20	
Light roast	4.34 ± 0.1	11.4
Medium roast	3.60 ± 0.1	26.5
Dark roast	3.12 ± 0.1	36.3
Yellow Caturra		
Green	5.07 ± 0.03	
Light roast	3.78 ± 0.08	25.4
Medium roast	3.38 ± 0.13	33.3
Dark roast	3.02 ± 0.04	40.4
Sarchimor		
Green	5.08 ± 0.06	
Light roast	4.29 ± 0.10	15.5
Medium roast	4.03 ± 0.01	20.6
Dark roast	3.03 ± 0.07	40.3

Values expressed on dry green bean basis (average of three separate samples ± SE).

Table 3
Changes to monosaccharide content of coffee polysaccharides during roasting

Variety and roasting stage	Amount (mg/10 g beans)							
	Rha	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
Catimor CIFC								
Green	13.5 ±2.0	396 ±14.5	22.0 ±1.0	2370 ±32.6	1176 ±45.1	725 ±15.0	136 ±17.1	66.5 ±5.5
Light	9.5 ±1.0	224 ±2.5	20.0 ±3.6	2224 ±62.7	1008 ±18.6	744 ±6.5	70.5 ±7.0	53.8 ±7.0
Medium	9.0 ±1.8	160 ±7.0	14.0 ±0.5	1867 ±19.0	779 ±5.9	679 ±7.0	63.0 ±16.0	37.0 ±3.1
Dark	5.0 ±1.5	94.0 ±2.9	12.8 ±0.2	1646 ±16.5	598 ±3.5	724 ±4.5	38.0 ±2.5	22.2 ±1.5
Yellow Caturra								
Green	17.5 ±1.5	429 ±13.0	36.5 ±6.0	2358 ±38.1	1210 ±20.0	709 ±16.5	154 ±2.5	64.0 ±1.9
Light	8.1 ±0.5	178 ±8.0	21.8 ±0.7	1986 ±12.0	838 ±24.1	610 ±34.6	119 ±2.9	34.0 ±1.1
Medium	7.0 ±0.8	132 ±3.0	12.2 ±4.3	1791 ±66.0	701 ±17.1	647 ±68.2	80.2 ±9.5	27.8 ±2.5
Dark	6.7 ±0.5	94.7 ±1.5	11.0 ±2.0	1565 ±40.1	597 ±6.5	693 ±9.5	34.3 ±2.0	22.6 ±1.2
Sarchimor								
Green	15.5 ±0.5	374 ±6.0	26 ±1.0	2489 ±68.3	1191 ±7.0	801 ±8.5	121 ±16.5	62.5 ±2.5
Light	7.5 ±2.0	191 ±15.5	21.4 ±13.1	2257 ±110	936 ±9.0	765 ±65.6	100 ±27.5	40.6 ±4.5
Medium	5.7 ±0.5	134 ±3.7	14.5 ±1.8	2162 ±55.0	791 ±15.8	829 ±31.7	64.5 ±13.8	36.3 ±4.1
Dark	4.1 ±0.4	74.1 ±2.5	14.5 ±1.5	1597 ±50.6	550 ±5.1	744 ±10.0	27.3 ±10.0	19.9 ±5.1

Values expressed on dry green bean basis (average of three separate samples ±SE).

Arabinogalactan (AG), mannan and cellulose show quite different sensitivities to roasting-induced degradation. It was apparent that the arabinose sidechains of the AG were particularly labile compared to the galactan backbone. Thus, between 43 and 58% of the arabinose sidechains were destroyed after a light roast and this increased to approximately 80% for all three varieties after a dark roast. For the galactan part of the polymer, there was as little as 6% degradation after a light roast (Catimor CIFC) although it was considerably higher in Yellow Caturra (30.8%). After a dark roast, these differences between varieties were much less apparent as approximately 50% of the galactan moiety of the AG were degraded in all three varieties. Differences between degradation levels among varieties was always greatest in the light roast which suggested that equilibration kinetics of the heated beans was more prone to variation in the earliest stages of the heating process.

The mannan backbone was less susceptible to degradation, showing less than 10% degradation after a light roast in the Catimor CIFC and Sarchimor beans, increasing to between 30 and 36% after a dark roast. Cellulose showed no apparent degradation even after a dark roast.

The four other polysaccharide derived monosaccharides were rhamnose, xylose, galacturonic acid and glucuronic acid. Rhamnose and galacturonic acid have been shown to be components of the small amount of pectin or rhamnogalacturonan in the coffee bean cell wall.[14] The pectin is substantially degraded at all stages of roasting. Glucuronic acid exists as terminal residues on the sidechains of the AG molecule.[14] Approximately 70% of the glucuronic acid was degraded after a dark roast in all three varieties. There is tentative evidence that xylose originates from small amounts of xylan in the cell wall.[19] It appeared to be more resistant to degradation than the AG, showing a degree of breakdown intermediate between the galactan and the mannan.

Changes to polysaccharide extractability.—The total polysaccharide fraction used for determining degrada-

Table 4
Yield of solubilised fractions from Arabica beans during isolation of CWM

Variety	Fraction (g/10 g dry beans)		
	PAW-soluble [a]	Dialysis-soluble	CWM
Catimor CIFC			
Green	0.09	0.08	5.5
Light	0.28	0.25	5.8
Medium	0.36	0.28	5.3
Dark	0.39	0.28	5.3
Yellow Caturra			
Green	0.07	0.06	5.7
Light	0.28	0.25	5.9
Medium	0.35	0.24	5.4
Dark	0.36	0.17	5.4
Sarchimor			
Green	0.09	0.09	5.6
Light	0.33	0.24	5.7
Medium	0.32	0.33	5.3
Dark	0.28	0.19	5.6

[a] During dialysis of the PAW-soluble fraction, a precipitate formed which was removed. It accounted for approximately 2.5% of the dry weight of the bean. It contained extremely low levels of polysaccharide and was mostly protein.

tion levels was not suitable for isolating and purifying individual polysaccharide components because it contained high levels of protein (12–15%) and lipid. A more purified preparation, the CWM, was isolated following sequential extraction of the bean with 80% ethanol, methanol–chloroform and PAW. The latter two solvents removed lipids and proteins, respectively. The CWM after this treatment was dialysed and during this time additional material was solubilised. The yield of each polysaccharide-containing fraction is given in Table 4 and the monosaccharide composition of the fractions from green beans and a dark roast is shown in Table 5. The resulting CWM contained between 4 and 6% protein. Its monosaccharide composition for the green beans and each roasting stage is given in Table 6.

The swelling and reported increase in the pore size of the coffee bean during roasting[6] suggests that some polysaccharides may be more readily extractable from the roasted beans. This is supported by a marked increase in the solubilised material in the PAW and water (during dialysis) fractions following a light roast (Table 4). In green beans, the PAW- and dialysis-soluble fractions accounted for $\sim 2\%$ of the polysaccharide but this increased fivefold after a dark roast (Table 5). Whereas in the green bean, AG was the predominant polysaccharide solubilised by the extractants, during roasting increasing amounts of mannan were solubilised.

Extractability in 8 M KOH.—Despite the increased extractability of the coffee polysaccharides by aqueous solvents, even after a dark roast, 85% of the AG and mannan remained water-insoluble and was recovered in the CWM (Table 5). In order to solubilise and purify more of the arabinogalactans and mannans from the CWM, it was necessary to extract the CWM in 8 M KOH. Following extraction, the mixture of AG and mannan was partially purified by precipitation of the mannan with $Ba(OH)_2$. The $Ba(OH)_2$ precipitation did not give a complete separation of AG and galactomannan because there is a degree of co-precipitation of the AG with the precipitating mannan. Nevertheless, enriched AG and GM fractions were obtained. The relative amounts of polysaccharide solubilised in the 8 M KOH and the amounts of AG- and GM-enriched fractions obtained following $Ba(OH)_2$ precipitation are shown in Fig. 1.

In all three varieties, there was an increase in the total extractable polysaccharide content as roasting progressed. In the green beans, 10% of the CWM was solubilised by 8 M KOH but this increased to 34–38% after a dark roast. Whereas in the green bean mostly AG was solubilised, in the roasted beans the proportion of mannan increased and it became the predominant solubilised polysaccharide after a dark roast. At the dark roasting level, the amounts of extracted AG decreased, because by this stage the AG had been considerably degraded (Table 3).

Changes to structural features during roasting.—Selected polysaccharide fractions were used to investigate changes to the average-molecular weight (M_{Wav}) and glycosyl-linkage composition. The former was done using calibrated gel-permeation columns and the latter by methylation analysis using GC–MS. The AG- and GM-enriched polysaccharide fractions were subjected to a second $Ba(OH)_2$ precipitation before gel-permeation chromatography to further reduce the amount of the AG in the GM fraction and vice versa.

Molecular weight changes.—The gel-permeation profiles of the AG- and GM-enriched fractions respectively extracted from green and roasted Sarchimor beans are shown in Fig. 2.

The M_{Wav} changes in the AG and GM fractions were quite different. The AGs showed a sharp decrease in their degree of polymerisation after the initial roast, which represented at least a tenfold reduction in M_{Wav}. Subsequent decreases in M_{Wav} after a medium and dark roast were not marked. The magnitude of the initial M_{Wav} decrease was more than could have been predicted from loss of arabinose residues from the sidechains and must therefore have resulted from fission of the galactan backbone.

The GM showed no decrease in M_{Wav} during roasting (Fig. 2). Although the elution profile showed a

decrease in the proportion of the first peak during roasting, this peak contained AG which was not completely removed by the $Ba(OH)_2$ precipitation. The second peak contained the GM and the increased amounts of GM solubilised at each roasting stage showed no change in the peak optimum of the elution profiles. The increased solubility of the GM during roasting therefore probably resulted from physical changes to the structure of the bean. Swelling of the cell-wall structure and increase in pore size may facilitate extraction of wall associated GM. A similar pattern of molecular weight change was shown by the AG- and GM-enriched fractions from Yellow Caturra (data not shown).

Because the M_{Wav} of the AG ($\sim$ 500 kDa) and GM ($\sim$ 10 kDa) in the green bean were so different they were clearly separated during gel-permeation chromatography. This allowed further purification of the GM and AG fractions. The extent of the separation was limited at the longer roasting times when the AG was depolymerised to a M_{Wav} similar to that of the GM.

Changes to structural features.—The monosaccharide compositions of the AG and GM fractions from Sarchimor and Yellow Caturra after gel-permeation chromatography are shown in Table 7.

The most significant result was an increase in the galactose/arabinose ratio of the AG as roasting proceeded. This is consistent with the preferential degradation of the arabinose sidechains. Little information could be gained on the degree of galactosylation of the GM fractions, which still contained amounts of degraded AG.

Selected AG and GM fractions from the Sarchimor beans were methylated and their glycosyl-linkage profiles determined by GC–MS analysis of their PMAAs (Table 8).

Table 5
Distribution of monosaccharides in polysaccharide fractions extracted from green and dark roasted Catimor CIFC, Yellow Caturra and Sarchimor coffee beans

Fraction	Amount (mg/10 g beans)							
	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GlcA
Catimor CIFC								
Green								
Paw-sol	3.0	14.6	1.5	10.1	13.3	5.2	1.4	1.0
Dialysis-sol	9.2	1.0	0.8	13.9	18.3	5.6	2.6	1.0
CWM	11.6	357.5	17.1	1980	1018	556	181	39
Dark roast								
Paw-sol	1.3	17.7	0.5	118.7	108.2	6.8	7.0	2.8
Dialysis-sol	0.2	3.3	0.14	157.2	20.7	12.7	2.4	0.6
CWM	5.3	100.7	16.4	1463	561.8	641.3	63.6	16.9
Yellow Caturra								
Green								
Paw-sol	2.5	8.8	0.7	3.2	8.6	1.8	0.7	05
CWM-sol	1.2	8.2	0.3	5.2	16.6	0.5	1.9	0.9
CWM	11.4	393.3	34.8	2234	1151	724	176.7	52.0
Dark roast								
Paw-sol	1.1	11.3	0.3	91.7	75.6	3.2	5.4	3.2
CWM-sol	0.1	1.3	0.1	117	8.9	0.9	1.2	0.3
CWM	4.3	102.6	16.2	1512	626	794	48.6	21.6
Sarchimor								
Green								
Paw-sol	0.4	7.3	0.1	0.4	0.8	0.4	0.1	0.5
Dialysis-sol	0.7	7.5	1.1	14.1	15.6	6.5	3.1	0.8
CWM	11.8	341.6	16.8	2144	1030	660	162.4	45.4
Dark roast								
Paw-sol	3.0	17.6	0.5	115.6	102.2	7.7	6.1	2.0
Dialysis-sol	0.1	2.2	0.1	103.5	13.7	8.4	1.6	0.4
CWM	6.7	89.6	23.5	1534	560	761	56.0	17.4

Table 6
Monosaccharide composition of CWM from green and roasted Arabica coffee beans

Variety and roasting stage	Monosaccharide composition (μg/mg)								
	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GlcA	Total (μg/mg)
Catimor CIFC									
Green	2.1	65	3.1	360	185	101	33	7.0	756
Light	1.2	33	3.0	372	145	128	18	7.0	707
Medium	0.7	25	4.0	341	125	137	16	5.1	653
Dark	1.0	19	3.1	276	106	121	12	3.2	541
Yellow Caturra									
Green	2.0	69	6.1	392	202	127	31	9.1	838
Light	1.0	30	4.2	340	136	120	20	5.1	656
Medium	1.1	23	3.4	336	124	147	11	5.0	650
Dark	0.8	19	3.0	280	116	147	9	4.0	578
Sarchimor									
Green	2.1	61	3.0	383	184	118	29	8.1	788
Light	1.0	28	3.1	377	142	146	16	6.0	719
Medium	1.4	22	4.3	351	115	137	16	5.2	651
Dark	1.2	16	4.2	274	100	136	10	3.1	544

The linkage data provides additional information on the mechanism of AG breakdown during roasting. The dramatic loss of arabinosyl residues is reflected by a decrease in both terminal and 5-linked arabinosyl residues. At the same time, there is an increase in the content of terminal galactosyl residues. This suggested that moderate amounts of galactosyl residues occurred in the AG sidechains as internally linked residues terminated by arabinose. With the removal of the arabinosyl residues, the disubstituted galactosyl residues become terminal residues. The most likely candidate is the 3,6-galactosyl residue, which is the only disubstituted galactosyl residue present in sufficient quantities in the green coffee AG. Apart from the degradation of the AG sidechains, there is also a decrease in the degree of branching of the galactan backbone illustrated by the increase in the ratio of 3- to 3,6-galactosyl residues during roasting.

Finally, there was a significant increase in the proportion of 6-galactosyl residues during roasting. If this resulted from breakdown of the AG sidechains, there would need to be significant amounts of triply substituted galactosyl residues in the sidechains. Such residues have been reported in the sidechains of the AG from *Lolium multiflorum* endosperm.[20] Sidechains of 6-galactosyl residues were reported which were also substituted in the O-3 position by single arabinosyl residue. Similar evidence has been presented for coffee AG[14] and therefore the preferential loss of the arabinosyl residue during roasting would result in an increase in O-6 linked galactosyl residues. Alternatively, if fission of the galactan backbone took place at the 3,6-linked galactose between the nonreducing end of the branched galactosyl residue and the reducing end of the adjacent 3-linked galactosyl residue, an increase in the proportion of O-6 linked galactosyl residues would occur.

There was a reduction in the proportion of 4,6-linked mannosyl residues in the GM fraction from the light roast compared to that in green coffee. This supports the finding of Nunes and Coimbra who found a similar trend in two varieties of Arabica coffee.[12] There was no change in the ratio of 4-man to 4,6-man between light and dark roast. Because the amount of GM solubilised in the green coffee represents such a low percentage of the total mannan in the bean, it cannot be assumed that all the GM in the roasted beans are less branched than in the green beans. There is evidence that a GM fraction, which is solubilised by mild-extraction methods, is more highly branched than the mannan which resists such reagents and which makes up the bulk of the mannan in the bean.[19] Nevertheless, it would be surprising if some loss of galactose residues did not take place from the GM during roasting.

Arabinogalactan-proteins in roasted coffee.—The major AG fraction was isolated from CWMs of the Yellow Caturra beans at three roasting stages using methods described elsewhere.[14] The AG was separated by anion exchange chromatography into two AG fractions with different galactose/arabinose ratios (F1 and F2)[14] and subjected to the β-glucosyl Yariv gel-diffusion assay for arabinogalactan proteins.[13] At each roasting stage, the AGs in F1 and F2 gave a positive assay for AGP (Fig. 3). Thus, to some extent, the

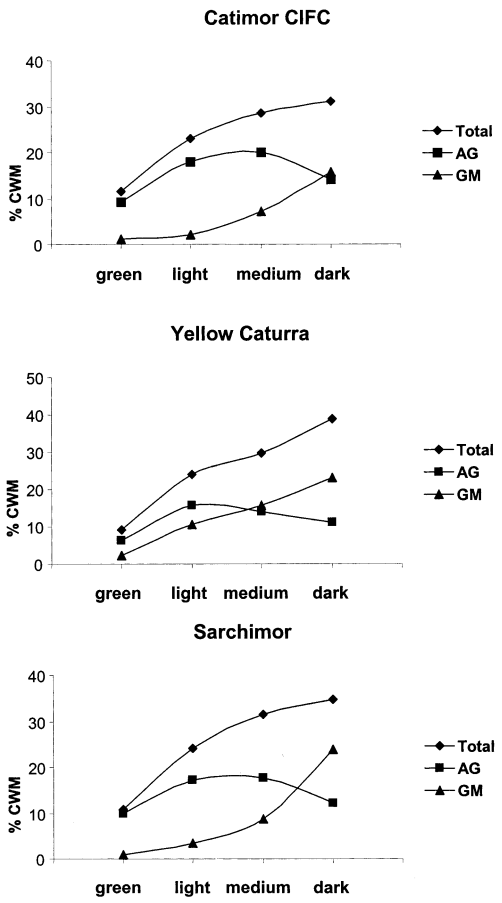

Fig. 1. Changes to the extractability of the 8 M KOH-soluble polysaccharide fractions from CWMs of three varieties of Arabica beans during roasting. CWM was extracted in 8 M KOH and a portion of the soluble-fraction then subjected to $Ba(OH)_2$ precipitation which separated the polysaccharides into two sub-fractions, an AG- and a GM-enriched fraction. After dialysis and freeze-drying, each fraction was weighed and the data plotted as weight % of CWM.

protein–polysaccharide linkage of the AGPs remained intact, even though extensive degradation of the AG moiety occurred after a dark roast.

Increase in non-polysaccharide component during roasting.—CWM isolated from green coffee beans normally consists of 80–85% polysaccharide, the remaining material being made up of mostly protein.[19] However, during roasting, the amount of material which could be attributed to polysaccharide decreased in the CWM. After the dark roast, non-polysaccharide material accounted for 42–45% of the CWM of the three varieties (Table 6). Much of this material was attributable to the formation of Maillard products or melanoidins, some of which may be derived from pyrolysis products of the polysaccharides. The formation of Maillard products between reducing sugars and proteins or amino acids is well known, but the extent to which polysaccharides in coffee can form covalent links between melanoidin compounds or proteins has not been shown. We have

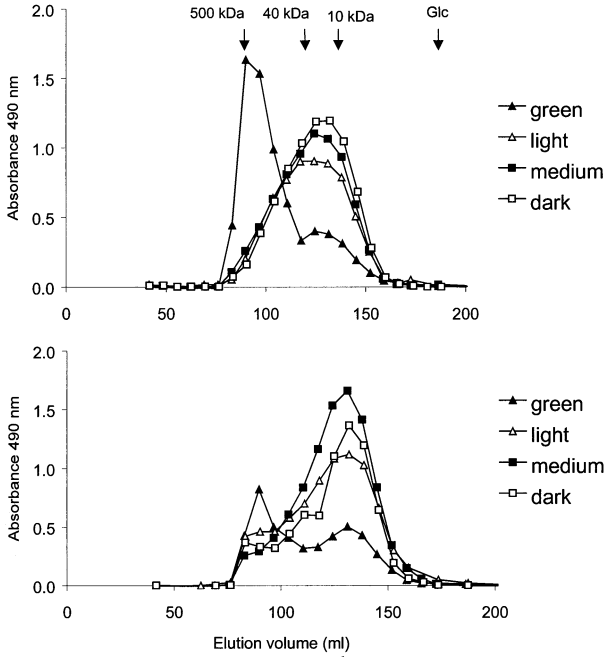

Fig. 2. Gel-permeation profiles on Sephacryl S-300 of AG- (upper profile) and GM-enriched (lower profile) fractions from green and roasted Sarchimor coffee beans.

preliminary evidence that some mannans after roasting are closely associated with the brown pigments, since they are retained on anion-exchange columns along with a fraction of the melanoidins. In green beans, the mannans are not retained which indicated a roasting-induced association between the neutral mannans and the anionic melanoidins.

Some of the non-polysaccharide components in the CWM were extremely insoluble, remaining resistant to solubilisation in 12 M sulfuric acid at room temperature and subsequent hydrolysis in 2N sulfuric for 3 h at 100 °C. The proportion of this insoluble fraction increased during roasting. After a light, medium and dark roast, 30, 42 and 60%, respectively, of the non-polysaccharide compounds in the CWM was such material.

4. Conclusion

In each of three varieties of Arabica coffee beans, between 12 and 24% of the polysaccharide content was degraded after a light roast and after a dark roast this increased to 35–40%. The thermal stability of individual polysaccharides supported earlier data,[7–9] which reported that the arabinogalactans were more susceptible to degradation than the mannans or cellulose. Studies have shown that pyrolysis of glucans is relatively insensitive to linkage position and orientation (α- or β-) of the glucose residues.[21] Presumably, differences in pyrolysis rates are more related to the nature of individ-

ual sugars and to the location of each polymer type in the cell wall structure. In the current study, values for the relative degrees of degradation for each monosaccharide component were higher than previously reported. Thaler and Arneth[7,9] reported degradation for arabinose, galactose and mannose components of the cell wall for a dark roast as 60, 30 and 34%, respectively. Corresponding figures for the present study were 80, 50, 35%.

In green coffee beans, the association of cellulose, mannan and arabinogalactan is largely resistant to solubilisation in aqueous solvents. The AGs, which normally are readily water-soluble, are retained in the coffee cell wall as part of the insoluble polysaccharide complex. After roasting however, increased amounts of both AG and GM could be extracted. The cell wall undergoes structural modifications during roasting as the increase in internal pressure caused by water vapour and CO_2 formation makes the cells expand. The increased solubility of the polysaccharides is undoubtedly due to both the loosening of the cell-wall structure as it swells, and the depolymerisation of the polysaccharides. Each of these phenomena may contribute to solubilisation of the AGs and GMs to differing degrees.

After a light roast, there was a 10–20-fold decrease in the molecular weight of the extractable AG compared to the AG in green coffee. The depolymerisation could be attributed to a loss of sidechain arabinose and a fission of the galactose backbone. Although longer roasting continued to degrade the polymer, the extent of further structural modification was only moderate compared to that which followed the short initial roast.

The GM in the cell wall after roasting showed no change in molecular weight from that of the GM extracted from the green bean, despite the fact that 35% of the cell-wall mannan was degraded during the dark roast. Several possibilities may account for this phenomenon. The GM extracted from the green bean in 8 M KOH was only a fraction of the total GM and therefore may not be representative of the true M_{Wav} of the total coffee GM. Secondly, mannan degradation across the wall may not be uniform. There may be sites in the wall where there is complete degradation of mannans and others where there is little or no breakdown (e.g., mannan closely associated with the cellulose). Finally, degraded mannans may interact with Maillard products during roasting to produce molecular complexes. The molecular weight profiles for GM

Table 7
Composition of AG- and GM-soluble fractions after gel-permeation chromatography on Sephacryl S-300

Variety and roasting stage	Monosaccharide composition (mol%)						
	Rha	Ara	Xyl	Man	Gal	Glc	Gal/Ara ratio
AG-fraction							
Yellow Caturra							
Green	3.0	28.3	1.7	0.7	65.1	1.2	2.3
Light	0.7	14.7	2.0	2.8	78.6	1.3	5.3
Medium	0.5	11.9	1.8	1.4	83.2	1.3	7.0
Dark	0.3	9.9	1.5	1.3	85.6	1.4	8.6
AG-fraction							
Sarchimor							
Green	1.4	26.3	2.1	2.0	67.3	1.0	2.6
Light		12.6	2.3	3.5	79.6	2.0	6.3
Medium		11.5	1.9	5.9	79.1	1.6	6.9
Dark		10.4	2.1	1.8	84.3	1.5	8.1
GM-fraction							
Yellow Caturra							
Green	1.4	8.6	7.2	50.0	21.7	11.2	
Light		3.4	1.3	80.1	12.1	3.1	
Medium	0.3	2.6	0.6	79.1	15.0	2.5	
Dark		3.2	0.9	77.8	15.6	2.5	
GM-fraction							
Sarchimor							
Green	0.8	7.7	10.6	50.8	17.3	12.9	
Light		3.2	2.4	58.7	29.0	6.8	
Medium		2.5	1.0	76.3	18.0	2.1	
Dark	0.1	2.3	1.4	71.2	16.0	8.9	

Table 8
Linkage composition of Sarchimor Arabica AG and GM fractions from green and roasted coffee

Sugar	Linkage	Linkage composition (mol%)					
		AG-fraction			GM-fraction		
		Green	Light roast	Dark roast	Green	Light roast	Dark roast
Rha*p*	Terminal	3.1	0.9	2.6			
Ara*f*	Terminal	17.1	12.0	8.8	4.3	1.5	1.1
	5-	8.0	2.3	2.3	1.6	0.5	
	2,5-	0.5	1.4	0.9			
	3,5-	0.4	0.1	0.3			
Xyl*p*	Terminal	0.6	0.3	0.3	2.0		
	4-	3.2	3.2	2.8	5.0	1.2	0.9
Gal*p*	Terminal	5.0	12.0	14.8	2.0	6.9	5.3
	3-	35.0	39.2	38.9	7.1	8.2	5.4
	4-	0.1	0.5	1.1			
	6-	1.2	4.5	5.4			
	3,4-	1.4	1.3	1.6			
	3,6-	21.7	19.3	18.1	4.4	8.7	2.7
Glc*p*	terminal				2.1	1.0	1.3
	4-	1.1	0.8	1.1	8.9	4.0	6.0
Man*p*	terminal				0.9		1.1
	4-man	1.7	2.0	1.1	57.5	65.2	73.1
	4,6-man				4.1	2.9	3.2

presented in Fig. 2 for the roasted samples may therefore represent GM-melanoidin complexes which, in part, could mask the extent of roasting induced depolymerisation of the mannans.

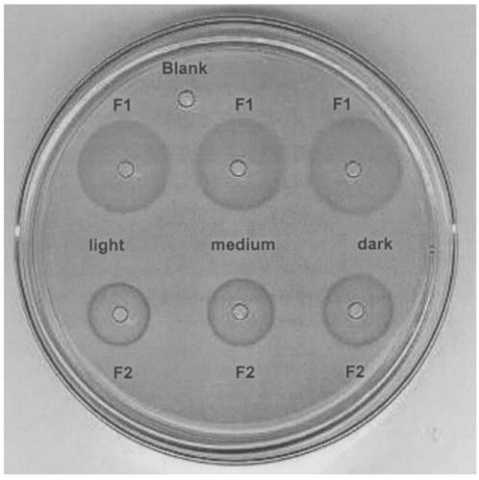

Fig. 3. β-Glucosyl-Yariv gel-diffusion assay for arabinogalactan-proteins in AG fractions isolated from three roasting stages of Yellow Caturra coffee beans. F1 (low Ara/Gal ratio); F2 (high Ara/Gal ratio). Blank is Sigma Larch AG.

There was no significant decrease in the cellulose content of the bean during roasting despite the fact that at least 10–17% of the bean is loss by pyrolysis. This suggests that the compact nature of the cellulose fibrils together with their location in the cell wall affords them maximum protection from degradation. Although the specific arrangement of the cell-wall polysaccharides is not known, it is likely that the cellulose fibrils, which account for 15% of the cell-wall polysaccharides are embedded in a matrix of mannan and AG. Such an arrangement could insulate the cellulose fibrils from the degradative effects of much of the roasting process. The cellulose content has been determined by the quantitation of glucose following acid hydrolysis of the CWM and it is possible that not all the glucose is derived from cellulose. Insoluble glucose containing polymers may form from condensation products during roasting and may compensate for glucose lost by the degradation of cellulose.

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