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Extraction and characterization of polysaccharides from green and roasted *Coffea arabica* beans

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Abstract

Polysaccharides were sequentially extracted from green and roasted *Coffea arabica* beans with water (90 °C), EDTA, 0.05, 1, and 4 M NaOH and characterized chemically. Additionally, the beans were subjected to a single extraction with water at 170 °C. Green arabica coffee beans contained large proportions of 1 → 4-linked mannans, of which on average 1 in every 23 mannopyranose residues was branched with single unit galactose side-chains at O-6. A part of these galactomannans could be extracted relatively easy with water and EDTA. These galactomannans were found to have a relatively high degree of branching (gal:man ~ 1:8) and a relatively low molecular weight in comparison to the remaining galactomannans (gal:man ~ 1:15–24). Additionally, 1 → 3-linked galactans, heavily branched at O-6 with side-chains containing arabinose and galactose residues, were present in the green coffee beans, as well as smaller amounts of pectins, cellulose, and xyloglucans.

Roasting resulted in a loss of 8% of the dry weight. This could be partly explained by the relatively high percentage of sugars which was lost during the roasting process, most probably as a result of conversion into, e.g. Maillard and pyrolysis products. After roasting the extractability of polysaccharides was increased significantly. A decrease in the degree of branching as well as a decrease in molecular weight of arabinogalactans, galactomannans, and xyloglucans was observed after roasting.

Keywords: *Coffea arabica*; Coffee beans; Green; Roasted; Arabinogalactans; Galactomannans

1. Introduction

Green arabica coffee beans consist for 48–60% of polysaccharides (Clifford, 1985; Wolfrom & Patin, 1965; Wolfrom, Plunket, & Laver, 1960). These polysaccharides play an important role in the formation of flavour compounds during roasting and also play a role in the foam stability of espresso coffee (Nunes & Coimbra, 1998).

Three types of polysaccharides are predominating in the green coffee bean: cellulose, arabinogalactan type II and galactomannans (Fischer, Reimann, Trovato, & Redgwell, 2001; Wolfrom et al., 1960). The arabinogalactans consist of a main chain of 1 → 3-linked galactose branched at O-6 with side-chains containing arabinose and galactose residues (Fischer, Reimann, Trovato, & Redgwell, 1999; Fischer et al., 2001). The ratio arabinose:galactose found in green arabica beans is approximately 0.4:1 (Fischer et al., 2001). The galactomannans in arabica beans are composed of a backbone of β-1 → 4-linked mannans with single unit galactose side-chains α-linked at O-6 (Fischer et al., 1999). In some studies these polysaccharides were described as mannans based on the low degree of branching of these polysaccharides (Bradbury & Halliday, 1990). The molecular weight range of water extractable carbohydrates (predominantly galactomannans and arabinogalactans) from green arabica beans was found to be up to 200,000 Da (Leloup & Liardon, 1993).

Roasting is an essential step in coffee production for the formation of flavour compounds (Steinhauser, Oestreich-Janzen, & Baltes, 1999). During roasting monosaccharides are released which may form precursors for flavour compounds during further processing. Also, the molecular weight of the water extractable polysaccharides decreases significantly (Leloup & Liardon, 1993) and the degree of branching of the arabinogalactans and galactomannans decreases (Leloup & Liardon, 1993; Nunes & Coimbra,

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2001). Additionally, the roasting process is significant for galactomannan solubilization (Leloup & Liardon, 1993).

The structural characteristics of water extractable polysaccharides from green and roasted coffee beans have been the subject of many studies (Fischer et al., 2001; Leloup & Liardon, 1993; Navarini, Gilli, Gombac, Abatangelo, Bosco, & Toffanin, 1999; Thaler, 1979; Wolfrom & Patin, 1965; Wolfrom et al., 1960). Additionally, some work has been directed towards the quantitative determination of the polysaccharides of green coffee beans (Fischer et al., 2001). However, in order to understand the solubilization and degradation of polysaccharides from coffee beans during the roasting process, it is important to study the structural characteristics of all the polysacharides present in green and roasted coffee beans. In the current investigation an overview is given of the polysaccharides present in green arabica coffee beans and the changes in polysaccharide composition which occur during the roasting process.

2. Experimental

2.1. Materials

Green *Coffea arabica* beans (Columbia) were kindly supplied by the coffee industry. Roasted coffee beans were used with a roasting loss of 8%.

2.2. Extraction of polysaccharides from green and roasted coffee beans

Ground coffee beans (green and roasted) were Soxhlet-extracted for 6 h with petroleum ether (40–60 °C) to remove the lipids. Defatted ground coffee beans (100 g) were sequentially extracted with 2000 ml of: water (90 °C, 1 h), 0.05 M EDTA in 0.05 M NaOAc buffer (pH 5.0, 70 °C, 1 h), 0.05 M NaOH (0 °C, 1 h), 1 M NaOH + 0.02 M $NaBH_4$ (25 °C, 3 h), 4 M NaOH + 0.02 M $NaBH_4$ (25 °C, 3 h). Additionally, 100 g of defatted ground coffee beans (green and roasted) were extracted with 2000 ml of water (170 °C, 15 min). All extracts were neutralized to pH 6, dialyzed and freeze dried.

2.3. Analytical methods

The uronic acid content was determined by the automated m-hydroxy biphenyl assay (Thibault, 1979). The neutral sugar composition was determined after hydrolysis with sulphuric acid and conversion into alditol acetates as described previously (Oosterveld, Beldman, Schols, & Voragen, 1996). The sugar linkage composition of the neutral sugars was determined using the methylation analysis as described previously (Oosterveld et al., 1996), using hydrolysis with 90% (v/v) formic acid (5 h, 100 °C). The partially methylated alditol acetates were identified by GC–MS and quantified by GLC. The degrees of methylation (DM) and acetylation (DA) of the pectins were determined as described by Voragen, Schols and Pilnik (Voragen, Schols, & Pilnik, 1986). Nitrogen content was determined using the combustion (Dumas) method on the NA 2100 Nitrogen and Protein Analyser (Inter Sciences, The Netherlands) according to the instructions of the manufacturer. A factor of 6.28 was used for the conversion of nitrogen content into protein content. Moisture contents were determined after drying overnight at 105 °C.

2.4. Chromatography

High-performance size-exclusion chromatography (HPSEC) was performed on three Bio-Gel TSK columns in series (60XL–40XL–30XL) as described (Oosterveld, Beldman, Schols, & Voragen, 2000) using 0.2 M $NaNO_3$ as eluens.

Preparative size-exclusion chromatography was performed on a column (75 × 2.6 cm) of Sephacryl S 500 (Pharmacia) using a Hiload system (Pharmacia). The sample (0.5 g) was eluted with 0.05 M NaOAc pH 5.0 at a flow rate of 2.5 ml/min. Fractions obtained by preparative size-exclusion chromatography were assayed for total neutral sugar (Tollier & Robin, 1979) and uronic acid (Thibault, 1979) content, using arabinose and galacturonic acid as standards. A correction was made for the response of uronic acids in the neutral sugar test. Pooled fractions were dialyzed and freeze-dried.

3. Results and discussion

3.1. Composition of green and roasted arabica coffee beans

The green arabica coffee beans under investigation consisted for 55%w/w of carbohydrates (Table 1). Besides carbohydrates the green arabica beans contained 11.3%w/w fat and 6%w/w protein. These results are in agreement with the values found in the literature for green arabica beans (Clifford, 1975; Ky, Noirot, & Hamon, 1997; Wolfrom &

Table 1
Composition of green and roasted arabica coffee beans (%w/w)

	Green bean	Roasted beans
Carbohydrate composition (mol%)		
Rhamnose	1	1
Arabinose	12	6
Xylose	2	0
Mannose	43	51
Galactose	23	21
Glucose	15	16
Uronic acid	4	4
Carbohydrates	54.8	48.1
Fat	11.3	15.9
Protein	5.8	6.8
Total	71.9	70.8

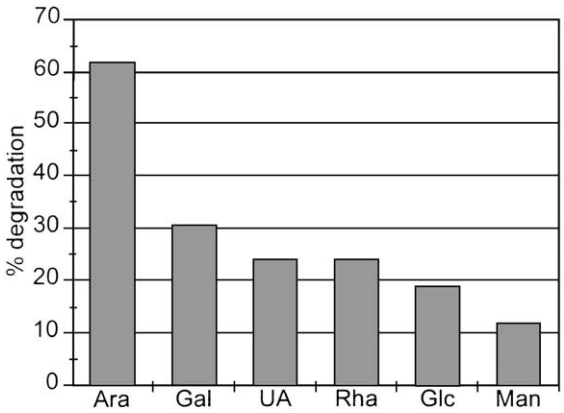

Fig. 1. Loss of sugar residues after roasting of arabica coffee beans expressed as percentage of the initial content of these sugars in green coffee beans.

Patin, 1965; Wolfrom et al., 1960). The remaining material consists of chlorogenic acids, minerals, lignin, amino acids, alifatic acids, and some trigonellin and caffein (Clifford, 1975). Mannose accounted for 44 mol% of the sugars present in the green beans. Galactose and arabinose together accounted for another 35 mol% of the sugars. These results show that approximately 80 mol% of the sugars in green beans are present as galactomannans and arabinogalactans, while cellulose and xyloglucans make up only 16 mol% of the polysaccharides. The uronic acid content in the green bean was only 4 mol%, indicating that the pectic backbone was not a predominant structural element in coffee beans.

During roasting the dry weight of the coffee beans decreased with approximately 8%. The carbohydrate content of the roasted arabica coffee beans decreased to 48%, probably as a result of the conversion of sugars to Maillard or pyrolysis products (Steinhauser et al., 1999). The fat content showed a relative increase probably as a result of the degradation of carbohydrates during roasting. The protein content showed a slight increase to 7%w/w.

Fig. 1 shows the degree of degradation for each type of sugar moiety after roasting and illustrates that 60% of the arabinose initially present in the green coffee bean is not recovered, probably as a result of the conversion into degradation products such as Maillard or pyrolysis products during the roasting process. Also, approximately 24–30% of the galactose, uronic acid, and rhamnose is converted into degradation products. The degradation of glucose was somewhat higher than for mannose, which may be caused by the degradation of some residual sucrose.

3.2. Extraction of polysaccharides from green and roasted coffee beans

In order to study structural differences in the individual polysaccharides present in green and roasted coffee beans, polysaccharide fractions were isolated by sequential extraction with water, EDTA, 0.05 M NaOH, 1 M NaOH, and 4 M NaOH. The yields and compositions of these extracts are shown in Table 2.

The sequential extraction of the green beans was not very effective: only 7% of the polysaccharides were extracted with the procedure used, while 68% of the polysaccharides were found in the residue (Fig. 2). This indicates that the majority of the polysaccharides in the green arabica bean are firmly embedded in the cell-wall as was also described by Fischer et al. (Fischer et al., 2001). Approximately 25% of the sugars were not recovered during the extraction procedure, which was partly caused by the removal of mono- and oligosaccharides during dialysis. More than 25% of the rhamnose and uronic acid residues were extracted in the polymeric fractions with the sequential extraction procedure, indicating that pectins were relatively easily released from the green coffee bean (Fig. 3). Arabinose and galactose residues were more difficult to extract (15–20% of these sugars could be extracted) while mannose and glucose were the most difficult sugar residues to extract (less

Table 2
Composition of extracts obtained from green and roasted arabica coffee beans

	Carbohydrate composition (mol%)							Sugars (%w/w)	Protein (%w/w)	Total (%w/w)	Yield (%w/w)
	Rha	Ara	Xyl	Man	Gal	Glc	UA				
Green beans											
Water	7	26	1	18	36	4	10	34	59	93	4.5
EDTA	7	18	0	8	31	6	31	39	39	78	1.7
0.05 M NaOH	7	27	1	2	40	4	18	30	25	55	2.2
1 M NaOH	2	26	3	2	50	5	11	52	41	93	0.9
4 M NaOH	2	20	6	21	39	8	5	45	24	69	0.6
Residue	0	11	1	47	22	17	4	79	6	85	46.3
Roasted beans											
Water	2	9	0	49	33	1	7	66	12	78	10.8
EDTA	1	8	0	46	32	1	11	41	23	63	2.7
0.05 M NaOH	2	14	0	7	67	1	9	44	23	67	3.9
1 M NaOH	1	11	0	29	51	1	8	45	24	69	4.8
4 M NaOH	1	6	1	59	29	2	5	56	10	66	2.9
Residue	0	4	0	56	12	26	2	69	12	81	33.8

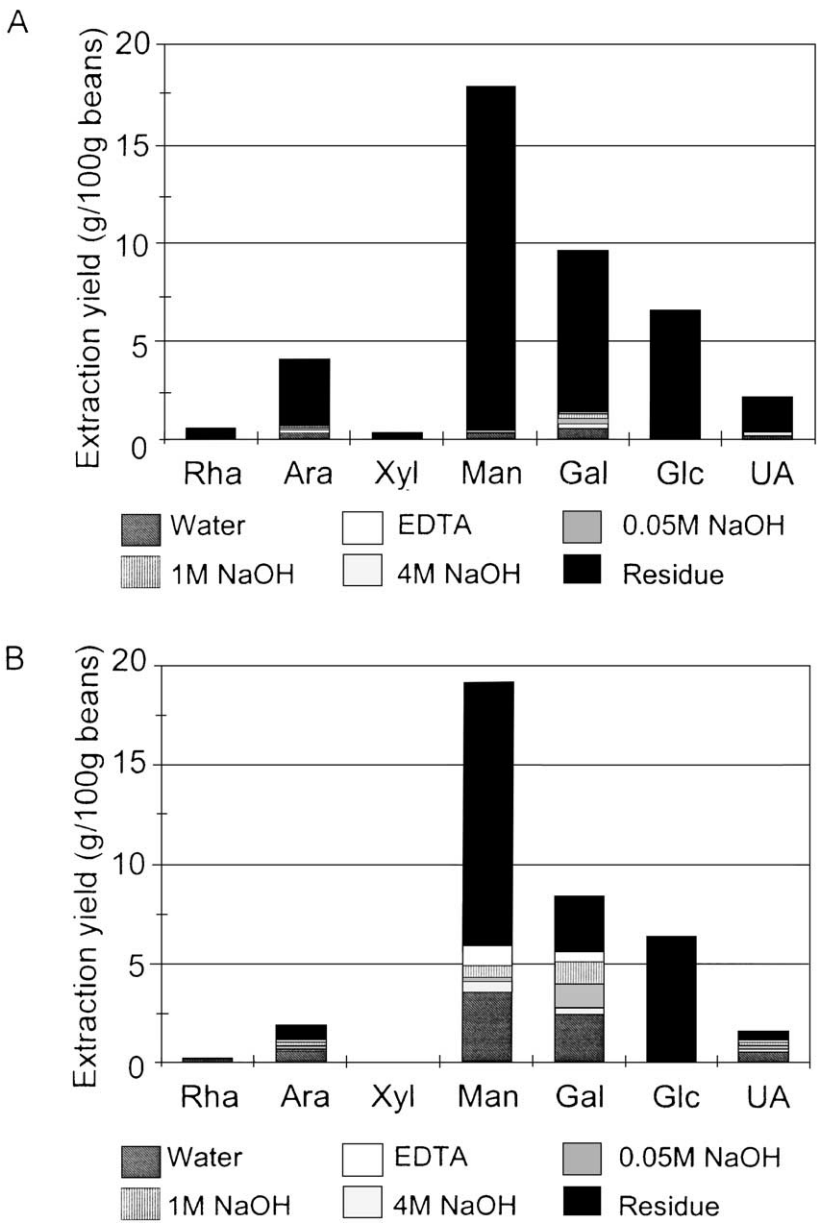

Fig. 2. Yields of individuals sugar residues in extracts obtained from green (A) and roasted (B) arabica coffee beans expressed as g/100 g initial bean.

than 5% of these sugars were found in the extracts). This shows that both galactomannans and cellulose are firmly embedded in the cell.

After roasting, the extractability increased significantly. Approximately 29% of the polysaccharides could be extracted. The extractability of the sugar residues had the following order (from high to low): rhamnose, uronic acid, galactose, arabinose, mannose and, glucose. The results indicated that mainly the solubility of pectin, arabinogalactans, and galactomannans increased during roasting (Fig. 3). Still, most of the polysaccharides from the roasted arabica bean were found in the residue.

The highest extraction yield for the green bean was obtained with water (4.8%w/w). Most of the water extract consisted of proteins, although the protein content may have been overestimated due to the presence of other nitrogen containing substances. The amount of polysaccharides in this extract (2.8%w/w of the polysaccharides present in the green bean) was comparable to the results found in literature (Amorim, Teixeira, Melo, Cruz, & Malavolta, 1974; Maria, Trugo, Moreira, & Werneck, 1994; Thaler & Arneth, 1968). The sugar composition shows that arabinose and galactose were the predominant sugar residues in the water extract of the green coffee beans (26 and 36 mol%, respectively) indicating that the extract contained mainly arabinogalactans. Additionally, 18 mol% of mannose was present in this extract, originating from galactomannans. The EDTA extraction was performed in order to extract the calcium sensitive pectins from the coffee beans. Indeed, the uronic acid content (31 mol%) was relatively high, just as

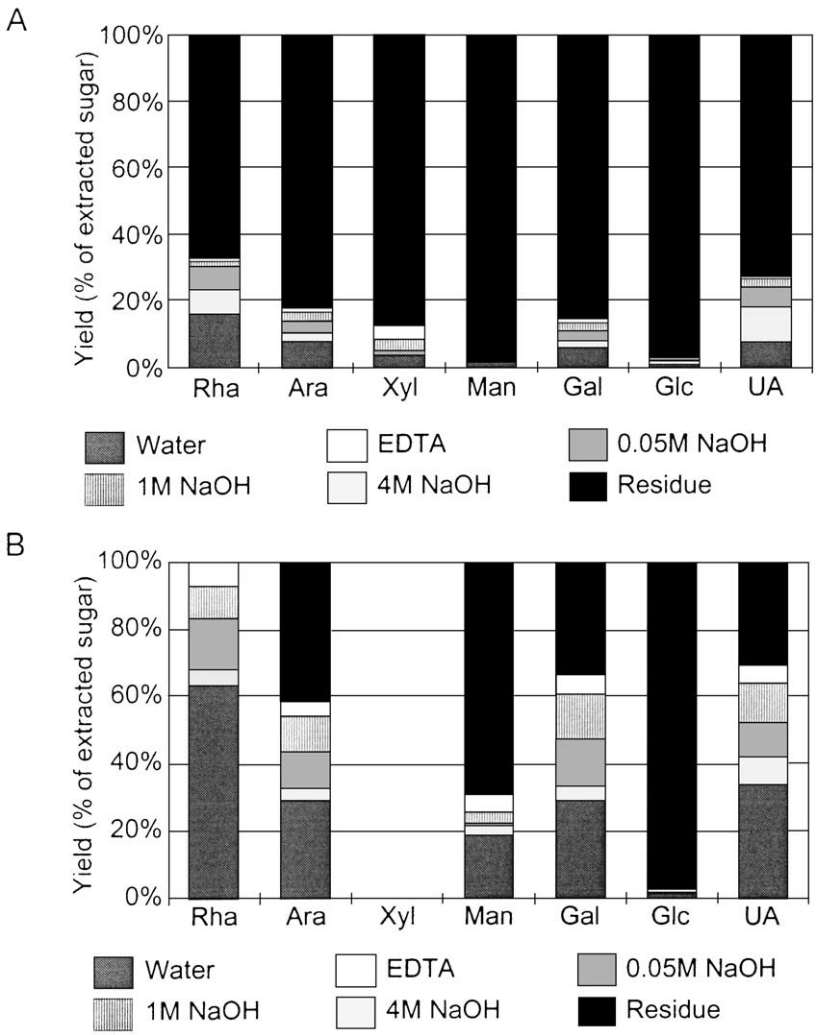

Fig. 3. Yields of individual sugar residues in extracts obtained from green (A) and roasted (B) arabica coffee beans expressed as percentage of the total amount of the referring sugar which was extracted.

the content of other pectin related sugar residues (rhamnose, arabinose, and galactose). From Fig. 3 it can be concluded that the EDTA extraction was the most effective treatment for the release of uronic acid containing polymers. The low mannose content in this extract indicates that hardly any galactomannans were extracted with EDTA. The 0.05 and 1 M NaOH extracts resulted in a yield of 2.4 and 0.9%, respectively, accounting for 1.7 and 1.1% of the polysaccharides present in the green bean. These extracts had comparable sugar compositions, consisting mainly of arabinose (26–27 mol%) and galactose (40–50 mol%). Very little galactomannans were extracted with these NaOH concentrations. The polysaccharide extractability using 4 M NaOH was very low (0.6%). Based on the sugar composition this extract consisted predominantly of arabinogalactans and galactomannans. The arabinose:galactose ratio of the extracts appeared to decrease with increasing extraction strength, indicating that the arabinose rich polysaccharides are relatively easy to extract or are not extracted at all (80%). Most of the polysaccharides were found in the residue. The sugar composition of the residue was almost similar to that of the initial green bean, which was expected, based on the low yields of the extractions.

While the (arabino)galactans were present in all extracts, a part of the galactomannans was released under mild extraction conditions with water and EDTA, while an other part could only be extracted with 4 M NaOH. Apparently a part of the galactomannans is loosely attached within the cell wall, while a major part of the galactomannans are embedded firmly in the cell wall. A similar distinction in the extractability of galactomannans from green arabica beans could be noted in the results of Fischer et al. (Fischer et al., 2001). It can be speculated that this difference in extractability of the galactomannans is a result of a different function of these polysaccharides in the cell wall.

In addition to the water extraction of green coffee beans at 90 °C, a water extraction at 170 °C was performed as well. In order to minimize the polysaccharide degradation during the sequential extraction of the green coffee bean, the water extraction at 170 °C was carried out separately. The total

Table 3
Composition of water extracts (170 °C) obtained from green and roasted arabica coffee beans (mol%)

	Green beans	Roasted beans
Rha	4	1
Ara	30	8
Xyl	0	0
Man	6	32
Gal	52	53
Glc	1	1
UA	7	6
Sugars (%w/w)	65	68
Protein (%w/w)	29	10
Total analyzed (%w/w)	94	78
Yield (%w/w)	7.9	10.5

yield of this extraction (7.9%) was much higher than for the extraction at 90 °C (4.5%; Table 3). This increase in yield was caused almost totally by the more efficient extraction of polysaccharides. The amount of protein extracted at 170 °C was comparable with that at 90 °C. Based on the sugar composition it can be concluded that especially the arabinogalactans were extracted in larger quantities at 170 °C, while the amount of galactomannans extracted was comparable with amounts extracted at 90 °C. This was in agreement with the results found by Leloup and Liardon (Leloup & Liardon, 1993) for the extraction of green arabica beans with water at 170 °C.

After roasting the distribution of the individual polysaccharides over the extracts changed significantly. In almost all extracts the arabinose content decreased, as well as the ratio arabinose:galactose. This indicates that the degree of branching of the arabinogalactans decreased during roasting. Also the uronic acid and rhamnose content in a number of extracts decreased, both in a relative as in an absolute way (Fig. 2). This appears to be a direct result of the conversion of a part of the sugar moieties in the coffee bean into, e.g. Maillard and pyrolysis products during roasting (Fig. 1). The mannose content in almost all extracts increased showing that solubilization of galactomannans was mainly responsible for the higher yields found for the extracts from roasted coffee beans (see also Fig. 2).

The water extract consisted for 66%w/w of polysaccharides. The amount of protein was much lower (1.3 g/100 g of coffee beans) in comparison with the water extract from green coffee beans, which was in agreement with results from Nunes and Coimbra (Nunes & Coimbra, 2001). The water extract consisted for 49 mol% of mannose, indicating that the extractability of the galactomannans increased during roasting. The decrease of the ratio arabinose:galactose in the water extract from 0.72 for green beans to 0.27 for roasted beans indicates that the arabinogalactans are linearized during the roasting process as was also shown by Nunes and Coimbra (Nunes & Coimbra, 2001. In the EDTA extract mannose was the predominant sugar (46 mol%). The absolute amount of uronic acid extracted with EDTA decreased significantly after roasting (Fig. 2), just as the arabinose content. The 0.05 M NaOH extract of roasted coffee beans appeared to consist mainly of arabinogalactans, but also a low amount of galactomannans was present based on the sugar composition. Also for this extract the ratio arabinose:galactose decreased from 0.68 for green coffee beans to 0.21 for roasted coffee beans, indicating a decrease of branching of the arabinogalactans. The mannose content in the 1 and 4 M NaOH extracts (29 and 59 mol%, respectively) was much higher than in the 0.05 M NaOH extract, indicating that a large part of the galactomannans were released using relatively harsh extraction conditions. For both the 1 and 4 M NaOH extract the ratio arabinose:galactose was much lower than in the same extracts obtained from green beans, ageing showing that the arabinogalactans were linearized during roasting.

It was found that also after roasting a part of the galactomannans was released under mild extraction conditions with water and EDTA, while a another part could only be extracted with 1 and 4 M NaOH. From this it was concluded that although the solubility of galactomannans is increased significantly after roasting, still a significant part of the galactomannans are embedded firmly in the cell wall.

For the roasted coffee beans a water extraction at 170 °C was performed as well. The total yield and the polysaccharide content of this extraction was comparable to that of the extraction at 90 °C (Table 3), this in contrast with the results found for the green beans. The sugar composition showed that a higher proportion of arabinogalactans were extracted at 170 °C, while the amount of galactomannans was lower compared to extraction at 90 °C. Apparently, a higher extraction temperature (170°) for a shorter time (20 min) resulted in higher levels of arabinogalactans and lower galactomannan levels. At 170 °C the ratio of arabinose:galactose decreased as compared to the extraction at 90 °C. Apparently arabinose residues are more susceptible to degradation at this temperature.

3.3. Sugar linkage composition of polysaccharides from green and roasted coffee beans

The sugar linkage composition of the green and roasted beans, and of the water, EDTA, and NaOH extracts thereof, were investigated in order to study the structural characteristics of the polysaccharides present in the green and roasted coffee beans (Tables 4 and 5). The sugar composition established after per-methylation were in good agreement with the values found before per-methylation (Tables 1 and 2), although in general the glucose content was somewhat higher.

In the green arabica coffee bean, mannose was predominantly present as 1 → 4-linked residues. The ratio 1 → 4,6-linked mannose:1 → 4-linked mannose shows that approximately one out every twenty mannose residues was substituted with galactose, which is in agreement with

Table 4
Sugar linkage analysis of extracts obtained from green arabica coffee beans (mol%)

Linkage	Bean	Water	EDTA	0.05 M NaOH	1 M NaOH	4 M NaOH	Residue
t-rha	3.1	0.0	1.7	0.0	0.0	0.6	0.3
1 → 2,4-rha	1.0	2.3	4.6	3.1	0.3	0.7	0.3
t-ara	3.6	11.9	16.1	19.1	22.7	18.7	5.3
1 → 5-ara	2.4	8.6	15.1	12.9	7.9	9.9	4.8
1 → 3,5-ara	0.0	0.0	4.9	2.4	0.0	8.0	0.0
1 → 2,3,5 ara	1.4	2.0	0.0	3.6	0.0	0.5	1.0
t-xyl	4.5	4.0	0.0	3.2	0.5	0.7	0.4
1 → 2-xyl	0.0	0.0	0.0	0.0	0.0	3.5	0.0
t-glc	0.0	0.0	1.6	2.2	0.0	0.0	4.5
1 → 4-glc	9.9	10.0	3.3	3.7	2.8	4.0	15.2
1 → 4,6-glc	6.1	0.0	0.0	0.0	0.7	2.0	4.2
1 → 2,3,4,6-glc	1.8	0.0	0.0	0.0	0.1	0.0	0.0
t-man	1.9	3.6	0.8	0.8	2.8	0.4	3.6
1 → 4-man	41.8	22.5	9.0	1.6	1.3	7.4	45.3
1 → 4,6-man	2.3	3.6	0.0	0.0	0.3	0.8	2.2
1 → 2,3,4,6-man	1.0	0.0	0.0	3.3	0.1	3.4	2.4
t-gal	1.1	4.3	1.6	6.6	2.6	4.1	1.7
1 → 6-gal	0.0	0.4	0.0	2.5	1.1	2.3	0.5
1 → 3-gal	4.1	13.1	27.6	14.1	33.1	15.9	7.4
1 → 4-gal	0.0	0.0	0.0	6.9	0.0	5.6	0.0
1 → 2,4-gal	1.3	0.5	0.0	0.0	0.0	0.6	0.0
1 → 3,4-gal	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1 → 3,6-gal	3.6	11.2	13.7	10.4	22.7	10.3	3.4
1 → 4,6-gal	0.0	2.0	0.0	0.0	0.0	0.0	0.9
1 → 2,3,4,6-gal	9.2	0.0	0.0	4.8	1.2	0.0	0.2

Table 5
Sugar linkage analysis of extracts obtained from roasted arabica coffee beans (mol%)

Linkage	Bean	Water	EDTA	0.05 M NaOH	1 M NaOH	4 M NaOH	Residue
t-rha	0.0	0.2	0.4	2.1	0.3	4.3	0.1
1 → 2,4-rha	3.3	2.8	2.2	1.6	1.4	3.0	2.6
t-ara	1.5	4.5	5.1	12.3	7.8	2.9	0.3
1 → 5-ara	1.0	1.2	1.5	5.9	1.7	1.5	2.6
1 → 2,3,5-ara	0.4	0.2	0.3	6.5	0.5	1.6	0.1
t-xyl	1.5	0.2	1.5	0.3	0.5	2.6	1.5
1 → 4-glc	3.3	1.4	4.8	6.6	3.2	10.0	17.0
1 → 4,6-glc	0.0	0.0	0.0	0.0	0.0	1.2	0.0
t-man	3.5	2.0	2.9	1.3	1.9	4.6	2.3
1 → 4-man	66.7	57.4	47.9	3.9	31.8	37.6	64.3
1 → 4,6-man	3.0	2.5	2.1	0.8	1.7	1.2	2.0
1 → 2,3,4,6-man	1.7	0.8	3.1	0.6	1.4	5.7	0.5
t-gal	3.7	5.8	5.9	1.0	8.0	2.7	1.8
1 → 6-gal	1.1	2.2	1.5	0.1	3.4	0.0	0.2
1 → 3-gal	6.1	10.8	13.0	31.8	22.4	12.4	3.3
1 → 2,4-gal	0.0	0.3	0.3	0.0	0.3	0.0	0.0
1 → 3,4-gal	0.0	0.0	0.0	0.0	0.3	0.0	0.4
1 → 3,6-gal	2.8	7.6	7.4	19.7	13.4	6.2	1.9
1 → 4,6-gal	0.0	0.1	0.0	0.0	0.0	0.0	0.3
1 → 2,3,4,6-gal	0.1	0.1	0.0	5.2	0.0	2.6	0.0

the results found by Fischer et al. (Fischer et al., 2001). Galactose was mainly 1 → 3- and 1 → 3,6-linked in the green coffee bean, indicating the presence of type II arabinogalactans as classified by Aspinall (Aspinall, 1973). Arabinose was found to be mainly terminally and 1 → 5-linked. The high proportion of 1 → 4,6-linked glucose, shows that glucose was not only present as a building block of cellulose. Assuming that coffee beans contain a type I cell wall according to Carpita and Gibeaut (Carpita & Gibeaut, 1993), it is probable that the 1 → 4,6-linked glucose originated from xyloglucans.

Based on the ratio 1 → 4,6-linked mannose:1 → 4-linked mannose in the water and EDTA extracts (1:8 and 1:11, respectively) of the green beans it can be concluded that the degree of substitution of the galactomannans was much higher in this extract as compared to the galactomannans present in the green bean. This may be an explanation for the good extractability of these galactomannans with water. In the 4 M NaOH extract and the residue obtained from the green bean the degree of branching of the galactomannans was more comparable to that of the whole bean. The molecular size of the galactomannans as determined by the ratio non-terminal mannose:terminal mannose was much lower for the water extract than for most other extracts, which may explain their good extractability in water. Based on the ratio 1 → 3,6-linked galactose:1 → 3-linked galactose the degree of branching of the arabinogalactans decreased with increasing extraction strength, which is in agreement with the results described in Section 3.2.

In the roasted coffee beans the molecular size of the galactomannans, based on the sugar linkage analysis is somewhat lower than in the green coffee bean. This shows that some degradation of the galactomannans occurred during roasting. In the extracts obtained from the roasted beans, however, the molecular size of the galactomannans is generally somewhat higher than in the green extracts. This indicates that during roasting high molecular weight galactomannans are solubilized. Also, in the roasted bean the degree of branching of the galactomannans is somewhat lower than in the green bean. This may explain part of the loss of galactose during the roasting process (Fig. 1). The lower degree of branching after roasting is seen in all extracts, but especially in the water extract. After roasting the degree of substitution of the arabinogalactans in the coffee beans decreased based on the proportion of branched galactose molecules and the amount of terminally linked arabinose residues. This change in the degree of substitution of the arabinogalactans was observed for all extracts. Additionally, it was observed that the degree of branching of the arabinogalactans decreased with increasing extraction strength. It was found that the proportion of 1 → 4,6-linked glucose was very low in the roasted coffee bean, in its residue and in the 1 and 4 M NaOH extracts. This observation, as well as the decrease of 1 → 2-linked and terminally linked xylose after roasting, indicates that the xyloglucans from coffee beans are debranched during roasting. The degradation of the xyloglucans, which are a part of the cellulose–xyloglucan network and are responsible for the strength of the cell wall, may be an important factor for the loss of firmness of the cell wall during roasting. This may result in an increased accessibility for the extractant, which will result in an increased solubilisation of other polysaccharides.

3.4. High-performance size-exclusion chromatography of the polysaccharides extracted from green and roasted coffee beans

The molecular size distribution of the extracts obtained from green and roasted arabica coffee beans is shown in Fig. 4. In the water extract obtained from the green beans a high Mw population (eluting at 20 min) and a low Mw population eluting at 30 min (Fig. 4) were observed. A similar Mw distribution for a water extract (90 °C) of green arabica coffee beans was found by Leloup and Liardon (Leloup & Liardon, 1993). The same populations were found in the EDTA, the 0.05 M NaOH, and the 1 M NaOH extracts, as well as an additional population eluting at 32 min. For all extracts, the proportion of the population eluting at 30 min decreased with increasing extraction strength. The large peak eluting at 33 min in the EDTA and 0.05 M NaOH extracts was caused by the presence of some residual EDTA. The Mw distribution of the 4 M NaOH extract indicates that most of the polysaccharides in this extract have a fairly low molecular weight. After roasting only a single population with a relatively low molecular weight was found in the water extract, which was in agreement with the results found by Leloup and Liardon (Leloup & Liardon, 1993). A similar molecular weight distribution was found for the other extracts, but the molecular weight appeared to increase somewhat with increasing extraction strength. Apparently, the lower molecular weight polysaccharides are relatively easy to extract and are released under mild extraction conditions. Also, these data indicate that the molecular weight of the polysaccharides in the extracts are lower after roasting, probably as a result of degradation of these polysaccharides. This appears to result in an increase in extractability of these polysaccharides, resulting in higher yields.

The molecular weight distributions of the 170 °C extracts from green and roasted arabica coffee beans are shown in Fig. 5. Three populations were present in the 170 °C extract obtained from green beans: two populations eluted at the same retention times (22 and 30 min) which were also found for populations in the water (90 °C), the EDTA, the 0.05 M NaOH, and the 1 M NaOH extracts, and an additional population eluting at approximately 25 min. Based on the sugar composition of these extracts it is speculated that this population consists predominantly of arabinogalactans. The average molecular weight of the 170 °C extract obtained

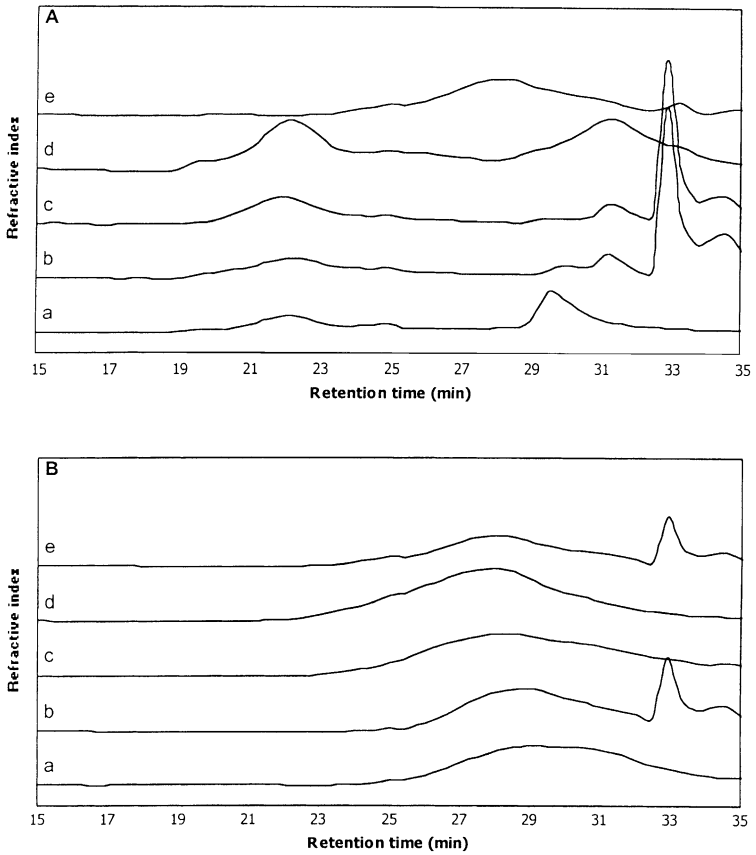

Fig. 4. Molecular size distribution (HPSEC) of extracts obtained with water (a), EDTA (b), 0.05 M NaOH (c), 1 M NaOH (d), and 4 M NaOH (e) from green (A) and roasted (B) arabica coffee beans.

from roasted beans was lower than that of the green been. In comparison with the water extract from green beans obtained at 90 °C, the molecular weight increased. This indicates that the use of higher temperatures and short times during the extraction of roasted coffee beans with water results in a higher molecular weight of the polysaccharides released than the use of lower temperatures in combination with longer extraction times.

3.5. Preparative size-exclusion chromatography of water-extractable polysaccharides

In order to study the nature of the polysaccharide populations found by HPSEC, the water (90 °C) extracts of the green and roasted coffee beans were separated by preparative SEC using Sephacryl S500 (Fig. 6). Two distinct populations could be distinguished for the water

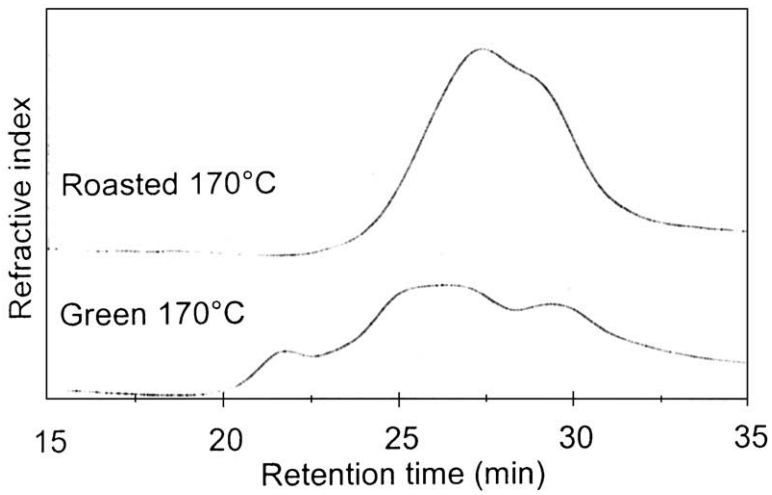

Fig. 5. Molecular size distribution (HPSEC) of water extracts (170 °C) obtained from green and roasted arabica coffee beans.

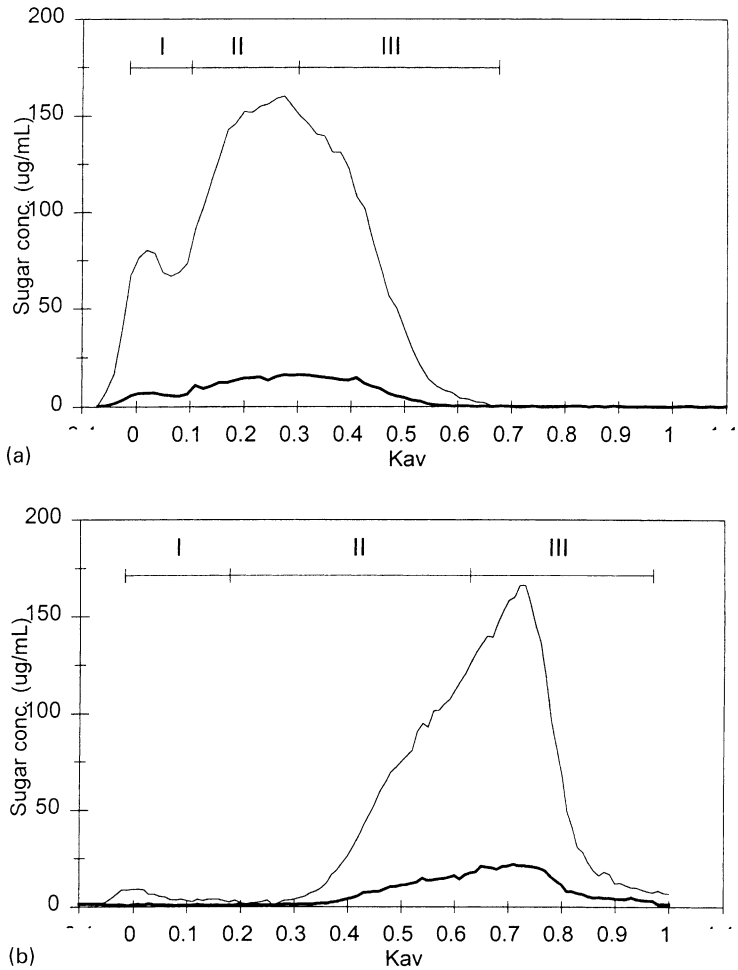

Fig. 6. (a) Preparative size exclusive chromatography of the water extract (90°) obtained from green arabica coffee beans. Thin line; neutral sugars; thick line: uronic acid. (b) Preparative size exclusive chromatography of the water extract (90°) obtained from roasted arabica coffee beans. Thin line; neutral sugars; thick line: uronic acid.

extract of the green bean: a high molecular size population eluting at Kav = 0 and a population with a lower molecular size (0.1 < Kav < 0.6) containing most of the polysaccharides. The hot water extract of the roasted bean also showed two populations, but the relative amount of the high molecular weight population (eluting at Kav = 0) was much lower for this extract. The molecular size of the second population (eluting between Kav = 0.3 and Kav = 0.9) was much lower than was found for the corresponding population of the hot water extract from green bean. The fractions were pooled as indicated in Fig. 6.

Pool I of the hot water extract of the green bean consisted mainly of arabinogalactans (40% Ara and 30% Gal, respectively; Table 6). However, compared to the parental water extract this pool was enriched in mannans. Pool II also consisted predominantly of arabinogalactans. In this pool the arabinose:galactose ratio was much lower. Compared to pool I the content of the pectic sugars rhamnose and uronic acid was still relatively high. Most of the mannose was recovered in pool III, indicating that most of the water extractable galactomannans have a fairly low molecular weight. This was confirmed by the data from the sugar linkage analysis (Table 4). Some arabinogalactans were also present in this fraction based on the sugar composition.

The high molecular weight population (pool I) of the hot water extract of the roasted coffee beans had a mannose content of 73 mol%, indicating that this pool consists of a relatively pure galactomannan. Pool II and III had very

Table 6
Carbohydrate composition of pools of the water extractable polysaccharides from green and roasted arabica coffee beans (%w/w) obtained after SEC using Sephacryl S-500

	Rha	Ara	Xyl	Man	Gal	Glc	Aua	Sugars (%)
Green beans								
Pool I	0	18	0	40	30	2	7	8
Pool II	6	29	1	7	51	1	6	45
Pool III	3	24	1	22	39	3	9	35
Roasted beans								
Pool I	1	4	1	73	9	7	5	38
Pool II	3	12	1	25	51	1	8	21
Pool III	2	12	0	21	53	1	10	9

similar sugar compositions. These pools consisted predominantly of arabinogalactans and galactomannans.

4. Concluding remarks

Characterization of the polysaccharides from green arabica coffee beans showed the presence of large proportions of 1 → 4-linked mannans, of which 1 out of 23 residues was branched with single unit galactose side-chains at O-6 and 1 → 3-linked galactans heavily branched at O-6 with side-chains containing arabinose and galactose residues, as well as smaller amounts of pectins, cellulose, and xyloglucans. Extraction with water, EDTA, and NaOH released less than 10% of the polysaccharides, indicating that the polysacharides in green coffee beans are very difficult to extract. This may be the result of a very dense architecture of the cell walls of the coffee beans. Especially galactomannans and cellulose were very difficult to extract, indicating that they are very closely associated in the cell wall. The galactomannans which were released under mild extraction conditions had a higher degree of branching and a lower molecular weight than the galactomannans which were released using stronger extraction media and the galactomannans present in the residue. This shows that there is a diversity of galactomannans present in green coffee beans, which vary in their degree of branching and possibly in their molecular weight. Although the function of these different galactomannans in the cell wall matrix is not known, it can be speculated that the degree of branching of these galactomannans affects the physico–chemical properties of the cell wall matrix.

Roasting resulted in a loss of 8% of the dry weight. Additionally, a relatively high percentage of sugars was lost during roasting, probably as a result of conversion processes such as the Maillard reaction and pyrolysis reactions. Especially arabinose was easily degraded, with a loss of approximately 60%. Mannose was the most resistant sugar. During roasting the extractability of arabinogalactans, pectins and galactomannans increased significantly. This may be a result of the conversion of high molecular weight polysaccharides into lower molecular weight polysaccharides, making them more easily extractable. Low molecular weight polysaccharides in their turn are converted into monosaccharides and eventually into maillard products, thus explaining the loss of sugars in the roasted coffee beans. It is expected that the degradation of xyloglucans during roasting plays an important role in the increase in accessibility of the cell wall matrix. It was also found that the hydrolysis reactions which occur during roasting result in a linearization of the arabinogalactans and the galactomannans. This may affect their entanglement in the cell wall matrix and thus result in a more open architecture of the cell wall, which also increases the extractability of the polysaccharides.

By giving an overview of the polysaccharide structures found after sequential extraction of green an roasted coffee beans we were able to recognize the most important processes which occur during the roasting of green coffee beans. Future research will include a more detailed investigation of the conversion of polysaccharides during the roasting process of coffee beans.

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