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Extractability and structure of spent coffee ground polysaccharides by roasting pre-treatments

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ABSTRACT

The coffee residue left after the preparation of the brew (spent coffee grounds – SCG) is very rich in polysaccharides, namely galactomannans and arabinogalactans, which are polymers that can be used as dietary fibre and present immunostimulatory activity. Considering the huge amount of SCG produced all over the world, the reutilisation of this by-product by its application as food ingredients is very promising. However, the yields of extraction of these polysaccharides tend to be very low, namely the galactomannans. Based on the observation that the yield of galactomannans extracted from the ground coffee to the brew increase when the coffee is roasted, in this study, with the aim of increasing the yield of these polysaccharides, the SCG was roasted and then extracted with hot water and alkali solutions. The roasting at 160 °C promoted an increment of 15% in the yield of galactomannan extractions and further improvement of the yield of extraction until 56% of all galactomannans was achieved by alkali extractions at 60 and 120 °C. In these samples the galactomannans still kept their characteristic structure, including the acetylation and branching, determined by sugar linkage analysis and mass spectrometry. The yield of extraction of arabinogalactans under these conditions was 54%.

1. Introduction

Consumable coffee is prepared after roasting of green coffee bean. The roasting of coffee beans promotes an increase of the bean volume and the appearance of larger cell wall micropores due to the opening the cell wall matrix (Schenker, Handschin, Frey, Perren, & Escher, 2000). These changes promote the increase of the amount of hot-water-soluble polysaccharides when the ground roasted coffee beans are used to prepare the coffee brew, namely, the galactomannans (Nunes & Coimbra, 2001). The higher the degree of roasting of the coffee, the higher the amount of galactomannans present in the coffee brews of both *Coffea arabica* (Nunes & Coimbra, 2002a) and *Coffea canefora* (robusta) (Nunes & Coimbra, 2002b). Also, the roasting of the coffee bean promotes the extraction of cell wall polysaccharides with NaOH solutions (Oosterveld, Harmsen, Voragen, & Schols, 2003).

The galactomannans are the main polysaccharides present in roasted coffee beans (Redgwell, Trovato, Curti, & Fischer, 2002; Oosterveld et al., 2003) as well as in coffee brew (Nunes & Coimbra, 2001). Coffee galactomannans are composed by a linear β-(1→4)-Man*p* backbone substituted at O-6 with single residues of α-D-Gal*p* (Navarini et al., 1999). The galactomannans are acetylated polysaccharides (Oosterveld, Coenen, Vermeulen, Voragen, & Schols, 2004), as acetyl groups have been observed at the O-2 or O-3 of mannose residues (Nunes, Domingues, & Coimbra, 2005; Simões, Nunes, Domingues, & Coimbra, 2010). Also, coffee galactomannans contain single arabinose residues as side chains (Nunes et al., 2005; Simões et al., 2010) and β-(1→4)-Glc*p* residues interspersed in the main backbone (Nunes et al., 2005). During the roasting process, the galactomannans can undergo depolymerization, debranching, Maillard reactions, caramelization, isomerisation, oxidation, decarboxylation, and melanoidins formation (Nunes, Reis, Domingues, & Coimbra, 2006). Recently, it was shown in mannotriose model compounds that roasting can also promote polymerisation reactions giving rise to molecules with different glycosidic linkage compositions (Moreira, Coimbra, Nunes, Simões, & Domingues, 2011). In contrast to cellulose, which is the polysaccharide less affected by the roasting process, coffee arabinogalactans are very vulnerable to degradation. Coffee arabinogalactans are polysaccharides composed by β-(1→3)-linked-galactosyl residues, some of which are substituted at O-6 with arabinose and galactose residues (Bradbury & Halliday, 1990). Even after a light roast, the arabinogalactans are depolymerized, showing a large decrease in molecular weight (Redgwell et al., 2002). The debranching of the arabinose side chains occurred more rapidly than the hydrolysis of the galactan backbone (Oosterveld et al., 2003).

The consumption of coffee all over the world is very high. Also, during coffee preparation, the spent coffee grounds (SCG) are left with no apparent valuation. The majority of the coffee polysaccharides remain associated to the cell wall matrix. These

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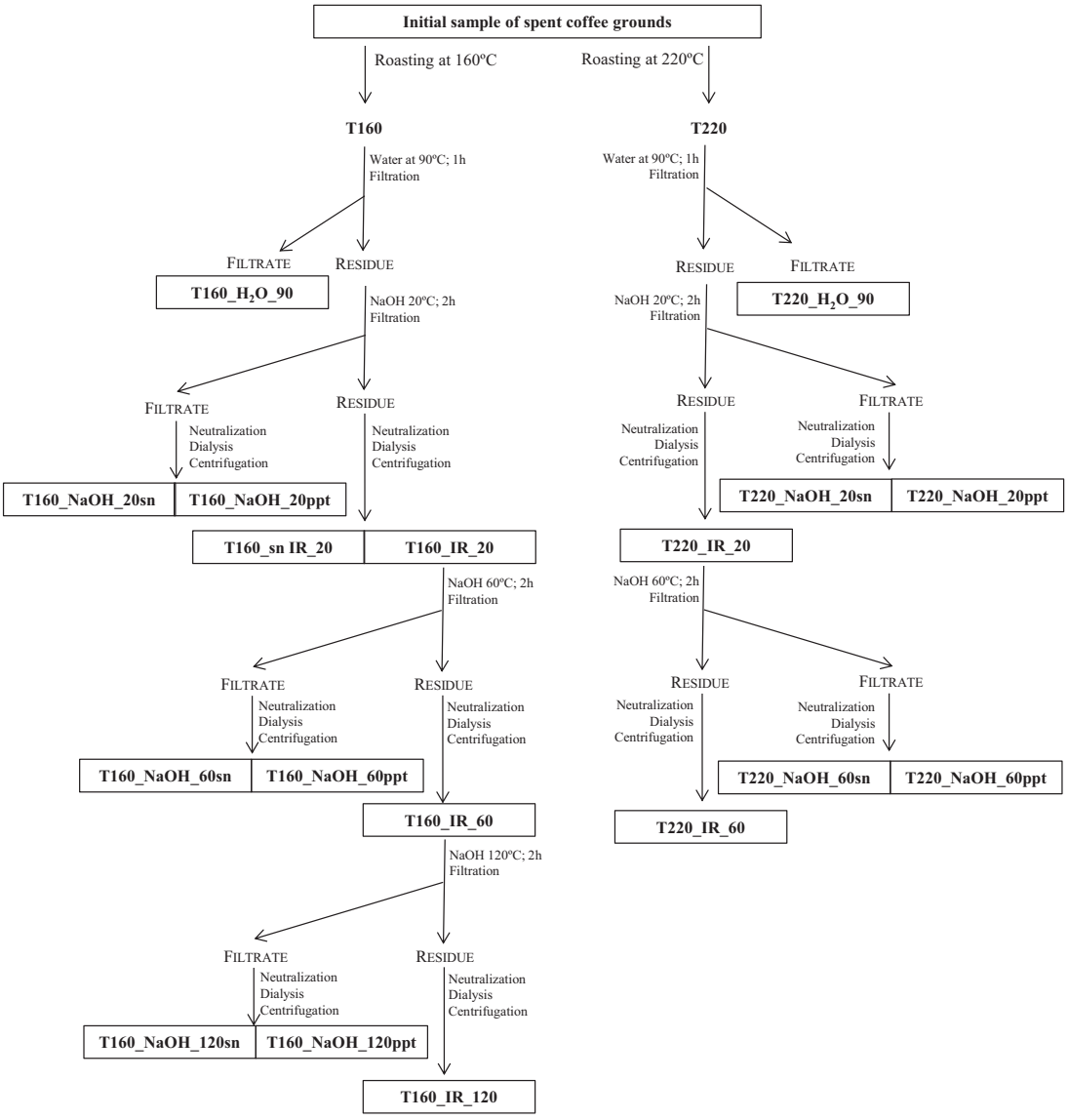
polysaccharides are the main components of SCG, after a coffee beverage was prepared (Simões et al., 2009). Coffee galactomannans (Simões et al., 2009) and arabinogalactans (Gotoda et al., 2006) have been shown to present *in vitro* immunostimulatory properties. One handicap for the utilisation of SCG as a source of these polysaccharides is its low yield of extraction, namely the galactomannans, which reaches only 20% even when 4 M NaOH reagents are used (Simões et al., 2009). Also, recently, the microwave superheated water extraction of poly and oligosaccharides from SCG was shown to be a feasible tool for this purpose (Passos & Coimbra, 2013). In order to continue the search for new tools to improve the extractability of SCG galactomannans, in this study, the SCG resultant from espresso coffee preparation was submitted to a roasting pre-treatment at two different temperatures, 160 °C and 220 °C for 2 h followed by extractions with hot water and with 4 M NaOH solutions at 20 °C, 60 °C, and 120 °C. The effect of the SCG roasting on coffee galactomannans structural features was investigated. The galactomannans were extracted with NaOH solutions and further submitted to a selective degradation by *Aspergillus niger endo*-β-D-mannanase and the oligosaccharides (OS) obtained were fractioned by size exclusion chromatography. Different chromatographic fractions were analysed by electrospray tandem mass spectrometry (ESI-MS/MS) allowing to obtain detailed information about the structure of these coffee galactomannans (Simões et al., 2010; Simões, Nunes, Domingues, & Coimbra, 2011).

2. Experimental

2.1. SCG samples, roasting pre-treatments, and extractions conditions

The SCG used to study the polysaccharide extractability was provided by the cafeteria of the Department of Chemistry of the University of Aveiro. It was obtained from dark roasted Arabica coffee beans, from Buondi, Portugal, used to prepare espresso coffee.

The SCG were collected, submitted to a roasting pre-treatment and then were sequentially extracted with hot water and alkali solutions (Scheme 1) in order to evaluate the possible increase of extraction yield of galactomannans. Samples of 500 g with 61% of moisture were submitted to a roasting procedure, one at 160 °C (T160) and another at 220 °C (T220). These roasting procedures were done in a pre-heated oven (Binder) with an internal volume of 115 L, during 2 h for the assay at 160 °C and during 1.5 h for the



Scheme 1. Roasting pre-treatment of SCG and extractions performed.

220 °C assays. After this period of time, at 220 °C, occurred carbonization of the SCG occurred. Both SCG samples submitted to a roasting pre-treatment were sequentially extracted with: (a) distilled water at 90 °C during 1 h, then (b) with 4 M NaOH at room temperature (20 °C), (c) 4 M NaOH at 60 °C and (d) 4 M NaOH at 120 °C. All NaOH extractions were performed during 2 h. To prevent peeling reactions and alkaline oxidation of the polysaccharides, the NaOH extractions were carried out under an inert atmosphere (N_2) with O_2 free solutions containing 0.02 M $NaBH_4$. The NaOH solutions were prepared using distilled water previously boiled for 20 min and cooled under a nitrogen atmosphere. After each extraction step the mixture was filtered, concentrated under reduced pressure, and dialysed for 3 days, with several changes of distilled water. The final insoluble residue (IR) obtained were also dialysed for 3 days, with several changes of distilled water. The alkali extracts and IR extract were previously acidified to pH 5.0 with glacial acetic acid. After dialysis, the extracts were centrifuged and the precipitates (ppt), if present, were recovered separately from the supernatants (sn). The IR dialysed could be in same cases separated from another fraction, a supernatants (sn IR). All samples were frozen and freeze-dried.

2.2. Sugar analysis

The individual sugars components of the polysaccharides were determined by gas chromatography (GC) as alditol acetates using 2-deoxyglucose as internal standard. The polysaccharides were treated with 72% (w/w) H_2SO_4 (10 mg/mL) during 3 h at room temperature with occasional stirring followed by hydrolysis for 2.5 h with 2 M sulfuric acid at 100 °C. Monosaccharides were reduced with $NaBH_4$ (15% in NH_3 3 M) during 1 h at 30 °C and subsequent acetylated with acetic anhydride (3 mL) in the presence of 1-methylimidazole (450 μL) during 30 min at 30 °C. Alditol acetate derivatives were separated with dichloromethane and analysed by GC with a FID detector and equipped with a 30 m column DB-225 (J&W Scientific, Folsom, CA, USA) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively. The oven temperature program used was: initial temperature 200 °C, a rise in temperature at a rate of 40 °C/min until 220 °C, standing for 7 min, followed by a rate of 20 °C/min until 230 °C and maintain this temperature 1 min. The injector and detector temperatures were, respectively, 220 and 230 °C. The flow rate of the carrier gas (H_2) was set at 1.7 mL/min (Nunes & Coimbra, 2001). The hydrolysis of all samples was performed in duplicate. The total sugars content was achieved by the sum of the individual contribution of each sugar residue to the total mass of the sample.

2.3. Linkage analysis

The sample was dissolved in 1 mL of anhydrous dimethylsulfoxide (DMSO), then powdered NaOH (40 mg) were added and samples were methylated with CH_3I (80 μL) during 20 min. Distilled water (2 mL) and dichloromethane (3 mL), were then added, and dichloromethane phase was washed three times by addition of distilled water (2 mL). The organic phase was evaporated to dryness and the material was remethylated using the same procedure (Nunes & Coimbra, 2001). The methylated material was hydrolyzed with TFA 2 M at 121 °C for 1 h, and then reduced and acetylated as previously described for neutral sugar analysis. The partially methylated alditol acetates (PMAA) were separated and analysed by gas chromatography–mass spectrometry (GC–MS) on an Agilent Technologies 6890N Network. The GC was equipped with a DB-1 (J&W Scientific, Folsom, CA, USA) capillary column (30 m length, 0.25 mm of internal diameter and 0.15 μm of film thickness). The samples were injected in splitless mode (time of splitless 5 min), with the injector operating at 220 °C, and using the following temperature program: 45 °C for 5 min with a linear increase of 10 °C/min up 140 °C, and standing for 5 min at this temperature, followed by linear increase of 0.5 °C/min up to 170 °C, and standing for 1 min at this temperature, followed by linear increase of 15 °C/min up to 280 °C, with further 5 min at 280 °C. The helium carrier gas had a flow rate of 1.7 mL/min and a column head pressure of 2.8 psi. The GC was connected to an Agilent 5973 mass quadrupole selective detector operating with an electron impact mode at 70 eV and scanning the range *m/z* 40–500 in a 1 s cycle in a full scan mode acquisition.

2.4. Scanning electron microscopy (SEM)

Samples preparation for the SEM assays was performed by fixed the SCG on steel supports and coated with 60% gold/40% palladium using a JEOL metalizer (FFC-1100, Tokyo, Japan) at 1100–1200 V, 5 mA for 10 min. SCG samples were observed in a scanning electron microscope (Hitachi, S4100, Tokyo, Japan) at 25 kV.

2.5. Enzymatic hydrolysis and size-exclusion chromatography (Bio-Gel P2)

Samples (15 mg) were hydrolysed with 1 U of a pure *endo*-β-(1→4)-D-mannanase preparation (Megazyme, EC 3.2.1.78) during 48 h at 37 °C with continuous stirring in a 100 mM Na-acetate buffer, pH 5.5, containing 0.02% sodium azide. The freeze-dried material was dissolved in water, and loaded on a XK 1.6/100 column containing Bio-Gel P-2 (Bio-Rad) previously equilibrated with water, and calibrated with DP4 (stachyose), DP2 (cellobiose), and monosaccharide (glucose), using a flow of 0.3 mL/min. Fractions (1 mL) were collected and assayed for sugars with evaporative light scattering detection. Collected fractions containing oligosaccharides were pooled and evaporated until all the eluent was removed. No freeze dried was performed since it has been shown that freeze dried promotes O-acetyl migration on galactomannans (Nunes et al., 2005).

2.6. Electrospray mass spectrometry

The fractions obtained from the Biogel P2 column were dissolved in water and further diluted in methanol/water/formic acid (50:49.9:0.1, v/v/v). Positive ion ESI-MS and MS/MS spectra were acquired using a LXQ linear ion trap mass spectrometer (ThermoFinningan) using following conditions: nitrogen sheath gas 30 psi, spray voltage 5 kV, heated capillary temperature 275 °C, capillary voltage 1 V, and tube lens voltage 40 V. The flow rate was set to 8 μL/min and the voltage applied was 5 kV. Nitrogen was used as nebulising and drying gas. Full scan mass spectra ranging from *m/z* 100 to 1500 were acquired. In the MS^2 experiments, collision energy varied between 15 and 25 of normalised collision. Data acquisition was carried out with Xcalibur data system.

3. Results and discussion

3.1. Yield of extraction of polysaccharides from roasted SCG and characterisation of fractions extracted with hot water and 4 M NaOH at 20 °C

The espresso SCG sample used was composed mainly by mannose (46%), galactose (27%), glucose (20%), and arabinose (7%) (Table 1). The higher abundance of mannose allows to infer that the galactomannans are the major polysaccharides present, accounting for approximately 50% of total carbohydrates. Performing a hydrolysis with 2 M of H_2SO_4 at 100 °C during 2.5 h, the total carbohydrates were observed to account for 60% of the dry weight material of SCG. This value was higher than the 45% estimated when

1 M of H_2SO_4 was used under the same conditions. However, the same relative proportions of sugars have been observed in the two hydrolysis conditions (data not shown). These observations allow to infer that the total polysaccharides can be underestimated when 1 M of H_2SO_4 is used. Comparable results were obtained when the SCG were hydrolysed using 2 M H_2SO_4 at 120 °C during 1 h (Passos & Coimbra, 2013).

Under the experimental conditions described for the roasting of SCG at 160 °C, during the first 10 min the temperature was below 160 °C due to the water evaporation. After 1 h, the material still retained 34% of water, but after 2 h, a weight loss of 1% was observed. For the roasting pre-treatment at 220 °C, after 1 h, the material still retained 11% of water. When the pre-treatment was prolonged for more 30 min, a weight loss of 35% was obtained. In order to evaluate the effect of these pre-treatments on the extent of SCG polysaccharides extractability, the two roasted residues were extracted with hot water at 90 °C during 1 h and then the solubilised material was dialysed. For the roasting at 160 °C, the amount of polymeric material recovered with hot water only accounted for 1.4% of total sample (Table 1), composed by 44% of sugars. The main sugar residue was galactose (51%), mannose accounting for only 33% of total sugars. This shows that the arabinogalactans were the polysaccharides preferably extracted under these conditions. For the roasting at 220 °C, the hot water polymeric extracted material accounted only for 0.2% of total SCG and was composed only by 21% of sugars, which was lower than that observed for the 160 °C pre-treatment. However, in these roasting conditions, the main sugar residue was mannose (51%), accounting galactose only for 37% of total sugars, showing that under these conditions, the arabinogalactans are not the main polysaccharide extracted.

Taking into account that SCG galactomannans are soluble and extractable in 4 M NaOH solutions (Simões et al., 2009), the residues left after the hot water extraction of the roasted material were suspended in a 4 M NaOH solution at room temperature during 2 h. Upon dialysis of the neutralised material, in both experiments, it was obtained a fraction of material that was insoluble in cold water (NaOH_20ppt) and a fraction of material that was soluble (NaOH_20sn). The material roasted at 160 °C was mainly recovered in the precipitate (9.8%) whereas the material roasted at 220 °C was mainly recovered in the supernatant (9.4%). The supernatants were, in both cases, richer in galactose (61% and 45%, respectively) and poorer in mannose (24% and 47%) than the precipitates (21% and 11% for galactose, and 73% and 76% for mannose, respectively). As the supernatants were also rich in arabinose, it can be inferred that they contain mainly arabinogalactans, thus indicating that arabinogalactans were removed by the alkali and remained soluble upon neutralisation. However, the extraction from the 220 °C residue also contained higher amount of soluble mannose, allowing to infer an increase of extraction of alkaline extracted water soluble galactomannans.

The insoluble residues obtained after the alkali extraction were suspended in water, neutralised, and dialysed. The material that was solubilised during dialysis was recovered in the supernatant after centrifugation (T160_snIR_20), separated from the insoluble material (T160_IR_20). The T160_snIR_20 fraction accounted for 4.6% of the starting material, on a dry basis, whereas 42% were sugars. The main sugar residue of this fraction was galactose (67%), accounting arabinose for 17% and mannose for only 14% of total sugars, suggesting the presence of arabinogalactans as the main polysaccharide. For the 220 °C roast, no material was obtained in the snIR_20 fraction. The T160_IR_20 fraction obtained from the 160 °C experiments, was mainly composed by mannose (52%) followed by glucose (24%), galactose (19%) and arabinose (5%), in a 75% of total sugar. This composition shows that a large proportion of galactomannans still remained in residue. A comparable sugars composition to T160_IR_20 was obtained for the T220_IR_20 residue obtained from the 220 °C experiments, although this material contained a lower amount of total sugars (15%).

Fig. 1 shows the scanning electron microscopy (SEM) analysis of the initial SCG (Fig. 1a), and of the residues left after the alkali extraction of roasted SCG at 160 °C (T160_IR_20, Fig. 1b) and 220 °C (T220_IR_20, Fig. 1c). Comparing with the initial sample, the T160_IR_20 residue showed the appearance of more defined and hollowed structures, possibly the cell walls. This may indicate that although some material was solubilised by the alkali reagent, some still remain in the matrix, confirming the results obtained by the sugar analysis of the residue. However, in T220_IR_20, these better defined structures are not so well visible, showing possibly the degradation of the matrix resultant from a roasting at this high temperature. This is accordance with the low sugars content (15%) of this residue.

Table 1
Sugars composition of initial SCG and fractions from the extraction with 4 M NaOH at different temperatures of roasted SCG.

	Yield of extraction (%)	Sugar composition (mol%)				Total Sugar (%)
		Ara	Man	Gal	Glc	
Initial sample of SCG	–	7	46	27	20	60.0
Roasting at 160 °C						
T160_H_2O_90	1.4	14	33	51	2	44.3
T160_NaOH_20sn	7.8	14	24	61	1	63.2
T160_NaOH_20ppt	9.8	4	73	21	2	65.9
T160_sn IR_20	4.6	17	14	68	1	42.1
T160_IR_20		5	52	19	24	75.0
T160_NaOH_60sn	2.3	16	12	71	1	51.0
T160_NaOH_60ppt	6.5	2	86	11	1	81.0
T160_IR_60		5	44	20	31	71.7
T160_NaOH_120sn	2.6	17	15	67	1	71.2
T160_NaOH_120ppt	5.7	2	89	7	2	80.1
T160_IR_120	20.0	6	45	24	25	57.1
Roasting at 220 °C						
T220_H_2O_90	0.2	8	51	37	4	21.2
T220_NaOH_20sn	9.4	6	47	45	2	52.6
T220_NaOH_20ppt	5.3	2	76	11	11	70.0
T220_ IR_20		4	42	24	30	15.0
T220_NaOH_60sn	4.4	6	59	31	4	48.6
T220_NaOH_60ppt	0.8	2	54	9	35	33.7
T220_IR_60	53.5	4	30	18	48	12.4

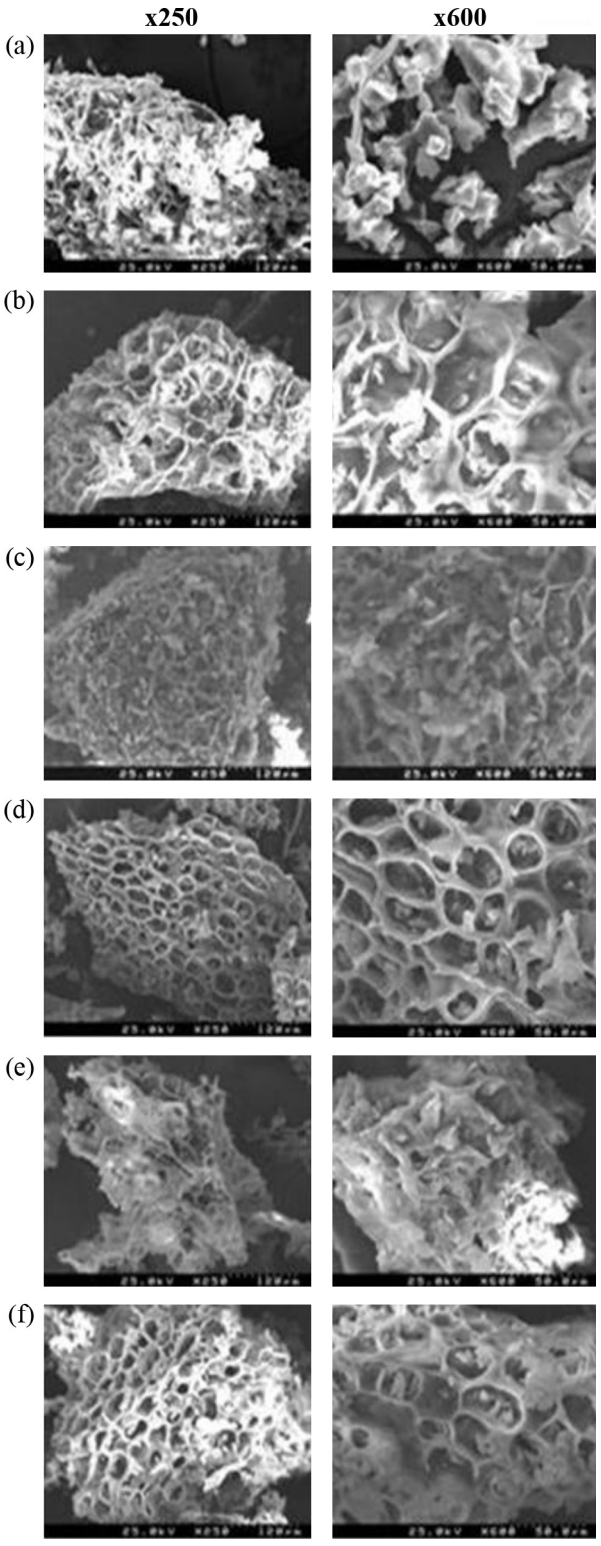


Fig. 1. Scanning electron microscopy (SEM) images at ×250 and ×600 of SCG. (a) Initial coffee sample, (b) T160_IR_20, (c) T220_IR_20, (d) T160_IR_60, (e) T220_IR_60, and (f) T160_IR_120.

3.2. Yield of extraction of polysaccharides from roasted SCG and characterisation of fractions extracted with 4 M NaOH at 60 and 120 °C

In order to further extract the polysaccharides still remaining in the residues T160_IR_20 and T220_IR_20, a 4 M NaOH extraction was performed at 60 °C (NaOH60). As observed for the material extracted at 20 °C, Table 1 shows that the material roasted at 160 °C and extracted with NaOH at 60 °C was mainly recovered as a precipitate (T160_NaOH_60ppt, 6.5% of the initial sample) whereas the material roasted at 220 °C was mainly recovered in the supernatant (T220_NaOH_60sn, 4.4%). Also, as observed for the material extracted at 20 °C, the supernatant of T160 was richer in galactose (71%) and poorer in mannose (12%) than the precipitates (11% and 86%, respectively), allowing to infer the occurrence of arabinogalactans as the main polysaccharides. However, both T220_NaOH_60sn and ppt, were richer in mannose than galactose, showing that, under these higher roasting conditions, the galactomannans were the main polysaccharide extracted. Considering that fraction T160_NaOH_60ppt was rich in sugars (81%), was the most abundant one, and was the richest in mannose, these conditions seem to be relevant for extraction of galactomannans from coffee residue. However, in contrast, fraction T220_NaOH_60ppt was not so rich in sugars, the yield was low, and was not rich in mannose. Also, the determination of 35% of glucose in this precipitated fraction shows that degraded cellulose should be also extracted under these extreme conditions.

The insoluble residues obtained after the alkali extraction ate 60 °C were suspended in water, neutralised, and dialysed, giving origin to the T160_IR_60 and T220_IR_60 residues. No material was recovered in the dialysis supernatant. For the 160 °C roast, the IR_60 fraction was mainly composed by mannose (44%) followed by glucose (31%), galactose (20%) and arabinose (5%), in a 72% of total sugar. This composition shows that a large proportion of galactomannans still remained in residue. On the contrary, the sugars composition obtained for the T220_IR_60 residue was poor in sugars (12%), and the main sugar residue was glucose (48%), accounting mannose only for 30% (Table 1). These results show that no relevant carbohydrate material was any longer present in T220_IR_60 residue.

To perform quantitative analysis of insoluble residues, the material should be previously treated with 72% sulphuric acid solution for a complete soaking of the polysaccharides (Selvendram, March, & Ring, 1979). However, when the sugars are loosely bound to the matrix, this treatment can be discarded. In order to observe if the galactomannans and cellulose were loosely bound in the cell wall matrix due to the roasting process, explaining the occurrence of the high amount of glucose in the T220_NaOH_60 fraction, the sugars of the two residues, T160_IR_60 and T220_IR_60, were analysed with and without this treatment. Fig. 1 shows the extra amount of mannose and galactose obtained when the 72% sulphuric acid treatment was performed. The amount of mannose tightly bound to the matrix accounted for 40% in T160_IR_60 and 25% in T220_IR_60. Also, the amount of cellulosic glucose was higher in the T160_IR_60 (90%) than in T220_IR_60 (70%). These results show that the roasting process of the SCG promotes degradation of the polysaccharides tightly bound to the cell wall matrix.

SEM analysis of T160_IR_60 (Fig. 1d) showed the appearance of even better defined cell walls of hollowed cells than those observed in T160_IR_20 (Fig. 1b), resultant from the removal of all surrounding material. However, in the SEM images of T220_IR_60 residue (Fig. 1e) it was not observed the well defined cell-wall matrix visible in the T160_IR_60 residue, possibly due to the extensive degradation of the material. These observations are in accordance with the high sugar content in T160_IR_60 residue and the lower sugar content of T220_IR_60 residue (Table 1).

In order to further extract the SCG polysaccharides that still remain in the T160_IR_60 residue, a 4 M NaOH extraction was performed at 120 °C. The total yield of the extract was 8.3% of the initial SCG. The material that precipitated upon neutralisation and recovered by dialysis (T160_NaOH_120ppt) accounted for 5.7% of the initial residue. It was composed by mannose (89%), galactose

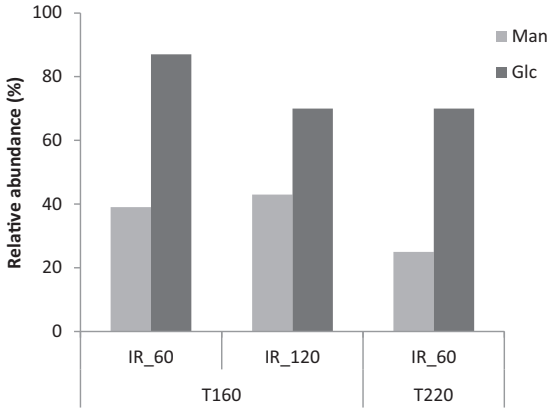


Fig. 2. Relative amount of total mannose and total glucose only released from the insoluble residues when a pre-hydrolysis was performed.

(7%), glucose (2%), and arabinose (2%), in 80% of total sugars, which are characteristics of a pure galactomannan. The material that remained in the supernatant upon neutralisation and dialysis (T160_NaOH_120sn) was composed mainly by galactose (66%) and arabinose (17%), in a fraction containing 71% of sugars, showing the presence of an arabinogalactan. The residue left (T160_IR_120) contained 45% of mannose. Taking into account the amount of yield of the extracts and their content in mannose, it can be estimated that only 16% of the mannose constituent of the SCG still remains in the final residue.

SEM analysis of the residue T160_IR_120 (Fig. 1f) showed defined cell walls of hollowed cells comparable to those observed in T160_IR_60 residue (Fig. 1d), although some compacted material seems to occur. According to Fig. 2, the amount of mannose and glucose tightly bound to the matrix of T160_IR_120 accounted for 40% and 70%, respectively. These results show that although degradation of the polysaccharides tightly bound to the cell wall matrix was higher than that observed in T160_IR_60, it was not so high as that observed in T220_IR_60.

3.3. Glycosidic linkage analysis of galactomannans from roasted SCG extracted with 4 M NaOH at 20, 60, and 120 °C

The results of methylation analysis of the fractions rich in mannose, recovered as precipitates upon extraction of roasted SCG by 4 M NaOH, are shown in Table 2. The predominance of the (1→4)-linked mannose residues, and the presence of (1→4,6)- and terminally-linked mannose residues, as well as terminally-linked galactose residues, confirm the presence of galactomannans.

The ratio of total Man/terminally-linked Man gives a direct indication of the polymer size. In the samples submitted to a roasting procedure at 160 °C and then extracted with NaOH at 20 °C, a value of 11 was obtained. This is lower than the value of 23 obtained previously for the galactomannan-rich extracts obtained from spent coffee gounds extracted with 4 M NaOH at 20 °C without the prior roasting pre-treatment (Simões et al., 2011). However, when a following extraction was performed at 60 °C by the same strength alkali solution, the galactomannans obtained presented a total Man/terminally-linked Man of 30, and this ratio further increased to 56 for the galactomannans obtained when a third extraction was performed at 120 °C. In the samples submitted to a roasting procedure at 220 °C, the ratio of total Man/terminally-linked Man was similar for the two extractions performed (21 and 20 for the extractions at 20 and 60 °C).

The ratio (1→4,6)-Man/Total Man allows to infer the degree of branching of the galactomannans. All galactomannan-rich fractions extracted from the SCG roasted at 160 °C had a similar degree of branching, showing an average of 1 branched residue in 100 Man residues, which is a very low degree of branching when compared with the SCG galactomannans previously extracted without the roasting pre-treatment (7.8). This lower branching of the galactomannans obtained from roasted SCG show that the roasting promoted the debranching of galactomannans, as previously stated by Nunes and Coimbra (2002a, 2002b). This observation is reinforced by the data obtained for the galactomannans extracted from the SCG roasted at 220 °C, where in one fraction (T220_NaOH_20ppt) no (1→4,6)-Man neither terminally-linked galactose were detected. In all samples, it was possible to detect (1→3)-Gal, (1 → 3,6)-Gal, and (1→5)-Ara, which are diagnostic linkages for the presence of arabinogalactans. The relative content of these residues tend to decrease when the temperature of the alkali reagent used for extraction increased.

3.4. Yield of extraction of polysaccharides

Based on the extraction yield of the different fractions and content of the different sugars and glycosidic-linkage composition, it was possible to estimate the relative amount of galactomannans and arabinogalactans extracted (Table 3). For the estimation of the galactomannans, it was assumed that the mannose obtained was all from galactomannans and that the branching of the galactomannans in SCG is, on average, 8% (Simões et al., 2010). Due to the lower content of glucose, arabinose and acetyl groups in these galactomannans, their contribution were not considered. Based on

Table 2
Glycosidic-linkage composition of SCG galactomannans submitted to a roasting procedure at 160 °C and 220 °C, extracted with 4 M NaOH solutions at different temperatures: 20 °C, 60 °C and 120 °C.

Linkage	Without roasting pre-treatment NaOH_20ppt[a]	T160_NaOH_ppt			T220_NaOH_ppt	
		20	60	120	20	60
T-Ara*f*	4.8	0.4	0.6	0.1	0.1	0.2
5-Ara*f*	2.9	0.5	1.3	0.5	0.0	1.1
T-Man*p*	3.0	7.5	2.9	1.7	3.9	2.3
4-Man*p*	60.8	70.6	84.6	90.1	77.3	42.2
4,6-Man*p*	5.4	1.0	0.9	1.5	0.0	0.9
T-Gal*p*	6.4	3.5	1.3	0.8	0.0	1.0
3-Gal*p*	8.5	9.7	0.7	1.1	2.1	1.4
3,6-Gal*p*	6.2	1.2	0.0	0.3	0.0	0.0
4-Glc*p*	1.2	5.5	7.6	4.0	16.6	48.6
Total Man*p*/T-Man*p*	23	11	30	56	21	20
(4,6-Man*p*/total Man*p*) × 100	7.8	1.3	1.0	1.6	0.0	2.1

[a] Values from Simões et al. (2010).

Table 3
Extraction yield (%) of SCG galactomannans and arabinogalactans extracted with 4 M NaOH at different temperatures from SCG submitted to a roasting pre-treatment at 160 °C or 220 °C.

	NaOH extraction temperature (°C)	Extraction yield (%)	
		Galactomannans	Arabinogalactans
T160	20	23	38
	60	17	7
	120	16	9
	Total	56	54
T220	20	18	14
	60	5	4
	Total	23	18

these assumptions, the amount of galactomannans in each extract was estimated by the sum of the amount of mannose plus 8% of the total amount of mannose, accounting for the galactose content.

Previous results by Simões et al. (2009) reported that the alkaline extraction of SCG galactomannans without any pre-treatment of roasting provided an extraction yield of 20%. The extraction of SCG galactomannans after a roasting pre-treatment at 160 °C (T160) using the same extraction conditions allowed to obtain an extraction yield of 23% of these polysaccharides. The extraction of galactomannans from SCG with NaOH solutions at 60 °C extracted more 17% and the extraction with NaOH at 120 °C, extracted extra 16%. These results showed that the roasting pre-treatment at 160 °C followed by successive NaOH extractions with increasing temperatures (20, 60, and 120 °C) improved the extractability of galactomannans from SCG to an aggregate of 56%. This value, although lower than the 66% of mannose recovered for the best condition when using microwave superheated water extraction from SCG (Passos & Coimbra, 2013), was totally composed by polymeric material (retained by 12 kDa pore dialysis), in contrast with the oligomeric compounds (recovered mainly as ethanol soluble material) when microwave extraction was used (Passos & Coimbra, 2013).

In case of the roasting pre-treatment of SCG at 220 °C (T220) followed by NaOH extraction at 20 °C, the galactomannans extraction yield was only 18%. The extraction with NaOH solutions at 60 °C allowed to obtain more 5%, showing that these conditions were too extreme to recover the galactomannans.

For the estimation of the arabinogalactans, it was assumed that all galactose, except an amount corresponding to 8% of total mannose, was component of the arabinogalactans. Also, it was assumed that all arabinose in fractions was from the arabinogalactans. The extraction arabinogalactans from roasted SCG at 160 °C with the NaOH solution at 20 °C allowed an yield of 38%, which is lower than the value of 43% reported for the extraction of arabinogalactans from SCG without any roasting pre-treatment (Simões et al., 2009). Also, the successive extractions of the SCG with NaOH solutions at 60 °C and 120 °C only allowed to obtain extra 7% and 9%, respectively, in a total of 54% (Table 3). These results show that, contrarily to what was observed for galactomannans, the roasting of the SCG does not allow to improve the extraction yield of the arabinogalactans. Also, the use of heated alkali solutions does not allow to obtain much more polysaccharides. This was confirmed in the case of the roasting pre-treatment at 220 °C, where the NaOH solution at 20 °C only allowed to extract 14% of the arabinogalactans, and the extraction at 60 °C only allowed to obtain an extra 4%.

3.5. Selective hydrolysis by an endo-β-(1→4)-D-mannanase

In order to evaluate the structural features of the roasted SCG galactomannans obtained from different roasting procedures and extraction conditions, they were selectively hydrolysed with an *endo*-β-(1→4)-D-mannanase. The hydrolysis of the β-(1→4) linked mannan backbone is hindered by the presence of substituted residues, allowing to obtain galactomannan oligosaccharides that contain structural details on the substituents of the galactomannan backbone (Dhawan & Kaur, 2007; Moreira & Filho, 2008; Simões et al., 2010, 2011). The oligosaccharides obtained were fractioned by size-exclusion.

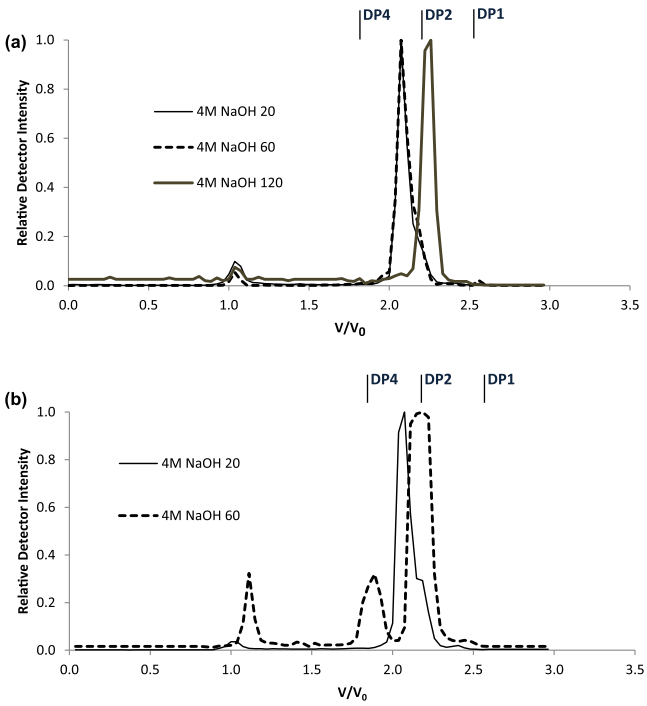


Fig. 3. Size-exclusion chromatography on Bio-Gel P2 of the OS obtained after enzymatic hydrolysis with an endo-β-D-mannanase of galactomannan obtained from SCG (a) T160_NaOH_20ppt, T160_NaOH_600ppt and T160_NaOH_120ppt (b) T220_NaOH_20ppt and T220_NaOH_600ppt. DP4 and DP2 correspond to the elution volume of DP4 and DP2 standard oligosaccharides, and DP1 corresponds to the elution volume of monomers.

The size-exclusion chromatograms of the mixture of OS obtained after hydrolysis of the SCG galactomannans submitted to roasting procedure at 160 °C, which was extracted with 4 M NaOH at 20 °C, and at 60 °C and at 120 °C (Fig. 3a). The hydrolysis of the galactomannan obtained after extraction with at 20 °C and at 60 °C, originated mainly trisaccharides (DP3) while in the case of the hydrolysis of galactomannan extracted at 120 °C, the major oligosaccharides observed were disaccharides (DP2). In these three extractions a very small amount of oligosaccharides of a higher degree of polymerisation were also observed. As the enzyme was able to completely degrade the galactomannans, it can be inferred that they keep their β-(1→4) linked mannan characteristics. The size-exclusion chromatogram of the SCG galactomannans submitted to roasting procedure at 220 °C and extracted with NaOH at 20 °C (Fig. 3b) had a similar pattern to those roasted at 160 °C, showing that significant backbone modification occurred in these polysaccharides. However, the galactomannans extracted at 60 °C showed higher molecular weight oligosaccharides, allowing to infer that these polysaccharides have been modified in their backbone.

3.6. Characterisation of galactomannans by mass spectrometry

In order to analyse in detail the structural features of the galactomannans extracted from the roasted SCG, different fractions obtained by the cleavage with an *endo*-β-mannanase were

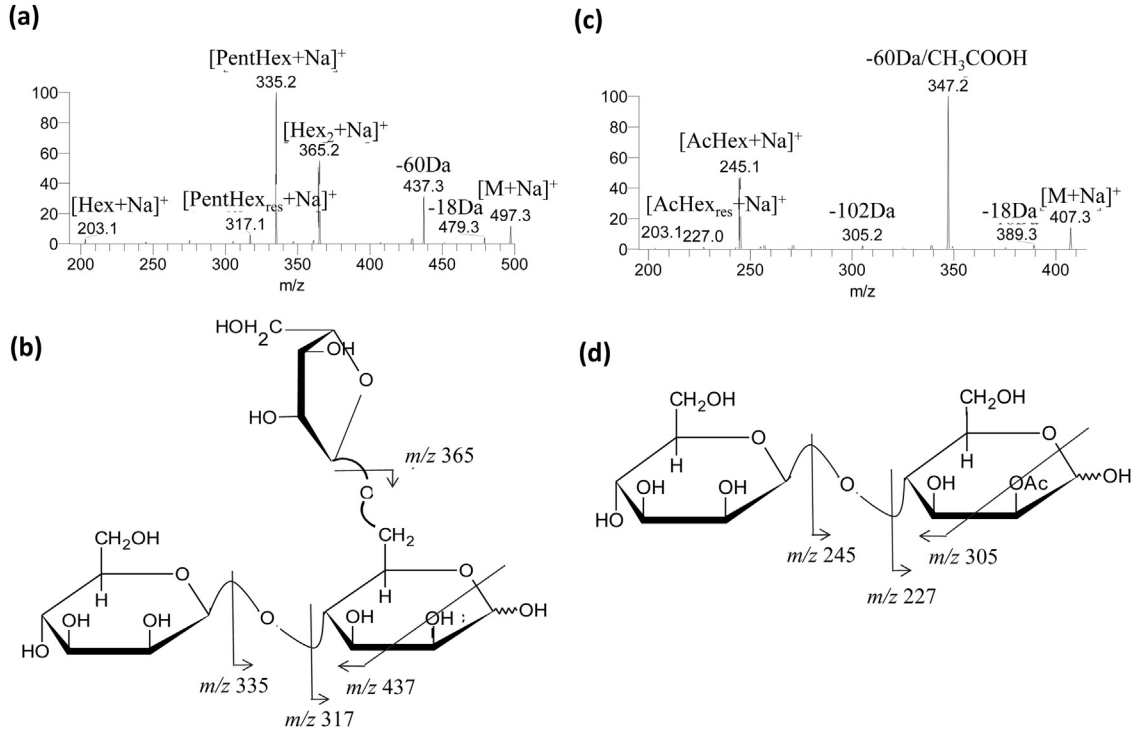


Fig. 4. Tandem mass spectrometry assays of oligosaccharides formed by *endo*-β-mannanase. (a) ESI-MS/MS spectrum ion at *m/z* 497, attributed to $[PentHex_2 + Na]^+$ and (b) correspondent fragmentation pattern and proposed structure. (c) ESI-MS/MS spectrum ion at *m/z* 407, attributed to $[AcHex_2 + Na]^+$ and (d) correspondent fragmentation pattern and proposed structure.

analysed by mass spectrometry with electrospray ionisation (ESI-MS). The OS recovered in the Biogel P2 collected fractions were identified in the ESI-MS spectra as $[M + Na]^+$ ions and confirmed by ESI-MS/MS. "Hex" represents a hexose, as the *m/z* of Man, Gal or Glc is the same; "Pent" represents a pentose, Ara because this was the only pentose detected by sugar analysis and methylation analysis, and "Ac" represents the acetyl groups.

The ESI-MS/MS spectrum of the ion at *m/z* 497, identified as $[PentHex_2 + Na]^+$ (Fig. 4a), shows the ions at *m/z* 365, *m/z* 335, *m/z* 317, and *m/z* 203, that can be attributed to losses of a pentose residue ($Pent_{res}$), a hexose residue (Hex_{res}), a hexose, and a Pent, respectively. These results allow to observe the occurrence of a pentose residue in the galactomannan structure (Fig. 4b), showing that even when the galactomannans are submitted to an extensive roasting treatment at 160 °C, and extracted with strong alkali solutions at temperatures up to 120 °C, the galactomannans maintain their branching characteristics. These structural features were also observed in the OS obtained from the roasting procedure at 220 °C, although in very small amount (data not shown).

The product ion spectrum (ESI-MS/MS) obtained for the ion at *m/z* 407, identified as $[AcHex_2 + Na]^+$, is present in all the three alkali galactomannan-rich samples extracted from the SCG roasted at 160 °C (Fig. 4c). The presence of product ions at *m/z* 245 and 227, which can be attributed to the ions $[AcHex + Na]^+$ and $[AcHex_{res} + Na]^+$, can be resultant from loss of one Hex_{res} (162 Da), and one Hex (180 Da), respectively, from the ion $[AcHex_2 + Na]^+$. Also, the presence of one acetyl group in this OS is also noticed by the abundant ion at *m/z* 347, resultant of loss of $-CH_3COOH$ (60 Da) (Reis et al., 2005; Simões et al., 2010). The occurrence of the ion at *m/z* 305, resultant from loss of 102 Da, attributed to a $^{0,2}A_3$ cross ring fragmentation containing the acetyl group at O-2 position (Nunes et al., 2005), shows that the acetyl group is linked to the O-2 of the hexose residue of the reducing end terminal (Fig. 4a and c). The observed fragmentation allowed to infer the presence of acetyl groups in roasting SCG, similarly as was observed from SCG without roasting procedure (Simões et al., 2010). Acetyl groups have been described to occur in green coffee galactomannans (Oosterveld et al., 2004), in coffee infusion from roasted coffee beans (Nunes et al., 2005) but not in SCG. These results show that even when the galactomannans are submitted to an extensive roasting treatment at 160 °C, and extracted with strong alkali solutions at temperatures up to 120 °C, the characteristic acetyl groups of galactomannans are still observed. The presence of acetyl groups were also observed in the OS obtained from the roasting procedure at 220 °C, although in very small amount (ESI-MS/MS spectrum similar to that presented in Fig. 4c), confirming their resistance to temperature and alkali reagents. A similar trend was observed by Pinto, Evtuguin, & Pascoal Neto (2005) in hardwood glucuronoxylans from *Eucalyptus globulus*, *Eucalyptus urograndis*, and *Betula pendula* after the kraft pulping process, using temperatures of 160 °C and highly concentrated solutions of sodium hydroxide and sodium sulphide. A percentage of 20% was reported to be retained in the polysaccharides, showing high resistance to hydrolysis of some polysaccharide acetyl groups to the strong alkali solutions, even at high temperatures.

4. Concluding remarks

The selective hydrolysis with *endo*-β-mannanase of roasted SCG mannans followed by the separation of the released oligosaccharides by size exclusion chromatography, and their analysis by ESI-MS/MS shows that the roasting process at 160 °C followed by 4 M NaOH extractions at 20 °C, 60 °C, and 120 °C applied to SCG improves the extractability of galactomannans without their apparent degradation, preserving their β-(1→4)-Man backbone, Gal and Ara side chains, and acetylation.

The extreme roasting process, at 220 °C, promotes the loss of a large extent of mannans. However, their structural features and even the small details like acetylation and Gal and Ara side chains are kept.

The roasting process at 160 °C complemented with extractions with NaOH at 20, 60, and 120 °C could be used to successfully extract galactomannans, valorising the SCG as a source of galactomannans. This procedure, although suitable for galactomannans, does not improve the extractability of the arabinogalactans.

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