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Sequential microwave superheated water extraction of mannans from spent coffee grounds

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ABSTRACT

The feasibility of using sequential microwave superheated water extraction (MAE) for the recovery of mannans from spent coffee grounds (SCG) was studied. Due to the high contents of mannose still present in the SCG residue left after two consecutive MAE, the unextracted material was re-suspended in water and submitted to a third microwave irradiation (MAE3) at 200 °C for 3 min. With MAE3, mannose recovery achieved 48%, increasing to 56% by MAE4, and reaching a maximum of 69% with MAE5. Glycosidic-linkage analysis showed that in MAE3 mainly galactomannans were recovered, while debranched galactomannans were recovered with MAE4 and MAE5. With increasing the number of extractions, the average degree of polymerization of the mannans decreased, as observed by size-exclusion chromatography and by methylation analysis. Scanning electron microscopy images showed a decrease on cell walls thickness. After final MAE5, the remaining un-extracted insoluble material, representing 22% of the initial SCG, was composed mainly by cellulose (84%).

1. Introduction

Coffee and coffee by-products are a good source of both arabinogalactans and galactomannans. These polysaccharides, namely the coffee type II arabinogalactans (Nosal'ova et al., 2011) and galactomannans (Simões et al., 2009) have been shown *in vitro* immunostimulatory activities. Type II arabinogalactans were also related with prebiotic effects for selective growth of *Lactobacillus* spp. (Robinson, Feirtag, & Slavin, 2001). Concerning the galactomannans, beyond their potential of innate immune system stimulation, the mannooligosaccharides (MOS) derived from these polysaccharides have also prebiotic activity for selective growth of *Bifidobacterium* spp., and *Lactobacillus* spp. (Gibson, Ottaway, & Rastall, 2000). The galactomannans have also been described to present anticoagulation and fibrinolytic activity (Hussein, Helmy, & Salem, 1998) and the MOS may prevent adherence of toxic bacteria to the intestinal wall, mediated by lectins, thus presenting anti-infectious potential (Gibson et al., 2000; Sharon & Ofek, 2000; Titapoka, Keawsompong, Haltrich, & Nitisinprasert, 2008). Furthermore, *in vivo* studies in pig and chicken have shown that both MOS and mannose reduce the incidence of *Salmonella enterica* and *Escherichia coli* (Naughton, Mikkelsen, & Jensen, 2001; Oyofo et al., 1989).

The exhaustive extraction of polysaccharides from coffee beans has been done using a sequential extraction with KOH with increasing concentrations until 8 M KOH (Fischer, Reimann, Trovato, & Redgwell, 2001). Similar sequential extractions have been proposed for the extraction of the spent coffee grounds (SCG) (Simões et al., 2009). However, due to the unextractability of these polysaccharides, alkali treatments have been combined with cellulase treatments (Iwai & Kasai, 2010) or with roasting of the SCG (Simões, Nunes, Domingues, & Coimbra, 2013). Microwave assisted extraction (MAE) has also been proved a feasible tool to extract SCG polysaccharides, with the advantage of using only water as solvent (Passos & Coimbra, 2013). With MAE, the SCG arabinogalactans were quantitatively obtained by two sequential extractions using a ratio of mass of SCG to water of 1:30 (g:mL). However, these conditions only allowed the extraction of 41% of the SCG mannose content. In order to try to improve the yield of extraction of polysaccharides, in the present work, a sequential MAE of up to 5 repeating cycles (MAE*n*) was performed. The polysaccharides obtained in each extract (MAE*n*Sn) were structurally characterized concerning the carbohydrates content, composition, linkages, and molecular size. The residues (MAE*n*R) were also monitored for their carbohydrates content and composition, and cell walls microstructure.

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2. Experimental

2.1. Samples

Spent coffee grounds (SCG) were obtained from a local cafeteria from a commercial batch of Delta Cafés Platina (Portugal), already used in a previous work (Passos & Coimbra, 2013). It contained a moisture of 61% and, on a dry weight basis, was composed by 66% carbohydrates, mainly mannose (45%), galactose (25%), glucose (24%), and arabinose (6%). The samples were stored at −20 °C previously to the analysis. All reagents used were of analytical grade or higher available purity.

2.2. Microwave irradiation

Microwave irradiation was performed with a EthosSYNTH Labstation (maximum output, 1 kW, 2.45 GHz; Milestone Inc., Shelton, CT) using a high pressure 100 mL reactor. The operating conditions were as described in Passos and Coimbra (2013) for a mass of SCG to water of 1:30 (g:mL) prepared in a total volume of approximately 80 mL and using two similar reactors standing opposite to each other. Microwave power was adjusted to attain 200 °C in 3 min, and maintain the temperature for 2 min. On the perspective of achieving the best condition for the maximization of the amount of polysaccharides obtained per batch, it has been shown that the 1:10 ratio was the best condition (Passos & Coimbra, 2013). However, in order to maximize the extraction of arabinogalactans from the initial SCG matrix, the 1:30 ratio was the optimum, leaving a residue after two sequential extractions that was only composed by galactomannans and cellulose.

After the first microwave assisted extraction (MAE1), the unextracted insoluble material was re-suspended in water and submitted to a second microwave assisted extraction (MAE2) under the same operating conditions. A total of four sequential cycles were performed. In the fifth cycle of heating (MAE5), the microwave power was adjusted to attain 230 °C (instead of 200 °C). In each new extraction, the reactors were cooled down to room temperature. All samples were centrifuged at 15,000 rpm, for 20 min, at 4 °C and the supernatant solution was filtered using MN GF-3 glass fibre filter, frozen, freeze-dried, and stored under an anhydrous atmosphere.

2.3. Sugar and glycosidic-linkage analysis

The individual neutral sugars were determined after acid hydrolysis, derivatization to alditol acetates, and analysis by GC-FID, as described by Passos and Coimbra (2013). The total sugars content was determined by the sum of the amount of the individual sugars, taking into account that the hydrolysis of a glycosidic linkage results in an addition of a water molecule into the sugar residue.

Glycosidic-linkage composition of polysaccharides was determined by methylation analysis. The partially methylated alditol acetates (PMAA) were separated and analyzed by gas chromatography–mass spectrometry (GC–MS) as described by Passos and Coimbra (2013).

2.4. Matrix-assisted laser desorption/ionization spectrometry (MALDI-MS)

MALDI mass spectra were acquired using a MALDI-TOF/TOF Applied Biosystems 4800 Proteomics Analyzer (Applied Biosystems, Framingham, MA) instrument equipped with a nitrogen laser emitting at 337 nm and operating in a reflectron mode. Full-scan mass spectra ranging from m/z 450 to 4000 were acquired in the positive mode. Dihydroxybenzoic acid (DHB) was used as matrix for MALDI-MS analysis and matrix solution and sample were prepared as described by Moreira, Coimbra, Nunes, Simões, and Domingues (2011).

Table 1
Operation parameters and chemical characterization of materials obtained after sequential MAE*n*.

	MAE*n*				
	n = 1	2	3	4	5
Temperature (°C)	200	200	200	200	230
Yield of extraction (%, w/w)	29.0	19.3	14.3	8.0	19.0
Carbohydrates content (%, w/w)					
MAE*n*Sn[a]	69.4	74.2	88.5	88.3	98.4
MAE*n*R[b]	61.4	74.3	79.3	72.4	74.2
Mw[c] (kDa)	17.4	5.8	4.0	4.4	1.7
Mn[c] (kDa)	8.7	4.5	3.3	3.7	0.8
PD[d] (M_w/M_n)	2.0	1.3	1.2	1.2	2.1

[a] Material recovered in the supernatant.
[b] Insoluble material remaining after the extraction.
[c] Weighted-average (M_w) and number-average (M_n) molecular weights.
[d] Polydispersity.

2.5. Scanning electron microscopy (SEM)

Samples were fixed on stainless steel supports and coated with gold using a JEOL metalizer (FFC-1100, Tokyo, Japan) at 1100–1200 V, 5 mA for 10 min. A scanning electron microscope (Hitachi, S4-70, Tokyo, Japan) at 15 kV was used (Nunes, Saraiva, & Coimbra, 2008).

2.6. Size exclusion chromatography (SEC)

About 4–5 mg of each soluble extract was dissolved in 500 μL of 0.1 M $NaNO_3$ aqueous solution at 20 °C during 60 min, reaching a sample concentration of ca. 1%. The obtained solution was filtered through a 0.4 μm PVDF filter. The SEC analysis was carried out using two PL aquagel-OH MIXED 8 μm 300 mm × 7.5 mm columns protected by a PL aquagel-OH Guard 8 μm pre-column on a PL-GPC 110 system (Polymer Laboratories, UK), as described by Mendes, Xavier, Evtuguin, and Lopes (2013). The columns, injector system, and the detector (RI) were maintained at 36 °C during the analysis. The eluent (0.1 M $NaNO_3$) was pumped at a flow rate of 0.9 mL/min. The columns were calibrated with pullulans (Polymer Laboratories, UK) in the range 0.7–48.0 kDa. The injected volume was 100 μL.

3. Results and discussion

3.1. Influence of MAEn on yield and sugar composition of SCG polysaccharides

The microwave assisted extraction of SCG (MAE1) yielded 29% of soluble material (MAE1Sn), of which 69.4% were sugars (Table 1). The re-extraction of the material left by the renewal of the water used as solvent (MAE2) yielded 19% of soluble material (MAE2Sn), which represented 14% of the initial SCG (Fig. 1), of which 74.2% were sugars. The sugar composition of MAE1Sn and MAE2Sn are shown in Fig. 1, which are in accordance with those previously described when the same operating conditions were used (Passos & Coimbra, 2013).

After two consecutive extractions, MAE2R still possess high sugar content (74.3%), especially Man (59 mol%) and Glc (38 mol%). To try to extract the mannans, MAE2R was re-suspended in water and submitted to a third MAE (MAE3) under the same operating conditions. MAE3 yielded 14.3% of soluble material (MAE3Sn, in Table 1, correspondent to 8% of the SCG, in Fig. 1). This extract had a sugar content of 88.5% (Table 1), and a molar composition where

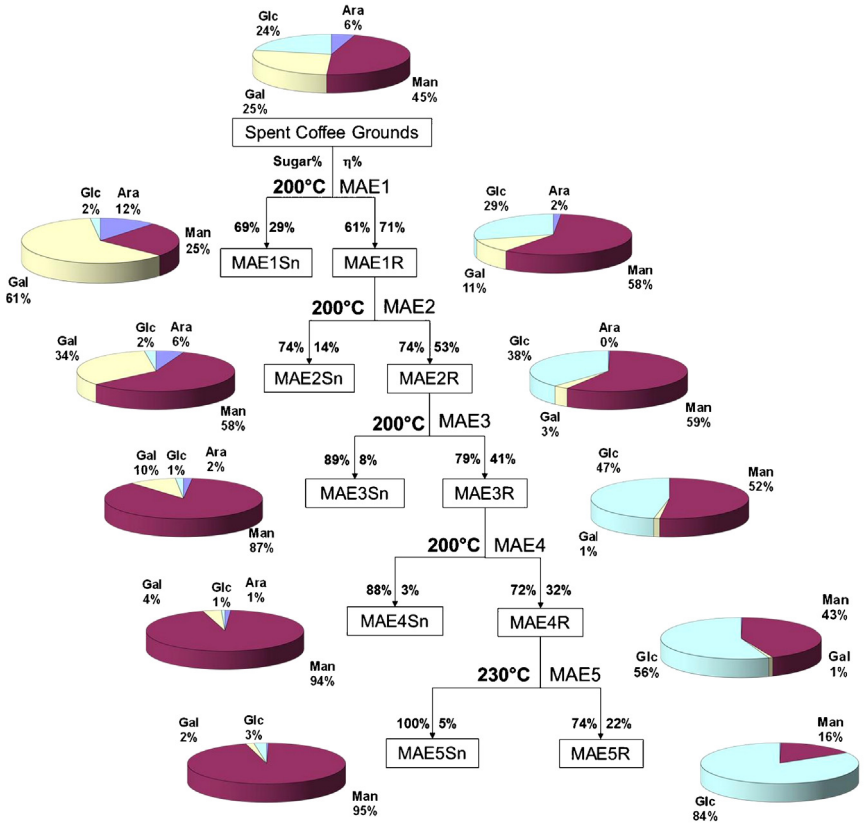

Fig. 1. Representation of the sequential MAE*n* procedure. Represented are the operating condition, with number of sequential extractions *n*, with *n* = 1–5 and temperature (°C), yield of extraction (η, %), sugar content (w, %), and molar compositions (%) for both soluble (MAE*n*Sn) and insoluble material (MAE*n*R).

Man (87 mol%) and Gal (10 mol%) were the major constituents (Fig. 1). With 3 sequential MAE, the total Man extracted accounted for 58% of SCG Man. This calculation was obtained taking into account the sum of the mol% of Man in the extracts multiplied by the percentage of sugars in the extract, and multiplied by the yield of each extraction, and dividing by the mol% of Man multiplied by the sugar content of SCG. Up to MAE3, the total Man extracted from SCG was calculated as follows: [(0.25 Man*0.69 sugars*0.29 yield of MAE1Sn + 0.58 Man*0.74 sugars*0.14 yield of MAE2Sn + 0.87 Man*0.89 sugars*0.08 yield of MAE3Sn)/0.45 Man*0.66 sugars of SCG]. The un-extracted material (MAE3R), accounting for 41% of the initial SCG, had a sugar content of 79% and was still rich in Man (52 mol%) (Fig. 1). In order to try to recover these mannans, MAE3R was re-suspended in water and submitted to a fourth MAE under the same operating conditions. The yield of extraction was 8% for MAE4 (Table 1), corresponding to 3% of the initial SCG mass. With MAE4, the Man extraction increased to 66% of total SCG Man.

As the MAE conditions used did not allow the total Man recovery from SCG, MAE4R was re-suspended in water and submitted to a fifth MAE, where the temperature of extraction was increased to 230 °C. With MAE5, the yield of extraction was 19%, allowing calculating from Fig. 1 data a total yield of 82% of SCG Man extracted. A high purity Man-rich fraction (95 mol%) (Table 1) was recovered in MAE5Sn. Still, about 22% of the SCG weight remained as insoluble material in MAE5R, of which 74% were sugars (16 mol% Man and 84 mol% Glc, Fig. 1). This residue represented a cellulosic-rich material corresponding to 13.7% of the initial SCG.

3.2. Molecular weight distribution

The molecular weights (M_w and M_n) and polydispersity (PD) of polysaccharides in MAE*n*Sn extracts were assessed by size exclusion chromatography (SEC) (Table 1). The highest M_w (17.4 kDa) was observed for MAE1Sn. For MAE2Sn the M_w was 5.8 kDa, while polysaccharides of MAE3Sn and MAE4Sn revealed M_w of 4.0 and 4.4 kDa, respectively. For MAE5Sn, possessing the molecular weight of 1.7 kDa, the M_w value was the lowest of the obtained extracts (Table 1). Hence, the molecular weight of the polysaccharides was decreased after each consecutive MAE*n*, although their polydispersities (Table 1) were rather significant, especially for MAE1Sn and MAE5Sn, indicating the structural heterogeneity of dissolved polysaccharides. With the smallest M_w value obtained in MAE5 (1.7 kDa), short-chain polysaccharides were obtained.

3.3. Linkage-composition of MAEnSn carbohydrates

The results of the methylation analysis to the polysaccharides recovered in MAE*n*Sn extracts are shown in Table 2. The presence of (1 → 3)-Gal and (1 → 3,6)-Gal residues are diagnostic of arabinogalactans (AG). Meanwhile, the presence of (1 → 4)-Man, (1 → 4,6)-Man, and terminally linked Man residues are diagnostic of galactomannans (GM). To estimate the amount of AG and GM from the glycosidic composition, it was taken into account that Man residues are components of GM and that an amount of Gal residues equal to the amount of (1 → 4,6)-Man are also a component of GM. Furthermore, all Ara and Gal residues (except the terminally linked Gal attributed to the GM) were assumed to be components of the AG (Nunes & Coimbra, 2002, 2007). A small amount of the (1 → 4)-Glc and the terminally linked Glc contents could be assigned respectively to GM (Nunes, Domingues, & Coimbra, 2005) and AG (Fischer et al., 2001).

Based on the sugar analysis and the glycosidic-linkage composition (Table 2), it can be inferred that MAE1 allowed to obtain an

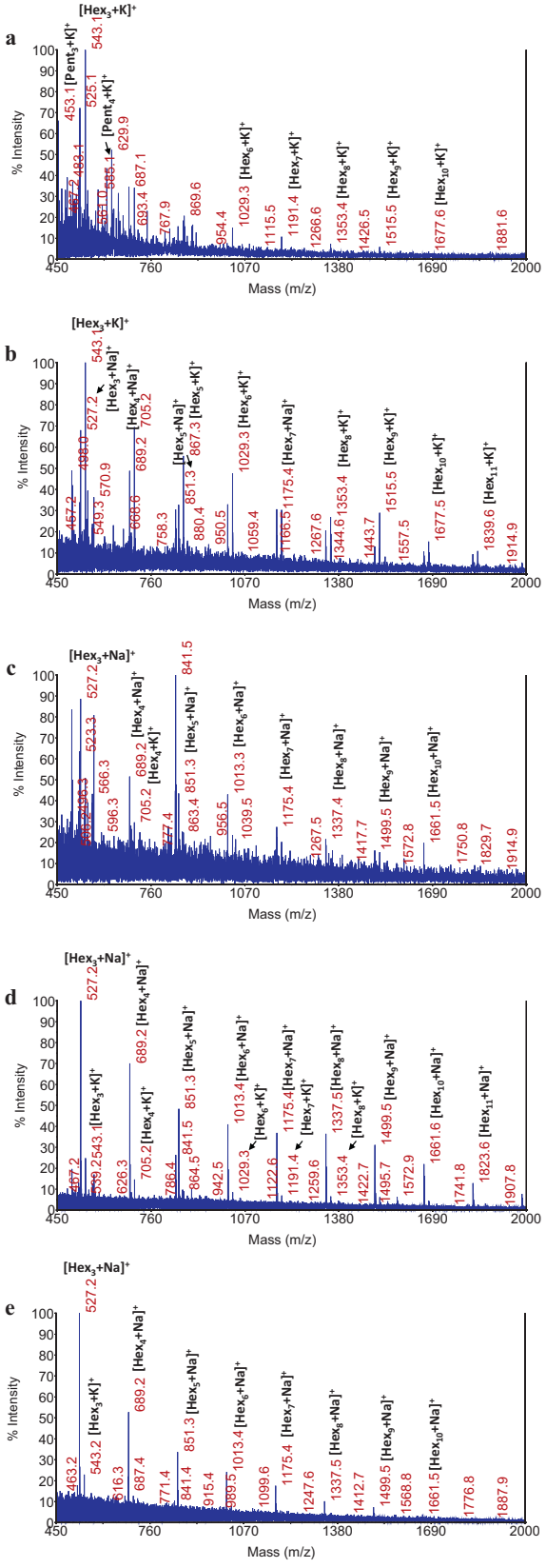

Fig. 2. MALDI-MS spectra acquired for the supernatants (MAE*n*Sn) recovered from MAE*n* (*n* = 1–5) (a–e).

Table 2
Glycosidic-linkage composition (mol%) of carbohydrates extracted during MAE*n*.

Linkage (%)	MAE				
	1	2	3	4	5
T-Ara*f*	2.9	0.5	0.5	0.0	0.0
5-Ara*f*	2.0	0.3	1.0	0.0	0.0
Total Ara	4.9 (12)	0.8 (6)	1.5 (2)	0.0 (1)	0.0 (0)
T-Man*p*	2.3	9.4	12.5	14.9	23.1
6-Man*p*	0.0	0.9	1.4	0.0	0.0
4-Man*p*	24.6	49.0	62.3	72.9	60.0
4,6-Man*p*	2.1	4.6	6.3	2.3	1.1
Total Man	29.0 (25)	63.9 (58)	82.5 (87)	90.1 (93)	84.2 (95)
T-Gal*p*	11.2	7.4	2.8	1.2	3.8
6-Gal*p*	11.8	5.3	1.9	0.0	0.0
3-Gal*p*	23.9	9.6	2.5	0.9	0.4
3,6-Gal*p*	16.2	7.1	1.9	0.0	0.0
Total Gal	63.1 (61)	29.4 (34)	9.1 (10)	2.1 (4)	4.2 (2)
T-Glc*p*	0.6	1.1	0.4	1.3	5.2
4-Glc*p*	2.4	4.8	6.4	6.5	6.5
Total Glc	3.0 (2)	5.9 (2)	6.8 (2)	7.8 (1)	11.7 (3)
AG (%)[a]	66	26	4	0	0
GM (%)[b]	31	69	89	92	88
Cellulose[c]	3	6	7	8	12

[a] Arabinogalactans derived material.
[b] Galactomannans derived material.
[c] Cellulose derived material.

extract (MAE1Sn) of water soluble polysaccharides composed of 2/3 AG and 1/3 GM and MAE2 an extract with approximately 1/3 AG and 2/3 GM. These results are in agreement with those previously reported by Passos and Coimbra (2013). An extract rich in (1 → 4)-Man (62.3%), terminally linked Man (12.5%), and (1 → 4,6)-Man (6.3%) was obtained by MAE3, indicating that only GM were recovered. The molecules recovered in both MAE4Sn and MAE5Sn extracts were composed by 2.3 and 1.1% of (1 → 4,6)-Man and terminally linked Gal were 1.2 and 3.8%, respectively, allowing to infer the presence of debranched GM.

The terminally linked Man content increased from 2.3% in MAE1Sn to 23.1% in MAE5Sn. Such tendency is consistent with the results given by SEC analysis that predicted a decreasing of the molecular weight of the polysaccharides solubilized by each new MAE*n* (Table 1). A further observation is the high content of (1 → 4)-Glc and terminally linked Glc recovered throughout MAE*n*, especially with increasing of *n*. From the 3.0 mol% of (1 → 4)-Glc + terminally linked Glc recovered in MAE1Sn, a maximum of 11.7% was quantified in MAE5Sn (Table 2). These residues may be hydrolysis products derived from SGC cellulose.

Table 3
Identification of sodium ([M+Na]$^+$) and potassium ([M+K]$^+$) adducts identified in the MALDI-MS spectra of the MAE*n*Sn material.

Compound [M]	*m/z*	
	[M+Na]$^+$	[M+K]$^+$
Hex_3	527	543
Hex_4	689	705
Hex_5	851	867
Hex_6	1013	1029
Hex_7	1175	1191
Hex_8	1337	1353
Hex_9	1499	1515
Hex_{10}	1661	1677
Hex_{11}	1823	1839
$Pent_3$	–	453
$Pent_4$	569	585

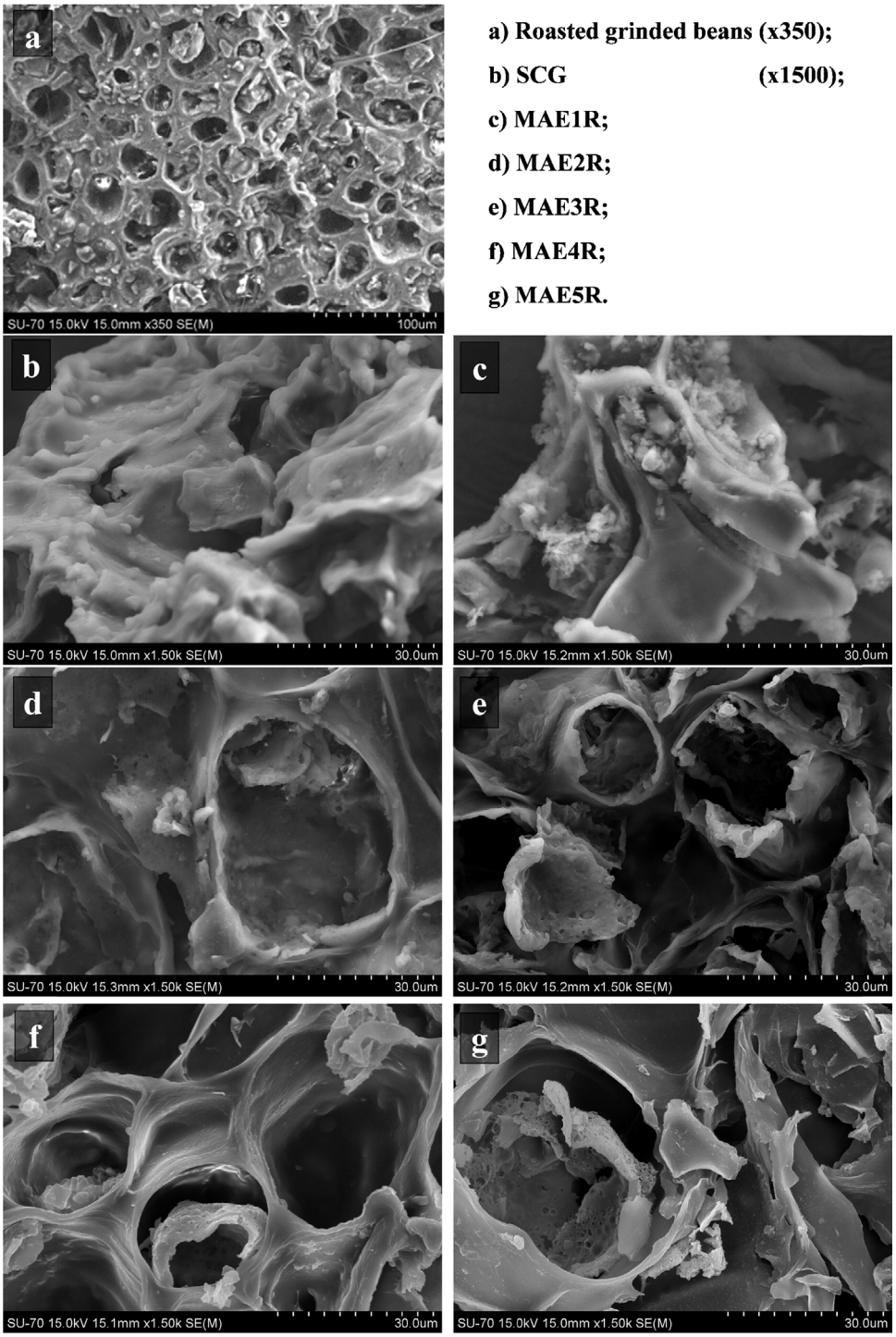

Fig. 3. SEM images: (a) Coffee grinded bean (before espresso preparation); (b) SCG (after espresso preparation); (c) MAE1R; (d) MAE2R; (e) MAE3R; (f) MAE4R; (g) MAE5R.

3.4. MALDI-MS analysis of the MAEnSn carbohydrates

MALDI-MS analyses were performed in each MAE*n*Sn conducted to gain information of it carbohydrate composition. Fig. 2 shows the MALDI-MS spectra of the 5 fractions studied that allowed the identification of several ions of oligosaccharides in the range between *m*/*z* 450 and 2000. Although these extracts are rich in polysaccharides, using this methodology, only GM- and AG-derived oligosaccharides can be detected. The oligosaccharides were identified as sodium $[M+Na]^+$ and potassium $[M+K]^+$ adducts (Table 3 and Fig. 2) belonging to series of Hex_n ($n = 3–11$) and $Pent_n$ ($n = 3–4$), where the Hex can derive from both mannosyl and galactosyl residues, and the Pent can derive from arabinosyl oligosaccharides (Table 2). Arabinosyl oligosaccharides were identified predominantly in MAE1Sn, while mannnosyl oligosaccharides were abundant in all fractions, which are in accordance with the results of the glycosidic-linkage analyses.

The MALDI-MS spectra of samples from MAE1Sn and MAE2Sn (Fig. 2a,b) showed the potassium adducts ($[M+K]^+$) with major relative abundances than the sodium adducts ($[M+Na]^+$). MALDI-MS

spectra of neutral oligosaccharides usually show $[M+Na]^+$ adducts in much higher abundance than the $[M+K]^+$ (Simões, Nunes, Domingues, & Coimbra, 2010, 2011). In the present study, the higher abundance of potassium adducts in MAE1Sn and MAE2Sn is a consequence of the high abundance of potassium ions in coffee beans and brews (Gillies & Birkbeck, 1983), as these samples were directly analyzed without any purification. These results show that the extracts obtained by MAE from SCG are a rich source of potassium.

3.5. SEM analysis of the MAEnR samples

Fig. 3a shows a SEM image of the ground coffee bean before espresso preparation. The initial ground coffee shows thick cell walls (light surfaces) when compared to the diameter of cell lumen (dark circular shape structures) under the magnification of 350×. The thickness of the cell walls remained high upon espresso coffee preparation (Fig. 3b), even in MAE1R (Fig. 3c). Such result is consistent with the extraction of AG, which are primarily components of the intercellular spaces (Fincher, Stone, & Clarke, 1983). However, after MAE2 (Fig. 3d), the cell walls become thinner after each consecutive extraction, as observed in MAE3R (Fig. 3e), MAE4R (Fig. 3f), and MAE5R (Fig. 3g). These observations are in agreement with the extraction extent of GM. The progressive damage of cell walls depicted in Fig. 3 with the increase of MAE*n* number corroborates with the microstructure changes that have been previously reported by Chen, Liu, Jiang, and Zeng (2005) in MAE of polysaccharides from *Solanum nigrum*. These observations are in agreement with the results on methylation analysis and the proposed cellulose degradation with partial solubilization of cellulose-derived oligosaccharides together with GM.

4. Concluding remarks

This work showed the effectiveness in the implementation of a sequential microwave assisted extraction (MAE*n*) procedure to obtain a series of galactomannans, which due to their unique structural differences, from high to low molecular weights, highly branched to complete de-branched structures, and different Man/Gal ratios, can be further used in different applications. Based on neutral sugars and linkage analyses, supported by SEC and MALDI-MS results, it was suggested that AG are recovered mainly in MAE1Sn, whereas GM are recovered in MAE2Sn and MAE3Sn. Mannans are recovered in the following MAE4Sn and MAE5Sn fractions, leaving an insoluble cellulose-rich residue.

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