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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