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Microwave superheated water extraction of polysaccharides from spent coffee grounds

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ABSTRACT

The spent coffee grounds (SCG) are a food industry by-product that can be used as a rich source of polysaccharides. In the present work, the feasibility of microwave superheated water extraction of polysaccharides from SCG was studied. Different ratios of mass of SCG to water, from 1:30 to 1:5 (g:mL) were used for a total volume of 80 mL. Although the amount of material extracted/batch (MAE1) increased with the increase of the concentration of the sample, the amount of polysaccharides achieved a maximum of 0.57 g/batch for 1:10. Glycosidic-linkage composition showed that all extraction conditions allowed to obtain mainly arabinogalactans. When the unextracted insoluble material was re-extracted under the same conditions (MAE2), a further extraction of polysaccharides was observed (0.34 g/batch for 1:10), mainly galactomannans. Also, a high amount of oligosaccharides, mainly derived from galactomannans, can be obtained in MAE2 (0.96 g/batch for 1:10). This technology allows to obtain galactomannans and arabinogalactans in proportions that are dependent on the operating conditions.

1. Introduction

The coffee bean is a rich source of polysaccharides, namely galactomannans, type II arabinogalactans, and cellulose (Bradbury and Halliday, 1990). Coffee galactomannans are high molecular weight low branched polysaccharides composed by a backbone of β-(1 → 4)-linked mannose residues, branched at O-6 by single α-(1 → 6)-linked galactose and single (1 → 5)-linked arabinose residues (Nunes, Domingues, & Coimbra, 2005). As low branched highly polymerized polysaccharides, the coffee galactomannans tend to be water insoluble (Simões, Nunes, Domingues, & Coimbra, 2010). Coffee type II arabinogalactans are high molecular weight highly branched polysaccharides composed by a backbone of β-(1 → 3)-linked galactose residues, branched at O-6 by β-(1 → 6)-linked galactose and/or α-(1 → 5)-linked arabinose residues (Nunes, Reis, Silva, Domingues, & Coimbra, 2008). The side chains contribute to the hydrophilic behaviour of the polymer increasing their solubility in water. Coffee galactomannans and arabinogalactans, among other properties, have been shown *in vitro* immunostimulatory activities (Nosal'ova et al., 2011; Simões et al., 2009).

Upon coffee roasting, during the beverage preparation, using hot pressurized water, galactomannans and type II arabinogalactans, are extracted to the brew (Nunes & Coimbra, 2001). However, the majority of these polysaccharides remain as insoluble material bounded to the SCG matrix (Mussatto, Carneiro, Silva, Roberto, & Teixeira, 2011; Simões et al., 2009). Coffee bean polysaccharides have been obtained by exhaustive sequential extractions, including KOH aqueous solutions with increasing concentrations, allowing the dissolution of polysaccharides associated by hydrogen bonds (Fischer, Reimann, Trovato, & Redgwell, 2001). However, the amount of polysaccharides recovered by these methods was very small. As an alternative, microwave irradiation is becoming a feasible method to extract polysaccharides using only hot compressed water (Adam, 2003) or dilute aqueous solutions (Benko et al., 2007). This has been reported to allow milder reaction conditions, low production costs, formation of cleaner products with higher yields, and minor wastes when compared with the use of alkali reagents (Wang & Weller, 2006; Yu, Lou, & Wu, 2007).

Temperature is one of the most important factors contributing to the recovery yield when using microwave assisted extraction (MAE). Generally, the higher temperature applied, the higher recovery yield is obtained. However, high temperatures may cause degradation of products (Wang & Weller, 2006), dependent of the structure of the polysaccharides (Ando et al., 2000; Tsubaki, Iida, Sakamoto, & Azuma, 2008; Yu et al., 2007). Furthermore, the solid/solvent ratio is also an important parameter when performing MAE, yielding higher recoveries when using more diluted conditions (Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011). The use of diluted conditions may be however, economically disadvantageous, increasing the costs of the process for the recovery of the polysaccharides from the liquid phase.

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The aim of this work was to study the feasibility of microwave superheated water extraction of polysaccharides from SCG. This was based on the amount of carbohydrates extracted for different ratios of mass of SCG to water in two consecutive microwave extractions. Furthermore, the structural features of the galactomannans and arabinogalactans recovered from SCG were assessed.

2. Experimental

2.1. 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 (HPR). The EthosSYNTH Labstation is a multimode microwave oven in which the real-time temperature inside the reactor is monitored with a thermometer. Heating temperature is controlled precisely with a PID (Proportional, Integral, Derivative) algorithm by changing the power of microwave irradiation. The suspension in the reactor is continuously stirred with a magnetic bar that minimizes the heterogeneous microwave heating. The reactor is made of polytetrafluoroethylene (PTFE) containing <1% perfluoropropyl vinyl ether (PPVE) modifier that can endure temperatures up to 250 °C and pressures up to 55 bar. Microwave energy is transmitted through the reactor and directly heats the compounds inside.

Each experiment was conducted in two similar reactors standing opposite to each other. Suspensions containing the proportion of 1:30, 1:20, 1:12, 1:10, 1:7, and 1:5 of spent coffee grounds (SCG) (dry weight, g) and water (mL) were prepared in a total volume of approximately 80 mL. Microwave power was adjusted to attain 200 °C in 3 min, and maintain the temperature for 2 min. Due to security measures the equipment was programmed to stop irradiating whenever the temperature overcame the one displayed and/or when pressure achieved 40 bar. 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.

2.2. Samples and general procedures

Espresso SCG were obtained in a local cafeteria from a commercial batch of Delta Cafés Platina (Portugal). The SCG samples were stored at −20 °C previously to the analysis. All reagents used were of analytical grade or higher available purity. The percentage of water content was determined in triplicate according to the ISO/DIS 11294-1993, by the method of oven drying at 105 °C during 4 h (Illy & Viani, 1995). Fat content was determined by Soxhlet using 30 g of dried SCG and 150 mL of *n*-hexane (Passos, Silva, Da Silva, Coimbra, & Silva, 2010) and total protein content was determined by GC-FID by the sum of the amount of the individual amino acids obtained after acid hydrolysis and derivatization to the heptafluorobutyrate derivatives (Coimbra, Nunes, Cunha, & Guiné, 2011). To determine the solubility of the extracts in water, an aliquot of 1 g of sample was dissolved in the minimum water possible until the persistent appearance of material in suspension during 10 min.

2.3. Fractionation by graded ethanol precipitation

Polysaccharides were recovered by ethanol precipitation. Each extract was dissolved in the minimum amount of water, stirring during 10 min at room temperature and then absolute ethanol was added to reach an aqueous solution containing 75% ethanol (v/v). The solution was then centrifuged at 15 000 rpm for 10 min at 4 °C. The supernatant (EtSN) and the residue (Et75) obtained were further dissolved in water, rotary evaporated at 40 °C to completely remove the ethanol, and then were frozen, and freeze dried.

2.4. Sugar and glycosidic-linkage analysis

Neutral sugars were determined by gas chromatography (GC) as alditol acetates. 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 structure. The polysaccharides were treated with 12 M H_2SO_4 during 3 h at room temperature with occasional stirring followed by hydrolysis for 1 h with 2 M sulfuric acid at 120 °C. Monosaccharides were reduced with $NaBH_4$ (15% in NH_3 3 M) during 1 h at 30 °C and subsequently 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 analyzed by GC with a FID detector (Perkin Elmer–Clarus 400) 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 programme 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 maintaining 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.

To determine the composition and the amount of monosaccharides, the samples were not submitted to the acid hydrolysis step prior to the derivatization to alditol acetates.

Glycosidic-linkage composition of polysaccharides was determined by methylation analysis (Coimbra et al., 1996; Nunes & Coimbra, 2001). The samples (1–2 mg) were dissolved in 1 mL of anhydrous dimethylsulfoxide (DMSO), then powdered NaOH (40 mg) were added under an argon atmosphere. The samples were methylated with CH_3I (80 μL) during 20 min with stirring, following by a second addition of 80 μL CH_3I and stirring for another 20 min. $CHCl_3$/MeOH (1:1, v/v, 3 mL) was added, and the solution was dialyzed (membrane with a pore diameter of 12–14 kDa) against 3 lots of 50% EtOH. The dialysate was evaporated to dryness and the material was remethylated using the same procedure. The remethylated material was hydrolyzed with 2 M TFA (1 mL) at 120 °C for 1 h (Harris, Henry, Blakeney, & Stone, 1984), and then reduced and acetylated as previously described for neutral sugar analysis (using $NaBD_4$ instead of $NaBH_4$). The partially methylated alditol acetates (PMAA) were separated and analyzed by gas chromatography–mass spectrometry (GC–MS) (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 6 min), with the injector operating at 220 °C, and using the following temperature programme: 50 °C with a linear increase of 8 °C/min up to 140 °C, and standing for 5 min at this temperature, followed by a linear increase of 0.5 °C/min up to 150 °C, followed by a linear increase of 40 °C/min up to 280 °C, with further 1 min at 280 °C. The helium carrier gas had a flow rate of 1.7 mL/min and a column head pressure of 14.4 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.

Table 1
Chemical characterization of spent coffee grounds (SCG) and unextracted material left after MAE1.

	SCG	Unextracted material left after MAE1					
		Mass of SCG (g): volume of water (mL)					
		1:5	1:7	1:10	1:12	1:20	1:30
Moisture (%)	60.76 ± 0.04[a]						
Fat[b] (%)	12.95 ± 0.04						
Protein[b] (%)	4.9 ± 0.6						
Carbohydrate[b] (%)	65.9 ± 6.5	54.7	60.3	54.8	58.3	61.9	60.8
Sugars composition							
Ara (mol%)	6	3	3	3	3	2	3
Man (mol%)	45	49	50	46	53	54	55
Gal (mol%)	25	21	17	20	14	11	12
Glc (mol%)	24	27	30	31	30	33	30

[a] Average ± standard deviation.
[b] Dry basis.

3. Results and discussion

3.1. Chemical characterization of spent coffee grounds (SCG)

The chemical composition of the SCG used as starting material is presented in Table 1. The moisture accounted for 61% of the SCG. On a dry weight basis, this material was composed by 66% of sugars. This value was obtained using 2 M H_2SO_4 for 1 h at 120 °C, a stronger acid hydrolysis than the 1 M H_2SO_4 for 2.5 h at 100 °C performed by Simões et al. (2009), recovering 35% of sugars, and the 0.5 M H_2SO_4 for 0.75 h at 121 °C performed by Mussatto et al. (2011), recovering 45.3%. The SCG was composed also by 13% of fat and 5% of proteins. Although the fat composition are in accordance with literature for SCG obtained after beverage preparation (Cruz et al., 2012), the protein content was lower than the 15% and 13.6%, reported by Cruz et al. (2012) and Mussatto et al. (2011), respectively, possibly because different methods were used. According to literature, the SCG obtained after beverage preparation contains much more unextractable material than those obtained from the industrial spent coffee (Cruz et al., 2012), which are richer in polysaccharides (data not shown). The remaining 16% were probably melanoidins, e.g. the brown compounds fraction formed upon the roasting of the coffee beans (Nunes & Coimbra, 2007), whose chemical composition has not yet been established (Nunes & Coimbra, 2010). The carbohydrate material was mainly mannose (45%), galactose (25%), glucose (24%), and arabinose (6%). Uronic acids occurred only in trace amounts (data not shown). Based on previous knowledge of coffee polysaccharides (Nunes & Coimbra, 2001, 2002; Nunes, Reis, Domingues, & Coimbra, 2006), it can be inferred that galactomannans accounted for near 50% of total SCG polysaccharides. The arabinogalactans and cellulose, in equal proportions, accounted for the other 50%. In order to extract the non-cellulosic polysaccharides using only water as solvent, microwave assisted extraction (MAE) was used varying the proportion of the mass of SCG to the volume of water used as solvent. For each experimental condition the extraction yield and the chemical characteristics of galactomannans and arabinogalactans obtained in the aqueous extracts were evaluated.

3.2. Influence of the proportion of SCG to the volume of water (r:w) on the extraction yield and carbohydrate composition

When 3.7 g of dried SCG were mixed with 65 mL of water and submitted to a microwave irradiation power of 900 W, under stirring, in a closed vessel of 100 mL, it was observed that after 3 min, the temperature of 200 °C was reached. This temperature was kept during additional 2 min, in a total of 5 min of MAE. Under these conditions, the pressure inside the vessel achieved 16 bar, which is slightly above the saturated vapour pressure of water at 200 °C (Wagner & Pruss, 1993). The ratio of 1 g of dried SCG to 20 mL of water (1:20), allowed a yield of extraction of 23.4% of water soluble material (0.87 g/batch), of which 64.9% were sugars (Table 2). The content of monosaccharides was 2.6% of the total mass of the extract, corresponding to 5.3% of total sugars extracted. The addition of 75% ethanol allowed to recover 70.9% of this material as a precipitate (Et75). Based on the yield of the fraction and its sugar content (69.9%), it could be estimated that the amount of polysaccharides extracted under these microwave conditions accounted for 87.3% of total sugars extracted, representing 0.49 g of polysaccharides/batch (Fig. 1a). The carbohydrates that did not precipitate in 75% ethanol solution, remaining in the supernatant fraction (EtSN), can be accounted as short chain polysaccharides, oligosaccharides, and monosaccharides(Nunes & Coimbra, 2001). Taking into account the amount of monosaccharides in this extract, the amount of oligomeric material (including

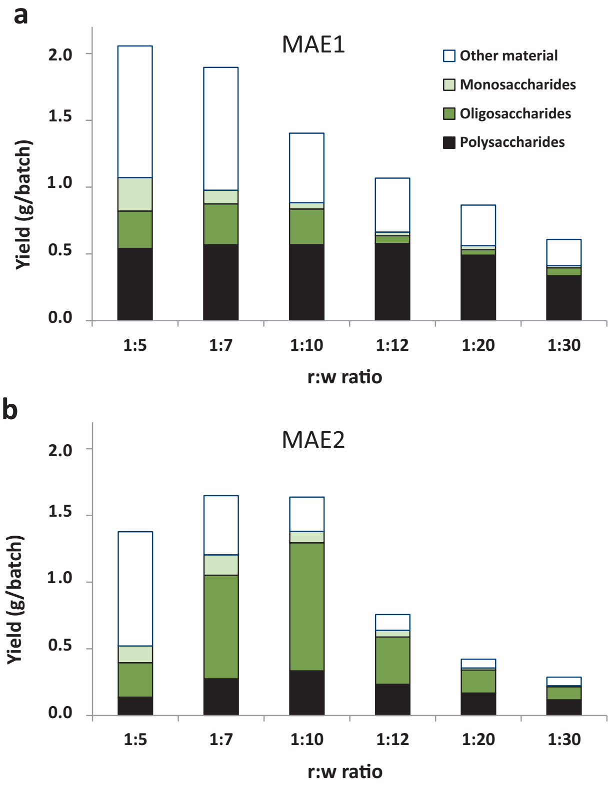

Fig. 1. Amount of water-soluble material recovered per batch for the different proportions of the mass of SCG (g) to the volume of water (mL) by (a) MAE1; (b) MAE2.

Table 2
Operational parameters and chemical characterization of MAE material.

Mass of SCG (g): volume of water (mL)	Sample					
	1:5	1:7	1:10	1:12	1:20	1:30
Temperature (°C)	170	180	200	200	200	200
Pressure (bar)	40	40	40	24	16	15
Material extracted (g/batch of 80 mL)	2.06	1.90	1.40	1.07	0.87	0.61
Yield of extraction (%, w/w)	14.8	20.4	19.5	19.4	23.4	29.0
Carbohydrates extracted (%, w/w)	52.1	51.5	62.9	62.1	64.9	67.8
Monosaccharides in extract (%, w/w)	11.8	5.7	3.1	2.5	2.6	2.2
Ara (mol%)	18	34	46	59	69	81
Man (mol%)	44	29	25	14	10	5
Gal (mol%)	26	21	20	20	16	9
Glc (mol%)	12	15	8	6	4	4
Yield of Et75 fraction (%, w/w)[a]	45.8	50.0	54.8	73.3	70.9	65.2
Et75 sugar content (%, w/w)	58.1	62.8	73.9	78.3	69.9	72.0
Ara (mol%)	6	6	6	5	6	5
Man (mol%)	36	25	33	38	28	29
Gal (mol%)	55	67	60	55	64	64
Glc (mol%)	3	2	1	2	1	1
Yield of EtSN fraction (%, w/w)[b]	54.2	50.0	45.2	26.7	29.1	34.8
EtSN sugar content (%, w/w)	46.1	45.0	45.8	28.4	21.4	28.7
Ara (mol%)	7	7	27	31	47	44
Man (mol%)	38	38	31	34	17	22
Gal (mol%)	51	51	34	30	27	27
Glc (mol%)	4	4	7	5	6	4
Solubility (g/mL of water)	1.00	0.50	0.33	0.33	0.20	0.20

[a] Material that precipitated in 75% ethanol (v/v).
[b] Material that remained soluble in 75% ethanol (v/v).

short chain polysaccharides) can be estimated as 7.4% of total sugars. The 1:20 material was completely solubilized when 0.2 g were dissolved in 1.0 mL of water. This solubility is comparable to that of lactose (Machado, Coutinho, & Macedo, 2000) and three times lower than that of sucrose (Cardoso, Carvalho, & Sabadini, 2012).

The yield of extraction of the water soluble material, when the ratio of dried SCG to the volume of water was 1:30 (g:mL), was 29.0% (0.61 g/batch, Table 2). Comparing to the 1:20 ratio, it was possible to observe an increase on the yield of extraction with a simultaneous decrease in the amount of material extracted/batch. The content of monosaccharides was 2.2% of the total mass of the extract, corresponding to 4.1% of total sugars extracted. The amount of polysaccharides extracted accounted for 81.2% of total sugars, which corresponded to 0.34 g/batch (Fig. 1a) and the amount of oligosaccharides can be estimated as 14.7% of total sugars (Table 2). Compared with 1:20, the amount of polysaccharides extracted/batch using a 1:30 ratio decreased 32%. Such result allows to conclude that although more efficient than the 1:20, under the 1:30 ratio, the amount of polysaccharides recovered was much lower.

Using a mass of SCG to the volume of solvent of 1:12 and 1:10 allowed yields of extraction of 19.4 and 19.5%, respectively, resulting in 1.07 and 1.40 g/batch (Table 2 and Fig. 1a). Although the yields of extraction were 17% lower than 1:20, the amount/batch was 23 and 62% higher, respectively, with a similar lower content of monosaccharides (4.2% and 5.3% of the total sugars, respectively). The material in Et75 fractions accounted for 73.3% and 54.8%, presenting very high sugar content (Table 2), with a relative percentage of polysaccharides in total carbohydrates of 87.2% and 64.6% for 1:12 and 1:10, respectively, corresponding to 0.57 g/batch for both conditions (Fig. 1a). The carbohydrates in EtSN fraction were 28.4% in 1:12, showing values comparable to the 1:20 condition. However, for the 1:10 condition, much more carbohydrates (45.8%) were recovered in the EtSN fraction. The amount of oligosaccharides was 8.6% and 30.2% of total sugars for 1:12 and 1:10, respectively. Although the 1:12 and 1:10 conditions present only a small difference on the amount of material and solvent used, a high difference in the vessel pressure was observed between these two conditions (24 bar for 1:12 and 40 bar for 1:10, Table 2). This pressure increase should explain the observed decrease in the relative content of polysaccharides and the increase of both the relative content of oligosaccharides and monosaccharides for the 1:10 ratio, which is diagnostic of depolymerisation. The increase in pressure observed for these two conditions when compared with 1:20 and 1:30, where the pressure corresponded to the saturated vapour pressure of water at 200 °C (15 bar), showed the contribution for the increase of the pressure of compounds other than water. After all MAE treatment conditions a pH decrease of two orders of magnitude, possibly due to the hydrolysis of chlorogenic and acetyl esters, has been observed, which may explain the higher extent of depolymerisation of the coffee polysaccharides. The material extracted in both conditions was completely solubilized when 0.33 g were dissolved in 1.0 mL of water, showing higher solubility than 1:20, confirming the presence of higher relative content of depolymerised carbohydrates. Using these two conditions it was possible to increase the amount of polysaccharides extracted per batch in 16% when compared with the 1:20 ratio. The 1:12 allowed to maximize the amount of polysaccharides extracted from the SCG. However, the 1:10 ratio resulted in a further recovery of 0.27 g/batch of oligosaccharides (Fig. 1a). These results show that depending on the aim of the extraction, different conditions should be used.

Using 1:7 and 1:5 ratios of mass of SCG to the volume of water the yields of extraction were 20.4% and 14.8% (Table 2) which was lower than those previously reported when more diluted solutions were used. Nevertheless, for the same batch, higher amount of material could be solubilized (1.90 and 2.06 g/batch, respectively, Fig. 1a). For these two conditions, the content of monosaccharides was much higher than for the previous conditions (10.5% and 23.4% of the total sugars extracted). The relative percentage of polysaccharides in total carbohydrates was 58.2% and 50.5% for 1:7 and 1:5, respectively (0.57 and 0.54 g/batch, Fig. 1a). These results show that the increase of the amount of SCG in relation to the volume of water did not result in a higher recovery of the polysaccharide content but only of the monosaccharides. The material extracted in 1:7 and 1:5 ratios was completely solubilized when, respectively, 0.50 and 1.0 g were dissolved in 1.0 mL of water, approaching the solubility of sucrose (Cardoso et al., 2012).

Table 3
Glycosidic-linkage composition (mol%) of the carbohydrates extracted using MAE.

Linkage (%)	Mass of SCG (g): volume of water (mL)					
	1:5	1:7	1:10	1:12	1:20	1:30
T-Ara*f*	2.8	3.2	1.8	1.8	2.3	1.9
5-Ara*f*	0.0	0.3	0.8	0.5	0.2	0.4
total Ara	2.8(9)	3.5(7)	2.6(10)	2.3(8)	2.5(11)	2.3 (9)
T-Man*p*	3.3	3.3	3.3	3.6	2.7	2.6
6-Man*p*	0.0	0.0	0.2	0.1	0.0	0.0
4-Man*p*	29.5	23.9	31.9	32.9	32.6	29.0
4,6-Man*p*	0.8	1.0	2.5	1.9	0.9	1.0
total Man	33.6(38)	28.2(38)	37.9(40)	38.5(36)	36.2(26)	32.6(28)
T-Gal*p*	16.9	17.8	11.9	14.2	14.8	15.2
6-Gal*p*	5.6	6.6	7.1	6.0	4.0	5.4
3-Gal*p*	27.5	30.0	23.1	26.6	30.3	33.7
3,6-Gal*p*	10.7	12.6	15.3	11.3	9.8	10.2
total Gal	60.7(50)	67.7(52)	57.4(48)	58.1(54)	58.9(61)	64.5(61)
T-Glc*p*	0.1	0.1	0.1	0.1	0.1	0.1
1,4-Glc*p*	0.0	1.2	2.1	1.1	2.1	0.7
total Glc	0.1(3)	1.3(3)	2.2(2)	1.2(2)	2.1(2)	0.8(2)
AG (%)[a]	66	71	60	60	63	66
GM (%)[b]	34	29	40	40	37	34

[a] Arabinogalactans.
[b] Galactomannans

Lower *r*:*w* ratios, namely (1:3) and (1:2), resulted in an overpressure of the reactor (pressure above 55 bar), not allowing its use for safety reasons.

For all conditions, the carbohydrates recovered in the Et75 were mainly composed by galactose (55–67 mol%, Table 2), which allowed to infer the presence in high amounts of arabinogalactans. The second highest content was due to mannose (25–38 mol%), which allowed to infer the presence in lower amounts of galactomannans. For the more diluted conditions (1:30, 1:20), the carbohydrates recovered in the EtSN were mainly composed by arabinose (44–47 mol%) and galactose (27 mol%) (Table 2). This sugar composition, allowed to infer the presence in high amounts of arabinogalactan derived sugars together with arabinose monosaccharides. When using the 1:12 and 1:10 conditions, the carbohydrates recovered in the EtSN were composed by similar proportions of mannose (31–34 mol%), galactose (30–34 mol%), and arabinose (27–31 mol%), which allowed to infer the presence of both arabinogalactan and galactomanan derived sugars, although the major part of the arabinose occur as monosaccharides. For the more concentrated conditions (1:7, 1:5), the carbohydrates recovered in EtSN were mainly composed by galactose (51 mol%), which allowed to infer the presence of arabinogalactan derived short chain polysaccharides or oligosaccharides, together with a higher content of mannose in the monosaccharide form (Table 2).

The sugar analysis of unextracted insoluble material left after MAE showed that the relative content of galactose and arabinose decreased when compared with the initial SCG, whereas an enrichment in mannose and glucose was observed (Table 1). These results are in accordance with the primary extraction of arabinogalactans other than galactomannans by the MAE performed, leaving a residue richer in galactomannans and cellulose.

3.3. *Influence of the proportion of SCG to the volume of water (r:w) on the glycosidic-linkage composition of the polysaccharides extracted*

The results of the methylation analysis of the polysaccharides recovered in the extracts obtained using different *r*:*w* ratios are shown in Table 3. The presence of $(1 \rightarrow 3)$-Gal and $(1 \rightarrow 3,6)$-Gal residues are diagnostic of the presence of arabinogalactans, while the presence of $(1 \rightarrow 4)$-Man, $(1 \rightarrow 4,6)$-Man, and terminally-linked mannose and galactose residues are diagnostic of the presence of galactomannans (Table 3). To estimate the amount of arabinogalactans and galactomannans from the glycosidic composition, it was taken into account that mannose residues are components of galactomannans and that an amount of galactose residues equal to the amount of $(1 \rightarrow 4,6)$-Man are also a component of galactomannans. Furthermore, all arabinose and galactose residues, except the terminally-linked galactose previously attributed to the galactomannans, were assumed to be components of the arabinogalactans (Nunes & Coimbra, 2002, 2007). These two polysaccharides are present in all extracts in a proportion that can be estimated as 2/3 arabinogalactans and 1/3 galactomannans (Table 3). Their glycosidic-linkage compositions are similar in all conditions and their proportions are similar to those observed for polysaccharides from coffee infusions (Nunes & Coimbra, 2001, 2007), which justify their solubility.

3.4. *Influence of a second and consecutive MAE on the extraction yield of SCG carbohydrates*

After the MAE performed to all conditions, the unextracted insoluble material resultant from each extraction condition was resuspended in water using the same *r*:*w* proportions and submitted to a second MAE under the same operating conditions. For the second MAE extraction, the temperature of 200 °C was reached in all experiments (Table 4), which was in accordance with the lower pressure observed for the highest concentrated suspensions when compared with MAE1, resultant from the removal of the majority of potential volatile compounds in MAE1. However, the concentrated samples (1:7 and 1:5) still presented higher pressures than the diluted ones.

The yield of extraction in MAE2 was higher for 1:10 ratio (25.2%, Table 4), which was even higher than the yield obtained in MAE1 (19.5%, Table 2). The amount of material recovered accounted for 1.64 g/batch, which was similar to that observed for the 1:7 ratio and even higher than that obtained for 1:5 (Table 4, Fig. 1b). These results contrast with that observed in MAE1 (Fig. 1a), where the amount of material extracted increased with the increase of the amount of SCG in the batch. Also, the material extracted was richer in carbohydrates in the 1:10 ratio (84.4%) whereas the material extracted from the more concentrated suspensions was much poorer (37.8% for 1:5). These results allow to infer that the 1:10 ratio promotes conditions able to extract the carbohydrates in higher yield as a compromise between the amount of material that can be loaded in a reactor and the efficiency of extraction of carbohydrates.

Table 4
Operational parameters and chemical characterization of the MAE2 material.

Mass of residue (g): volume of water (mL)	Sample					
	1:5	1:7	1:10	1:12	1:20	1:30
Temperature (°C)	200	200	200	200	200	200
Pressure (bar)	21	24	18	16	15	N.D.[a]
Material extracted (g/batch of 80 mL)	1.38	1.65	1.64	0.76	0.42	0.29
Yield of extraction (%, w/w)	12.3	23.9	25.2	19.4	18.3	20.5
Carbohydrates extracted (%, w/w)	37.8	73.0	84.4	84.5	84.4	77.7
Monosaccharides in extract (%, w/w)	6.5	7.7	4.1	6.3	3.2	2.9
Ara (mol%)	6	10	38	79	73	56
Man (mol%)	52	56	38	8	11	23
Gal (mol%)	24	25	20	11	14	14
Glc (mol%)	17	9	3	2	2	6
Yield of Et75 fraction (%, w/w)[b]	13.3	17.6	18.9	34.4	41.2	46.6
Et75 sugar content (%, w/w)	53.5	78.3	84.7	83.1	83.2	86.3
Ara (mol%)	1	1	2	2	1	2
Man (mol%)	51	67	58	47	47	55
Gal (mol%)	45	30	38	49	49	42
Glc (mol%)	3	2	2	1	1	1
Yield of EtSN fraction (%, w/w)[c]	86.7	82.4	81.1	65.6	58.8	53.4
EtSN sugar content (%, w/w)	23.0	56.7	61.6	75.6	65.7	68.8
Ara (mol%)	3	1	3	7	8	4
Man (mol%)	59	69	62	56	55	57
Gal (mol%)	29	26	32	32	35	36
Glc (mol%)	8	3	3	3	2	2
Solubility (g/mL of water)	0.5	0.25	0.13	0.1	0.07	0.07

[a] N.d. – not determined.
[b] Material that precipitated in 75% ethanol (v/v).
[c] Material that remained soluble in 75% ethanol (v/v).

For the 1:10 ratio, the material recovered in Et75 fraction was only 18.9%, of which 84.7% were sugars (Table 4), allowing to estimate the relative percentage of polysaccharides as 24.3% of the total carbohydrate content extracted by MAE2, which corresponded to the maximum of 0.34 g/batch recovered with MAE2 (Fig. 1b). It is possible that for the lower concentrations than 1:10, the amount of polysaccharides recovered tended to decrease due to the lower amount of material loaded, whereas for the higher concentrations, the amount of polysaccharides recovered tended to decrease due to the lower amount of solvent used (Fig. 1b).

Taking into account the amount of monosaccharides in 1:10 extract (4.1% of the extract mass, representing 6.2% of total sugars) the amount of oligomeric material can be estimated as 69.5% of total sugars. This amount represented a recovery of 0.96 g/batch of oligosaccharides, which was the condition where the highest oligosaccharides content/batch was recovered (Fig. 1b). As observed for the polysaccharides, and for the same reasons, the content of oligosaccharides/batch recovered tend to decrease as the concentration of the suspension decreased from 1:10, and tend to decrease as the concentration of the suspension increased.

The amount of polysaccharides/batch obtained by MAE2 was lower than that obtained by MAE1, with values that vary from 40% lower in 1:10 to 74% and 65% in 1:5 and 1:30, respectively. On the contrary, the amount of oligosaccharides recovered in MAE2, except for 1:5, was higher in MAE2. For 1:10 the amount of oligosaccharides recovered in MAE2 was 3.6 times higher than in MAE1.

The solubility of the material extracted in MAE2 varied from 0.07 for 1:30 to 0.5 for 1:5, showing an increase in solubility with the increase of the concentration of the suspension. Although the tendency observed was similar to that observed in MAE1 the values for MAE2 were 2–3 times lower than those observed for MAE1.

For all extracts, the carbohydrates recovered in the Et75 and EtSN were mainly composed by mannose (47–67 and 55–69 mol%, respectively), which allowed to infer the presence in high amounts of galactomannans, both as polysaccharides or as galactomannan derived oligosaccharides (Table 4). The presence of a high content of galactose shows the occurrence of also high amounts of arabinogalactans in the more diluted conditions. Mannose was the major sugar quantified in the monosaccharide form in the more concentrated conditions (56 and 52 mol% for 1:7 and 1:5, respectively), whereas in the more diluted conditions, arabinose was the major monosaccharide quantified (56, 73 and 79 mol% for 1:30, 1:20 and 1:12, respectively). The presence of high contents of the more labile monomeric arabinose in the diluted conditions and of the monomeric mannose in the concentrated conditions shows that the concentrated conditions promoted a higher extension of degradation of the SCG polysaccharides.

3.5. Linkage-composition of MAE2 polysaccharides

For MAE2, both the glycosidic-linkage composition and their proportions varied according to the operating conditions used, which contrasted with the similar glycosidic-linkage composition and their proportions observed for all MAE1 extracts. For the more diluted conditions (1:30, 1:20, and 1:12) the relative content of the total galactose and its different glycosidic-linkages decreased, whereas the total mannose and its different glycosidic-linkages increased (Table 5). Based on these results, it can be estimated that the relative content of the arabinogalactans in relation to the total polysaccharides in the extract decreased from 65% to 37% for the more diluted conditions. On the contrary, for the more concentrated conditions (1:10, 1:7, and 1:5), the sugar analysis and the glycosidic-linkage composition showed that the majority of the polysaccharides were recovered as galactomannans (53–67%, Table 5). The higher relative content of galactomannans obtained in MAE2 may justify the lower solubility of the extracts obtained in MAE2 (Table 4) when compared to the MAE1 (Table 2). The lower solubility of the extracts obtained with the more diluted conditions (1:30 and 1:20), showed to have high relative contents of arabinogalactans, allowed to infer the occurrence of higher molecular weight polysaccharides, resultant from these milder conditions.

4. Concluding remarks

Table 6 summarizes the yields of extraction of SCG for the different ratios of residue to water using two consecutive MAE. The

Table 5
Glycosidic-linkage composition (%) of the carbohydrates extracted using MAE2.

Linkage (%)	Mass of residue (g): volume of water (mL)					
	1:5	1:7	1:10	1:12	1:20	1:30
T-Ara*f*	0.5	0.4	0.4	1.2	1.1	1.0
5-Ara*f*	0.0	0.0	0.0	0.0	0.6	0.5
total Ara	0.5(2)	0.4(2)	0.4(5)	1.2(7)	1.7(7)	1.5(5)
T-Man*p*	11.0	12.0	9.3	3.5	4.6	6.8
6-Man*p*	0.8	1.1	0.0	0.5	0.3	0.0
4-Man*p*	36.2	45.8	51.0	26.3	33.8	50.5
4,6-Man*p*	2.7	3.8	2.7	3.4	3.3	4.0
total Man	50.7(51)	62.7(64)	63.0(62)	33.7(31)	42.0(36)	61.3(52)
T-Gal*p*	9.3	8.0	8.2	10.0	9.6	8.2
6-Gal*p*	6.7	5.7	4.7	12.8	9.3	6.1
3-Gal*p*	16.2	11.2	13.5	17.3	17.5	7.9
3,6-Gal*p*	10.9	8.5	8.0	23.3	18.0	11.7
total Gal	43.1(37)	33.4(31)	34.4(31)	63.4(58)	54.4(56)	33.9(40)
T-Glc*p*	0.9	0.5	0.2	0.2	0.3	0.5
4-Glc*p*	4.9	3.2	2.0	0.2	1.7	2.8
total Glc	5.8(7)	3.7(3)	2.2(2)	0.4(2)	2.0(1)	3.3(2)
AG (%)[a]	47	34	34	63	55	35
GM (%)[b]	53	67	66	37	45	65

[a] Arabinogalactans.
[b] Galactomannans

Table 6
Synoptic table of MAE (MAE1 + MAE2) of SCG: extraction of mass of material and sugars per sample.

Mass of SCG (g): volume of water (mL)	Sample					
	1:5	1:7	1:10	1:12	1:20	1:30
Material extracted (g)	3.44	3.55	3.04	1.83	1.29	0.90
Yield of extraction (%, w/w)	25	38	42	33	35	43
Yield of carbohydrates (%, w/w)	13	34	55	42	41	50
Yield of Ara (%, w/w)	16	24	61	56	69	69
Yield of Man (%, w/w)	12	41	66	31	27	41
Yield of Gal (%, w/w)	23	48	74	88	90	98
Yield of Glc (%, w/w)	2	5	6	4	3	4

higher the concentration used the higher the amount of material was extracted. However, considering the yield of extraction, the maximum occurred for the more diluted condition (1:30) and also for the intermediate condition (1:10). These were the two conditions where the higher yield of sugars has also been observed, 50% and 55%, respectively. The galactose was almost completely extracted in the condition 1:30. With the increase of the concentration of the sample the amount of galactose extracted tends to decrease. At 1:10, 74% of total galactose was recovered. This condition was the one that allowed a better recovery of mannose (66%) and also of galactomannans, 0.23 and 0.22 g/batch in MAE1 and MAE2, respectively (Fig. 2). Higher concentrations of SCG than 1:10 or lower resulted in lower amounts of galactomannans recovered by the two consecutive MAE. The 1:10 ratio seems also to be the optimum concentration for extraction of SCG arabinogalactans by MAE, although 1:7 results in a slightly higher recovery by MAE1 and 1:12 results in a slightly higher recovery by MAE2.

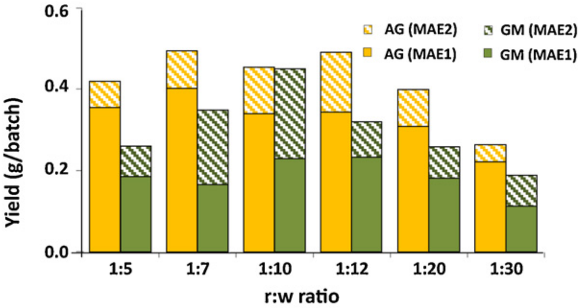

Fig. 2. Amount of arabinogalactans (AG) and galactomannans (GM) recovered during the first (MAE1) and second (MAE2) microwave assisted extraction procedures.

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