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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.