Carbohydrate Polymers 214 (2019) 53–61

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural features of spent coffee grounds water-soluble polysaccharides: Towards tailor-made microwave assisted extractions

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ARTICLE INFO

Keywords:
Coffee residue
Arabinogalactans
Galactomannans
Polysaccharides
Methylation analysis
Response surface methodology

ABSTRACT

This work studies the microwave-assisted extraction conditions for recovery of polysaccharides from spent coffee grounds, including their effect on arabinogalactans and galactomannans polymerization and branching structural features. *Temperature* (140, 170, and 200 °C) has the most significant impact on total extracted mass ($\eta_{\text{total soluble solids}}$) and sugars yield ($\eta_{sugars}$), arabinogalactans ($\eta_{AG}$) and galactomannans ($\eta_{GM}$), and polysaccharide mass ratio (η_{AG}/η_{GM}). *Time* (2, 5, and 10 min) and *alkali* (diluted 0.1 M NaOH) treatments have less influence. *Alkali* treatment and shorter *time* (2 min) provided a protective effect against polysaccharides degradation. At 170 °C, the yield of arabinogalactans was found to be significantly higher than that of galactomannans ($\eta_{AG}/\eta_{GM} > 1$). Increasing temperature to 200 °C leads to decrease the polymerization of polysaccharides, promoting the formation of debranched polysaccharides and oligosaccharides. This study shows that the optimum conditions for polysaccharides extraction cannot be selected only by mass yield but need to be defined according to the desired structural features for the specific applications.

1. Introduction

Within a green extraction perspective, the microwave assisted extraction has been considered a feasible tool to extract polysaccharides and/or oligosaccharides from various sources using only pressurized water (Benko et al., 2007; Coelho, Rocha, Saraiva, & Coimbra, 2014; Passos & Coimbra, 2013; Passos, Moreira, Domingues, Evtuguin, & Coimbra, 2014; Passos et al., 2015; Tsubaki, Iida, Sakamoto, & Azuma, 2008) or dilute alkali solutions (Benko et al., 2007; Lundqvist et al., 2003). Diluted acid conditions have also been reported for carbohydrates extraction (Yuan et al., 2018), mostly for the conversion of biomass-derived carbohydrates into monosaccharides (Fan et al., 2014; Fischer & Bipp, 2005).

Temperature has been described as the most important parameter contributing to the high recovery of carbohydrates in aqueous solutions. Generally, the higher the temperature applied, the higher the recovery yield. However, higher temperature leads to autohydrolysis of the polysaccharides resulting in the recovery of oligosaccharides, which are eventually transformed into monosaccharides (Benko et al., 2007; Tsubaki et al., 2008; Tsubaki, Oono, Hiraoka, Onda, & Mitani, 2016). From a structural point of view, high temperature conditions affect the polysaccharides molecular structures, including *e.g.* molecular weight distribution, as reported for galactoglucomannans (Lundqvist et al., 2003) and arabinogalactans (Tsubaki et al., 2008). Galactomannans are stable at temperatures ≤200 °C, even during long term exposure (> 3 h), but arabinogalactans start to degrade at 180 °C under similar exposure conditions. For the spent coffee grounds insoluble matrix, which contains galactomannans, arabinogalactans, and cellulose, the thermal behaviour is modulated by the presence of all existent polysaccharides (Simões, Maricato, Nunes, Domingues, & Coimbra, 2014). One of the main advantages of the microwave technology, when compared to other technological solutions for extraction of polysaccharides, is the short operating time. Nevertheless, even small differences when time is combined with other important operational parameters, such as temperature, can highly affect the final results (Tsubaki et al., 2008). Another important effect is the change of pH of the medium, which decreased after microwave assisted extraction (MAE) treatments of spent coffee grounds (SCG) (Passos & Coimbra, 2013) due to the hydrolysis of chlorogenic and acetyl esters initially bounded to the polysaccharides matrix (Moreira et al., 2015). Because polysaccharides are more susceptible to degradation at high temperatures under acidic conditions (Selvendran, March, & Ring, 1979; Yuan et al., 2018), the

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https://doi.org/10.1016/j.carbpol.2019.02.094
Received 21 December 2018; Received in revised form 26 February 2019; Accepted 26 February 2019
Available online 28 February 2019

use of dilute alkali conditions moderates this effect (Benko et al., 2007; Coelho et al., 2014; Lundqvist et al., 2003).

Several health-related properties have been associated with coffee polysaccharides. Arabinogalactans have been shown a potential towards *in vitro* immunostimulatory activity due to the presence of terminal arabinose units (Ferreira et al., 2018), which may be favored in the presence of a higher degree of branching. The immunostimulatory potential of coffee galactomannans was associated with the presence of acetyl groups (Simões et al., 2009). This is, however, a structural feature not present in SCG galactomannans when obtained under alkali-treated extraction conditions (Simões, Nunes, Domingues, & Coimbra, 2010). The mannooligosaccharides, as those resultant from coffee galactomannans, are resistant to the gastrointestinal track enzymes (human salivary α-amylase, artificial gastric juice, porcine pancreatic enzymes, and rat intestinal mucous enzymes). When reaching the colon, they are prone to be fermented by the faecal bacteria, originating acetic, propionic and *n*-butyric acids, which represent a prebiotic effect (Asano, Hamaguchi, Fujii, & Iino, 2003).

The first attempts to extract polysaccharides from SCG using microwave technology were done using different ratios of mass of SCG to water at constant temperature (200 °C), resulting in the recovery of arabinogalactans as the major polysaccharides (Passos & Coimbra, 2013). These experiments showed that diluted conditions allow to yield higher ratios of polysaccharides to oligosaccharides, although total mass yields were lower. The arabinogalactans and galactomannans that remain in the SCG residue resultant from the microwave assisted extraction can be obtained in oligomeric form by using five consecutive microwave extraction cycles at 200 °C, leaving an insoluble cellulose-rich residue (Passos, Cepeda et al., 2014; Passos, Moreira et al., 2014). When applying a lower temperature (170 °C) for microwave extraction of SCG polysaccharides, the content of arabinose in arabinogalactans is higher (Passos et al., 2015), allowing to hypothesize that defining specific microwave operating conditions for carbohydrates extraction from SCG may allow the recovery of compounds with specific characteristics for different applications. In this work, three experimental factors: temperature (*T*), time of irradiation (*t*), and addition of alkali (*alkali*) to SCG suspensions were varied according to a full factorial experimental design and their effect on the extraction yield as total mass of soluble solids (η_{mass}, $g_{extracted}/100\ g_{SCG}$), sugars content ($\eta_{sugars}$, %), arabinogalactans ($\eta_{AG}$) and galactomannans content (η_{GM}), ratio of arabinogalactans to galactomannans (η_{AG}/η_{GM}), arabinogalactans degree of branching (DB_{AG}), and galactomannans degree of polymerization (DP_{GM}) was assessed.

2. Experimental

2.1. Samples

Spent coffee grounds (SCG), which is the residue left after espresso coffee preparation, were obtained from a commercial lot composed mainly by Arabica varieties of Delta Cafés Platina (Portugal), after beverage preparation in a local cafeteria. The SCG presented a moisture content of 63%. To remove the water, the SCG samples were oven dried at 105 °C for 8 h according to the ISO/DIS11294-1993 method (Illy & Viani, 1995). On a dry weight basis, SCG were composed by 65% carbohydrates, namely mannose (45%), galactose (26%), glucose (22%), and arabinose (6%), determined as alditol acetates by GC-FID after acid hydrolysis, as previously described (Passos, Cepeda et al., 2014; Passos, Moreira et al., 2014). The SCG composition was also constituted by 12% of oil (Barbosa, De Melo, Coimbra, Passos, & Silva, 2014) and 1–2% of free chlorogenic acids (Passos et al., 2015). The samples were stored at −20 °C prior to the analysis. All reagents used were of analytical grade or higher available purity.

Table 1
Set of operating variables for optimization of MAE extraction process defined using a full factorial design.

Run	*T* (°C)	*alkali* (0.1 M NaOH)	*t* (min)
1	140	−	2
2	140	−	5
3	140	−	10
4	140	+	2
5	140	+	5
6	140	+	10
7	170	−	2
8	170	−	5
9	170	−	10
10	170	+	2
11	170	+	5
12	170	+	10
13	200	−	2
14	200	−	5
15[a]	200	−	10
16	200	+	2
17	200	+	5
18[a]	200	+	10

[a] Data for these conditions were not obtained due to equipment limitations (maximum pressure of 55 bar exceeded).

2.2. Microwave irradiation

A MicroSYNTH Labstation (Milestone srl., Bergamo, Italy) equipment with a maximum output delivery power of 1000 W was used for the microwave experiments using two high pressure reactors of 100 mL capacity each. Reactor A was the one that incorporate the pressure and temperature sensors. The operating conditions were as described in Passos and Coimbra (2013): the dried SCG samples were suspended in water using a ratio of 1:10 g/mL to obtain a total volume of 70 mL *per* reactor. After extraction, the reactors were cool down to room temperature. After centrifugation (15,000 rpm for 20 min at 4 °C) the supernatant solution was filtered using a MN GF-3 glass fibre filter, frozen, and freeze-dried. The total solids content was determined as the total weight of the freeze-dried extracts.

2.3. Design of experiments and response surface methodology

The influence of 3 factors was studied: temperature (*T*), time of exposure to microwave irradiation (*t*), and the use of water/or alkali (0.1 M NaOH) treatment (*alkali*) were considered as independent variables and use in accordance with the design of experiments prepared (Table 1). Three levels for temperature (*T*) and time of exposure (*t*): (*T*) = 140, 170, and 200 °C, and (*t*) = 2, 5, and 10 min, respectively. Two levels were considered for alkali treatment: (*alkali*) = 0 M or 0.1 M. A total of 18 experiments were conducted as described in Table 1, each condition setting represented by two duplicates correspondent to reactor A and B.

2.4. Sugar and glycosidic-linkage analyses

Individual neutral sugars were quantified after acid hydrolysis, followed by derivatization to alditol acetates, and detection by GC-FID (Nunes & Coimbra, 2001; Passos & Coimbra, 2013). Sugars were determined in duplicate. The sugars yield (η_{sugars}) is the account of sugars *per* total solids mass. In cases where the major sugars had higher than 5% variability a third analysis was performed. Methylation analysis was also performed for determination of glycosidic-linkage composition of the polysaccharides. Prior to the GC–MS analyses the sugars were derivatized to partially methylated alditol acetates (PMAA) (Nunes & Coimbra, 2001; Passos & Coimbra, 2013).

Coffee galactomannans (GM) are high molecular weight low

Table 2
Chemical characterization of water-soluble material obtained under microwave assisted conditions using aqueous/or dilute alkali treatments at 140 °C. The data includes total soluble solids yield [$\eta_{\text{total soluble solids}}$, (%, w/w)]; total sugars yield (η_{sugars}, %); arabinogalactans (AG) sugar content [η_{AG}, (mg_{AG}/g_{SCG})] and (η_{AG}, %); galactomannans (GM) sugar content [η_{GM}, (mg_{GM}/g_{SCG})] and (η_{GM}, %); degree of polymerization (DP); and degree of branching (DB).

t (min)		Aqueous						NaOH					
		2 min		5 min		10 min		2 min		5 min		10 min	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
$\eta_{\text{total soluble solids}}$ (%)[a]		9.0	10.3	8.7	8.3	13.8	8.9	8.4	8.5	7.3	7.9	8.5	8.9
η_{sugars} (%)		40.0	43.5	64.7	42.0	47.6	54.7	36.4	28.1	36.0	34.0	43.6	41.8
Linkage (%)													
T-Ara*f*		2.6	3.8	4.5	2.4	3.7	3.2	1.8	2.6	3.0	5.0	2.9	3.3
5-Ara*f*		0.0	0.8	1.7	0.9	1.9	1.2	1.0	1.5	1.1	0.6	1.2	0.9
Total Ara (M)		**2.6**	**4.6**	**6.2**	**3.3**	**5.6**	**4.4**	**2.8**	**4.1**	**4.0**	**5.6**	**4.1**	**4.2**
(A)		**(10.9)**	**(11.1)**	**(11.2)**	**(11.0)**	**(11.1)**	**(10.5)**	**(10.7)**	**(8.7)**	**(9.1)**	**(10.1)**	**(9.2)**	**(10.7)**
T-Man*p*		1.2	1.4	2.0	1.6	2.1	1.5	1.5	2.2	1.1	1.2	1.7	1.4
4-Man*p*		69.5	64.6	56.3	59.6	50.9	53.2	52.5	52.3	38.4	48.7	46.9	41.6
4,6-Man*p*		1.7	1.6	3.3	2.5	3.2	2.2	2.7	4.3	1.8	1.2	2.3	2.1
Total Man (M)		**72.4**	**67.5**	**61.6**	**63.7**	**56.3**	**56.9**	**56.7**	**58.8**	**41.3**	**51.0**	**50.8**	**45.1**
(A)		**(46.5)**	**(47.0)**	**(47.7)**	**(47.3)**	**(42.8)**	**(43.8)**	**(46.2)**	**(46.7)**	**(42.1)**	**(45.0)**	**(43.1)**	**(44.1)**
T-Gal*p*		3.7	4.3	6.0	4.7	6.8	4.7	12.8	4.7	8.1	6.5	6.1	4.9
6-Gal*p*		1.6	1.4	2.9	2.3	3.6	2.8	2.3	4.1	3.8	1.9	3.3	2.7
3-Gal*p*		12.4	12.9	11.9	14.4	15.3	18.9	12.8	12.7	25.7	20.6	18.1	19.1
3,6-Gal*p*		6.1	8.3	10.3	10.9	10.8	11.4	9.6	12.2	15.6	12.8	12.8	13.3
Total Gal (M)		**23.6**	**26.9**	**31.1**	**32.3**	**36.6**	**37.8**	**37.6**	**33.7**	**53.1**	**41.7**	**40.4**	**40.0**
(A)		**(37.1)**	**(37.3)**	**(36.7)**	**(37.1)**	**(40.8)**	**(41.3)**	**(37.0)**	**(38.5)**	**(44.7)**	**(39.7)**	**(42.9)**	**(40.7)**
T-Glc*p*		0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.3	0.1	0.0	1.0	0.0
4-Glc*p*		1.3	1.0	1.1	0.7	1.4	0.9	2.9	2.8	1.5	1.6	3.6	10.8
Total Glc (M)		**1.3**	**1.0**	**1.2**	**0.7**	**1.6**	**0.9**	**3.0**	**3.1**	**1.6**	**1.6**	**4.7**	**10.8**
(A)		**(3.8)**	**(3.0)**	**(2.9)**	**(3.1)**	**(3.6)**	**(2.9)**	**(4.5)**	**(4.7)**	**(2.5)**	**(3.5)**	**(3.3)**	**(3.1)**
AG	**η_{AG}** (mg_{AG}/g_{SCG})	7.8	13.4	19.0	11.6	25.5	19.4	11.6	8.0	14.5	12.3	15.6	15.5
	η_{AG} (%)[b]	25	30	34	33	39	40	38	34	55	46	42	42
	DP_{AG}	6.0	5.9	4.6	6.4	4.9	7.6	2.7	6.2	6.4	6.3	6.2	7.7
	DB_{AG}	0.28	0.33	0.37	0.37	0.33	0.32	0.28	0.42	0.30	0.32	0.34	0.35
GM	**η_{GM}** (mg_{GM}/g_{SCG})	23.7	30.9	36.3	23.1	39.0	28.7	18.2	15.0	11.3	14.0	19.6	17.4
	η_{GM} (%)[c]	74	69	65	66	60	59	59	63	43	52	53	47
	DP_{GM}	62.3	48.8	30.6	39.5	26.3	38.2	37.9	26.6	39.2	43.3	30.8	32.1
	DB_{GM}	0.02	0.02	0.05	0.04	0.06	0.04	0.05	0.07	0.04	0.02	0.05	0.05
AG/GM		0.3	0.4	0.5	0.5	0.7	0.7	0.6	0.5	1.3	0.9	0.8	0.9

Samples A and B are the duplicate samples respectively obtained at reactor A and B in each microwave run. (M) Glycosidic-linkage composition of polysaccharides was determined as partially methylated alditol acetated by methylation analysis with GC–MS. (A) Sugar composition determined by derivatization to alditol acetates and analysis by GC-FID. [a]$g_{extracted}$ /100 g $_{SCG}$. [b][AG/(AG + GM)]. [c][GM/(AG + GM)]. DP – Degree of polymerization. DB – Degree of Branching.

Table 3
Sources of variation in the ANOVA models for: total soluble solids, [$\eta_{\text{total soluble solids}}$ (%), ($g_{extracted}$/100 g $_{SCG}$)]; sugars content, [η_{sugars} (%), ($g_{carbohydrates}$/100 g $_{SCG}$)]; content of arabinogalactans AG [η_{AG}, (mg_{AG}/g_{SCG})]; content of galactomannans GM [η_{GM}, (mg_{GM}/g_{SCG})]; degree of branching (DB) for arabinogalactans (DB_{AG}) and galactomannans (DB_{GM}), and galactomannans degree of polymerization (DP_{GM}). Operating parameters are: temperature (*T*, °C), time (*t*, min), and the use of alkali (*alkali*).

			Parameters (p-Values)					
Effects	**$\eta_{\text{total soluble solids}}$ (%)**[a]	**η_{sugars} (%)**	Arabinogalactans (AG)		Galactomannans (GM)			**η_{AG}/η_{GM}**
			η_{AG} (mg/g_{SCG})	**DB_{AG}**	**η_{GM} (mg/g_{SCG})**	**DB_{GM}**	**DP_{GM}**	
T	**0.000**	**0.004**	**0.000**	**0.021**	**0.000**	0.067	**0.000**	**0.000**
t	**0.000**	**0.011**	**0.000**	0.988	**0.025**	0.702	**0.012**	**0.001**
alkali	0.082	0.354	0.738	0.687	**0.013**	0.710	0.271	0.365
T*t	**0.002**	0.278	**0.000**	0.950	0.196	0.903	0.408	**0.020**
T*alkali	0.181	**0.019**	0.503	0.498	**0.006**	0.527	0.159	0.054
alkali*t	0.598	0.851	0.698	0.361	0.477	0.839	0.949	0.399

Significant sources of variation ($p < 0.05$) are marked in **bold**.
[a] $g_{extracted}$/100 g $_{SCG}$.

branched polysaccharides composed mainly by a backbone of (β1→4)-linked mannose residues, branched at O-6 by single residues of galactose or arabinose residues, although other residues can also occur in small amount (Nunes, Domingues, & Coimbra, 2005). For quantification of galactomannans, all manose linkage contributions including terminally-linked Man (T-Man*p*), 4-Man*p*, and 4,6-Man*p* individual abundances were considered together in the Eq. (1). Further, the 4,6-Man*p* abundance was added once more to account for the side chain single sugar residues occurring in galactomannans exactly at O-6 position (Nunes & Coimbra, 2002b). Identification of linkages and relative abundances can be found in Table 2, representing the experiments performed at 140 °C. The data for 170 °C and 200 °C, respectively can be found in Data in Brief (Passos et al., submitted).

Table 4
Regression coefficients and adjusted R^2 of the ANOVA models represented in Table 3.

			Regression coefficients					
	$\eta_{\text{total soluble solids}}$ (%)[a]	η_{sugars} (%)	Arabinogalactans (AG)		Galactomannans (GM)			η_{AG}/η_{GM}
			η_{AG} (mg/g_{SCG})	DB_{AG}	η_{GM} (mg/g_{SCG})	DB_{GM}	DP_{GM}	
Constant	−11.4	0.230	−57.3	45.51	−0.061	−0.039	126.9	−1.39
T	0.134	0.001	0.475	−0.083	0.150	0.029	−0.559	0.014
t	−3.01	−0.033	−22.85	−0.134	−3.33	−0.079	−4.34	−0.435
Alkali	2.35	−0.281	12.45	−1.49	−31.0	1.30	−14.7	1.45
T*t	0.023	0.000	0.173	0.001	0.027	0.001	0.019	0.003
T*Alkali	−0.020	0.002	−0.071	0.014	0.159	−0.009	0.083	−0.008
Alkali*t	0.069	−0.001	−0.277	−0.234	0.281	0.026	−0.093	−0.023
$R2_{adj}$	0.87	0.39	0.87	0.05	0.56	0.05	0.65	0.71

[a] $g_{extracted}/100\ g_{SCG}$.

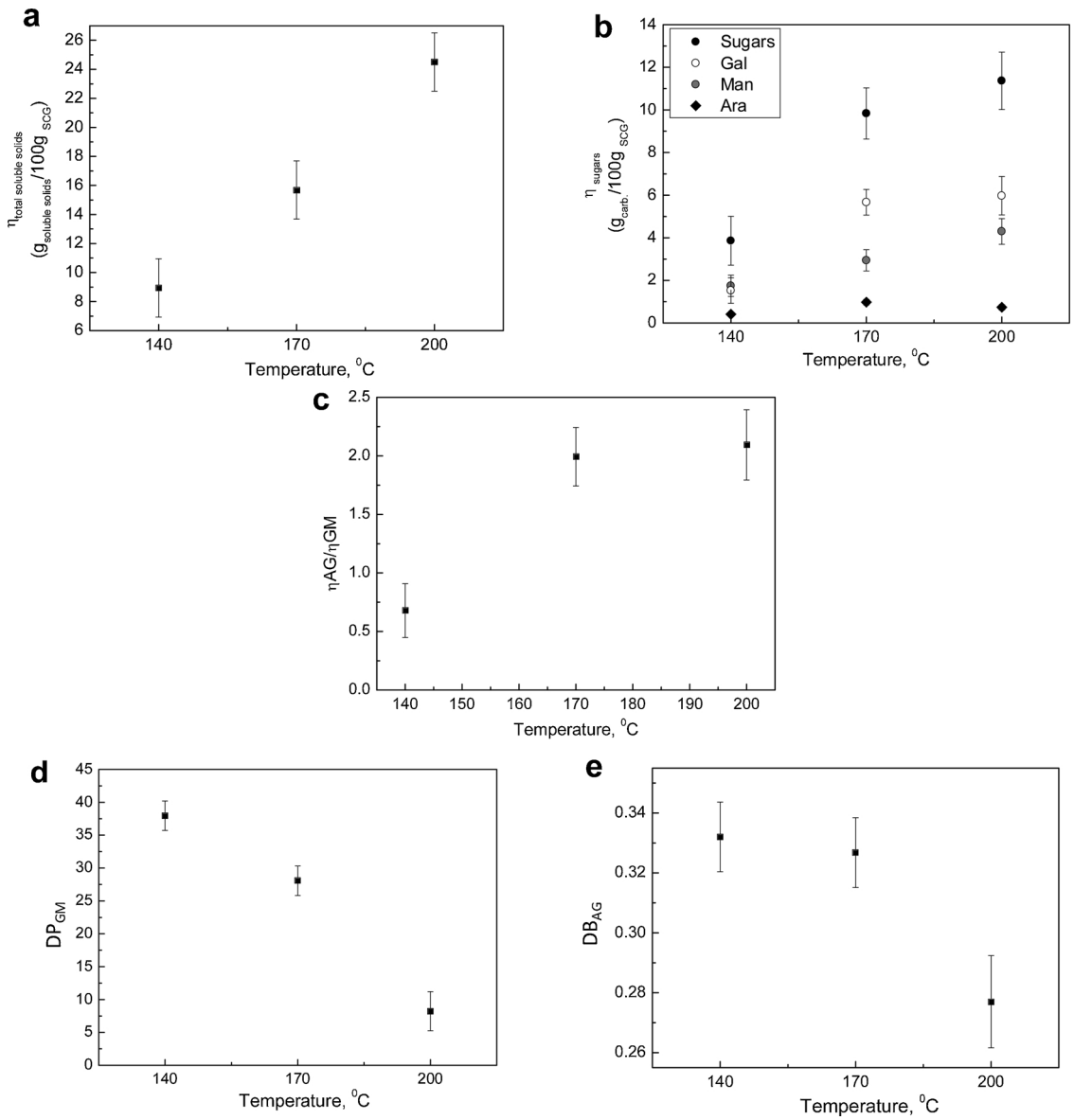

Fig. 1. Comparison on the solely temperature effect on yield and structural features: a) total soluble solids yield [$\eta_{\text{total soluble solids}}$, ($g_{extracted}/100\ g_{SCG}$)]; b) Arabinose (Ara) [$\eta_{Ara}$, ($g_{extracted}/100\ g_{SCG}$)], Mannose (Man) [$\eta_{Man}$, ($g_{extracted}/100\ g_{SCG}$)], Galactose (Gal) [$\eta_{Gal}$, ($g_{extracted}/100\ g_{SCG}$)], and total sugars content [η_{sugars}, ($g_{carbohydrates}/100\ g_{SCG}$)]; c) ratio of arabinogalactans to galactomannans, (η_{AG}/η_{GM}); d) galactomannans degree of polymerization (DP_{GM}); and e) arabinogalactans degree of branching (DB_{AG}). Each point represents an average of all data values for each temperature level with confidence intervals, representing a total of 6 values for 140 °C and 170 °C and 4 values for 200 °C, respectively.

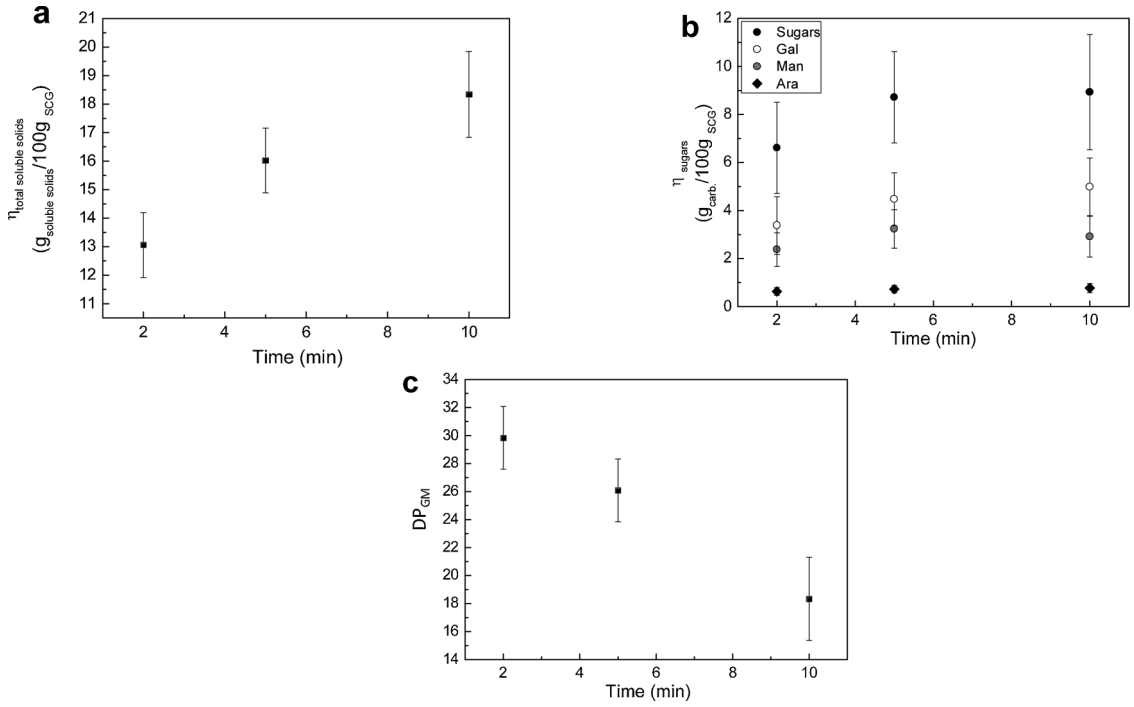

Fig. 2. Comparison on the individual effect of time (*t*) on: a) total soluble solids [$\eta_{\text{total soluble solids}}$, ($g_{\text{extracted}}$/100 g $_{SCG}$)]; b) Arabinose (Ara) [$\eta_{Ara,}$ ($g_{\text{extracted}}$/100 g $_{SCG}$)], Mannose (Man) [$\eta_{Man,}$ ($g_{\text{extracted}}$/100 g $_{SCG}$)], Galactose (Gal) [$\eta_{Gal,}$ ($g_{\text{extracted}}$/100 g $_{SCG}$), and total sugars content [η_{sugars}, ($g_{\text{carbohydrates}}$/100 g $_{SCG}$)]; and c) galactomannans degree of polymerization (DP_{GM}). Each point represents an average of all data values for each time level with confidence intervals, representing a total of 6 values for 2 and 5 min and 4 values for 10 min, respectively.

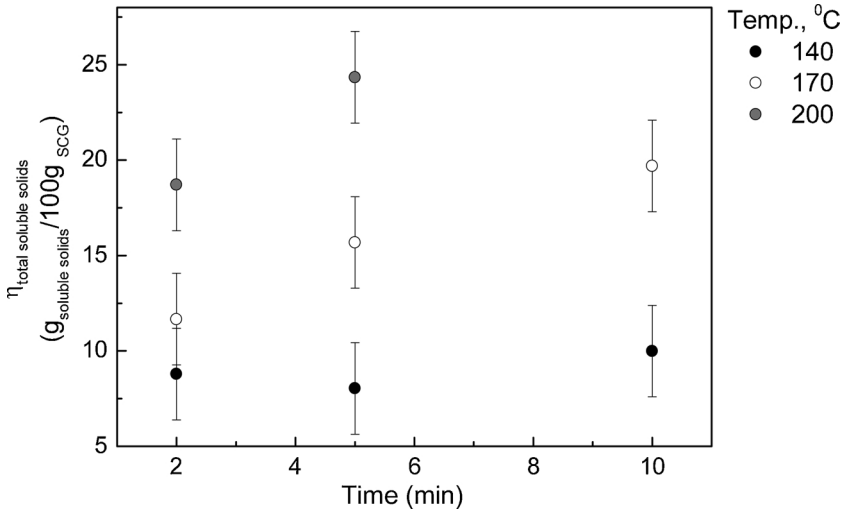

Fig. 3. Representation of the interrelation between the operating conditions time (*t*) and temperature (*T*) *versus* total soluble solids yield [$\eta_{\text{total soluble solids,}}$ ($g_{\text{extracted}}$/100 g $_{SCG}$)]. Each point represents an average of all data points for each temperature and time level combination with confidence intervals, representing each point a total of 2 values.

$$\text{GM}\,(\%) = [(T-Manp)+(4-Manp)+(4,6-Manp)] + [\text{T}{-}Galp\ (\text{equivalent to})\ 4,6-Manp] \quad (1)$$

The ratio of total mannose to terminally-linked Man (T-Man*p*) (Eq. (2)) was used to estimate galactomannans degree of polymerisation (DP_{GM}). However, the DP_{GM} value may be underestimated as it is also possible that mannose residues at the side chains exist (Mandal & Das, 1980).

$$\text{GM Degree of Polymerization } (DP_{GM}) = \frac{[(T-Manp)+(4-Manp)+(4,6-Manp)]}{(T-Manp)} \quad (2)$$

The ratio of O-6 branched Man residues (4,6-Man*p*) to total mannose (Eq. (3)) can be used to estimate galactomannans degree of branching (DB_{GM}) (Nunes & Coimbra, 2002a; Simões et al., 2010).

$$\text{GM Degree of Branching } (DB_{GM}) = \frac{(4,6-Manp)}{[(T-Manp)+(4-Manp)+(4,6-Manp)]} \quad (3)$$

Coffee type II arabinogalactans (AG) are high molecular weight highly branched polysaccharides composed mainly by a backbone of (β1→3)-linked D-galactose residues, branched at O-6 with short chains of (β1→6)-linked D-galactose residues, and further substituted with various combinations of arabinose, rhamnose, and glucuronic acid residues (Nunes, Reis, Silva, Domingues, & Coimbra, 2008). Therefore, the ratio presented in Eq. (4) may be used as a diagnostic of the DB_{AG} for arabinogalactans (Nunes & Coimbra, 2002b).

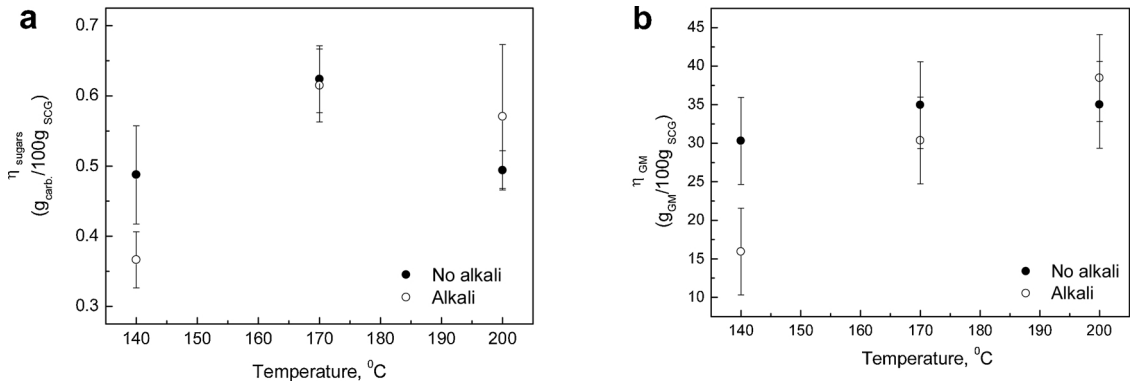

Fig. 4. Comparison on the interrelations between temperature (*T*) and the use of alkali conditions on: a) sugars content [η_{sugars}, (%, $g_{carb.}$/100 g $_{SCG}$)] and b) content of galactomannans [η_{GM}, (g_{GM}/100 g $_{SCG}$)]. Each point represents an average of all data values for each temperature level with confidence intervals for the *alkali* and *No alkali* (aqueous) sequences, respectively representing a total of 2–3 values for each combination of temperature and alkali treatment.

$$\text{AG Degree of Branching } (DB_{AG}) = \frac{(3,6-Galp)}{[(T-Gal)+(6-Gal)+(3-Gal)+(3,6-Gal)]-(4,6\,Manp)} \quad (4)$$

To quantify arabinogalactans in coffee extracts 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, 2002b). The glucose linkages (terminally-linked Glc*p* and 4-Glc*p*) were excluded from AG or GM quantification as their contribution was about 1–3% and mostly related to cellulose degradation.

2.5. Size exclusion chromatography (SEC)

Size exclusion chromatography was applied as described by Passos, Cepeda et al. (2014) as an adaptation of the methodology in Mendes, Xavier, Evtuguin, and Lopes (2013) using a PL-GPC 110 system (Polymer Laboratories, UK) equipped with an RI detector. The system used two PL aquagel-OH MIXED (8 µm 300 × 7.5 mm) columns protected by a PL aquagel-OH Guard 8 µm pre-column, with an eluent (0.1 M $NaNO_3$) flow rate of 0.9 mL/min. The columns were calibrated using pullulans in the range 0.7–1000.0 kDa (Polymer Laboratories, UK).

2.6. Statistical analysis

Statistical significance of the effects of 3 factors: temperature, time of exposure and use of water/or alkali treatments for extraction and their interactions was done using analysis of variance (ANOVA). Total mass yield, yield of sugars and contents in arabinogalactans and galactomannans as well as the degrees of polymerization and branching of arabinogalactans and galactomannans were used as response variables. Pair-wise comparison of group means for all factors and their interactions was done using multiple comparison test with critical values from t distribution with Bonferroni adjustment.

The ANOVA model including all main effects and their interaction was calculated according to the Eq. (5).

$$x_i = \mu + a + b + c + (ab) + (ac) + (bc) + e, \quad (5)$$

Where μ is an offset, *a* is a main effect of temperature, *b* is a main effect of time, c is a main effect of alkali treatment, *(ab), (ac)* and *(bc)* are their interactions, e is a residual error.

As some of the experimental data points were missing, analysis of effects of the data set was carried out according to the literature recommendations for analysis of unbalanced designs. The recommendations are to select analysis approach depending on the data structure and design objective. Taking into account the relevance of the main effects and the fact that different sequential models led to similar conclusions, ANOVA calculations were done using Type I sum of squares (Hector, Felten, & Schmid, 2010). All calculations were made in Matlab 9.5 (R2018b).

3. Results and discussion

To evaluate the feasibility of the recovery of compounds with specific characteristics using microwave operating conditions for spent coffee grounds (SCG) arabinogalactans and galactomannans, in this work, different *temperature* conditions (200 °C, 170 °C, and 140 °C), *time* (2, 5, and 10 min), and the presence of *alkali* (water or 0.1 M NaOH) were established.

In these experiments, two reactors were used, where one incorporated the pressure and temperature sensor. It was observed that this device affects the extraction conditions, allowing a high variability in the data gathered. For this reason, it was decided to use both experiments (A and B) independently, not the average. The data of total soluble solids [$\eta_{total\ soluble\ solids}$, (%, w/w)] and sugars ($\eta_{sugars}$, %) yield, the sugar and glycosidic-linkage analysis obtained by methylation, as well as arabinogalactans (AG) [η_{AG}, (mg_{AG}/g_{SCG}), and η_{AG} (%)]; galactomannans (GM) [η_{GM}, (mg_{GM}/g_{SCG}) and η_{GM} (%)] sugar content, degree of polymerization (DP); and degree of branching (DB) can be found in Table 2 representing the experiments performed at 140 °C. This is an example of the data that can be found in Data in Brief (Passos et al., submitted) as Tables 1–3, for 140 °C, 170 °C, and 200 °C, respectively. The impact of the experimental conditions was evaluated in terms of the total content of soluble material recovered, accounting for the percentage of sugars and individual yields for arabinogalactans and galactomannans (Table 3). According to ANOVA results, the lower the *p-values* the higher the influence on the parameters under study, where only the results with **p < 0.05** (in bold) are considered statistically significant. The results for the multiple comparisons with Bonferroni adjustment for ANOVA models can be found in Data in Brief (Passos et al., submitted) as Tables 4–7, respectively for total mass yield, total sugar yield, arabinogalactans yield, and galactomannans yield. The factor that affected most carbohydrate extraction was *temperature*, *temperature* with *time*, and *temperature* with application of *alkali* (Tables 3 and 4). The only exception was the galactomannans degree of branching, DB_{GM}, which was not significantly affected by any MAE experimental conditions. Based on this observation, the DB_{GM} is no longer discussed when considering the influence of the operating conditions on the different parameters.

3.1. Effect of temperature

The effect of temperature on the type of polysaccharides being

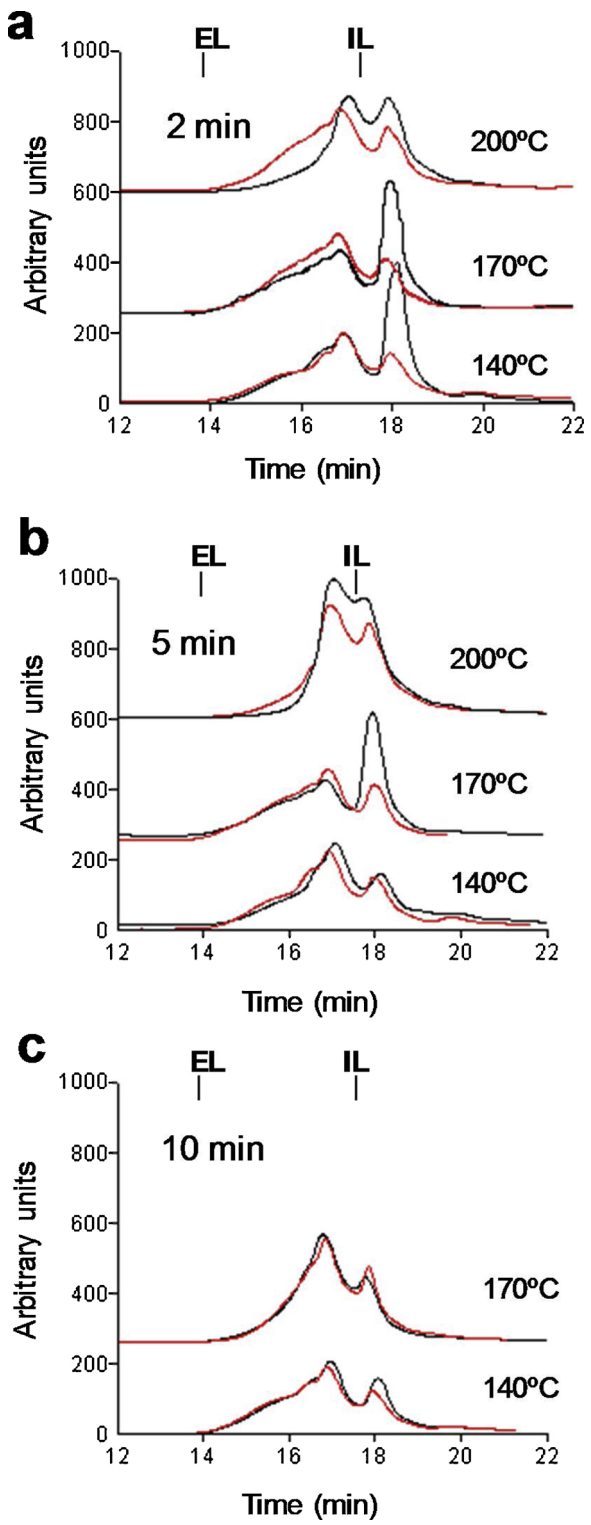

Fig. 5. Size exclusion chromatography profile of the different polysaccharide extracts obtained under different operating conditions: variable temperature conditions (140 °C, 170 °C, 200 °C) at constant time of: a) 2 min; b) 5 min; and c) 10 min. Black line represents extraction with water; red line represents extraction under alkali conditions. EL – exclusion limit; IL – inclusion limit for monosaccharides existent in the sample in comparison with glucose retention time (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

extracted and, more specifically, the impact of the treatment reflected on their molecular weight, becomes evident at temperatures above 200 °C, when degradation began to occur and most of the extracted compounds are monosaccharides (> 90%) (Yu, Lou, & Wu, 2008). For this reason, the maximum temperature tested was 200 °C.

The total soluble solids yield, $\eta_{\text{total soluble solids}}$ (%, $g_{\text{extracted}}/100\ g_{\text{SCG}}$), recovered at 140 °C was 8.9%, reaching 15.7% at 170 °C and 21.5% at 200 °C. A linear relationship could be inferred ($R^2 = 0.9983$) between the temperature and the yield (Fig. 1a). Individually, mannose content in the recovered material increased linearly ($R^2 = 0.9988$) from 1.7% to 4.3% with the increase of the temperature of extraction from 140 °C to 200 °C. Galactose content had a high increase from 1.5% to 5.7% when the temperature increase from 140 °C to 170 °C, contributing to half of the total sugars content at 170 °C (Fig. 1b). While mannose yield continued to increase at 200 °C, galactose content showed no significant differences for extraction made at 170 °C and 200 °C (Fig. 1b), which is in accordance with the phenomenon of autohydrolysis process described for spent coffee grounds using an hydrothermal pressurized system at 160 °C (Ballesteros, Teixeira, & Mussatto, 2017) or more specifically with autohydrolysis of galactose at temperatures above 170 °C (Tsubaki et al., 2008).

While the mannose quantified is directly related with the extraction of galactomannans, and the arabinose is mostly related to the extraction of arabinogalactans, the galactose is a component of both galactomannans and arabinogalactans (Nunes et al., 2005). To reveal the origin of these sugar residues by the identification of the glyosidic linkages, a methylation analysis was performed. Information on glycosidic-linkage identification and quantification obtained at different conditions with a constant temperature of 140 °C can be found in Table 2 as example. Fore more information considering the data at 140 °C, 170 °C and 200 °C can be found in Data in Brief (Passos et al., submitted) as Figs. 1–3 for identification and Tables 1–3 for quantification. As more than 50% of polysaccharides are constituted by 4-Man*p*, plus 1–2% of T-Man*p* and 1–3% of 4,6-Man*p*, it can be inferred that most of the polysaccharides extracted at 140 °C are galactomannans.

The continuous increase of terminally-linked mannose residues concomitant with galactomannans' chain length decrease was nearly proportional ($R^2 = 0.968$) to the temperature applied (Fig. 1d) and presents the same trend of the mannose sugar content (Fig. 1b). At 200 °C, with an average DP_{GM} of 11 residues, the polymers recovered achieved the oligomeric/polymeric boundary limits. This effect has been observed also for other polysaccharides, as *e.g.*, increasing temperature from 160 °C to 210 °C increased xylans extraction yield from 10% to 30% at the expense of molecular weight decrease to about half (Benko et al., 2007).

At 170 °C, more than 44% of extracted polysaccharides were constituted by galactose residues (total Gal), calculated as the sum of all galactose detected linkages: 3-Gal*p*, 3,6-Gal*p*, T-Gal*p*, and 6-Gal*p* (in the order of decreasing abundance). Although a small amount of galactose residues derived from galactomannans, as inferred by the presence of 1–4% of branched mannose residues (4,6-Man*p*) where T-Gal*p* is linked, the majority of the polysaccharides extracted are derived from arabinogalactans. As a result, while the lower temperature of 140 °C favoured the recovery of galactomannans with proportion $\eta_{AG}/\eta_{GM} < 1$, a relatively higher amount of arabinogalactans ($\eta_{AG}/\eta_{GM} > 1$) was recovered at 170 °C and 200 °C (Fig. 1c). Methylation analysis also revealed structurally distinct features of arabinogalactans extracted at 200 °C, with a decrease of the proportion of 3,6-Gal*p* residues. Comparatively, with a lower sugars yield reported for 140 °C, the same average degree of branching (DB_{AG}) of 0.33 was reported for both 140 °C and 170 °C conditions, while at 200 °C the DB_{AG} was 0.28, showing the debranching effect on arabinogalactans occurring specifically at 200 °C (Fig. 1e). These results are in accordance with the recovery of a maximum polysaccharides content at 179 °C described by Getachew, Cho, and Chun (2018) after extraction from SCG and ethanol

precipitation. The results presented in this work show the increase of the amount of the extraction of galactomannans combined with the decrease of their chain-length with the increase of the temperature. In addition, a significant degradation of arabinogalactans with a significant loss of side chains occur at temperatures above 170 °C.

3.2. Effect of treatment time

From a practical point of view, shorter times are associated with minimal processing with also reduction of the costs involved in the extraction process, which is the main advantage of the use of microwave assisted technology (Wang & Weller, 2006). *Time* (*t*) as an individual parameter, according to ANOVA results (Table 3), affected the amount of soluble solids recovered $\eta_{\text{total soluble solids}}$, the content of sugars η_{sugars}, arabinogalactans content η_{AG}, galactomannans content η_{GM}, and galactomannans degree of polymerization (DP_{GM}). A positive linear correlation with *time* (*t*) was observed for all described parameters (Fig. 2a and b), with exception for DP_{GM}, where the correlation was negative (Fig. 2c). The total soluble solids yield $\eta_{\text{total soluble solids}}$ (%, $g_{extracted}/100\ g_{SCG}$) reached 13% after 2 min, 16% after 5 min, and 18% for a maximum of 10 min. These results correspond to a positive linear response ($R^2 = 0.958$), although the increase in total soluble solids ($\eta_{\text{total Soluble Solids}}$) was only statistically significant from 2 to 5 min (Fig. 2a). Fig. 2b shows that there was no significant increase in the amount of extracted individual sugars when the time increased from 5 min to 10 min. Apart from galactomannans η_{GM} extraction yield, *time* (*t*) also affected galactomannans DP_{GM} (Table 3), which decreases with longer extraction time. Significance in DP_{GM} was observed between 5 and 10 min (Fig. 2c). Under the more drastic conditions of 10 min of extraction, the galactomannans DP_{GM} are lowered, on average, from 30 to 19.

When compared separately, both *temperature* or *time,* under the most severe conditions, namely higher *T* or longer *t*, yielding a DP_{GM} decrease to, respectively, 11 or 19, showing a more preponderant effect towards *temperature* in accordance with the lower *p-values* given in Table 3.

3.3. Interrelation between temperature and time

The use of contour plots can add additional information by defining areas of similar applicability, an example can be found for total soluble solids recovery and for the recovery of arabinogalactans in Data in Brief (Passos et al., submitted, Fig. 4a and Fig. 4b, respectively). This observation may have practical implications on the selection of the operating conditions, as a lower temperature with longer extraction time may ensure the maximum yield under more easily applicable operating conditions.

3.4. Influence of alkali addition

At high concentrations, the use of alkali conditions destroys hydrogen bonding, facilitating polysaccharide extraction (Simões et al., 2010). In this work, the use of diluted alkali treatments (*alkali*) showed only a specific effect on the yield for galactomannans [η_{GM}, ($g_{GM}/100\ g_{SCG}$)]. On the interaction of *alkali* treatment with *temperature* (Table 3), significant differences occurred only at the lowest temperature condition of 140 °C for η_{GM} (Fig. 4a) and sugars content (η_{sugars}) (Fig. 4b) obtaining a lower extraction when using alkali conditions.

The impact of alkali conditions on polysaccharides structure was highlighted by analysing the size exclusion chromatography profile (Fig. 5). The comparison of the aqueous extracts obtained at different temperatures and different times reveals a decrease of the molecular weight at the higher temperatures, as observed for ulvans polysaccharides under microwave conditions (Tsubaki et al., 2016). The combination of the alkali treatment and the lowest temperature of 140 °C yielded the highest molecular weight material at all conditions. Additionally, the use of alkali conditions decreased the fraction of lower molecular weight material at the inclusion limit and for a shorter time limit of 2 min (Fig. 5a). With increase of the temperature to 170 °C, the alkali protection was extended to 5 min (Fig. 5b). At 200 °C, a protective effect was only observed when using a 2 min exposure *time*, evidenced by a higher recovery of the higher molecular weight material only when using the alkali treatment (Fig. 5a, between 14–17 min). At 5 min, this protective effect was moderate (Fig. 5b). For the exposure time of 10 min, no differences were observed between water or *alkali* treatments at 140 °C or 170 °C (Fig. 5c).

At 200 °C/10 min condition, the safety pressure limit was achieved (55 bar), not allowing to complete the experiment. According to the results obtained, operating at higher *temperature* and for longer *time* periods, result in higher extraction yields of lower DP poly- and oligosaccharides. As temperature increases, the release of acetyl groups is able to decrease the pH of the aqueous solution and, consequently further promote the polysaccharides hydrolysis, as observed for xylans (Benko et al., 2007). The presence of alkali mitigates this effect by preventing the depolymerization of the polysaccharides, at least for shorter periods of time and especially at lower temperatures. On the other hand, higher temperatures and longer times are ideal to obtain low molecular weight polysaccharides at a higher yield.

4. Concluding remarks

In this work, microwave technology for extraction of galactomannans and arabinogalactans from spent coffee grounds has been applied under a broad range of operational conditions, which have been shown to strongly influence the structural features of the extracted polysaccharides. To extract higher amounts of arabinogalactans, higher temperatures are desirable. However, to maintain a high degree of branching (DB > 0.33), temperatures should be kept equal or lower than 170 °C. The use of alkali treatments may confer protection to high molecular weight polysaccharides at the expenses of a lower yield. On the other hand, while the extraction of galactomannans is favoured at lower temperatures, galactomannans' chain length was shown to decreased proportionally to the temperature increase. Thus, for the extraction of galactomannan-derived mannooligosaccharides, temperatures equal or higher than 170 °C would be desirable. The polysaccharides recovered at 200 °C had an average DP of 11 residues, which is at the boundary limits of the definitions of oligomeric/polymeric carbohydrates.

This study shows that the optimum conditions for carbohydrate extraction from spent coffee grounds cannot be selected only by mass yield but defined according to the desired structural features of the polysaccharides to be obtained for the specific application. These results present a contribution towards the development of industrial microwave assisted extraction processes for recovery of carbohydrate polymers from agrofood-waste material.

Acknowledgements

This work was financially supported by the project "PulManCar" - POCI-01-0145-FEDER-029560- funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/QUI/00062/2019) through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638) thanks FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. The financial support of CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through

the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement is also greatly acknowledged. Cláudia Passos (SFRH/BPD/107881/2015) and Alisa Rudnitskaya (SFRH/BPD/104265/2014) were supported by post-doc grants by FCT, while Guido Lopes (SFRH/BD/104855/2014) was supported by a doctoral grant by FCT. This work was also funded by national funds (OE), through FCT, in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. Thanks are also due to Prof. Artur Silva from the Department of Chemistry of the University of Aveiro for the assessment to the microwave facilities and assistance.

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