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Thermal stability of spent coffee ground polysaccharides: Galactomannans and arabinogalactans

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ABSTRACT

In order to better understand the thermal stability of spent coffee grounds (SCG) galactomannans and arabinogalactans and the reactions that can occur upon roasting, long term isothermal exposures, up to 3 h, were performed at 160, 180, 200, 220, and 240 °C. The resultant products were analysed according to the sugars and linkage composition and also by electrospray mass spectrometry. Galactomannans did not loss mass at $T \leq 200$ °C during exposures up to 3 h whereas the arabinogalactans showed that thermal stability only for $T \leq 180$ °C. This was in accordance with the estimated activation energies of their thermal decomposition of 138 kJ/mol and 94 kJ/mol, respectively. The roasting of galactomannans promoted the formation of new glycosidic linkages, with occurrence of 2-, 6-, 2,3-, 2,6-, 3,6-, 2,3,6-, 3,4,6-linked mannose residues, 3,4,6-linked galactose residues, and terminally-linked glucose residues, observed by methylation analysis. Depolymerisation and formation of anhydrohexose residues at the reducing end and mannose–glucose isomerisation were also observed. The roasting of galactomannans at 200 °C promoted their solubility in water upon alkali extraction and neutralisation.

1. Introduction

Roasted coffee bean polysaccharides are mainly galactomannans, type II arabinogalactans, and cellulose. During the preparation of coffee brew, a part of these polysaccharides, mainly galactomannans and also arabinogalactans, are extracted (Nunes & Coimbra, 2001). However, the majority of the galactomannans, as well as the arabinogalactans, remains associated to the cellulose-rich cell wall matrix. The galactomannans are the main components of spent coffee grounds (SCG) (Simões et al., 2009). These polysaccharides are composed by a linear (1→4)-linked mannopyranose residues backbone substituted at O-6 with single residues of galactopyranose, as well as single arabinose residues (Simões, Nunes, Domingues, & Coimbra, 2010). The galactomannans present in coffee infusions contain also β-(1→4)-D-glucopyranose residues interspersed in the main backbone (Nunes, Domingues, & Coimbra, 2005), a structural feature that may also occur in all other coffee galactomannans, as inferred by the occurrence of small amounts of (1→4)-linked glucose residues and by the presence of oligosaccharides derived from galactomannans resistant to endo-β-(1→4)-D-mannanase (Simões et al., 2010). Coffee arabinogalactans are branched polysaccharides composed by a main backbone of β-(1→3)-linked galactose residues with side chains of β-(1→6)-linked galactose oligosaccharides attached to the O-6 position of the main chain. The β-(1→6)-linked side chains are substituted at O-3 with arabinose and arabinose oligosaccharide residues presenting a rhamnose residue as non-reducing terminal (Nunes, Reis, Silva, Domingues, & Coimbra, 2008). Some populations of arabinogalactans also contain single glucuronic acid residues in the terminal position of the β-(1→6)-linked side chains (Redgwell, Curti, Fischer, Nicola, & Fay, 2002).

Coffee arabinogalactans are the most vulnerable of the coffee polysaccharides to degradation during roasting, especially the more labile arabinose residues (Moreira, Coimbra, Nunes, & Domingues, 2013; Nunes & Coimbra, 2002a, 2002b; Redgwell, Trovato, Curti, & Fischer, 2002) present as side chains. Also, the arabinogalactans depolymerize during roasting and a huge decrease in molecular weight is observed even at after a light roast (Redgwell, Trovato, et al., 2002). The debranching of the arabinose side chains seems to occur more rapidly than the hydrolysis of the galactan backbone (Oosterveld, Harmsen, Voragen, & Schols, 2003). Although coffee bean galactomannans are known to be more resistant than arabinogalactans (Oosterveld et al., 2003), it has been shown that during the roasting of the coffee beans, the galactomannans can undergo depolymerisation, debranching, Maillard reactions, caramelization, isomerisation, oxidation,

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decarboxylation, and melanoidins formation (Nunes, Reis, Domingues, & Coimbra, 2006). These changes promote the increase in coffee galactomannans extractability to the coffee brew (Nunes & Coimbra, 2001). Also, the higher the degree of roast, the higher the amount of galactomannans present in the coffee brews of both *Coffea arabica* (Nunes & Coimbra, 2002a) and *Coffea canefora* (robusta) (Nunes & Coimbra, 2002b). Also, the roasting of the coffee bean promotes the easy of extraction of cell wall polysaccharides with NaOH aqueous solutions (Oosterveld et al., 2003). Using (1→4)-linked manno-oligosaccharide model compounds, Moreira, Coimbra, Nunes, Simões, and Domingues (2011) showed that the roasting can also promote polymerisation reactions by transglycosilation, giving rise to molecules with (1→2)- and (1→6)-linked mannose residues, isomerisation reactions by the presence of (1→4)-linked glucose residues, and the occurrence of anhydrohexoses by the identification of mono- and tridehydrated derivatives. In order to better understand the thermal stability of SCG galactomannans and arabinogalactans and the reactions that can occur upon roasting, the thermal study of these SCG polysaccharides was performed. The thermograms from 20 to 600 °C at a heating rate of 10 °C/min were obtained and compared with other polysaccharides, namely cellulose, locust bean gum, and Gum Arabic. The coffee polysaccharides were also submitted to an isothermal heating at different temperatures (160, 180, 200, 220, and 240 °C) with long time of exposure, up to 3 h. The activation energies of thermal degradation were determined using both methods. The resultant products of thermal heating were analysed according to the sugars and linkage composition and also by electrospray mass spectrometry.

2. Experimental

2.1. Samples

Galactomannans (GM) and arabinogalactans (AG) were isolated from espresso spent coffee grounds (SCG) obtained after a commercial espresso coffee preparation (Simões, Nunes, Domingues, & Coimbra, 2013). SCG carbohydrates were polymeric mannose (46%), galactose (27%), glucose (20%) and arabinose (7%). The arabinogalactan-rich sample was obtained by SCG extraction with a 4 M NaOH solution. This fraction remained soluble upon neutralisation of the extract and was mainly composed by galactose (67%), arabinose (17%), mannose (15%), and glucose (1%) in a total sugars content of 71%. The galactomannan-rich sample was recovered with 4 M NaOH and became insoluble upon neutralisation of the extract. This material was mainly composed by mannose (89%), galactose (7%), arabinose (2%), and glucose (2%), in a total sugars content of 80%.

The LBG galactomannans used were obtained and purified from a commercial sample, HG M200-INDAL-Faro, Portugal, provided by Prof. José A. Lopes da Silva, from the University of Aveiro. LBG galactomannan was mainly composed by mannose (77%), galactose (22%), glucose (1%), and traces of arabinose (Simões, Nunes, Domingues, & Coimbra, 2011).

Gum Arabic and cellulose used were commercial samples. Gum Arabic was an arabinogalactan from acacia tree (Sigma), and cellulose was microcrystalline, obtained from J.T. Baker.

2.2. Roasting of coffee galactomannans and arabinogalactans

2.2.1. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was carried out using a thermogravimetric analyser (Shimadzu-50 automatic analyser, Tokyo, Japan). Assays of SCG, Gum Arabic, cellulose, LBG galactomannan, and coffee galactomannan were taken and warmed up from ambient to 600 °C at a heating rate of 10 °C/min in a dynamic (20 mL/min) air atmosphere. Thermogravimetric (TG) curves were analysed using Shimadzu TASYS software.

2.2.2. Thermal stability of coffee polysaccharides

To study the thermal stability of coffee polysaccharides, samples (15 ± 0.1 mg) of coffee galactomannans and arabinogalactans were used in experiments under a controlled air flow of 20 mL/min, and an isothermical heating at 160, 180, 200, 220, and 240 °C, during 3 h, using a thermogravimetric analyser (Shimadzu-50 automatic analyser, Tokyo, Japan).

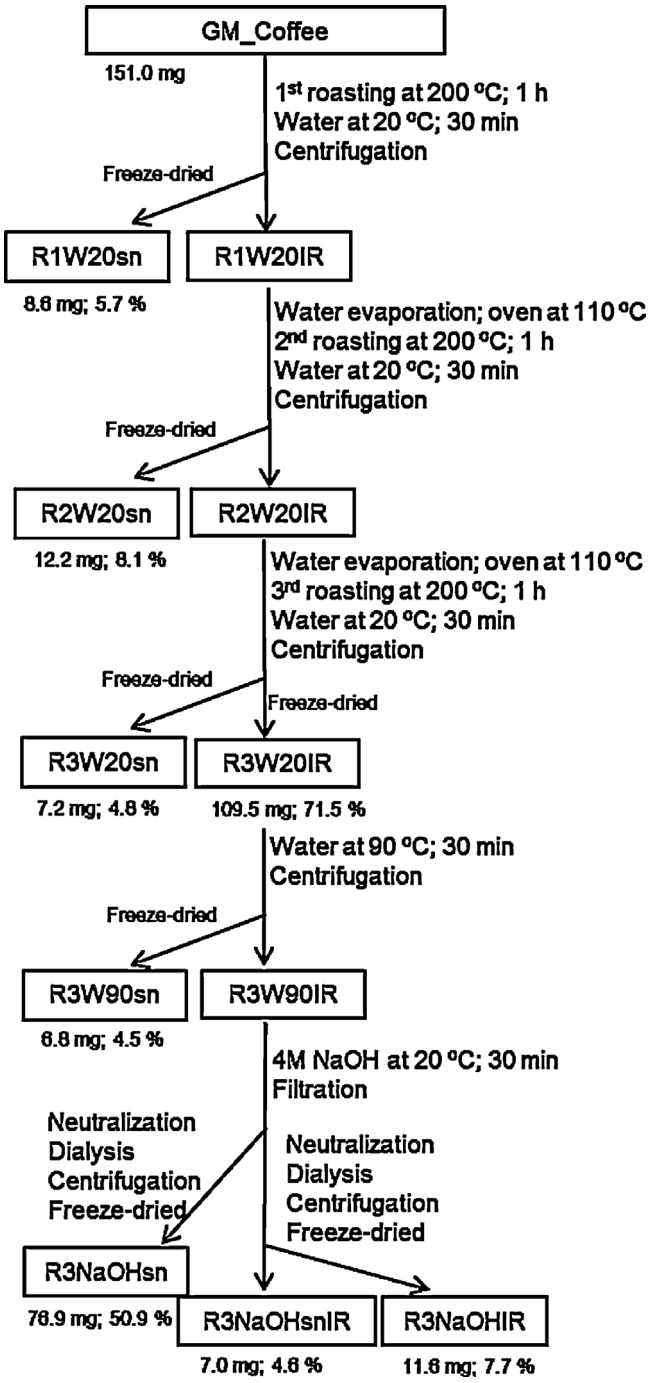
2.2.3. Roasting of manno-oligosaccharides

Oligosaccharide samples of β-(1→4)-D-mannotriose (Man_3) and β-(1→4)-D-mannotetraose (Man_4) were used (Megazyme). The Man_3 and Man_4 samples (3–5 mg) were submitted to a 160 °C and 200 °C roasting procedure. These roasting procedures were done during 2 h in a pre-heated oven (Binder) with an internal volume of 115 L.

2.2.4. Sequential roasting procedures and solubilisation of galactomannans

In order to try to convert the insoluble galactomannans recovered from SCG into cold water soluble compounds, the water insoluble coffee galactomannan rich-fraction (1.0 g) was submitted to a roasting treatment at 160 °C, in a pre-heated oven (Binder) with an internal volume of 115 L during 1 h. After the roasting procedure, the material was suspended in 100 mL of water at room temperature (20 °C) with stirring during 1 h. The suspension was then centrifuged and the supernatant was separated from the insoluble residue and freeze-dried. The residue obtained was then dried at 110 °C and submitted to another roasting treatment at 160 °C during 1 h. After the second roasting procedure at 160 °C, the material was suspended in 100 mL of water at room temperature (20 °C) with stirring, during 1 h. This repetitive process of roasting at 160 °C plus solubilisation in water were done in a total of 8 times.

The water insoluble coffee galactomannan rich-fraction (151 mg) was also submitted to a roasting treatment at 200 °C during 1 h. After the roasting procedure, the material was suspended in 50 mL of water at room temperature (20 °C) with stirring, during 1 h, as described for the 160 °C. The suspension was then centrifuged and the supernatant (R1W20sn) was separated from the insoluble residue (R1W20IR) and freeze-dried. These assays were schematically registered in Scheme 1, together with the indication of the mass balance and respective yields of extraction. The residue obtained was then dried at 110 °C and submitted to another roasting treatment at 200 °C during 1 h. After the second roasting procedure at 200 °C, the material was suspended in 50 mL of water at room temperature (20 °C) with stirring, during 1 h, allowing to obtain a new soluble fraction (R2W20sn) and a remaining insoluble residue (R2W20IR). This repetitive process of roasting at 200 °C plus solubilisation in water at 20 °C was repeated a third time allowing to obtain the R3W20sn fraction and the R3W20IR insoluble residue. The residue was then suspended in 100 mL of water at 90 °C and was kept at 90 °C under stirring during 30 min, allowing to obtain a soluble material (R3W90sn) that was separated from the residue (R3W90IR) by centrifugation. This residue was then dispersed in 100 mL of 4 M NaOH solution containing 0.02 M $NaBH_4$ at room temperature and left under stirring 30 min. The suspension was filtered and the alkali-soluble extract (R3NaOHsn) and the residue, after suspension in water, were acidified to pH 5.0 with glacial acetic acid and dialyzed for 3 days with several changes of distilled water. After dialysis of the residue, the supernatant solution that remained inside the dialysis membrane (R3NaOHsnIR) was separated by centrifugation from



Scheme 1. Spent coffee ground galactomannans solubilisation by consecutive roasting procedure at 200 °C and followed solubilisation in water and NaOH.

the insoluble material (R3NaOHIR) and both fractions were frozen and freeze dried (Coimbra, Waldron, & Selvendran, 1994).

2.3. Glycosidic-linkage analysis

The sample was dissolved in 1 mL of anhydrous dimethylsulfoxide (DMSO), then powdered NaOH (40 mg) was added and samples were methylated with CH_3I (80 μL) during 20 min. Distilled water (2 mL) and dichloromethane (3 mL) were then added, and dichloromethane phase was washed three times by addition of distilled water (2 mL). The organic phase was evaporated to dryness and the material was remethylated using the same procedure (Nunes & Coimbra, 2001). The methylated material was hydrolysed with TFA 2 M at 121 °C for 1 h, and then reduced and acetylated as previously described for neutral sugar analysis. The partially methylated alditol acetates (PMAA) were separated and analysed by gas chromatography–mass spectrometry (GC–MS) on an 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 5 min), with the injector operating at 220 °C, and using the following temperature programme: 45 °C for 5 min with a linear increase of 10 °C/min up 140 °C, and standing for 5 min at this temperature, followed by linear increase of 0.5 °C/min up to 170 °C, and standing for 1 min at this temperature, followed by linear increase of 15 °C/min up to 280 °C, with further 5 min at 280 °C. The helium carrier gas had a flow rate of 1.7 mL/min and a column head pressure of 2.8 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.

2.4. Mass spectrometry

The roasted tri- and mannotetraose oligosaccharides were dissolved in water and further diluted in methanol/water/formic acid (50:49.9:0.1, v/v/v). Positive ion ESI-MS and MS/MS spectra were acquired using a LXQ linear ion trap mass spectrometer (ThermoFinningan) using following conditions: nitrogen sheath gas 30 psi, spray voltage 5 kV, heated capillary temperature 275 °C, capillary voltage 1 V, and tube lens voltage 40 V. The flow rate was set to 8 μL/min and the voltage applied was 5 kV. Nitrogen was used as nebulising and drying gas. Full scan mass spectra ranging from m/z 100 to 1500 were acquired. In the MS/MS experiments, collision energy varied between 15 and 25 of normalised collision. Data acquisition was carried out with Xcalibur data system (Simões et al., 2011).

3. Results and discussion

3.1. Polysaccharides thermal behaviour

Fig. 1a shows the plot of the thermogravimetic analysis, performed at 10 °C/min until 600 °C, of spent coffee grounds (SCG) obtained from espresso coffee preparation. According to the first derivative plot (Fig. 1b), the major weight losses occurred at 309 °C (56%), 439 °C (19%), and 497 °C (17%). The total sample mass was lost at 510 °C, allowing to infer that the SCG did not contain significant amount of minerals. When the same experiment was performed using a coffee galactomannan-rich fraction (GM_Coffee), the major mass loss (48%) was observed at 323 °C, which is a higher temperature and lower mass loss than that observed for the SCG. This galactomannan-rich fraction showed to be more resistant to weight loss at higher temperatures than the SCG. At 540 °C all material was lost. Comparing the thermal behaviour of coffee galactomannan with locust bean gum (LBG) galactomannan, where the later contains a Man/Gal ratio of 3.5, which is lower than that verified for coffee galactomannan (12.7), it is possible to observe that the galactomannan from LBG starts degrading first than coffee galactomannans. This observation allows to infer that the SCG galactomannan was less heat labile than LBG galactomannan, possibly due to the lower degree of branching, resulting in a higher inter-chain hydrogen bonding and a material with higher ordered structure. The LBG galactomannans presented a higher decrease in mass loss (72%) than coffee galactomannans (48%) at a narrow temperature gap (317 and 323 °C, respectively), showing that the coffee galactomannan was less homogeneous than the one from

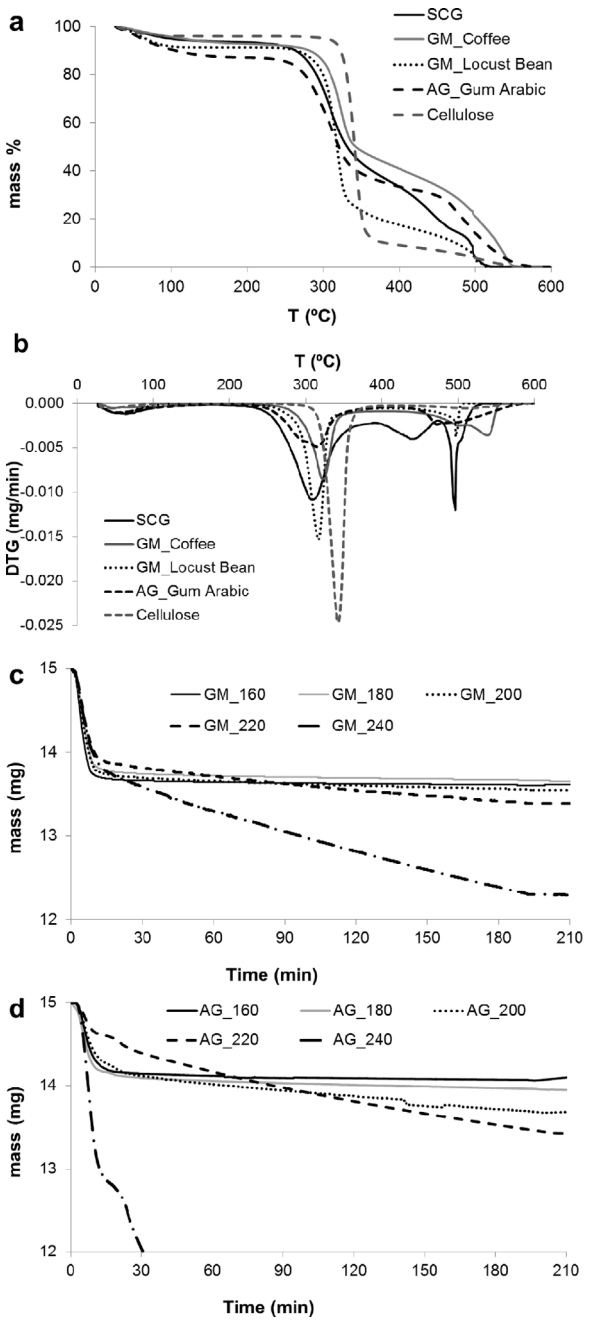


Fig. 1. (a) Thermograms heating rate of 10 °C/min and (b) respective 1st derivative of the weight loss curve of samples: SCG, GM_Coffee, GM_Locust Bean and AG_Gum Arabic. Thermograms at different isothermal (160, 180, 200, 220 and 240 °C) of (c) GM_Coffee (d) AG_Coffee.

LBG. Both galactomannan samples showed a higher temperature for the highest mass loss when compared with the SCG. Comparing the thermal degradation of all material analysed with the cellulose thermal degradation, it was observed that cellulose had the highest mass loss (86%) as well as the highest temperature of degradation (343 °C), confirming the higher homogeneity and resistance of this unbranched and highly polymerised polysaccharide, promoting higher number of intra- and inter-chain hydrogen bonds. As the SGC is composed by a mixture of galactomannans, cellulose, and arabinogalactans, the difference observed may be due to the presence of the arabinogalactans, which has been reported to be more heat labile polysaccharides (Redgwell, Trovato, et al., 2002).

In order to analyse the thermal behaviour of a pure arabinogalactan, thermogravimetric analysis of the polysaccharide from Gum Arabic was performed (Fig. 1a). The major mass loss was observed at 315 °C, which was lower when compared to the galactomannans. These results show that the thermal behaviour of SCG was modulated by the presence of all three polysaccharide families: galactomannans, cellulose, and arabinogalactans. The galactomannans and cellulose confer more thermal resistance to the coffee structure than the arabinogalactans.

According to Fig. 1a and b, it was observed that the galactomannans were degraded at temperatures approaching 300 °C. However, in long term exposures they should degrade at lower temperatures. In order to evaluate the long term thermal resistance of coffee galactomannans, experiments were performed using isothermals at 160, 180, 200, 220, and 240 °C during 3 h. Fig. 1c presents the different isothermal weight loss curves of a coffee galactomannan along the time. It is possible to observe an initial mass loss stage from 0 to 10 min, which can be attributed to the loss of sorbed water. This loss was approximately 8% for all isothermals except for 220 °C, that was slightly lower (6%). The rate of mass loss ranged between 0.120 mg/min at 160 °C and 0.096 mg/min at 240 °C. After this mass loss, no variation was observed for the experiments performed at temperatures below 200 °C during the 3 h. However, mass losses were observed at 220 °C (4%) and 240 °C (10%), ranging from 0.0028 mg/min to 0.0083 mg/min, respectively. These results show that the galactomannans are thermally stable at temperatures lower or equal to 200 °C during long term exposures up to 3 h. Taking into account that the coffee roasting process is usually performed at temperatures around 200 °C, it is expectable that during the roasting of the coffee beans the galactomannans keep their thermal stability. The thermal behaviour of the galactomannans at 220 °C shows a decrease of mass loss rate for the shorter period of temperature exposure and an increase of the long term degradation. It is possible that at this temperature less water was released due to its consumption in hydrolysis reactions, forming more thermally resistant compounds.

Fig. 1d presents the different isothermal weight loss curves of a coffee arabinogalactan along time, using the same procedure described for galactomannans (Fig. 1c). It is possible to observe, as for galactomannans, two main mass loss stages. The first one occurred from 0 to 6 min, which can be attributed to the loss of water, approximately 5% for all isotherms, except for 220 °C and 240 °C. At 200 °C it is possible to observe a slight decrease of the amount of water lost, possibly due to its consumption in hydrolysis reactions, as observed for the galactomannans. This effect is highly visible at 220 °C when only one third of the water was lost in the first minutes of heating. The rate of mass loss was 0.133 mg/min for 160 °C, 180 °C, and 200 °C assays. At 220 °C, the mass loss was not so well defined as the previous ones, possibly due to the simultaneous degradation of the polysaccharides at this stage. At 240 °C it was observed a higher extent of mass loss (23%) in the first 30 min of roasting. The mass loss that occurred from 6 to 210 min for all temperatures may be attributed to polysaccharide decomposition. This loss was approximately 6% for the experiments performed at 160 °C and 180 °C, 9% and 10% for the experiments performed at 200 °C and 220 °C, and 46% for the experiments performed at 240 °C. The rate of mass loss ranged between 0.005 mg/min at 160 °C and 0.033 mg/min at 240 °C. These results show that the coffee arabinogalactans are thermally stable at temperatures lower or equal to 180 °C during long term exposures up to 3 h, which is a temperature lower than the 200 °C observed for coffee galactomannans. Taking into account that the coffee roasting process is usually performed at temperatures around 200 °C, it is expectable that during the roasting of the coffee the arabinogalactans are degraded, contrarily to the galactomannans that keep their thermal stability.

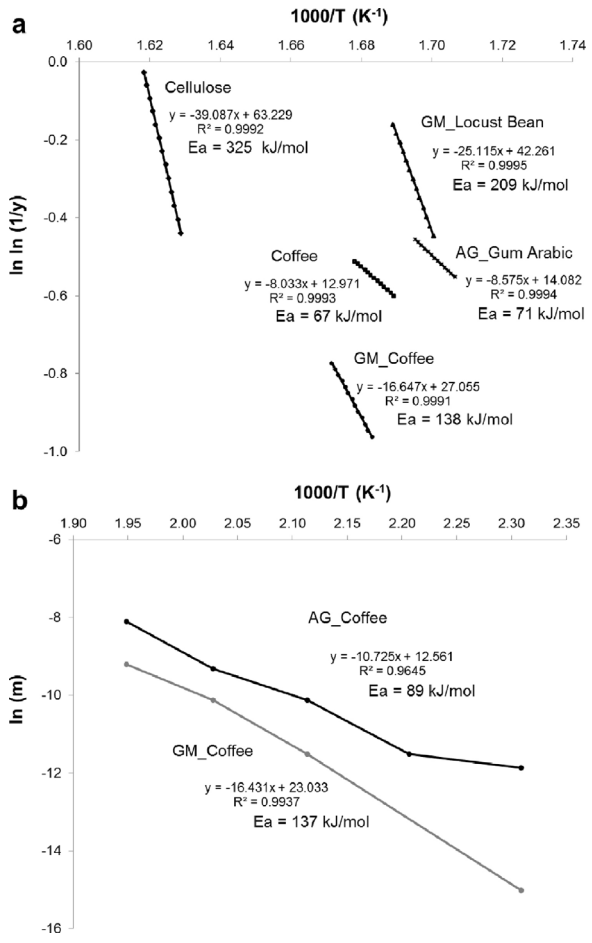


Fig. 2. Determination of activation energy of thermal decomposition.

3.2. Activation energy of thermal decomposition

The acceleration of thermal degradation can be quantitatively represented by determining the apparent activation energy of thermal decomposition (Ea) for different stages of degradation of the material. The Ea of thermal decomposition of coffee galactomannan- and arabinogalactan-rich fractions, as well as SCG, LBG galactomannan, cellulose, and arabinogalactan from Gum Arabic, were determined through the thermograms obtained. The activation energies were calculated by the integral method of Broido (Broido, 1969). The Broido equation is $\ln[\ln(1/y)] = (\mathrm{Ea}/R)/T + \text{constant}$, where y is defined by the relationship $y = (\mathrm{Wt} - \mathrm{Wf})/(\mathrm{Wi} - \mathrm{Wf})$, where Wt is the weight of the sample at any time, Wi and Wf stand for sample initial and final weight, respectively, and T is the absolute temperature (K), recorded on the thermogram. Thus, a plot of $\ln[\ln(1/y)]$ vs. $1/T$ gives a straight line whose slope is related to the Ea. The plot $\ln[\ln(1/y)]$ vs. $1000/T$ was used to calculate Ea, in kJ/mol, based on the rates of mass loss when the temperature was continuously increased at a constant rate of 10 °C/min (Fig. 2a).

The Ea of thermal decomposition of coffee galactomannan was 138 kJ/mol, which is a value lower than the 209 kJ/mol determined for LBG galactomannan. This difference could be explained by the higher molecular weight of LBG when compared to the coffee galactomannan (Simões et al., 2011). In literature no references to coffee galactomannans Ea were found and for LBG an Ea value of 110 kJ/mol was reported, although reporting only one temperature measurement, at 121 °C (Kok, Hill, & Mitchell, 1999), which is hardly comparable to the range of temperatures used in this work. As discussed by Vasile et al. (2011), depending on the heating rate and experimental conditions used to examine the kinetics, different values can emerge. The Ea values obtained for galactomannans were however lower than the Ea determined for cellulose (325 kJ/mol), which is in accordance with the thermogram profiles discussed previously. In contrast, the Ea value determined for Gum Arabic arabinogalactan (71 kJ/mol) was much lower than that observed for the galactomannans and cellulose, allowing to infer a higher heat lability of this arabinogalactan, which is in accordance with its thermogram profile shown in Fig. 1a. According to the literature, the Ea for Gum Arabic presents values varying between 122 kJ/mol (Cozic, Picton, Garda, Marlhoux, & Cerf, 2009; Zohuriaan & Shokrolahi, 2004) and 155 kJ/mol (Cozic et al., 2009). These values are higher than the ones obtained in this work probably due to the different origin/structure of the samples. The SCG presented also a lower Ea value (67 kJ/mol), showing that the arabinogalactans may have a high influence in the overall behaviour upon heating of this material. As this Ea of thermal decomposition of SCG was lower than that of the coffee arabinogalactan-rich fraction, it is possible that other compounds may be involved. As the cell wall material of roasted coffee was shown be able to generate a large amount of melanoidins (Nunes, Cruz, & Coimbra, 2012), it is possible that these compounds, still remaining in the SCG, are responsible for the higher thermal lability of the SCG.

Using the thermograms obtained by the isothermal thermogravimetric analysis (Fig. 1c and d), it was possible also to determine the Ea using the plot $\ln(m)$ vs. $1/T$ of the equivalent equation: $\ln(m) = -(\mathrm{Ea}/R)/T + \text{constant}$, where m is the slope obtained in different thermograms, T is the temperature documented on the thermogram, in K, an R is the gas constant (8.314×10^{-3} kJ K^{-1} mol^{-1}). The Ea of thermal decomposition estimated for coffee galactomannan was 137 kJ/mol, confirming the Ea value previously obtained. In this experiment it was also possible to estimate the Ea of a coffee arabinogalactan-rich fraction, showing an Ea of 89 kJ/mol, which was lower than that determined for the galactomannan. This difference allows to conclude that the coffee galactomannans are more thermal resistant than coffee arabinogalactans. Also, comparing with the Gum Arabic arabinogalactan determined in the previous experiment, the Ea of coffee arabinogalactan was slightly higher, possibly due to the higher thermal resistance that could have been conferred during the roasting process to the less branched polysaccharides left.

3.3. Structural changes of galactomannans and arabinogalactans after thermal treatments

3.3.1. Coffee galactomannans

In order to analyse the changes on the structural features of galactomannans due to the thermal procedures, a linkage analysis of galactomannan samples submitted to different temperatures of roasting during 3 h was performed (Table 1). Galactomannans are characterised by the predominance of the (1→4)-linked mannose residues (56–90 mol%), the presence of (1→4,6)- and terminally-linked mannose residues (2–9 and 2–11 mol%, respectively), as well as terminally-linked galactose residues. The relative abundance of (1→4)-linked mannose residues decreased as the temperature of roasting increased whereas the (1→4,6)- and terminally-linked mannose residues increased with the increase of the roasting temperature. As the terminally-linked Man gives a direct indication of the polymer size, it can be inferred that the polysaccharide degree of polymerisation decreased with the increase of the roasting temperatures up to 240 °C. The relative abundance of the (1→4,6)-Man residues are usually used to infer the degree of branching of the galactomannans (Nunes & Coimbra, 2001). However, the presence of 1,6-anhydromannose residues can be formed at the reducing end of manno-oligosaccharides upon roasting (Moreira et al., 2011).

Table 1
Glycosidic-linkage composition (mol%) of SCG galactomannans rich fraction submitted to different thermal treatment at 160, 180, 200, 220, and 240 °C.

Linkage	GM	GM_160	GM_180	GM_200	GM_220	GM_240
T-Ara*f*	0.1	0.4	0.4	0.3	0.3	0.1
5-Ara*f*	0.5	1.1	2.2	1.7	1.1	1.6
T-Man*p*	1.7	3.7	4.6	6.4	9.7	10.8
2-Man*p*	0.0	0.0	0.0	0.0	0.1	0.3
4-Man*p*	90.1	81.4	71.9	71.3	64.3	55.8
6-Man*p*	0.0	0.3	0.7	1.2	2.8	5.3
2,3-Man*p*	0.0	0.0	0.0	0.0	0.1	0.3
2,6-Man*p*	0.0	0.0	0.0	0.0	0.8	1.6
3,6-Man*p*	0.0	0.0	0.0	0.0	0.3	0.7
4,6-Man*p*	1.5	5.2	6.1	7.5	9.3	9.4
2,3,6-Man*p*	0.0	0.0	0.0	0.0	0.0	1.8
3,4,6-Man*p*	0.0	0.0	0.0	0.0	0.3	0.5
T-Gal*p*	0.8	1.6	1.8	1.9	1.7	1.1
3-Gal*p*	1.1	1.2	1.5	1.2	1.1	0.7
6-Gal*p*	0,0	0.6	0.7	0.8	0.8	0.7
3,6-Gal*p*	0.3	1.5	1.0	0.8	0.7	0.4
3,4,6-Gal*p*	0.0	0.0	0.0	0.0	0.1	0.2
T-Glc*p*	0.0	0.1	0.3	0.2	0.2	0.2
4-Glc*p*	4.0	2.7	8.7	6.4	6.3	8.7

Because these residues form the same partially methylated derivative diagnostic of (1→4,6)-Man residues, the interpretation of the methylation analysis results should take into consideration this artefact.

In the galactomannan-rich fraction without any thermal treatment (GM), a relative abundance of 1.5 mol% was observed for (1→4,6)-Man whereas an increase to 5.2, 6.1, 7.5, 9.3, and 9.4 mol% was observed for (1→4,6)-Man for GM_160, GM_180, GM_200, GM_220, and GM_240. As the roasting of coffee promotes the debranching of galactomannans (Nunes & Coimbra, 2002a, 2002b), it is possible that their depolymerisation allowed the formation of reducing end sugar units that, in these roasting conditions, promoted the formation of 1,6-anhydromannose residues. According to Moreira et al. (2011), the roasting also promotes the formation of new linkages and polymerisation. An increase of the formation of (1→6)-Man (0–5 mol%) and, in a small extent, (1→2)-Man residues (0.0–0.3 mol%) was observed with the increase of the temperature of roasting. These new linkages were more abundant in GM_220 and GM_240. According to Moreira et al. (2011) they may be resultant from depolymerisation together with the formation of new linkages (transglycosylation). Formation of new linkages such as (1→2,6)- (0.8–1.6 mol%), (1→3,6)- (0.3–0.7 mol%), and (1→2,3)-Man (0.1–0.3 mol%) were also observed in GM_220 and GM_240. Also, at higher temperatures it is noticed the occurrence of the formation of (1→2,3,6)- (1.8 mol%) and (1→3,4,6)-Man (0.3–0.5 mol%).

It was observed the increase of (1→4)-linked Glc along the increment of the roasting temperatures (4–10 mol%). Although (1→4)-linked Glc is part of the structure of coffee galactomannans (Nunes et al., 2005), these linkages may also occur due to isomerisation reactions upon roasting at the reducing terminal of galactomannans (Nunes et al., 2006). The occurrence of terminally-linked-Glc was observed in all samples (0.1–0.3 mol%), possibly formed by isomerisation and transglycosylation.

In all samples, it was possible to detect small amounts of (1→3)-Gal, (1→3,6)-Gal, and (1→5)-Ara, which are diagnostic linkages for the presence of arabinogalactans. It is possible that this small portion of arabinogalactan structural features associated to the galactomannans is resultant from polymerisation reactions between arabinogalactans and galactomannans (Nunes & Coimbra, 2007).

3.3.2. Model studies with mannooligosaccharides

In order to understand the effect of a long term roasting on the β-(1→4)-linked mannose residues and confirm the results obtained on roasting of the galactomannans, a model study with mannooligosaccharides were used. Mannotriose (Man_3) and mannotetraose (Man_4) oligosaccharides were roasted at 160 °C and 200 °C during 2 h and were analysed by methylation analysis (Table 2) and mass spectrometry with electrospray ionisation (ESI-MS). No weight loss was observed in these assays, although the colour of the oligosaccharides turned from white to pale yellow at 160 °C and to brittle caramel dark brown at 200 °C.

The glycosidic-linkage composition of Man_3 and Man_4 samples roasted at 160 °C and 200 °C, when compared with the non-roasted oligosaccharides, showed a decrease in the relative abundance of (1→4)-linked mannose residues from 58 to 48 and 18 mol%, respectively, for Man_3, and from 63 to 61 and 29 mol%, respectively, for Man_4. On the contrary, the terminally-linked mannose residues increased with the increase of the roasting temperature from 34 to 46 and 52 mol%, respectively, for Man_3, and from 29 to 31 and 49 mol%, respectively, for Man_4. These results show that the cleavage of (1→4)-linked residues and the appearance of higher relative proportion of terminally-linked residues, possibly

Table 2
Glycosidic-linkage composition (mol%) of mannotriose and mannotetraose with and without thermal treatment at 160 °C and 200 °C.

Linkage	Man_3	Man_3_160	Man_3_200	Man_4	Man_4_160	Man_4_200
T-Man*p*	33.7	46.4	52.1	29.1	31.4	49.2
2-Man*p*	0.0	0.0	0.1	0.0	0.0	0.1
4-Man*p*	58.1	47.8	18.4	63.3	60.7	28.7
4,6-Man*p*	3.5	1.6	7.9	2.7	1.9	5.3
6-Man*p*	0.4	0.4	17.2	0.7	0.7	7.6
T-Gal*p*	3.1	2.5	0.8	2.4	2.2	1.3
4-Glc*p*	1.0	1.2	3.5	1.4	2.3	7.3

Table 3
Glycosidic-linkage composition (mol%) of galactomannan-rich solubilised fractions.

Linkage	GM	R1W20sn	R2W20sn	R3W20sn	R3W90sn	R3NAOHsn	R3NAOHsnIR	R3NAOHIR
T-Ara*f*	0.1	0.6	0.6	0.7	0.4	0.4	0.5	0.9
5-Ara*f*	0.5	0.2	0.2	0.1	0.0	0.2	0.2	0.3
T-Man*p*	1.7	9.7	11.8	17.0	9.2	6.9	6.8	12.8
2-Man*p*	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.2
4-Man*p*	90.1	77.1	71.5	63.5	80.7	74.4	67.3	53.9
6-Man*p*	0.0	0.5	1.3	2.5	0.9	1.3	1.1	1.0
4,6-Man*p*	1.5	4.5	6.8	8.7	5.1	7.2	6.8	4.4
T-Gal*p*	0.8	2.4	2.7	3.2	1.5	2.3	3.5	6.0
3-Gal*p*	1.1	1.8	1.8	1.3	0.5	2.4	4.5	6.9
6-Gal*p*	0.0	0.7	0.9	0.8	0.2	1.0	1.7	1.9
3,6-Gal*p*	0.3	1.0	0.9	0.5	0.1	1.6	3.2	2.8
T-Glc*p*	0.0	0.1	0.1	0.1	0.7	0.1	0.3	0.7
4-Glc*p*	4.0	1.5	1.6	1.6	0.7	2.1	4.1	8.3

resultant of depolymerisation, are promoted in higher extent with the increase of the roasting temperature. It can also be noticed that the oligosaccharides roasted at 200 °C presented higher relative amounts of (1→6)-linked mannose residues, both as (1→6) or as (1→4,6). For Man_3, the proportion of these glycosidic linkages increased from 0.4 and 3.5 to 17 and 8 mol%, respectively, and for Man_4 the increase was from 0.7 and 2.7 to 8 and 5 mol%. Also, it was observed the increase of (1→4)-linked Glc from 1.0 and 1.4 mol% in Man_3 and Man_4, respectively, to 3.5 and 7.3 mol% after roasting at 200 °C for 2 h. These results are in accordance with the formation of new glycosidic linkages by transglycosilation, the formation of 1,6-anhydromannose residues, and the occurrence of isomerisation reactions similar to those observed for the coffee galactomannans submitted to the different roasting temperatures.

In order to identify the products formed when the oligosaccharides are roasted at 160 and 200 °C, they were analysed by ESI-MS. Fig. 3 shows the spectra of the oligosaccharides obtained from mannotriose after the roasting at 160 °C (Fig. 3a) and at 200 °C (Fig. 3b). At 160 °C, the major ions were observed at *m*/*z* 527, corresponding to $[Hex_3+Na]^+$, and at *m*/*z* 1031, corresponding to $[2Hex_3+Na]^+$, whereas at 200 °C, the major ions were observed at *m*/*z* 347, 509, 671, and 833, attributed to the anhydrohexose-containing ions $[Hex_{1-4}AnHex+Na]^+$. Also, although in lower abundance, this spectrum shows the ions at *m*/*z* 995, 1157, and 1319, attributed to the ions $[Hex_{5-7}AnHex+Na]^+$. The ion at *m*/*z* 527, corresponding to $[Hex_3+Na]^+$, had an abundance similar to the ion at *m*/*z* 365, corresponding to $[Hex_2+Na]^+$, showing that the formation of the polymerised anhydrous forms is also accompanied with depolymerisation reactions. These reactions occur at 200 °C but not at 160 °C, even at a long exposure of 2 h.

3.3.3. Coffee arabinogalactans

Glycosidic-linkage analysis of the arabinogalactan-rich material roasted at 160, 180, and 200 °C (Table 4) showed that the linkages diagnostic of the arabinogalactans do not show a consistent pattern, even after repetition of the methylation procedures. This may indicate a heterogeneous thermal lability of the arabinogalactans contrasting with the thermal stability of the galactomannans at these temperatures observed by the (1→4)-Man, (1→4,6)-Man, and T-Man residues also present in these samples. A consistent increase of (1→4)-Glc with the increase of the roasting temperature was also observed. This may be explained by the degradation of the sugars and isomerisation reactions that may take place (Nunes et al., 2006). No sugars were observed in the samples AG_220 and AG_240 (data not shown), possibly due to the complete transformation of the arabinogalactan related sugars or insolubilization of the arabinogalactans related material during the roasting process.

3.4. Approaches to increase water solubility of roasted galactomannans

The coffee galactomannans isolated with alkali solutions are insoluble in water. Because the roasting process can promote depolymerisation and the formation of new glycosidic linkages and, consequently, solubilisation of galactomannans, the galactomannans were roasted at different temperatures and their solubility in water was tested. Upon roasting at 160 °C during 1 h, it was possible to solubilise at room temperature only 0.7% of the insoluble galactomannans. After 8 consecutive roasting and solubilisation

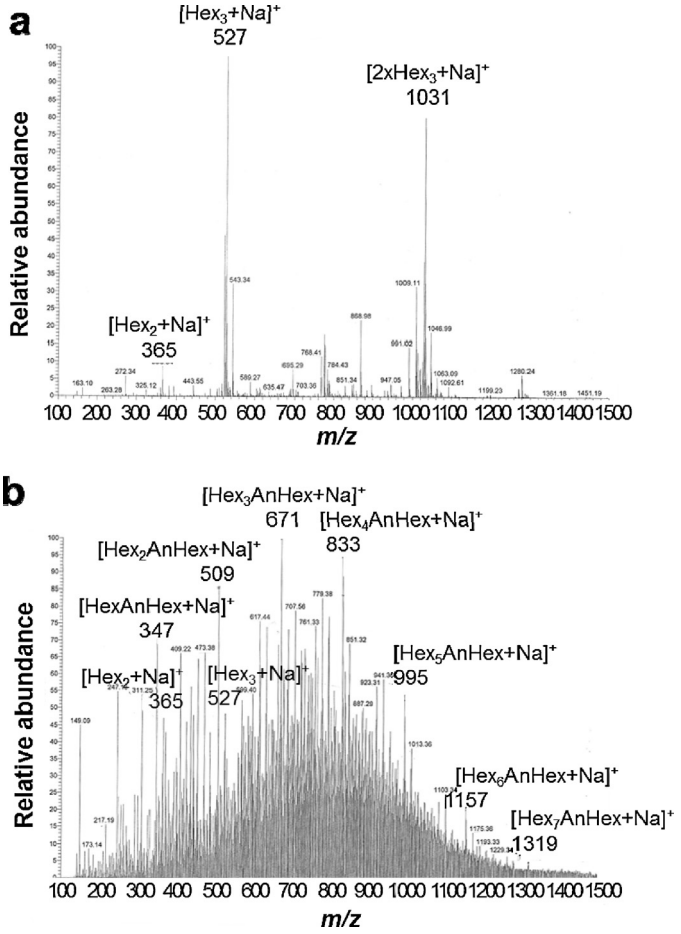


Fig. 3. ESI-MS spectra of (a) Man_3 submitted to a roasting procedure at 160 °C (b) Man_3 submitted to a roasting procedure at 200 °C.

Table 4
Glycosidic-linkage composition (mol%) of SCG arabinogalactans rich fraction submitted to different thermal treatment at 160, 180 and 200 °C.

Linkage	AG	AG_160	AG_180	AG_200
T-Ara*f*	15.4	30.6	2.6	9.8
5-Ara*f*	2.7	3.0	2.5	0.0
T-Man*p*	1.6	2.0	1.7	0.0
4-Man*p*	23.5	22.1	21.3	17.6
4,6-Man*p*	0.3	0.0	0.0	0.0
T-Gal*p*	11.0	14.8	9.2	13.3
3-Gal*p*	29.6	21.2	20.9	15.2
6-Gal*p*	3.4	1.5	6.0	0.0
3,6-Gal*p*	11.2	2.0	12.5	0.0
4-Glc*p*	1.3	6.7	23.2	53.9

procedures, only 3.3% of galactomannans have been solubilised. Thus, this low solubilisation in water confirms the high thermal stability of galactomannans at 160 °C.

In order to improve the yield of solubilisation of galactomannans, three consecutive roasting at 200 °C during 1 h and solubilisation in water at room temperature (20 °C) steps were performed (Scheme 1), allowing to obtain the fractions R1W20sn, R2W20sn, and R3W20sn. With this procedure, 18.5% of galactomannans become soluble in water at room temperature. These soluble fractions, when compared to the initial insoluble galactomannan, were shown to be composed by a higher amount of terminally-linked Man residues and a lower amount of (1→4)-linked Man residues (Table 3), indicating the presence of material with lower molecular weight. These three soluble fractions also showed higher relative amounts of (1→4,6)-linked Man residues, allowing to infer the occurrence of more branched compounds as well as the presence of 1,6-anhydromannose residues formed at the reducing end of manno-oligosaccharides upon roasting. The roasting at 200 °C promoted the formation of small amounts of (1→6)-Man residues, as well as traces of (1→2)-Man and T-Glc. The amount of these residues tends to increase with the increase of the number of consecutive roastings. In these fractions, although in a small extent, it was observed the presence of (1→3,6)-linked and (1→3)-linked Gal residues, which may represent co-solubilisation of arabinogalactans or, more probably, chimeric structures formed upon roasting between galactomannans and arabinogalactans. When the remaining insoluble material was submitted to a solubilisation in water at 90 °C it was possible to solubilise more 4.5%. Based on the glycosidic-linkage composition of this hot water solubilised galactomannan, a higher polymerisation degree than those solubilised in water at 20 °C, was observed. Also, in this solubilised material, it was observed the presence of a small amount of arabinogalactan structural features.

Because a large extent of galactomannans still remained insoluble in water, a 4 M NaOH solubilisation was done, followed by neutralisation and dialysis, allowing to obtain a fraction that remained soluble in water at room temperature, representing 51% of the initial insoluble material (R3NaOHsn). The glycosidic-linkage composition of R3NaOHsn was similar to the previous fractions, namely those obtained with water at 20 °C, except for the higher amount of (1→3,6)- and (1→3)-Gal, diagnostic of the presence of arabinogalactan structural features. The insoluble residue that remained after the NaOH solubilisation, which accounted for 12.3% of initial GM, was neutralised and dialysed, allowing to obtain another water soluble galactomannan-rich faction (R3NaOHsnIR). The remaining insoluble material (R3NaOHIR) was composed by galactomannans with a small portion of arabinogalactans, and presented a higher amount (8.6 mol%) of (1→4)-Glc. The reason for the insolubility of these polysaccharides was not disclosed.

4. Conclusions

The thermogravimetric study performed showed that the roasting at 200 °C up to 3 h promotes structural changes of the coffee residue galactomannans with no apparent weight loss. Compared with the coffee residue arabinogalactans, the galactomannans are more resistant to weight loss at this temperature. However, at 180 °C the arabinogalactans are also thermally stable up to 3 h. This different thermal stability is in accordance with their activation of energy for thermal degradation, which was estimated as 138 kJ/mol for coffee galactomannan and 94 kJ/mol for coffee arabinogalactan fractions. This higher heat lability of arabinogalactans should the responsible for the lower activation energy of thermal decomposition of SCG, while the galactomannans and cellulose should confer higher resistance. Although not leading to significant mass loss, the roast of coffee galactomannans promotes transglycosylation, as inferred by the formation of new linkages such as (1→2)- and (1→6)-linked mannose residues, formation of anhydrohexose residues at the reducing end of the polysaccharides, depolymerisation, and isomerisation reactions by formation of (1→4)-linked glucose residues. These modifications of coffee galactomannans allow their solubility in water upon alkali extraction and neutralisation.

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References

Broido, A. (1969). A simple, sensitive graphical method of treating thermogravimetric analysis data. *Journal of Polymer Science*, *7*, 1761–1773.
Coimbra, M. A., Waldron, K. W., & Selvendran, R. R. (1994). Isolation and characterization of Cell Wall Polymers from Olive Pulp (*Olea europaea* L.). *Carbohydrate Research*, *252*, 245–262.
Cozic, C., Picton, L., Garda, M. R., Marlhoux, F., & Cerf, D. (2009). Analysis of arabic gum: Study of degradation and water desorption processes. *Food Hydrocolloids*, *23*, 1930–1934.
Kok, M. S., Hill, S. E., & Mitchell, J. R. (1999). Viscosity of galactomannans during high temperature processing: Influence of degradation and solubilisation. *Food Hydrocolloids*, *13*, 535–542.
Moreira, A. S. P., Coimbra, M. A., Nunes, F. M., & Domingues, M. R. M. (2013). Roasting-induced changes in arabinotriose, a model of coffee arabinogalactan side chains. *Food Chemistry*, *138*, 2291–2299.
Moreira, A. S. P., Coimbra, M. A., Nunes, F. M., Simões, J., & Domingues, M. R. M. (2011). Evaluation of the effect of roasting on the structure of coffee galactomannans using model oligosaccharides. *Journal of Agricultural and Food Chemistry*, *59*, 10078–10087.
Nunes, F. M., & Coimbra, M. A. (2001). Chemical characterization of the high molecular weight material extracted with hot water from green and roasted arabica coffee. *Journal of Agricultural and Food Chemistry*, *49*, 1773–1782.
Nunes, F. M., & Coimbra, M. A. (2002a). Chemical characterization of galactomannans and arabinogalactans from two Arabica coffee infusions as affected by the degree of roast. *Journal of Agricultural and Food Chemistry*, *50*, 1429–1434.
Nunes, F. M., & Coimbra, M. A. (2002b). Chemical characterization of the high-molecular-weight material extracted with hot water from green and roasted Robusta coffees as affected by the degree of roast. *Journal of Agricultural and Food Chemistry*, *50*, 7046–7052.
Nunes, F. M., & Coimbra, M. A. (2007). Melanoidins from coffee infusions. Fractionation, chemical characterization, and effect of the degree of roast. *Journal of Agricultural and Food Chemistry*, *55*, 3967–3977.
Nunes, F. M., Cruz, A. C. S., & Coimbra, M. A. (2012). Insight into the mechanism of coffee melanoidin formation using modified "in bean" models. *Journal of Agricultural and Food Chemistry*, *60*, 8710–8719.
Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2005). Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. *Carbohydrate Research*, *340*, 1689–1698.
Nunes, F. M., Reis, A., Domingues, M. R., & Coimbra, M. A. (2006). Characterization of galactomannan derivatives in roasted coffee beverages. *Journal of Agricultural and Food Chemistry*, *54*, 3428–3439.

Nunes, F. M., Reis, A., Silva, A. M. S., Domingues, M. R. M., & Coimbra, M. A. (2008). Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains as structural features of coffee arabinogalactans. *Phytochemistry*, *69*, 1573–1585.

Oosterveld, A., Harmsen, J. S., Voragen, A. G. J., & Schols, H. A. (2003). Extraction and characterization of polysaccharides from green and roasted *Coffea arabica* beans. *Carbohydrate Polymers*, *53*, 285–296.

Redgwell, R., Curti, D., Fischer, M., Nicola, P., & Fay, L. (2002). Coffee arabinogalactans: Acidic polysaccharides covalently linked to proteins. *Carbohydrate Research*, *337*, 239–253.

Redgwell, R. J., Trovato, V., Curti, D., & Fischer, M. (2002). Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans. *Carbohydrate Research*, *337*, 421–431.

Simões, J., Madureira, P., Nunes, F. M., Domingues, M. R., Vilanova, M., & Coimbra, M. A. (2009). Immnunostimulatory properties of coffee mannans. *Molecular Nutrition & Food Research*, *53*, 1036–1043.

Simões, J., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2010). Structural features of partially acetylated coffee galactomannans presenting immunostimulatory activity. *Carbohydrate Polymers*, *79*, 397–402.

Simões, J., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2011). Demonstration of the presence of acetylation and arabinose branching as structural features of locust bean gum galactomannans. *Carbohydrate Polymers*, *86*, 1476–1483.

Simões, J., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2013). Extractability and structure of spent coffee ground polysaccharides by roasting pre-treatments. *Carbohydrate Polymers*, *97*, 81–89.

Vasile, C., Popescu, C. M., Popescu, M. C., Brebu, M., & Willfor, S. (2011). Thermal behaviour/treatment of some vegetable residues. IV. Thermal decomposition of eucalyptus wood. *Cellulose Chemistry and Technology*, *45*, 29–42.

Zohuriaan, M. J., & Shokrolahi, F. (2004). Thermal studies on natural and modified gums. *Polymer Testing*, *23*, 575–579.