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# Transglycosylation reactions between galactomannans and arabinogalactans during dry thermal treatment

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ABSTRACT

Aiming to investigate the possible occurrence of transglycosylation reactions between galactomannans and side chains of arabinogalactans during coffee roasting, mixtures of β-(1→4)-D-mannotriose and α-(1→5)-L-arabinotriose were subjected to dry thermal treatments at 200 °C. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis allowed identifying polysaccharides composed by pentose and hexose residues with a degree of polymerization up to 18 residues. Methylation analysis showed the occurrence of new types of glycosidic linkages in all thermally treated mixtures, as well as the occurrence of terminally and 5-linked ribose, possibly formed from arabinose isomerization. Also, xylose and lyxose were identified and proposed to be formed from mannose. These results support the occurrence of transglycosylation reactions promoted by roasting involving both oligosaccharides in the starting mixtures, resulting in arabinan and mannan chimeric polysaccharides. These structural features were also found in roasted coffee polysaccharide samples.



## 1. Introduction

The two most abundant polysaccharides in green coffee beans are galactomannans and type II arabinogalactans. They comprise, respectively, 22% and 14–17% of the beans' dry weight (Bradbury & Halliday, 1990). The galactomannans which are extractable with hot water are composed by a main backbone of β-(1→4)-linked D-mannose residues, some of them substituted at *O*-6 by single residues of α-D-galactose or L-arabinose and at *O*-2 and/or *O*-3 by acetyl groups. Also, β-(1→4)-linked D-glucose residues are components of the mannan backbone (Nunes, Domingues, & Coimbra, 2005). The arabinogalactans which are extractable with hot water are composed by a main backbone of β-(1→3)-linked D-galactose residues, some of them substituted at *O*-6 with short chains of β-(1→6)-linked D-galactose residues. The galactose residues of these short chains are substituted with various combinations of α-L-arabinose, α-L-rhamnose, and β-D-glucuronic acid residues. Specifically, side chains composed by α-(1→5)-linked L-arabinose residues are present (Nunes, Reis, Silva, Domingues, & Coimbra, 2008).

During the coffee roasting process, galactomannans and arabinogalactans undergo structural modifications, namely they undergo depolymerization and debranching. However, arabinogalactans are the most susceptible to roasting-induced modifications, especially the arabinose residues present in their side chains (Nunes & Coimbra, 2002; Oosterveld, Harmsen, Voragen, & Schols, 2003; Oosterveld, Voragen, & Schols, 2003; Redgwell, Trovato, Curti, & Fischer, 2002). Depending on the degree of roast of coffee beans, 43–80% of these residues were shown to be degraded (Redgwell et al., 2002). The lower thermal stability of the arabinose side chains was also evidenced when a structurally related oligosaccharide, α-(1→5)-L-arabinotriose ($Ara_3$), and oligosaccharides structurally related to galactomannans, namely β-(1→4)-D-mannotriose ($Man_3$), were individually subjected to dry thermal treatments mimicking coffee roasting conditions. Despite the different thermal stability of these oligosaccharides, in both studies, the identification of polymerized products and new types of glycosidic linkages allowed inferring the occurrence of transglycosylation reactions (Moreira, Coimbra, Nunes, & Domingues, 2013; Moreira, Coimbra, Nunes, Simões, & Domingues, 2011). Also, the roasting of spent coffee grounds (SCG) galactomannans promoted the formation of new types of glycosidic linkages (Simões, Maricato, Nunes, Domingues, & Coimbra, 2014). Together, these studies support the hypothesis of the occurrence of transglycosylation reactions during coffee roasting, namely between

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galactomannans and arabinose side chains of arabinogalactans. The galactomannans extracted with hot water from roasted coffee beans were found to contain less arabinose residues than those extracted from green beans (Nunes et al., 2005). This finding, however, does not exclude the presented hypothesis, because galactomannans containing a higher amount of arabinose residues can occur in the unextractable polysaccharide fraction, as supported by sugar and methylation analyses of the galactomannans recovered from SCG (Simões, Nunes, Domingues, & Coimbra, 2010). Also, the possible products formed during coffee roasting by transglycosylation reactions between galactomannans and arabinogalactans can be incorporated in melanoidin structures, since these polysaccharides are known to be involved in melanoidin formation (Moreira, Nunes, Domingues, & Coimbra, 2012).

Aiming to investigate the possible occurrence of transglycosylation reactions between galactomannans and arabinose side chains of arabinogalactans during coffee roasting, mixtures of $Ara_3$ and $Man_3$ were subjected to dry thermal treatments at 200 °C, using the experimental conditions used in the previous studies with each individual oligosaccharide (Moreira et al., 2011, 2013). As the distribution of galactomannans and arabinogalactans in the coffee bean cell wall was shown to be heterogeneous (Sutherland, Hallett, MacRae, Fischer, & Redgwell, 2004) and these polysaccharides have a different vulnerability to roasting-induced degradation, three mixtures with different molar proportions of $Man_3$ and $Ara_3$ were used to mimic possible regions within the cell wall with a distinct polysaccharide composition. The products formed during thermal treatment of each oligosaccharide mixture were analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), and also according to the sugar and glycosidic linkage compositions. In order to support the formation of chimeric polysaccharide structures of arabinogalactans and galactomannans upon roasting, roasted coffee polysaccharide-rich fractions were also treated with specific glycosidases.

## 2. Materials and methods

### 2.1. Oligosaccharide samples

α-(1→5)-L-Arabinotriose ($Ara_3$, syrup) and β-(1→4)-D-mannotriose ($Man_3$, powder), having a purity ≥95%, were purchased from Megazyme (County Wicklow, Ireland). The syrup sample was diluted with ultrapure water and freeze-dried. The resulting material was ground with an agate mortar and pestle and then stored at room temperature in a desiccator under a phosphorous pentoxide environment until to be used in the preparation of the oligosaccharide mixtures.

### 2.2. Preparation of the oligosaccharide mixtures and thermal treatments

Solutions of $Ara_3$ (53.3 mg, 0.129 mmol) and $Man_3$ (64.9 mg, 0.129 mmol) in 200 mL of ultrapure water (Millipore Synergy System, Billerica, MA) were prepared. Using these solutions, three mixtures were prepared containing different molar proportions of $Ara_3$ and $Man_3$ as follows: 25% of $Ara_3$ and 75% of $Man_3$, 50% of $Ara_3$ and 50% of $Man_3$, and 75% of $Ara_3$ and 25% of $Man_3$. These mixtures are designated as A25M75, A50M50, and A75M25, respectively. After freeze-drying, the solid mixtures were powdered with an agate mortar and pestle, and then stored at room temperature in a desiccator under a phosphorous pentoxide environment.

The oligosaccharide mixtures were submitted to the same temperature programs used in the previous studies with $Man_3$ (Moreira et al., 2011) and $Ara_3$ (Moreira et al., 2013), using a TGA-50 thermogravimetric analyser (Shimadzu, Kyoto, Japan). Three samples of each solid mixture (3–8 mg) were heated from room temperature up to 200 °C at a heating rate of 10 °C/min in a dynamic air atmosphere (20 mL/min), and maintained at 200 °C for different periods of time (0, 30, and 60 min). These treatments are respectively designated as T1, T2, and T3, whereas the untreated mixtures are designated as T0. The thermally treated samples were recuperated, weighed, and dissolved with ultrapure water to give a concentration of about 5 mg/mL. To facilitate their dissolution, they were then stirred at 37 °C for 3 h. The water-soluble fractions were separated and kept frozen at −20 °C until analysis by MALDI-MS. Solutions (1 mg/mL) of each untreated mixture were prepared and stored under the same conditions.

### 2.3. Spent coffee ground polysaccharides and enzymatic hydrolysis

A galactomannan-rich fraction containing arabinogalactans was isolated from spent coffee grounds (SCG) obtained after a commercial espresso coffee preparation. This fraction was recovered with 4 M NaOH and became insoluble upon neutralization of the extract (Simões, Nunes, Domingues, & Coimbra, 2013).

In order to convert the insoluble material recovered from SCG into water soluble, it 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, as described by Simões et al. (2013). 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. These processes of roasting at 160 °C plus solubilization in water were repetitively done in a total of 7 times.

A sample of the solubilized coffee polysaccharides (15 mg) was hydrolysed with 1 U of a α-galactosidase from Guar seed (Megazyme, EC 3.2.1.22) during 24 h at 40 °C with continuous stirring in a 100 mM Na-acetate buffer, pH 4.5, containing 0.02% sodium azide. The coffee polysaccharide sample (15 mg) was also hydrolysed with 1 U of a β-galactosidase from *Escherichia coli* (Sigma, EC 3.2.1.23) during 24 h at 37 °C with continuous stirring in a 100 mM Na-acetate buffer, pH 7.3, containing 0.02% sodium azide. The hydrolysed materials were dialyzed (MW cut-off 12–14 kDa) for 3 days, with several changes of distilled water. The high molecular weight materials were collected, frozen, and freeze-dried for further linkage analysis.

### 2.4. Instant coffee sample, enzymatic hydrolysis, and fractionation by ethanol precipitation

The instant coffee powder was obtained in a local supermarket from a commercial batch (Néscafe, Nestlé). A sample (400 g) was dissolved in 1 L of distilled water at 80 °C, and stirred during 1 h. The solution was cooled down at room temperature, and then was placed at 4 °C for a period of 48 h, prior to the decantation. The cold water insoluble material recovered was discarded. The supernatant was then centrifuged at 15,000 rpm for 15 min at 4 °C and the precipitate and supernatant recovered were frozen and freeze-dried.

The precipitate (5 g) was partially hydrolysed with 11.25 U of a pure *endo*-β-(1→4)-D-mannanase preparation (Megazyme, *Aspergillus niger*, EC 3.2.1.78) during 1 h at 37 °C with continuous stirring in 25 mL of 10 mM phosphate buffer, pH 5.5. The precipitate and supernatant recovered after hydrolysis were named respectively as Mannanase_Ppt and Mannanase_Sn. The Mannanase_Sn (40 mg in 1 mL of distilled water) was loaded on a XK26-100 column (Pharmacia) containing Bio-Gel P-30 (Bio-Rad, 2.5–40 kDa fractionation range) using 0.1 M NaCl as eluent. The collected fractions were assayed for sugars according to the phenol-sulphuric acid

method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The Mannanase_Sn (150 mg in 15 mL of distilled water) was also fractionated by graded ethanol precipitation. First, 15 mL of absolute ethanol were added to reach an aqueous solution containing 50% ethanol (v/v). The solution was then centrifuged at 15,000 rpm for 10 min at 4 °C. The precipitate recovered was named as Et50. To the supernatant was added absolute ethanol to reach an aqueous solution containing 75% ethanol (v/v). After centrifugation, the precipitate (Et75) and supernatant (Sn) were recovered.

### 2.5. Sugar and linkage analyses

Neutral sugars were converted to alditol acetates and analyzed by gas chromatography (GC) on a PerkinElmer Clarus 400 chromatograph (Waltham, MA) equipped with a flame ionization detector (FID) and a DB-225 column with 30 m of length, 0.25 mm of internal diameter, and 0.15 μm of film thickness (J&W Scientific, Folsom, CA). 2-Deoxyglucose was used as internal standard. To check the presence of other sugars not identified by GC-FID, alditol acetate derivatives from the untreated and thermally treated oligosaccharide mixtures were also analyzed by gas chromatography–mass spectrometry (GC–MS) on an Agilent Technologies 6890N Network (Santa Clara, CA) equipped with a DB-1 column with 30 m of length, 0.25 mm of internal diameter, and 0.1 μm of film thickness (J&W Scientific, Folsom, CA) and connected to an Agilent 5973 Network Mass Selective Detector. For linkage analysis, sugars were converted to partially *O*-methylated alditol acetates (PMAAs) and analyzed by GC–MS (Nunes et al., 2012).

### 2.6. Matrix-assisted laser desorption/ionization mass spectrometry

Untreated and thermally treated oligosaccharide mixtures, previously dissolved in water, were prepared for MALDI-MS analysis by mixing 5 μL of each sample to 5 μL of 2,5-dihydroxybenzoic acid matrix (10 mg/mL in methanol). From this mixture, 0.5 μL were deposited on the MALDI plate, allowed to dry at ambient conditions. Positive full-scan mass spectra were acquired over the *m*/*z* range 450–4000 on a MALDI-TOF/TOF Applied Biosystems 4800 Proteomics Analyser (Framingham, MA) instrument equipped with a nitrogen laser emitting at 337 nm and operating in a reflectron mode.

## 3. Results and discussion

### 3.1. Identification of roasting-induced products by MALDI-MS

To identify the structural modifications promoted by dry thermal treatment, untreated and thermally treated oligosaccharide ($Man_3$ and $Ara_3$) mixtures were analyzed by MALDI-MS in the positive-ion mode.

The MALDI-MS spectra of the thermally untreated mixtures (A50M50 in Fig. 1A) showed the ions at *m*/*z* 527 and 543, attributed to $[Man_3+Na]^+$ and $[Man_3+K]^+$, respectively. As previously reported for MALDI-MS analysis of $Man_3$ (Moreira et al., 2011), the $[M+Na]^+$ ion showed higher abundance than $[M+K]^+$ ion. Also, the ion at *m*/*z* 453 ($[Ara_3+K]^+$) was observed. It was not possible to observe the corresponding sodium adduct at *m*/*z* 437 ($[Ara_3+Na]^+$), because the spectra were only acquired from *m*/*z* 450 due the presence of background matrix ions at lower *m*/*z* values.

In addition to the aforementioned ions, new ions were observed in the MALDI-MS spectra of the thermally treated oligosaccharide mixtures. The spectrum of the A50M50 mixture heated to 200 °C (T1) is shown in Fig. 1B as an example. Considering all the MALDI-MS spectra acquired from the three different mixtures (A25M75, A50M50, and A75M25) submitted to the three different treatments (T1, T2, and T3), it was possible to observe several ions at *m*/*z* up to 2147, which is attributable to the sodium adduct of a polysaccharide composed by 13 hexose (Hex) residues. As part of $[Hex_m+Na]^+$ series were also identified the ions at *m*/*z* 689, 851, 1013, 1175, 1337, and 1499 (*m* = 4–9), as well as the ion at *m*/*z* 1823 (*m* = 11). These spectra also showed several ions at *m*/*z* up to 2813, which is attributable to the sodium adduct of a polysaccharide composed by 21 pentose (Pent) residues. As part of $[Pent_n+Na]^+$ series were also observed the ions at *m*/*z* 569, 701, 833, 965, 1097, 1229, 1361, 1493, and 1625 (*n* = 4–12). These results are in accordance with the data previously reported for $Man_3$ (Moreira et al., 2011) and $Ara_3$ (Moreira et al., 2013) when individually subjected to the same thermal treatments, confirming the occurrence of transglycosylation reactions involving the same type of sugar residues, and the formation of polysaccharides from the oligosaccharide mixtures submitted to dry heating conditions.

The MALDI-MS spectra of the thermally treated mixtures also showed several new ions attributable to $[M+Na]^+$ adducts of polysaccharides composed by both Pent and Hex residues, with *m*/*z* up to 2603, attributable to $PentHex_{15}$ (Table 1). As part of the same series were also identified the ions at *m*/*z* 497, 659, 821, 983, 1145, 1307, 1469, and 1631, attributable to $PentHex_{2-9}$, and at *m*/*z* 1955 ($PentHex_{11}$). Similarly, combinations of structures containing 2–15 Pent residues and single to 13 Hex residues were identified (Table 1). These results support the hypothesis of the occurrence of transglycosylation reactions involving sugar residues from different origins, and the formation of chimeric polysaccharides from the oligosaccharide mixtures when submitted to dry heating conditions.

As previously observed, when the $Man_3$ (Moreira et al., 2011) and $Ara_3$ (Moreira et al., 2013) were individually subjected to the same thermal treatments, or when $Man_3$ was roasted at 200 °C during 2 h (Simões et al., 2014), the formation of dehydrated derivatives was observed, particularly in the mixtures subjected to the longer thermal treatments (T2 and T3). Some of these derivatives have the same nominal mass (calculated by adding the mass of the predominant isotope of each element contributing to the molecule rounded to the nearest integer value) of non-modified oligo- or polysaccharides, as for example the ion observed at *m*/*z* 1229, identified as $[Pent_9+Na]^+$, but also attributable to $[Pent_3Hex_5\text{-}H_2O+Na]^+$. Thus, for the oligosaccharide mixtures subjected to the T2 or T3 treatment, the coexistence of different isobaric compounds, i.e. compounds of the same nominal mass but of different elemental composition, cannot be excluded. In order to gain additional structural information about the intact carbohydrate polymers formed under the dry heating conditions, the sugar compositions and their glycosidic linkages were analyzed.

### 3.2. Sugar and glycosidic linkage compositions

The results of sugar and glycosidic linkage analyses obtained for both untreated and thermally treated mixtures are shown in Table 2. For thermally untreated ones (T0), the molar percentages of arabinose (Ara) and mannose (Man) (28.5 and 69.2% for A25M75; 56.7 and 41.8% for A50M50; and 75.9 and 22.9% for A75M25, respectively) obtained by GC-FID analysis of the alditol acetate derivatives are in line with the proportions of $Ara_3$ and $Man_3$ used in the preparation of the oligosaccharide mixtures. It should be taken into account that the percentages of T-Ara*f*, 5-Ara*f*, T-Man*p*, and 4-Man*p*, determined by direct quantification of the areas of the PMAA derivatives given by GC–MS, are not in accordance with such proportions, possibly due to the differences in the response factors of the partially methylated hexitol acetates and partially methylated pentitol acetates.

For all thermally treated mixtures, new types of glycosidic linkages (absent in the untreated ones) were identified, corroborating

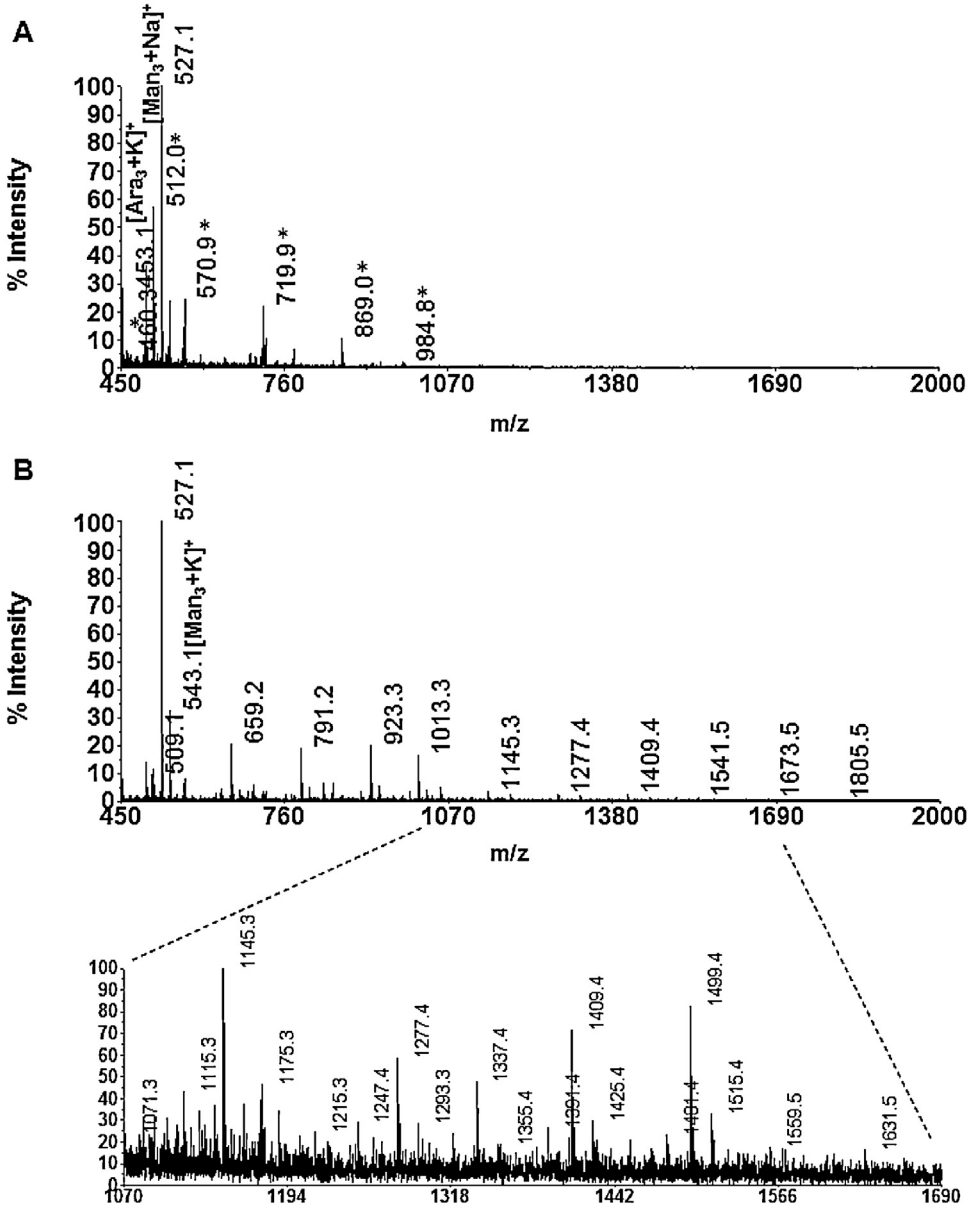


**Fig. 1.** MALDI-MS spectra of the A50M50 mixture (A) before and (B) after thermal treatment T1 ($m/z$ 1070–1690 in inset). The ions marked with an asterisk (*) were attributed to impurities.

**Table 1**
Oligo- and polysaccharides composed by pentose and hexose residues ($Pent_nHex_m$) identified in the MALDI-MS spectra of the thermally treated mixtures as $[M+Na]^+$ ions with the indication of the $m/z$ value and the proposed composition.

| $n$ | $m$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 13 | 15 |
| 1 | | 497 | 659 | 821 | 983 | 1145 | 1307 | 1469 | 1631 | 1955 | | 2603 |
| 2 | 467 | 629 | 791 | 953 | 1115 | 1277 | 1439 | | | | 2411 | |
| 3 | 599 | 761 | 923 | 1085 | 1247 | 1409 | | | 1895 | | | |
| 4 | 731 | 893 | 1055 | 1217 | 1379 | 1541 | 1703 | 1865 | 2027 | | | |
| 5 | 863 | 1025 | 1187 | 1349 | 1511 | 1673 | | | | | | |
| 6 | 995 | 1157 | 1319 | 1481 | 1643 | 1805 | 1967 | | | | | |
| 7 | 1127 | 1289 | 1451 | | | | | | | | | |
| 8 | 1259 | 1421 | 1583 | 1745 | | | | | 2555 | | | |
| 9 | 1391 | 1553 | 1715 | | | | | | | | | |
| 11 | 1655 | 1817 | | | | | | | | | | |
| 12 | | 1949 | | | | | | | | | | |
| 13 | | 2081 | | | | | | | | | | |
| 15 | | | 2507 | | | | | | | | | |

**Table 2**
Sugar and glycosidic linkage compositions of the oligosaccharide mixtures before (T0) and after thermal treatments (T1, T2, and T3).

| Linkages | A25M75 | | | | A50M50 | | | | A75M25 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T0 | T1 | T2 | T3 | T0 | T1 | T2 | T3 |
| T-Ara*f* | 5.4 | 11.6 | 12.9 | 15.0 | 12.5 | 13.8 | 17.3 | 14.6 | 27.6 | 24.7 | 38.7 | 23.9 |
| 2-Ara*f* | | | 0.8 | 1.7 | | 0.4 | 2.2 | 2.3 | | 0.7 | 3.0 | 4.1 |
| 3-Ara*f* | | | 0.9 | 1.3 | | 0.4 | 3.1 | 3.7 | | 0.9 | 4.3 | 7.2 |
| 5-Ara*f* | 7.4 | 8.5 | 7.6 | 7.8 | 20.3 | 20.4 | 14.9 | 15.2 | 38.0 | 35.3 | 28.6 | 25.0 |
| 2,5-Ara*f* | | | 0.5 | 0.8 | | 0.6 | 2.4 | 3.3 | | 1.0 | 2.2 | 6.3 |
| 3,5-Ara*f* | | | 0.4 | 0.5 | | 0.4 | 2.1 | 3.4 | | 1.0 | 2.0 | 6.2 |
| *Total* | 12.8 (28.5)[a] | 20.1 (23.7) | 23.1 (28.8) | 27.1 (29.8) | 32.8 (56.7) | 36.0 (50.1) | 42.0 (55.4) | 42.5 (57.3) | 65.6 (75.9) | 63.6 (74.9) | 78.8 (76.5) | 72.7 (76.4) |
| T-Rib*f* | | | 0.3 | 0.4 | | | 0.3 | 0.4 | | | 0.6 | 0.7 |
| 5-Rib*f* | 0.2 | 0.2 | 0.8 | 0.3 | 0.4 | 0.6 | 0.4 | 0.7 | 1.2 | 1.1 | 0.6 | 1.8 |
| *Total* | 0.2 (t) | 0.2 (0.5) | 1.1 (0.8) | 0.7 (0.8) | 0.4 (0.2) | 0.6 (1.2) | 0.7 (1.2) | 1.1 (1.3) | 1.2 (0.4) | 1.1 (1.7) | 1.2 (2.0) | 2.5 (3.7) |
| T-Pent*p*[b] | 0.2 | 0.2 | 0.8 | 1.0 | 0.3 | 0.2 | 0.9 | 1.4 | 0.9 | 0.4 | 1.5 | 2.1 |
| 4-Pent*p* | 0.1 | 0.3 | 0.4 | 0.4 | 0.3 | 0.6 | 0.4 | 0.2 | 0.2 | 0.8 | 0.9 | 0.3 |
| *Total* | 0.3 (t) | 0.5 (0.6) | 1.2 (0.3) | 1.4 (0.2) | 0.6 (t) | 0.8 (1.1) | 1.3 (1.2) | 1.6 (0.2) | 1.1 (t) | 1.2 (0.2) | 2.4 (0.2) | 2.4 (0.2) |
| T-Man*p* | 34.3 | 46.8 | 35.5 | 33.6 | 27.6 | 26.9 | 17.6 | 14.9 | 14.6 | 14.9 | 9.5 | 6.7 |
| 2-Man*p* | | | 1.8 | 1.8 | | 0.3 | 1.6 | 1.8 | | 0.2 | 0.1 | 0.6 |
| 4-Man*p* | 46.7 | 29.3 | 16.2 | 12.4 | 33.2 | 28.3 | 12.6 | 9.9 | 15.6 | 14.9 | 5.6 | 4.2 |
| 6-Man*p* | | 1.0 | 11.5 | 10.6 | | 2.5 | 9.9 | 11.0 | | 1.5 | 1.5 | 4.9 |
| 2,3-Man*p* | | | 0.2 | 0.4 | | | 0.3 | 0.3 | | | | |
| 2,4-Man*p* | | | 0.4 | 0.4 | | 0.4 | 1.1 | 0.8 | | | | 0.3 |
| 2,6-Man*p* | | | 0.5 | 0.8 | | | 1.2 | 2.1 | | | | 0.7 |
| 3,4-Man*p* | | | 0.1 | 0.2 | | | 0.2 | 0.2 | | | | |
| 3,6-Man*p* | | | | 0.2 | | | 0.4 | 0.6 | | | | 0.3 |
| 4,6-Man*p* | 2.0 | 0.4 | 4.8 | 4.9 | 1.7 | 2.4 | 7.3 | 8.2 | 0.6 | 1.2 | 0.5 | 3.4 |
| 2,3,6- and 2,4,6-Man*p* | | | 0.2 | 0.7 | | | 1.4 | 2.1 | | | | |
| 3,4,6-Man*p* | | | | | | | | 0.2 | | | | |
| *Total* | 83.0 (69.2) | 77.5 (72.5) | 71.2 (65.4) | 66.0 (64.9) | 62.5 (41.8) | 60.8 (44.8) | 53.6 (40.1) | 52.1 (38.7) | 30.8 (22.9) | 32.7 (22.0) | 17.2 (18.2) | 21.1 (17.9) |
| T-Gal*p* | 1.8 | 0.9 | 0.5 | 0.3 | 1.4 | 0.9 | 0.4 | 0.4 | 0.7 | 0.5 | t[c] | 0.1 |
| 6-Gal*p* | | | | | | | 0.1 | 0.1 | | | | |
| *Total* | 1.8 (2.0) | 0.9 (1.8) | 0.5 (2.7) | 0.3 (2.9) | 1.4 (1.1) | 0.9 (1.4) | 0.5 (1.4) | 0.5 (1.6) | 0.7 (0.7) | 0.5 (0.6) | t (0.9) | 0.1 (0.8) |
| T-Glc*p* | 0.6 | 0.1 | 0.8 | 0.8 | 0.8 | 0.9 | 0.1 | 0.2 | 0.2 | 0.2 | t | t |
| 4-Glc*p* | 1.3 | 0.7 | 2.0 | 3.6 | 1.5 | 0.1 | 2.0 | 2.3 | 0.4 | 0.7 | 0.4 | 1.1 |
| *Total* | 1.9 (0.3) | 0.8 (1.0) | 2.8 (2.0) | 4.4 (1.4) | 2.3 (0.2) | 1.0 (1.4) | 2.1 (0.7) | 2.5 (0.9) | 0.6 (0.1) | 0.9 (0.6) | 0.4 (2.2) | 1.1 (1.0) |

[a] Values in brackets are the molar percentages obtained by GC-FID analysis of the alditol acetate derivatives. The other values were obtained by GC–MS analysis of the PMAA derivatives.
[b] Pent*p* = Lyx*p* or Xyl*p*.
[c] traces (<0.1%).

the occurrence of transglycosylation reactions inferred by MALDI-MS (Table 2). The new Ara*f* linkages are in accordance with the previous observations of 2-, 3-, 2,5-, and 3,5-Ara*f* formed when $Ara_3$ was subjected to the same thermal treatments (Moreira et al., 2013). The formation of 2-, 6-, 2,3-, 2,4-, 2,6-, 3,4-, 3,6-, 2,3,6-, 2,4,6-, and 3,4,6-Man*p* was also observed for the thermally treated oligosaccharide mixtures. The transglycosylation reactions are exemplified in Fig. 2A for the formation of 6-linked Man*p* by reaction of a reducing Ara*f* with a Man*p*. Revisiting the results obtained when $Man_3$ was submitted to the same thermal treatments (Moreira et al., 2011), beyond the 2- and 6-Man*p* previously identified, the other new linkages were also identified. These new Man*p* linkages, except 2,4- and 3,4-linkages, were likewise observed for the roasted coffee polysaccharide data (Simões et al., 2014).

An increase of glucose (Glc*p*), namely of 4-Glc*p*, was also observed after thermal treatment of the oligosaccharide mixtures. D-Glc*p* can be formed from D-Man*p* via isomerization at C2 (Fig. 2B) (Moreira et al., 2011; Simões et al., 2014). Similarly, L-Rib*f* should be formed from L-Ara*f* via isomerization at C2 (Fig. 2C). To disclose this hypothesis, the alditol acetates of all untreated and thermally treated oligosaccharide mixtures were analyzed by GC-FID and GC–MS, allowing identifying as ribose a peak eluting immediately before the arabinose. Accordingly, a peak characteristic of 5-Pent*f* was observed eluting close to 5-Ara*f* in the GC–MS chromatograms of the PMAA derivatives of all samples (Table 2), hypothesized as 5-Rib*f*, resulting from the isomerization of 5-Ara*f*. This isomerization can be promoted by the alkali extraction of the arabinan (calcium hydroxide solution at 90 °C, according to Megazyme) used to obtain the commercial $Ara_3$ standard, explaining the presence of 5-Rib*f* in the unroasted samples. Thus, although the relative proportion of this linkage tends to increase upon roasting when compared to the unroasted samples, the presence of 5-Rib*f* cannot be attributed to a roasting promoted reaction. However, a peak characteristic of terminally linked Pent*f* was also observed in heated samples but not in the untreated ones. This result should evidence the transglycosylation reactions that can be also expected to occur with 5-Rib*f*, giving origin to T-Rib*f* (Table 2). Accordingly, the peaks corresponding to T- and 5-Rib*f* were also found revisiting the linkage analysis chromatograms of the thermally treated $Ara_3$ (Moreira et al., 2013).

In the GC–MS chromatograms of the PMAA derivatives of the thermally treated mixtures, two peaks were observed with the retention times and mass spectra similar to those obtained for T-Pent*p* and 4-Pent*p*. These peaks can be attributed to T-Xyl*p* and 4-Xyl*p*, using β-(1→4)-D-xylobiose as standard. The 4-Xyl*p* can be formed by the oxidative decarboxylation at C6 of the 4-Glc*p* at the

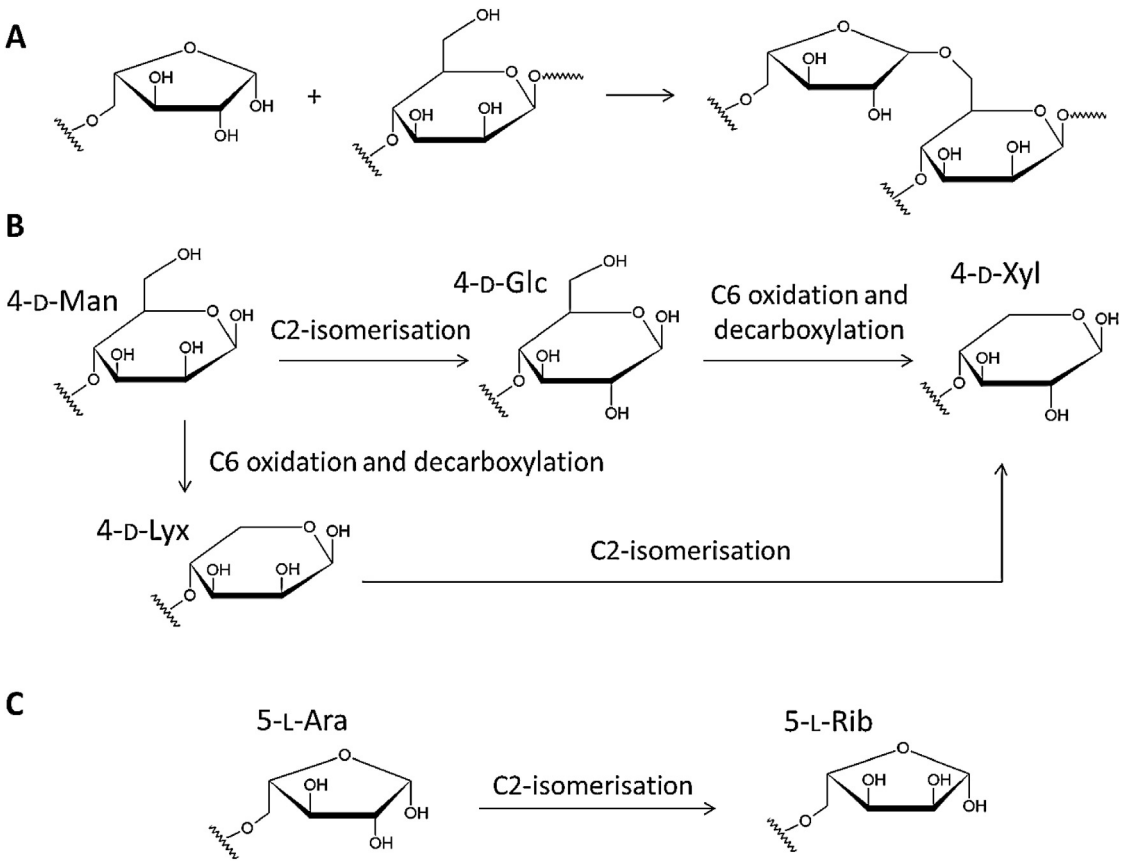


**Fig. 2.** Reactions proposed to occur during the roasting of the carbohydrates, originating new carbohydrate structures. (A) Transglycosylation reaction involving arabinose and mannose residues forming a 6-Man linkage; (B) reactions of isomerization and oxidative decarboxylation resulting in the formation of glucose, xylose, and lyxose; (C) reaction of isomerization of arabinose with concomitant formation of ribose.

reducing end, formed from 4-Man*p* isomerization, as suggested in Fig. 2B. T-Xyl*p* can be formed from 4-Xyl*p* via transglycosylation reactions, similarly as described for T-Rib*f*, formed from 5-Rib*f*. However, the T-Pent*p* residues can also be attributed to terminally linked lyxose (Lyx) residues, resulting from the oxidative decarboxylation at C6 of the Man located at the non-reducing end. Because the lyxitol acetate used as standard has the same structure of the arabinitol acetate, they could not be distinguished by the sugar analysis performed. Also, when the D-lyxose used as standard was methylated, giving the derivative T-Lyx*p*, it was observed the same retention time of T-Xyl*p* even using different chromatographic columns (DB-1 and FFAP). Because 4-Lyx*p* can also be formed from 4-Man*p* by oxidative decarboxylation, as suggested in Fig. 2B, T- and 4-Pent*p* were the designations used in Table 2 to refer the Xyl and Lyx derivatives.

### 3.3. Evidence of transglycosylation reactions in roasted coffee polysaccharides

Revisiting the methylation analysis chromatograms of the galactomannan-rich spent coffee grounds (SCG) fractions submitted to dry heating at 160–240 °C for 3 h (Simões et al., 2014), it was possible to observe, although in trace amounts, 2- and 3-Ara*f* linkages, as well as the 2,4- and 3,4-Man*p* linkages, not previously identified (Fig. 3). In accordance with the results obtained for the model oligosaccharides, it was also possible to find in these samples T- and 4-Pent*p* residues, which should correspond mainly to T- and 4-Lyx*p*, respectively, formed upon the dry heat treatment of the SCG polysaccharides. In contrast to the oligosaccharide samples, there is a low probability for the formation of Xyl*p* from 4-Glc*p* formed via isomerization of reducing 4-Man*p*, since a very low amount of 4-Man*p* residues in the SCG samples are present as reducing ends. In this work, the occurrence of transglycosylation reactions, particularly between galactomannans and arabinogalactans, was also corroborated with enzymatic treatment of coffee polysaccharide-rich fractions obtained from SCG as well as from instant coffee.

Table 3 shows the glycosidic-linkage composition of a polysaccharide fraction soluble in cold water obtained from dry thermal treatment of a water insoluble SCG galactomannan-rich fraction (Simões et al., 2013). It was characterized by the predominance of 4-Man*p* (63.2%), the presence of 4,6- and T-linked Man*p* (4.5 and 9.8%, respectively), as well as T-Gal*p* (4.9%). In accordance with the occurrence of new glycosidic linkages formed by thermal processing, it was also found 6-, 2,3-, 2,4-, 3,4-, 2,3,6-, and 3,4,6-Man*p* (Table 3). In this sample, it was also possible to detect small amounts of 3-Gal*p* (3.9%), 3,6-Gal*p* (3.9%), and 5-Ara*f* (0.4%), which are diagnostic linkages for the presence of arabinogalactans. However, according to the extraction procedures used to isolate the galactomannans, it was not expected to obtain arabinogalactans derived from a water insoluble galactomannan-rich fraction. The occurrence of 2- and 3-Ara*f* residues is also in accordance with the formation of new linkages by transglycosylation promoted by the roasting. When this fraction was selectively hydrolysed with a α-galactosidase, which is known to cleave the α-Gal side chains from galactomannans, the resultant glycosidic linkage composition showed the decrease of the relative content of T-Gal*p* and 4,6-Man*p* when compared with the non-hydrolysed polysaccharides. These results confirm the occurrence of T-α-Gal*p* residues linked to the 4,6-Man*p* residues, which is a structural characteristic of galactomannans. Also, the relative amount of the linkages diagnostic for

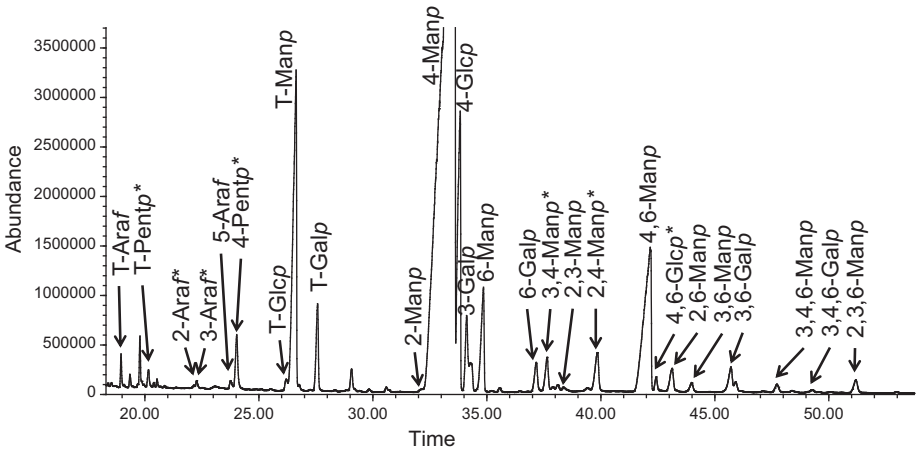


**Fig. 3.** GC–MS chromatogram of the PMAA derivatives obtained from the SCG galactomannans submitted to dry thermal treatment at 220 °C for 3 h. The peaks marked with an asterisk (*) were identified by revisiting the data previously obtained (Simões et al., 2014).

**Table 3**
Glycosidic linkage composition of the SCG polysaccharide fraction before and after enzymatic hydrolyses with α-galactosidase and β-galactosidase.

| Linkages | SCG polysaccharide fraction | | |
|---|---|---|---|
| | Non-hydrolysed | α-Galactosidase | β-Galactosidase |
| T-Ara*f* | 0.7 | 1.1 | 0.3 |
| 2-Ara*f* | t[a] | t | t |
| 3-Ara*f* | 0.1 | 0.1 | t |
| 5-Ara*f* | 0.4 | 0.5 | 0.5 |
| T-Pent*p* | 0.2 | 0.2 | |
| 4-Pent*p* | 0.7 | 1.2 | 2.1 |
| Pent*p* = Lyx*p* or Xyl*p* | | | |
| T-Man*p* | 9.8 | 6.9 | 10.3 |
| 4-Man*p* | 63.2 | 65.8 | 67.5 |
| 6-Man*p* | 1.0 | 0.7 | 0.8 |
| 2,3-Man*p* | 0.1 | 0.1 | |
| 2,4-Man*p* | 0.9 | 0.6 | 0.7 |
| 3,4-Man*p* | 0.5 | 0.4 | 0.7 |
| 4,6-Man*p* | 4.5 | 0.8 | 5.2 |
| 2,3,6-Man*p* | t | t | |
| 3,4,6-Man*p* | t | | |
| T-Gal*p* | 4.9 | 3.0 | 2.1 |
| 3-Gal*p* | 3.9 | 6.5 | 2.0 |
| 6-Gal*p* | 1.6 | 2.5 | 1.5 |
| 3,6-Gal*p* | 3.9 | 4.5 | 2.2 |
| 3,4,6-Gal*p* | 0.1 | 0.2 | t |
| T-Glc*p* | 0.2 | 0.4 | 0.1 |
| 4-Glc*p* | 3.2 | 4.1 | 3.8 |
| 4,6-Glc*p* | 0.1 | 0.2 | 0.1 |

[a] Traces (<0.1%).

the presence of arabinogalactans (T-, 3-, 6-, and 3,6-Gal*p*) decreased when the polysaccharide was hydrolysed with β-galactosidase, an enzyme that cleaves the β-Gal residues from the arabinogalactans. These results confirm the presence of arabinogalactan and galactomannan structural features, and new glycosidic linkages, possibly resultant from transglycosylation reactions between galactomannans and arabinogalactans promoted by the dry heat process.

Transglycosylation reactions between galactomannans and arabinogalactans were also recognized in instant coffee polysaccharides. Table 4 shows the sugar composition of a commercial sample of instant coffee. The main sugar residue was Gal (53.6 mol%), followed by Man (36.9 mol%), and Ara (8.2 mol%). In agreement with the literature for instant coffee (Passos et al., 2014; Wolfrom & Anderson, 1967), the higher percentage of Gal allowed inferring that the arabinogalactans were the most abundant polysaccharides present. Upon cooling to 4 °C, an insoluble fraction, rich in Man (62.3 mol%) and containing Gal, was recovered, suggesting that the galactomannans were the most abundant polysaccharides present. The partial hydrolysis with an *endo*-β-(1→4)-D-mannanase, which cleaves β-(1→4) linkages between the mannose residues in the mannan backbone, allowed a partial solubilization of these carbohydrates, giving fraction Mannanase_Sn, with a sugar composition rich in Gal (53.7 mol%), whereas Man and Ara accounted for 32.4 and 10.1 mol%, respectively. The solubilization of arabinogalactan-derived carbohydrates upon treatment with the *endo*-β-(1→4)-D-mannanase suggested the presence of chimeric polysaccharides derived from both arabinogalactans and galactomannans, possibly formed via the transglycosylation reactions, as previously reported to occur in roasted oligosaccharides

**Table 4**
Sugar composition of the initial instant coffee sample, precipitates, and supernatants.

| | Yield (mass%) | Sugar composition (mol%) | | | | | | Total sugar (%) |
|---|---|---|---|---|---|---|---|---|
| | | Rha | Ara | Xyl | Man | Gal | Glc | |
| Initial sample of instant coffee | | 0.4 | 8.2 | 0.9 | 36.9 | 53.6 | | 50 |
| *Precipitation by cooling to 4 °C, decantation, and centrifugation* | | | | | | | | |
| Supernatant | 79 | 0.9 | 9.5 | 5.3 | 26.6 | 55.7 | | 47 |
| Precipitate | 18 | 0.5 | 6.1 | 0.8 | 62.3 | 30.2 | | 66 |
| *Partial enzymatic hydrolysis of the cold water insoluble material* | | | | | | | | |
| Mannanase_Sn | 32 | 0.9 | 10.1 | 0.4 | 32.4 | 53.7 | 2.5 | 54 |
| Mannanase_Ppt | 68 | 0.3 | 3.5 | | 77.0 | 18.5 | 0.7 | 71 |
| *Fractionation of the mannanase supernatant by graded ethanol precipitation* | | | | | | | | |
| Et50 | 7 | 0.3 | 3.9 | 1.5 | 74.2 | 0.7 | | 80 |
| Et75 | 14 | 1.1 | 8.1 | 0.5 | 3.9 | 85.2 | 1.1 | 77 |
| Sn | 78 | 1.2 | 17.6 | | 48.3 | 28.3 | 4.6 | 22 |

and polysaccharides. The size-exclusion chromatography using BioGel P30 of the Mannanase_Sn fraction showed that the material was split in two distinct fractions, one at the void volume, and another at the exclusion volume, allowing to infer the presence of low and high molecular weight material. Also, the graded ethanol precipitation of the Mannanase_Sn fraction allowed to obtain a small fraction rich in galactomannans (Et50) and a fraction, accounting for 14% of the Mannanase_Sn material, rich in arabinogalactans (Et75), in accordance with previous studies (Nunes & Coimbra, 2001, 2002). The fraction soluble in 75% ethanol (Sn) was mainly composed by Man (48.3 mol%), and also Gal and Ara (Table 4), a composition explained by the action of the *endo*-β-(1→4)-D-mannanase on galactomannan moieties, forming low molecular weight compounds rich in Man and an arabinogalactan fraction precipitated in 75% ethanol, obtained from the water insoluble chimeric polysaccharides.

Instant coffee is prepared using highly processed thermally treated coffee beans when compared with regular coffee used to prepare coffee brews. However, revisiting the data already published on coffee brew polysaccharides (Nunes, Reis, Silva, Domingues, & Coimbra, 2006; Nunes & Coimbra, 2007), it was observed that it was not possible to separate the galactomannan structural features from those of the arabinogalactans even after exhaustive fractionations, namely, by combined graded ethanol fractionation, anion exchange chromatography on Q-sepharose FF, and phenylboronic acid sepharose affinity chromatography or copper affinity chromatography. Based on the data of the present study, it can be inferred that chimeric coffee brew polysaccharides could also be formed by transglycosylation reactions.

## 4. Concluding remarks

The dry thermal treatment of the mixtures containing arabinosyl and mannosyl oligosaccharides ($Ara_3$ and $Man_3$) promoted the formation of polysaccharides composed by both hexose and pentose residues, mainly Man and Ara, corroborating the hypothesis of the occurrence of transglycosylation reactions between galactomannans and arabinose side chains of arabinogalactans during coffee roasting. This hypothesis was also corroborated by the finding of chimeric polysaccharides structures of arabinogalactans and galactomannans obtained from roasted coffee matrices and presenting the same characteristics found for the standards used as models.

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