Roasting-induced changes in arabinotriose, a model of coffee arabinogalactan side chains

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Abstract

Thermal processing can promote reactions that change the structure of food constituents, often by unknown mechanisms, such as those occurring in arabinose residues of coffee arabinogalactan side chains. Aiming to know more about these modifications, the structurally related \(\alpha-(1 \rightarrow 5)\)-arabinotriose was roasted at 200°C and the products obtained were identified by ESI-MS and MALDI-MS and characterised by ESI-MS. Depolymerised and polymerised oligosaccharides with up to 16 residues and new types of linkages were formed. Also, products resulting from dehydronation, oxidation, and cleavage of a carbon–carbon bond at the reducing end of the corresponding non-modified oligosaccharide were formed, probably promoting the release of formaldehyde, formic acid, glycolaldehyde, glyoxal, acetic acid, glycolic acid, glyceraldehyde, 2-hydroxypropanedialdehyde and lactic acid. As many of these compounds have been reported to occur in roasted coffee beans and/or brews, it can be suggested that the degradation of coffee arabinogalactan side chains can contribute to their formation upon roasting.

1. Introduction

In the food industry, thermal processing remains the most widely used industrial procedure (Sun, 2006). However, high temperatures can promote reactions that change the structure of food constituents, often with the formation of unknown compounds and, consequently, no knowledge of the properties of the newly formed compounds and their potential effects on human health. Thus, the identification of structural modifications occurring in food constituents as a consequence of thermal processing is of great interest. In the coffee industry, roasting green coffee beans is a long-established thermal treatment of great importance since it is responsible for the development of the characteristic aroma, taste and colour of coffee brews (Illy et al., 1995). During this treatment, several chemical reactions take place inside the beans, thus changing the structure of their polysaccharide components (Nunes & Coimbra, 2002; Nunes, Reis, Domingues, & Coimbra, 2006; Oosterveld, Harmsen, Voragen, & Schols, 2003a; Oosterveld, Voragen, & Schols, 2003b).

Polysaccharides comprise about 50% of the dry weight of green coffee beans, in which 14–17% comprise type II arabinogalactans (Bradbury & Halliday, 1990; Illy et al., 1995). Hot water extractable green coffee arabinogalactans are composed by a main backbone of \(\beta-(1 \rightarrow 3)\)-linked \(\alpha\)-galactose residues, some of them substituted at O-6 with short chains of \(\beta-(1 \rightarrow 6)\)-linked \(\alpha\)-galactose residues. The galactose residues of these short chains are substituted with various combinations of \(\alpha\)-L-arabinose (38 mol%), \(\alpha\)-L-rhamnose (8 mol%), and \(\beta\)-D-glucuronic acid (2 mol%) residues. In particular, some of the galactose residues are substituted at O-3 by single \(\alpha\)-L-arabinose residues or disaccharides of \(\alpha-(1 \rightarrow 5)\)-linked \(\alpha\)-arabinose residues (Nunes, Reis, Silva, Domingues, & Coimbra, 2008). During coffee roasting, the arabinose is the sugar residue most sensitive to degradation (Oosterveld et al., 2003b). Accordingly, \(\alpha-(1 \rightarrow 5)\)-linked \(\alpha\)-arabinose residues of coffee brews were observed to decrease with higher degrees of roast (Wei et al., 2012). Arabinose is involved in melanoidin formation occurring during the roasting of coffee beans (Bekedam, Loots, Schols, Van Boekel, & Smit, 2008; Moreira, Nunes, Domingues, & Coimbra, 2012; Nunes, Cruz, & Coimbra, 2012). In addition, based on the roasting of model compounds, it has also been suggested that arabinose residues of arabinogalactan side chains might play a role in acid formation, including that of formic, acetic, glycolic and lactic acids (Ginz, Balzer, Bradbury, & Maier, 2000).

In spite of the data already available, structural modifications promoted by roasting in arabinose residues of coffee arabinogalactan side chains are not well elucidated. In an aim to identify these structural modifications, \(\alpha-(1 \rightarrow 5)\)-arabinotriose \((\text{Ara}_3)\) was subjected to dry thermal treatments at 200°C for different periods. In order to obtain information concerning their sugar and glycosidic-linkage compositions, sugar and linkage analyses were performed in untreated and thermally treated samples. Mass spectrometry...
(MS), particularly tandem mass spectrometry (MS^2), has been used successfully to identify structural modifications induced by dry thermal treatment in mono-, oligo- and polysaccharides (Golon & Kuhnert, 2012; Moreira, Coimbra, Nunes, Simões, & Domingues, 2011; Nunes et al., 2006). Thus, electrospray ionisation mass spectrometry (ESI-MS), matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) and electrospray ionisation tandem mass spectrometry (ESI-MS^2) were also used for detailed analysis of structural modifications promoted by dry thermal processing of Ara3.

2. Materials and methods

2.1. Materials

\(\alpha-(1\rightarrow5)\)-L-arabinotriose (Ara\(_3\)), having a purity \(\geq 95\%\), was purchased from Megazyme (County Wicklow, Ireland). The syrup sample commercially provided was diluted with ultrapure water (Millipore Synergy system, Billerica, MA) and freeze-dried. The resulting material was ground with an agate mortar and pestle and then stored in a desiccator containing \(\text{P}_2\text{O}_5\) until used.

2.2. Thermal treatments

Ara\(_3\) was submitted to the temperature programs used in a previous study with mannosyl and galactomannosyl oligosaccharides (Moreira et al., 2011) using a TGA-50 thermogravimetric analyser (Shimadzu, Kyoto, Japan). Its thermal stability was studied by submitting it to a temperature program from ambient temperature to 600 °C. Also, in a different set of experiments, Ara\(_3\) was heated from room temperature up to 200 °C and maintained at 200 °C for different periods (0, 30 and 60 min). These three different thermal treatments are respectively designated as T1, T2 and T3, whereas the untreated sample is designated as T0. The thermally treated samples were recovered, weighed and suspended in ultrapure water (5 mg sample/mL solvent). The samples were stirred at 37 °C for 3 h and then centrifuged. The water-soluble fraction (WSF) was separated from the insoluble fraction (WIF) and both fractions were then kept frozen at \(-20\) °C until analysis. A solution (1 mg/mL) of untreated Ara\(_3\) was prepared and stored using the same procedure described for thermally treated samples.

2.3. Fractionation of water-insoluble fractions

A portion (approx. 2 mg) of each WIF resulting from T2 and T3 treatments was completely dissolved in 0.5 mL of anhydrous dimethyl sulfoxide. The most hydrophobic material was precipitated by addition of distilled water (5 mL). The supernatant was recovered after centrifugation and the precipitate was washed two times with 2 mL of distilled water. To remove the dimethyl sulfoxide, the combined supernatants were dialyzed (MW cutoff 1 kDa) over 8 h against 300 mL of distilled water, with two water renewals. The retentate was divided in two parts; one used for sugar analysis and the other for linkage analysis.

2.4. Sugar and linkage analyses

Neutral sugars were converted to alditol acetates and then analysed by gas chromatography on a chromatograph equipped with a DB-225 column (30 m length, 0.25 mm of internal diameter, and 0.15 μm of film thickness) from J&W Scientific (Folsom, CA) and a flame ionisation detector. For linkage analysis, sugars were converted to partially methylated alditol acetates, and then analysed by gas chromatography–mass spectrometry on an Agilent Technologies 6890 N Network chromatograph (Santa Clara, CA) equipped with a DB-1 column (30 m length, 0.25 mm of internal diameter, and 0.15 μm of film thickness) from J&W Scientific and connected to an Agilent 5973 mass analyser (Nunes et al., 2012).

2.5. \(^{18}\text{O}\)-labelling of the reducing sugar residue

To label the carbonyl oxygen at the reducing end of the oligosaccharide with oxygen-18, 50 μL of Ara\(_3\) solution (1 mg/mL) were dried and re-dissolved in 50 μL of \(^{18}\text{O}\)-enriched water (H\(_2\)^{18}O, 97%, Sigma-Aldrich, St. Louis, MO). This solution was stirred for 4 h at 37 °C in a sealed vial and was then frozen at \(-20\) °C until analysis by mass spectrometry.

2.6. Electrospray ionisation mass spectrometry

For all ESI-MS analyses, samples were diluted in methanol/formic acid (99.9:0.1, v/v) and MS spectra were acquired in the positive mode scanning the mass range from \(m/z\) 100 to 1500.

2.6.1. LIT instrument

ESI-MS and -MS\(^2\) studies were carried out on a LXQ linear ion trap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) using the operating conditions previously described (Moreira et al., 2011). ESI-MS\(^2\) spectra were acquired with the energy collision set between 20 and 37 (arbitrary units). Data acquisitions were carried out on an Xcalibur data system (version 2.0).

2.6.2. Q-TOF instrument

The ESI-MS spectrum of Ara\(_3\) heated to 200 °C (T1) was also acquired on an ESI-Q-TOF2 mass spectrometer (Micromass, Manchester, U.K.) using the operating conditions previously described (Moreira et al., 2011). This spectrum was processed using MassLynx software (version 4.0) to determine exact mass and elemental composition of the ions assigned as modified pentose oligosaccharides identified after the thermal treatment. For exact mass measurements, the lock mass used for each ion was the calculated monoisotopic mass of the corresponding non-modified pentose oligosaccharide in sodiated form ([M+Na]\(^+\)).

2.7. Matrix-assisted laser desorption/ionisation mass spectrometry

MALDI-MS spectra were acquired using a MALDI-TOF/TOF Applied Biosystems 4800 Proteomics Analyser (Applied Biosystems, Framingham, MA) instrument equipped with a nitrogen laser emitting at 337 nm and operating in a reflectron mode. Full-scan mass spectra ranging from \(m/z\) 500 to 4000 were acquired in the positive mode. Sample preparation for MALDI-MS was performed using 2,5-dihydroxybenzoic acid as matrix (Moreira et al., 2011).

3. Results and discussion

3.1. Thermal stability of \(\alpha-(1\rightarrow5)\)-L-arabinotriose

Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of \(\alpha-(1\rightarrow5)\)-L-arabinotriose (Ara\(_3\)) obtained from room temperature to 600 °C showed that it starts to degrade before 200 °C (Supplementary Fig. 1). These results confirmed that Ara\(_3\) has a lower thermal stability than that observed for \(\beta-(1\rightarrow4)\)-α-mannosidase (Man\(_\beta_3\)) under the same conditions (Moreira et al., 2011). The total relative mass loss observed when Ara\(_3\) was heated to 200 °C (T1) and maintained at this temperature for 30 min (T2) and 60 min (T3) was 6.0%, 19.4% and 21.7%, respectively. The longer the thermal treatment, the more intense the brown colouration observed, which was darker than that observed for Man\(_\beta_3\) subjected to the same treatment (Moreira et al., 2011). In relation to the
water-solubility, the material resulting from T1 treatment of Ara3 was completely soluble in water. Nevertheless, and contrarily to that observed for Man3 (Moreira et al., 2011), the materials resulting from T2 and T3 treatments were only partially soluble in water (35.3% for T2 and 24.6% for T3). To further characterise the structural changes associated with each thermal treatment, sugar and linkage analyses were performed.

3.2. Changes in sugar and glycosidic-linkage compositions

The sugar and glycosidic-linkage compositions of Ara3 before and after thermal processing are presented in Table 1. For T0, methylation analysis revealed the presence of terminally-linked Araf (T-Araf, 29.3%) and (1 \→\ 5)-linked Araf (5-Araf, 69.1%) approximately in the proportion of 1 to 2 and small amounts of 3,5- and 2,5-Araf (0.1%), T-Xylp (0.3%), 4-Xylp (0.4%), and 4-GlcP (0.5%). These results and those obtained by sugar analysis led to the conclusion that the unroasted sample was mainly composed by α-(1 \→\ 5)-\-arabinobiose, galactosyl-arabinobiose (\>98%). The percentage of T- and 5-Araf (12.9% and 68.6%, respectively) decreased from T0 to T1, whereas 3,5- and 2,5-Araf (2.8% and 3.0%, respectively) increased and 2-Araf (1.2%) and 3-Araf (2.3%) were formed. The percentage of total sugars decreased from 99.4% to 60.5%. The material resulting from T1 treatment was completely water soluble. However, for T2 and T3, a water insoluble fraction (WIF) was recovered for T2 and T3 treatments when compared with T0, the WSF from T2 and T3 treatments when compared with T0 showed a decrease of T- and 5-Ara

the percentage of total sugars in WIF was much lower than in T0 and after thermal processing inferred they had degraded. Also, the identification of new types of glycosidic linkages inferred the occurrence of trans-glycosylation reactions. A more detailed analysis of structural modifications was made by mass spectrometry (MS).

3.3. MS analysis of α-(1 \→\ 5)-\-arabinobiose

In order to identify the products formed by dry thermal processing of Ara3, an unroasted sample was firstly analysed by ESI-MS in the positive mode, without any salt addition. Under these conditions neutral oligosaccharides ionise preferentially as sodium adducts ([M+Na]+) (Moreira et al., 2011; Reis, Coimbra, Domingues, Ferrer-Correa, & Domingues, 2004; Tudella et al., 2011). Accordingly, the ESI-MS spectrum of unroasted sample (Supplementary Fig. 2a) showed a predominant ion at m/z 437, attributed to [Ara3+Na]+, and minor ions at m/z 453 and 851, attributed to [Ara3+K]+ and [2Ara3+Na]+, respectively.

The typical product ions observed in ESI-MS spectra of sodiated oligosaccharides ([M+Na]+) result from the cleavage of glycosidic linkages, cross-ring cleavages (cleavage of two bonds within the sugar ring) and loss of water. Their relative abundance depends on structural details, such as monosaccharide composition, type of glycosidic linkages and anomeric configurations (Asam & Glish, 1997; Simões et al., 2007). Thus, ESI-MS experiments were initiated to study the fragmentation pattern of an unroasted sample. In order to mass-discriminate the reducing end product ions from those derived from the non-reducing end of the oligosaccharide, 18-O-labelled at the carbonyl group of the reducing sugar residue was performed (Asam & Glish, 1997; Fang & Bendiak, 2007; Fang, Zirroli, & Bendiak, 2007; Hofmeister, Zhou, & Leary, 1991; Konda, Bendiak, & Xia, 2012). The ESI-MS spectra of [M+Na]+ ions of unlabelled (m/z 437) and 18O-labelled (m/z 439) Ara3 are shown in Fig. 1.

The MS2 spectrum of unlabelled Ara3 (Fig. 1a) showed two predominant product ions, at m/z 377 and 347, resulting from cross-ring cleavages with the loss of C2H4O2 (60 Da) and C3H6O2 (90 Da), respectively, from the precursor ion. Also, the product ion observed at m/z 407 can result from a cross-ring cleavage between two adjacent bonds with the loss of C2H4O2 (30 Da). Glycosidic cleavage product ions were also observed at m/z 305, formed by loss of one pentose residue (Pentres, \>132 Da); at m/z 287, formed by loss of a pentose sugar (Pent, \>150 Da); at m/z 173, formed by the loss of two Pentres; and at m/z 155, formed by combined loss of Pent and Pentres. The product ion observed at m/z 419 was formed by loss of H2O (\>18 Da). Minor abundant product ions were also found at m/z 245 (\>192 Da) and 215 (\>222 Da), formed by combined loss of Pentres and C3H6O2 or C4H8O3.

The MS2 spectrum of 18O-labelled Ara3 (Fig. 1b) showed two predominant product ions at m/z 377 and 347, formed by loss of C2H4O2 (62 Da) and C3H6O2 (92 Da), respectively. Also, the product ion at m/z 407, formed by loss of CH2,18O (\>32 Da), showed a higher relative abundance compared to that at m/z 409, formed by loss of CH2O (\>30 Da). These observations allowed the conclusion that cross-ring cleavages occur predominantly at

Table 1
Sugar and glycosidic-linkage composition of Ara3 before (T0) and after thermal processing (T1, T2 and T3).

<table>
<thead>
<tr>
<th>Linkage</th>
<th>T0 (%)</th>
<th>T1 (%)</th>
<th>T2 - wsf (%)</th>
<th>T3 - wsf (%)</th>
<th>T2 - wif (%)</th>
<th>T3 - wif (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Araf</td>
<td>29.3</td>
<td>12.9</td>
<td>16.6</td>
<td>24.7</td>
<td>29.6</td>
<td>29.8</td>
</tr>
<tr>
<td>2-Araf</td>
<td>–</td>
<td>1.2</td>
<td>4.3</td>
<td>8.6</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td>3-Araf</td>
<td>–</td>
<td>2.3</td>
<td>8.4</td>
<td>11.6</td>
<td>6.8</td>
<td>6.4</td>
</tr>
<tr>
<td>5-Araf</td>
<td>69.1</td>
<td>68.6</td>
<td>37.5</td>
<td>32.7</td>
<td>44.4</td>
<td>41.8</td>
</tr>
<tr>
<td>3,5-Araf</td>
<td>0.1</td>
<td>2.8</td>
<td>12.7</td>
<td>7.3</td>
<td>8.6</td>
<td>8.7</td>
</tr>
<tr>
<td>2,5-Araf</td>
<td>0.1</td>
<td>3.0</td>
<td>13.7</td>
<td>8.7</td>
<td>5.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Total</td>
<td>98.6 (99.5)</td>
<td>90.8 (99.4)</td>
<td>93.2 (98.8)</td>
<td>93.6 (99.0)</td>
<td>97.5 (99.9)</td>
<td>96.2 (99.0)</td>
</tr>
<tr>
<td>T-Xylp</td>
<td>0.3</td>
<td>0.1</td>
<td>1.0</td>
<td>1.7</td>
<td>2.4</td>
<td>3.7</td>
</tr>
<tr>
<td>4-Xylp</td>
<td>0.4</td>
<td>2.3</td>
<td>1.2</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>0.7 (0.2)</td>
<td>2.4 (0.3)</td>
<td>2.2 (0.4)</td>
<td>3.0 (0.4)</td>
<td>2.4 (0.1)</td>
<td>3.7 (0.1)</td>
</tr>
<tr>
<td>4-GlcP</td>
<td>0.5 (0.3)</td>
<td>6.7 (0.2)</td>
<td>4.6 (0.8)</td>
<td>3.6 (0.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>%Total sugars</td>
<td>99.4</td>
<td>60.5</td>
<td>nd</td>
<td>nd</td>
<td>47.2</td>
<td>46.8</td>
</tr>
</tbody>
</table>

a wsf, water-soluble fraction.
b wif, water-insoluble fraction.
c Molar percentage obtained by linkage analysis.
d Molar percentage (values in brackets) and total sugar percentage obtained by sugar analysis.
e Not determined.
the reducing terminal residue with loss of the carbonyl oxygen. In other words, and using the nomenclature proposed by Domon and Costello (1988), the product ions at m/z 407, 377, and 347 in the MS² spectrum of unlabelled Ara3 (Fig. 1a) are mainly attributable to ⁰⁴Å⁺, ⁰₂Å⁺, and ⁰₃Å⁺ ions, respectively. In the MS² spectrum of ¹⁸O-labelled Ara3 (Fig. 1b), the higher relative abundance of the product ion at m/z 307 (−132 Da) compared to that at m/z 305 (−134 Da), and the occurrence of the product ion at m/z 287 (−152 Da) instead of the m/z 289 (−150 Da), allowed the conclusion that glycosidic cleavages occur preferentially (but not exclusively) between the anomeric carbon and the glycosidic oxygen. Also, the higher abundance of the product ion at m/z 419, formed by loss of H₂¹⁸O (−20 Da), compared to that at m/z 421 (−18 Da) suggests that the water loss occurs preferentially at the reducing terminal residue with loss of the carbonyl oxygen. All these conclusions are in agreement with previous studies on the fragmentation pattern of ¹⁸O-labelled glucopyranose disaccharides (Asam & Glish, 1997; Hofmeister et al., 1991).

3.4. MS analysis of roasted samples

After heating Ara3 to 200 °C (T1), the ESI-MS spectrum (Supplementary Fig. 2b) showed that the ion at m/z 437 remained the most abundant, but several new ions were also shown. All of these ions are summarised in Table 2, presenting the m/z values and relative abundances of all ions identified. For longer thermal treatments (T2 and T3), the same ions were observed, although with different relative abundances (Supplementary Fig. 2c-d and Table S1). To obtain additional information about their structures and to confirm the proposed structural assignments, ESI-MS² (n = 2–3) experiments were also performed for all ions identified after thermal processing of Ara3 (T1, T2, and T3 treatments).

All ion series identified after thermal processing of Ara3 (Table 2) and their fragmentation patterns are described below, organised in the following sections grouping each series according to the different types of products formed: (a) Products of depolymerisation and polymerisation; (b) Dehydration products; (c) Oxidation products; and (d) Products of carbon–carbon bond cleavage. For each series, the MS² spectrum of the ion corresponding to the sodium adduct of the triose derivative (Pentₙ with n = 3 in Table 2) acquired from T1 sample is shown as an example.

3.4.1. Products of depolymerisation and polymerisation
The ions at m/z 173, 305, 347, 569, 701, 833, 965, 1097 and 1229 (Table 2) were attributed to [Pentₙ⁺Na]+. The identification of pentose and pentose oligosaccharides with a lower and higher degree of polymerisation (DP) than 3 is an indication of the occurrence of depolymerisation and polymerisation reactions. These reactions were previously reported to occur during T2 and T3 treatments of mannosyl and galactomannosyl oligosaccharides, while T1 treatment promoted almost no structural modifications (Moreira et al., 2011). This can be explained by the lower thermal stability of Ara3 when compared with that of Manₙ, which was also supported by the thermogravimetric data obtained. Depolymerisation of coffee polysaccharides is a well-known reaction occurring during roasting (Nunes & Coimbra, 2002; Oosterveld et al., 2003a). The occurrence of polymerisation has also been supported using model compounds (Moreira et al., 2011).

To evaluate the presence of oligosaccharides with higher DP than those observed by ESI-MS, MALDI-MS analysis was performed. The ions at m/z 569, 701, 833, 965, 1097, 1229, 1361, 1493, 1625, 1757, 1889, 2021, and 2153, attributed to [Pentₙ⁺⁶⁰Na]+, were observed in the MALDI-MS spectrum acquired from the T1 sample (Supplementary Fig. 3), as well as in those acquired from T2 and T3 (data not shown).

The ESI-MS² spectra of all ions of the [Pentₙ⁺Na]+ series with n ≥ 2 showed the product ions resulting from glycosidic cleavages, cross-ring cleavages and water loss. The product ions observed for the ion at m/z 437 ([Pent₂⁺Na]+, Supplementary Fig. 4) were the same as those observed for the untreated sample (Fig. 1a). However, the relative abundance of the product ions at m/z 377 (−60 Da) and 347 (−90 Da) was, respectively, lower and higher in the MS² spectrum of the roasted sample. Studies with hexose disaccharides have demonstrated that the relative abundance of cross-ring cleavage ions is linkage-dependent (Asam & Glish, 1997; Simões et al., 2007). Thus, the change in the relative abundance of these product ions suggests, in accordance with the results obtained by methylation analysis, that new types of glycosidic linkages are formed during thermal processing.

Other series of ions identified by ESI-MS after thermal processing of Araₙ were identified as resulting from modification of the [Pentₙ⁺Na]+ series (Table 2). The elemental compositions of all these ions were confirmed by exact mass measurement and elemental composition determination from the ESI-MS spectrum of T1 sample acquired using a Q-TOF instrument (Supplementary Table 2). For all these ions, the differences (in absolute values) between the observed and calculated masses ranging from 0.0 to 20.4 Da, which correspond to relative errors between 0.0 and 47.7 ppm, corroborate with high confidence the proposed modifications that will be described below. However, the coexistence of different isomers cannot be excluded.

3.4.2. Dehydration products
The series of ions with minus 18, 36 and 54 Da compared with the corresponding ion of the [Pentₙ⁺Na]+ series can be assigned as sodium adducts of pentose oligosaccharides modified by dehydration, due to the loss of one, two, or three water molecules, respectively. Dehydration products resulting from the loss of one or three water molecules, but not those resulting from the loss of two water molecules, were also formed during dry thermal processing of mannosyl and galactomannosyl oligosaccharides (Moreira et al., 2011). More recently, dehydration products resulting from the loss
of two water molecules were reported based on the MS analysis of sugar samples heated for 2 h at 140 °C in the case of fructose and at 180 °C for glucose and sucrose (Golon & Kuhnert, 2012).

The most abundant product ion observed in the ESI-MS<sup>2</sup> spectra (Supplementary Fig. 5) of the ions at m/z 419 ([Pent<sub>7</sub>H<sub>2</sub>O+Na]<sup>+</sup>) and 401 ([Pent<sub>7</sub>2H<sub>2</sub>O+Na]<sup>+</sup>) was formed by loss of a Pent<sub>7</sub>+. As glycosidic cleavages were shown in the <sup>18</sup>O-labelling experiment to occur preferentially between the anomic carbon and the glycosidic cleavage, the occurrence of this product ion suggests that the dehydration products have a non-modified Pent<sub>7</sub>+ located at the non-reducing end. For the ions at m/z 419 and 401, the MS<sup>3</sup> spectrum of the [M-Pent<sub>7</sub>+Na]<sup>+</sup> ion (Supplementary Fig. 5) also showed that the product ion that resulted from loss of a Pent<sub>7</sub>+ was the most abundant, indicating that the loss of one and two water molecules occurred at the reducing end of the sugar residue of the corresponding non-modified oligosaccharide. The MS<sup>2</sup> spectrum of the dehydration product resulting from the loss of three water molecules ([Pent<sub>7</sub>3H<sub>2</sub>O+Na]<sup>+</sup>) showed the product ions at m/z 287 and 269 that can be formed by loss of (Pent<sub>7</sub>res-2H<sub>2</sub>O) and (Pent-2H<sub>2</sub>O), respectively. Because a non-modified Pent<sub>7</sub>res was shown to be located at the non-reducing end, the presence of these ions suggests the loss of two water molecules at the reducing end and another at the middle Pent<sub>7</sub>res of the oligosaccharide.

### 3.4.3. Oxidation products

The series of ions with 16 Da more than the corresponding ion of the [Pent<sub>7</sub>+Na]<sup>+</sup> series can be assigned as sodium adducts of pentose oligosaccharides modified by oxidation containing an extra oxygen atom. These products can result from the formation of a pentonic acid moiety at the reducing end sugar residue, similar to that occurring during coffee roasting at the reducing end of galactomannans with formation of mannonic acid (Nunes et al., 2006). Oxidation products containing an additional oxygen atom and possessing an acid character were also identified by ESI-MS after oxidation of mannansyl and galactomannansyl oligosaccharides induced by the hydroxyl radicals generated by the Fenton reaction (Tudella et al., 2011). Nevertheless, according to the results of elemental composition determinations (Supplementary Table 2), [Pent<sub>7</sub>+O+Na]<sup>+</sup> is the second most probable assignment for some

| Table 2 |
| Summary of the ions observed in the ESI-MS spectrum of Ara<sub>7</sub>, heated to 200 °C (T1). |

<table>
<thead>
<tr>
<th>n</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
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<tbody>
<tr>
<td><strong>Products of depolymerisation and polymerisation</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>[Pent&lt;sub&gt;n&lt;/sub&gt;+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>173&lt;sup&gt;a&lt;/sup&gt;</td>
<td>305</td>
<td>437</td>
<td>569</td>
<td>701</td>
<td>833</td>
<td>965</td>
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<td>1229</td>
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<tr>
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<td>63 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.5 ± 2.7</td>
<td>100%</td>
<td>42.8 ± 1.5</td>
<td>30.9 ± 5.2</td>
<td>23.0 ± 3.9</td>
<td>11.5 ± 1.4</td>
<td>5.3 ± 0.6</td>
<td>2.2 ± 0.6</td>
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<td><strong>Dehydration products</strong></td>
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<tr>
<td>[Pent&lt;sub&gt;n&lt;/sub&gt;-18 Da+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>287</td>
<td>419</td>
<td>551</td>
<td>683</td>
<td>815</td>
<td>947</td>
<td>1079</td>
<td>1211</td>
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<td></td>
<td>8.3 ± 0.8</td>
<td>28.5 ± 1.1</td>
<td>19.5 ± 0.3</td>
<td>12.2 ± 1.1</td>
<td>9.9 ± 0.4</td>
<td>5.6 ± 1.2</td>
<td>1.3 ± 1.0</td>
<td>1.0 ± 0.8</td>
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<tr>
<td>[Pent&lt;sub&gt;n&lt;/sub&gt;-36 Da+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>269</td>
<td>401</td>
<td>533</td>
<td>665</td>
<td>797</td>
<td>929</td>
<td>1061</td>
<td>1101</td>
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<td>1.4 ± 0.2</td>
<td>5.5 ± 0.7</td>
<td>5.7 ± 0.2</td>
<td>4.6 ± 1.2</td>
<td>4.0 ± 0.6</td>
<td>2.6 ± 0.4</td>
<td>1.3 ± 0.8</td>
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<tr>
<td>[Pent&lt;sub&gt;n&lt;/sub&gt;-54 Da+Na]&lt;sup&gt;+&lt;/sup&gt;</td>
<td>383</td>
<td>515</td>
<td>647</td>
<td>779</td>
<td>911</td>
<td>1043</td>
<td>1185</td>
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<td>2.0 ± 0.5</td>
<td>3.6 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>3.7 ± 1.3</td>
<td>2.6 ± 0.8</td>
<td>1.3 ± 1.1</td>
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<td><strong>Oxidation products</strong></td>
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<tr>
<td>[Pent&lt;sub&gt;n&lt;/sub&gt;-16 Da+Na]&lt;sup&gt;+&lt;/sup&gt; (and [Pent&lt;sub&gt;n&lt;/sub&gt;+K]&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>321</td>
<td>453</td>
<td>585</td>
<td>717</td>
<td>849</td>
<td>981</td>
<td>1113</td>
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<td>4.7 ± 1.1</td>
<td>14.4 ± 5.8</td>
<td>6.9 ± 1.8</td>
<td>6.4 ± 1.3</td>
<td>5.8 ± 1.9</td>
<td>3.7 ± 1.5</td>
<td>1.6 ± 1.0</td>
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<tr>
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<td>435</td>
<td>567</td>
<td>699</td>
<td>831</td>
<td>963</td>
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<td></td>
<td>8.3 ± 1.5</td>
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<td>5.9 ± 1.7</td>
<td>6.0 ± 2.9</td>
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<sup>a</sup> m/z: Values of the ions are depicted in bold.
<sup>b</sup> Mean ± standard deviation of three replicate spectra acquisitions made in different days.
ions of this series, the first corresponding to the potassium adducts of pentose oligosaccharides ([Pent₃+K⁺]). Also, the increase in the relative abundance of the ion at m/z 453 ([Pent₃+O+Na⁺] and [Pent₃+K⁺]) in the ESI-MS spectrum acquired from T1 sample compared to that in the ESI-MS spectrum of untreated sample (Supplementary Fig. 2a-b) re-inforces that these two ion assignments are possible.

The ESI-MS² spectrum of the ion at m/z 453 acquired from the T1 sample (Supplementary Fig. 6b) showed product ions at m/z 409, 407, 305 and 287. The ions at m/z 305 and 287 were formed by loss of (Pent₃+O) and (Pent+O), confirming the presence of an additional oxygen. The ions at m/z 409 and 407 were formed by loss of HCOOH and CO₂, confirming the presence of a carboxylic acid group. The relative abundance of the ions at m/z 407 and 409 was higher in the MS² spectrum acquired from T2 and T3 samples (Supplementary Fig. 6c-d), suggesting an increase in the proportion of [Pent₃+O+Na⁺] ions in respect to [Pent₃+K⁺] ions for longer times of treatment.

![Fig. 2. Summary of the products resulting from the cleavage of a carbon–carbon bond.](image)

Fig. 2. Summary of the products resulting from the cleavage of a carbon–carbon bond.

![Fig. 3. Proposed mechanism for the formation of the products with 28 and 30 Da less than that of the corresponding non-modified oligosaccharide.](image)

Fig. 3. Proposed mechanism for the formation of the products with 28 and 30 Da less than that of the corresponding non-modified oligosaccharide.
As shown in Table 2, a series of ions with 2 Da less than those of the corresponding ion of the [Pent₃+Na⁺]⁺ series was also identified. Oxidation products with 2 Da less than the corresponding non-modified oligosaccharide due the formation of a keto group, also identified after oxidation of mannosyl and galactomannosyl oligosaccharides with hydroxyl radicals (Tudella et al., 2011), are well-known products of sugar oxidation (de Lederkremer & Marino, 2003). The ESI-MS² spectrum (Supplementary Fig. 7) of the ion at m/z 435 ([Pent₃-2H+Na⁺]) showed the product ions at m/z 303 and 171, formed by loss of one and two Pent₃res, at m/z 287, formed by loss of (Pent-2H), and at m/z 155, attributed to [Pent₃+Na⁺], supporting the modification of the sugar residue located at the reducing end.

### 3.4.4. Products of carbon–carbon bond cleavage

Several series of ions designated as products of carbon–carbon bond cleavage were also observed after thermal processing of Ara₃ (Table 2). These series are summarised in Fig. 2 and described below, grouped according to the proposed C–C bond undergoing cleavage.

#### 3.4.4.1. C1–C2 cleavage

The ions with 28, 30, and 46 Da less than those of the corresponding ion of the [Pent₃+Na⁺]⁺ series were attributed to [Pent₃-CO+Na⁺], [Pent₃-CH₂O+Na⁺], and [Pent₃-CH₂O₂+Na⁺], respectively. The formation of the [Pent₃-28 Da+Na⁺]⁺ series can be explained by hydrolytic cleavage of the C1–C2 bond of an 1,2-dicarbonyl intermediate (Fig. 3), as proposed to occur during dry thermal treatment (240 °C for 15 min) of glucose (Ginz et al., 2000). Also, the formation of the [Pent₃-46 Da+Na⁺]⁺ series can be explained by the hydrolytic cleavage of the C1–C2 bond of a 1,2-dicarbonyl intermediate (3-deoxypentosone) formed by dehydration of the 1,2-dienediol intermediate (Ginz et al., 2000). Both proposed mechanisms include the formation of formic acid (CH₂O₃), suggesting that the degradation of arabinose residues contributes to the formation of the formic acid emitted from green coffee beans during roasting (Yeretzian, Jordan, Badoud, & Lindinger, 2002; Yeretzian, Jordan, & Lindinger, 2003). The formation of the [Pent₃-30 Da+Na⁺]⁺ series can be explained by loss of formaldehyde (CH₂O, Fig. 3), another volatile organic compound known to be released from green coffee beans during roasting (Yeretzian et al., 2002).

The ESI-MS² spectrum of the ion at m/z 409 ([Pent₃-28 Da+Na⁺], Fig. 4a) showed the product ion at m/z 277, formed by loss of a Pent₃res. Also, its MS³ spectrum (data not shown) showed the product ion at m/z 145, formed by loss of another Pent₃res, supporting the conclusion that cleavage occurred at the sugar residue located at the reducing end. The ion at m/z 377, formed by loss of CH₃OH (-32 Da), due to its high abundance, should probably result from the reducing end. The ESI-MS² spectra of the ions at m/z 407 ([Pent₃-30 Da+Na⁺], Fig. 4b) and 391 ([Pent₃-46 Da+Na⁺], Fig. 4c) are product ions formed by loss of one and two Pent₃res, also supporting the probable cleavage of the sugar residue located at the reducing end. This assumption is also supported by the presence of the product ion at m/z 287, formed respectively by loss of (Pent-30 Da) and (Pent-46 Da) from the reducing end. The loss of CH₃OH (-32 Da) from the non-reducing end was also observed in both spectra.

#### 3.4.4.2. C2–C3 cleavage

The ions with 58 and 60 Da less than the corresponding ion of the [Pent₃+Na⁺]⁺ series were attributed to [Pent₃-C₃H₄O₂+Na⁺] and [Pent₃-C₃H₅O₃+Na⁺], respectively. The formation of [Pent₃-58 Da+Na⁺]⁺ series can be explained by hydrolytic cleavage of the C2–C3 bond of an 2,3-dienediol intermediate with the additional formation of glycolic acid (C₂H₄O₃). The products with 58 Da less than the corresponding non-modified pentose oligosaccharide can also be formed by loss of glycolal (C₃H₄O₂). The formation of the [Pent₃-60 Da+Na⁺]⁺ series can be explained by hydrolytic cleavage of the C2–C3 bond of a 2,3-dicarbonyl intermediate (1-deoxypentosone) formed by dehydration of the 2,3-dienediol intermediate. According to this mechanism, acetic acid (C₂H₄O₂) is also released from the hydrolytic cleavage (Ginz et al., 2000). The formation of products with 60 Da less than the corresponding non-modified oligosaccharide can also be explained.

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**Fig. 4.** ESI-MS² spectra of the ions at m/z (a) 409 ([Pent₃-28 Da+Na⁺]), (b) 407 ([Pent₃-30 Da+Na⁺]), and (c) 391 ([Pent₃-46 Da+Na⁺]) acquired from Ara₃ heated to 200 °C (T1).
by loss of glycolaldehyde (C2H4O2). The series of ions with 76 and 112 Da less than that of the corresponding ion of the [Pent+Na]+ series, attributed to [Pent-C4H6O2+Na]+ and [Pent-C6H8O2+Na]+, can be formed by dehydration from the products belonging to the [Pent-58 Da+Na]+ series with loss of one and three water molecules, respectively. Similarly, the ions of the [Pent-78 Da+Na]+ series can be formed by loss of one water molecule from the products belonging to the [Pent-60 Da+Na]+ series. On other hand, the ions of the [Pent-44 Da+Na]+ series, attributed to [Pent-C4H4O+Na]+, can be explained by oxidation from those belonging to the [Pent-60 Da+Na]+ series with the formation of a carboxylic acid group.

The ESI-MS² spectra (and casually MS³ spectra) acquired for the ion series derived from C2–C3 cleavages are shown in the Supporting Information (Supplementary Figs. 8–13). All spectra, except those acquired for the [Pent-112 Da+Na]+ series, supported the modification of only one of the three pentose residues, as well as the location of the modified residue at the reducing end. The MS³ spectrum (Supplementary Fig. 11b) of the ion at m/z 193 ([Pent-112 Da+Na]+) showed the product ion at m/z 137, formed by loss of (Pent-58 Da-2H2O) and attributed to [Pent-C3H4O+Na]+, suggesting the presence of two modified residues: (Pent-H2O) and (Pent-58 Da-2H2O).

Glycolic acid (Galli & Barbas, 2004), glyoxal (Daglia et al., 2007) and acetic acid (Yeretzian et al., 2002, 2003) are known to be released from green coffee beans during roasting. Glyoxal and glycolaldehyde were also identified as oxidation products of laminaran under pyrolysis conditions (Ovalle et al., 2001). Also, glycolaldehyde was shown to be released predominantly from the C1–C2 of glucose monomers during pyrolysis of 13C-labelled glucans (Ponder & Richards, 1993).

4. Conclusion

This work showed that α-(1–5)-linked arabinosyl trisaccharides are extensively modified by dry thermal processing. Together with the formation of depolymerised and polymerised products, the formation of dehydration and oxidation products was also observed, along with products of carbon–carbon bond cleavages with probable release of formaldehyde, formic acid, glycolaldehyde, glyoxal, acetic acid, glycolic acid, glyceraldehyde, 2-hydroxypropanedioaldehyde and lactic acid. The roasting of arabinotriose as a model of coffee arabinogalactan side chains showed that the arabinose residues can be a source of aldehydes, di-aldehydes and acids previously reported to occur in coffee brews.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.11.130.

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