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Carbohydrate Research 337 (2002) 917–924

CARBOHYDRATE RESEARCH

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Structural characterisation of the olive pomace pectic polysaccharide arabinan side chains

Susana M. Cardoso,[a,b] Artur M.S. Silva,[a] Manuel A. Coimbra[a,]*

[a] *Departamento de Química, Universidade de Aveiro, P-3810-193 Aveiro, Portugal*
[b] *Escola Superior Agrária de Bragança, P-5301-855 Bragança, Portugal*

Received 10 January 2002; accepted 15 March 2002

Abstract

An arabinan (97% of Ara and 3% of hexuronic acid) was isolated from the alcohol-insoluble residue (AIR) of olive pomace by treatment with 0.02 M HNO_3, at 80 °C, followed by graded precipitation with ethanol. It was separated from acidic pectic polysaccharides by anion-exchange chromatography, and by size-exclusion chromatography its molecular weight was estimated as 8.4 kDa. By methylation analysis, the linkage composition was established as 5:4:3:1 for (1 → 5)-Ara*f*, T-Ara*f*, (1 → 3,5)-Ara*f* and (1 → 3)-Ara*f*, respectively. ^{13}C NMR spectroscopy confirmed this linkage composition, and allowed to assign the α anomeric configuration for the arabinofuranosyl residues, except for some terminally linked ones, that were seen to occur as T-β-Ara*f*. By 2D NMR spectroscopy (^{1}H and ^{13}C), it was possible to conclude that the T-β-Ara*f* was (1 → 5)-linked to a (1 → 5)-Ara*f* residue. Also, in the arabinan (1 → 5)-Ara*f* backbone, the branched (1 → 3,5)-Ara*f* residues were always adjacent to linear (1 → 5)-Ara*f* residues. A tentative structure is proposed.

Keywords: Olive pomace; Pectic polysaccharides; Arabinan; Methylation analysis; NMR

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1. Introduction

Olive pomace is an industrial byproduct of the olive oil production process, that is obtained by squeezing the olive pulp without any chemical treatment. By water addition, three phases occur: oil, water and pomace. Pomace is usually furthered extracted with *n*-hexane yielding olive pomace oil. For environmental reasons, addition of water is now avoided in most industries. This technology change results in a very wet pomace due to retention of water from the fruit in the residue. This residue has no significant value because of the energy required for the drying process.[1,2] To add value to this byproduct, we have been studying its cell-wall polysaccharides in order to define new applications.

The pectic polysaccharides of fruits have many industrial applications.[3] They are used mainly in the food industry as gelling agents.[4] They are also useful in pharmaceutical and cosmetic industries for the manufacture of encapsulated drugs and skin-care products.[5] Three major pectic polysaccharides have been isolated and characterised: homogalacturonan, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II).[6,7] The homolacturonan is a linear polymer composed of 100–200 (1 → 4)-α-D-Gal*p*A units; RG-I is a linear polymer of near 100 disaccharide repeating units of (1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A; and RG-II is a branched polymer with a backbone of around nine (1 → 4)-α-D-Gal*p*A units substituted by four heteropolymeric side chains containing eleven different sugars.[6,7] Some of the Rha*p* residues of RG-I are substituted at C-4 with L-arabinosyl and D-galactosyl side chains. The L-arabinose-rich polysaccharides are named "arabinans". These polymers have a main structure of α-(1 → 5)-linked L-arabinofuranose units, substituted at either O-2 or O-3 or at both of these positions.[8] However, unusual structures have also been described. In arabinan fragments isolated from dehulled rapeseed, (1 → 5)-linked arabinose was not found in a great extent. Instead, terminal arabinosyl groups and units substituted at both O-2 and O-5 predominated.[9] Also, some

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* Corresponding author. Fax: + 351-234-370084.
E-mail address: mac@dq.ua.pt (M.A. Coimbra).

PII: S0008-6215(02)00082-4

terminal pyranosyl units were detected in purified arabinans from *Cajanus cajan* cotyledon.[10]

One third of the olive-pulp cell walls is arabinose-rich pectic polysaccharides.[11] To learn more about the structure of olive pectic polysaccharides, a pectic polysaccharide-rich extract from olive pomace was purified. The arabinan-rich fraction was fractionated into two arabinans. The structure of the larger arabinan was then investigated by methylation analysis and NMR spectroscopy.

2. Experimental

Samples origin.—Olive (*Olea europaea*, L.) pomace was collected at Prolagar, Mirandela, Portugal. The pomace was obtained by crushing the olives (90% *Verdial* variety) below 40 °C, with a residence period on the sequential extractor of 45 min. The pomace was separated from the oil by centrifugation.

Preparation of cell-wall material.—Fresh olive pomace (150 g) was dispersed in EtOH at a final concentration of 85% (v/v) and boiled for 10 min. The mixture was cooled, filtered through a glass fibre filter (Whatman GF/C) and the residue was then extracted twice with 2:1 $CHCl_3$–MeOH (3 × initial fresh weight) for 30 min under reflux to remove the oil. The resulting residue was washed with Et_2O, acetone and allowed to dry at rt. The particles of the broken seed were removed (31 g), and the remaining material, composed of olive-pulp cell walls and powdered olive seed, is referred to as "alcohol insoluble residue" (AIR).

Isolation of pectic material.—The AIR (40 g) was sequentially extracted with 1 L of water at 20 °C for 2 h, 2 L of water at 80 °C for 2 h and 2 L of 0.02 M HNO_3 at 80 °C for 2 h. After each extraction, the solubilised polymers were separated from the residue by filtration through a glass fibre filter (Whatman GF/C) and, with exception for the hot acid extract, were concentrated by rotary evaporation at 37 °C, frozen and freeze-dried. The material solubilised by the hot acid was dialysed against distilled water until neutrality before being freeze-dried.

Graded precipitation with EtOH.—The 0.02 M HNO_3 extract (650 mg) was dispersed in water (730 mL), stirred overnight at 4 °C and submitted to graded precipitation with 50%, 65% and 80% (v/v) EtOH. Each fraction was held at 4 °C for 1 h with gentle mixing and the precipitate was collected by centrifugation at 23,000*g*, 20 min. To remove all the EtOH, each precipitate and the 80% EtOH supernatant (EtOH 80% supt.) was dissolved in water, concentrated under reduced pressure at 37 °C, frozen and freeze-dried.[12]

Anion-exchange chromatography.—The 80% EtOH supernatant (167 mg) was suspended in water and stirred at rt. The insoluble residue was removed by centrifugation and sodium phosphate buffer (pH 6.5) was added to the supernatant to a final concentration of 50 mM and 1 mg/mL of material. The solution was then passed through a column of DEAE-Trisacryl (1 mL of resin/7.5 μmol of uronic acid) in the phosphate form, at 10 mL/h. The fractions were eluted sequentially with the same volume of buffer and buffer containing 0.125, 0.250, 0.5 and 1 M NaCl. Fractions (2.5 mL) were collected and assayed at λ 280 nm for phenolics and by the phenol–H_2SO_4 method for carbohydrate.[12] Appropriate fractions (A, B, C, D) were pooled, dialysed and freeze-dried.

Size-exclusion chromatography.—Size-exclusion chromatography was used for the fractionation of the first fraction (A) from the anion-exchange chromatography. This was performed with a column (100 × 2.6 cm) of Sephacryl S200 equilibrated with 200 mM of sodium phosphate buffer at a flow rate of 150 mL/min. The sample (56.5 mg) was dissolved in 0.95 mL of the same sodium phosphate buffer (pH 6.5), and the solution was then passed through the column. Fractions (2.5 mL) were assayed for phenolics and carbohydrates as described above. The appropriate fractions (A1, A2, A3, A4 and A5) were combined, dialysed and freeze-dried. The average molecular weight of the polymers of each fraction was established after a calibration of the column with dextrans of average molecular weights 5.22, 11.6, 23.8, 48.6 and 80.9 kDa. The inner volume of the column was determined with glucose and the void volume calculated using blue dextran 2000.

Monosaccharide composition and content of uronic acids.—Neutral monosaccharides were determined by gas–liquid chromatography after sulfuric acid hydrolysis[13] and conversion to their alditol acetates[14,15] in a Carlo–Erba 6000 gas chromatograph equipped with a split injector (split ratio 1:60) and a FID detector. The column was a DB-225 (J&W) with 30 m × 0.25 mm and film thickness of 0.25 μm and the oven temperature program was: 220 °C for 5 min and then the temperature was raised at 20 °C/min to 230 °C and maintained this temperature for another 6 min. The flow rate of the carrier gas (H_2) was set at 1 mL/min at 220 °C. The injector temperature was 220 °C and the flame ionisation detector temperature was 230 °C. Uronic acids were determined colorimetrically by a modification[12] of the method of Blumenkrantz and Asboe-Hansen.[16] Samples were prepared by hydrolysis in 0.2 mL 72% H_2SO_4 for 3 h at 20 °C followed by 1 h in 1 M H_2SO_4 at 100 °C. Calibration was made with D-galacturonic acid.

Methylation analysis and GC–MS.—Polysaccharides in Fraction A3 were treated with powdered NaOH and methylated with CH_3I[17,18] as described by Coimbra et al.[12] The partially methylated alditol acetates (PMAA) were analysed by GC-FID on an OV-1 capillary column (30 m length, 0.32 mm of internal diameter and

0.25 μm of film thickness) and characterised by GC–MS. The samples were injected in splitless mode (time of splitless 0.75 min), with the injector and detector operating at 210 and 220 °C, respectively, using the following temperature program: 55 °C for 0.75 min with a linear increase of 45 °C/min up to 140 °C, and standing for 1 min at this temperature, followed by a linear increase of 2.5 °C/min up to 218 °C, with further 37 min at 218 °C. For quantification, the molar response factors of Sweet et al.[19] were used. The linear velocity of the carrier gas (H_2) was set at 50 cm/s at 218 °C. GC–MS analysis was performed in a HP series 2 gas chromatograph and Trio-1S VG mass-lab with scans between 400–35 m/e/s with a 70 eV ionisation energy. The chromatographic conditions used were as described for GC-FID. Linear velocity of the carrier gas (He) was set at 40 cm/s at 200 °C, with a solvent delay of 4 min.

NMR studies.—^{1}H and ^{13}C NMR spectra were recorded in D_2O on a Bruker DRX 500 spectrometer operating at 500.13 and 125.77 MHz, respectively; the chemical shifts are expressed in δ (ppm) values relative to TSS as external reference. 2D COSYPR (homonuclear shift correlation with presaturation during relaxation delay) spectrum was recorded with 200 transients over 256 increments (zero-filled to 1 K) and 1 K data points with spectral widths of 1200 Hz. The repetition time was 1.8 s. These data were processed in the absolute-value mode. The phase sensitive 1H-detected (^{1}H,^{13}C) *g*HSQC (heteronuclear single quantum coherence, using gradient pulses for selection) spectrum was recorded with 200 transients over 256 increments (zero-filled to 1 K) and 1 K data points with spectral widths of 1280 Hz in F_2 and 7000 Hz in F_1. The repetition time was 2.3 s. A cosine multiplication was applied in both dimensions. The delays were adjusted according to a coupling constant ^1J(CH) of 149 Hz. The *g*HMBC (heteronuclear multiple quantum coherence, using gradient pulses for selection) spectrum was recorded with 200 transients over 256 increments (zero-filled to 1 K) and 1 K data points with spectral widths of 1280 Hz in F_2 and 7000 Hz in F_1. The repetition time was 2.3 s. A sine multiplication was applied in both dimensions. The low-pass J-filter of the experiment was adjusted for an average coupling constant ^1J(CH) of 149 Hz and the long-range delay utilised to excite the heteronuclear multiple quantum coherence was optimised for 7 Hz.

3. Results and discussion

Isolation of the olive pomace arabinan.—The residue insoluble in ethanol represented 34.2% of the wet olive pomace. From it, the smashed hard seeds were removed, and the residue obtained was named AIR (Table 1). The main component monosaccharides of the AIR were hexuronic acids (HexA, 31%), glucose (Glc, 29%), xylose (Xyl, 16%) and arabinose (Ara, 14%). This composition in monosaccharides shows the presence of pectic polysaccharides rich in Ara, cellulose, glucuronoxylans and xyloglucans, as already described to occur in olive pulp.[11,20,21]

Pectic polysaccharides from olive pomace were extracted sequentially with water at 20 and 80 °C, and 0.02 M HNO_3 at 80 °C. The total material solubilised was 23.2% of AIR, the most part of it extracted with water at 20 °C (Table 1). The water extracts contained 45–47% of sugars, composed of HexA, Ara and Glc, possibly resulting from pectic polysaccharides and loosely bound glucans. The occurrence of water-soluble glucans has been previously reported to occur in olive pulp,[11,20] although no starch was observed by the reaction with iodine–KI.[11] The acid extraction gave a fraction very rich in sugars, composed mainly by HexA and Ara, in a ratio near 1:1, that accounted for 2.5% of the AIR. The higher relative amount of Ara in this extract, when compared to the other Ara-rich extracts, can be explained by the hot acid treatment, that promotes the cleavage of the arabinan side chains and the cleavage of the galacturonan backbone, producing pectic polysaccharides rich in Ara.[22]

The pectic polysaccharides in the acid extract were separated into four fractions, according to their solubility in aqueous ethanol solutions. The fractions precipitated in 50, 65 and 80% ethanol had a ratio HexA/Ara of 2.4, 3.5, and 2.3, respectively. However, the material that remained soluble in 80% ethanol had a ratio HexA/Ara of 0.05, indicating the presence of Ara-rich polymers (Table 1). The elution of this Ara-rich fraction by anion-exchange chromatography on DEAE-Trisacryl allowed to obtain a neutral fraction (Fraction A), very rich in sugars, with 93 mol% of Ara and 6 mol% of HexA.

The size-exclusion chromatography of Fraction A on Sephacryl S200HR (Fig. 1 and Table 1) resulted in two main fractions (A3 and A4), very rich in Ara (97 and 96 mol%), with estimated molecular weights of 8.4 and 4.6 kDa, respectively.

Methylation analysis.—Methylation analysis of Fraction A3 showed that the arabinosyl-linkage composition was 5:4:3:1 for (1 → 5)-Ara*f*, T-Ara*f*, (1 → 3,5)-Ara*f* and (1 → 3)-Ara*f*, respectively (Table 2). This showed a good correlation between terminal and branched residues for the low degree of polymerisation of this polymer. These four types of residues are denoted in Scheme 1 as A, D as well as E, B and C, respectively. The relative amount of (1 → 3,5)-Ara*f* residues indicated that the arabinan had, on average, one branching point for each four residues. This arabinan was less branched than the arabinan moiety found in olive pulp pectic-polysaccharides of Douro variety.[11,22] Also, the amount of (1 → 3)-Ara*f* residues was lower. This assay detected

Table 1
Sugar composition of the olive pomace extractions and fractions

Fraction	Recovery (%)	Cell wall sugars (mol%)								Total sugar [a] (mg/g)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
AIR	13.5 [b]	3	1	14	16	2	3	29	31	490
Water 20 °C	15.5 [c]	3	t	22	3	1	5	13	52	448
Water 80 °C	5.5 [c]	4	t	15	4	3	5	21	48	474
0.02 M HNO_3	2.2 [c]	2	t	50	1	t	4	1	41	996
EtOH 50%	11.4 [d]	2		26	2	-	7	3	61	580
EtOH 65%	2.1 [d]	1		20	t	t	6	2	70	548
EtOH 80%	50.8 [d]	2		27	2	t	7	2	61	768
EtOH 80% supt.	31.8 [d]			93	t	t	1	1	5	552
Fraction A	44.1 [e]		1	93					6	928
A1	9.0 [f]	14		17				24	45	30
A2	5.0 [f]			76				9	15	103
A3	36.0 [f]			97				t	3	992
A4	14.0 [f]			96				t	4	917
A5	7.0 [f]	t	1	81				3	15	162
Fraction B	6.7 [e]	2	1	43	1		6	3	44	417
Fraction C	3.7 [e]	2	1	37	1		4	6	49	352
Fraction D	2.7 [e]	2	1	48	1		5	4	39	420
Insoluble residue	24.1 [c]	2	1	6	26	2	2	37	26	504

[a] Values are expressed as mg of anhydrosugar/g dry polymer.
[b] Recovery is expressed as a percentage of fresh olive pomace.
[c] Recovery is expressed as a percentage of AIR.
[d] Recovery is expressed as a percentage of the 0.02 M HNO_3 dry material.
[e] Recovery is expressed as a percentage of EtOH 80% supt. dry material.
[f] Recovery is expressed as a percentage of Fraction A dry material. t, trace amounts.

trace amounts of glucose (0.7%); (1 → 2,3,5)-Ara*f* and (1 → 2,3,4)-Ara*p* were not detected. T-Ara*p* residues accounted for less than 0.03% of the total residues analysed. Uronic acids were not analysed, as they were not carboxyl-reduced, due to their low content in the sample.

^{13}C NMR.—The ^{13}C NMR spectrum of A3 arabinan is shown in Fig. 2. Based on the literature data published for spectra of arabinofuranosides[23], arabinans,[24–27] and pectins,[9,28–30] it was possible to identify T-α-L-Ara*f*, (1 → 5)-α-L-Ara*f*, (1 → 3,5)-α-L-Ara*f* and (1 → 3)-α-L-Ara*f* (Table 3). Also, the well-defined signals at 102.3, 75.1 and 63.9, could be attributed to C-1, C-3 and C-5, respectively, of T-β-L-Ara*f*. These resonances are in accordance with those reported for T-β-L-Ara*f* in hydroxyproline arabinosides and Me-β-L-Ara*f*.[23] The presence of T-β-Ara*f* in olive pulp pectic-polysaccharides was previously reported by Coimbra et al.[12] in Douro variety.

HSQC.—The one-bond correlation of the ^{13}C and the ^{1}H NMR resonances is shown in Fig. 3. The HSQC spectrum presented five H-1–C-1 cross peaks. H-1 signal at $\delta_{H\text{-}1}$ 4.90 was correlated with carbon resonances for C-1 at 102.3 and 108.4. The former was assigned to C-1 of T-β-Ara*f*, while the latter was attributed to that of (1 → 5)-α-L-Ara*f*. As the H-1 frequencies of sugars were influenced by their C-1 linkage,[9] it could be inferred that the β-terminal residue is (1 → 5)-linked (link-

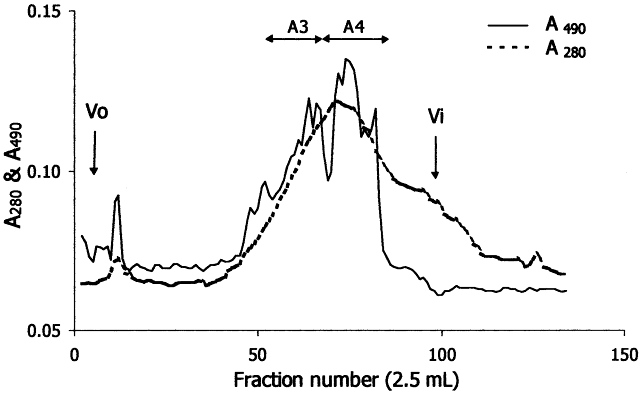
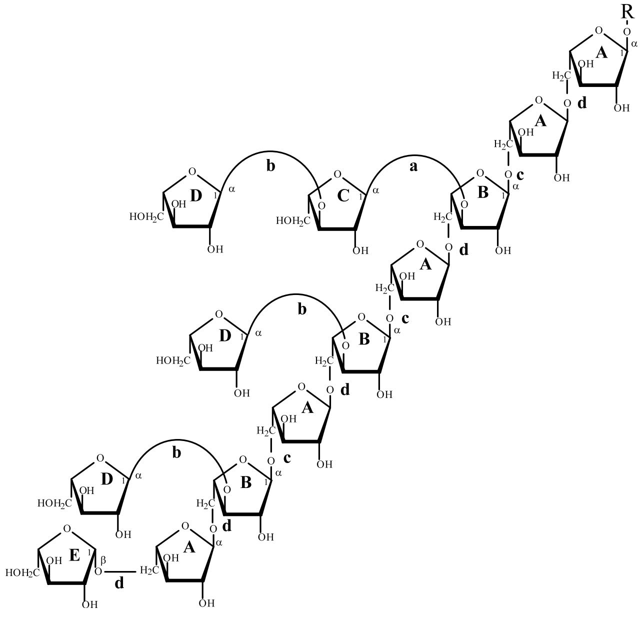


Fig. 1. Size-exclusion chromatography in Sephacryl S-200 of Fraction A.

Table 2
Glycosyl-linkage composition of Fraction A3

Linkage	Relative abundance (%)
T-Ara*f*	31.1
T-Ara*p*	<0.03
(1→5)-Ara*f*	34.3
(1→3)-Ara*f*	8.2
(1→3,5)-Ara*f*	26.5
(1→4)-Glc*p*	0.4
(1→6)-Glc*p*	0.3



Scheme 1. Tentative structure of the olive arabinan.

age *d* in Scheme 1). The C-1 signal of the (1→3,5)-α-L-Ara*f* residues ($\delta_{\text{C-1}}$ 108.3) that was also (1→5)-linked, was correlated with the H-1 resonance at δ 4.93 (linkage *c*), while those of T-α-L-Ara*f* ($\delta_{\text{C-1}}$ 108.1) and (1→3)-α-L-Ara*f* ($\delta_{\text{C-1}}$ 108.0), which were (1→3)-linked, were correlated with $\delta_{\text{H-1}}$ 4.96 (linkage *b*) and 4.99 (linkage *a*), respectively. The HSQC presented a large number of H-5–C-5 cross peaks. The C-5 of T-α-L-Ara*f* and (1→3)-α-L-Ara*f* ($\delta_{\text{C-5}}$ 62.0) was correlated with protons at $\delta_{\text{H-5}}$ 3.66–3.63 and 3.55–3.51. These H-5 resonances appeared as double doublets due to coupling each other, there is a chiral C-4 in the Ara residues, and with H-4. The C-5 signal of T-β-L-Ara*f* was correlated with the resonances at $\delta_{\text{H-5}}$ 3.63–3.62 and 3.55–3.54 and C-5 of (1→5)-α-L-Ara*f* and (1→3,5)-α-L-Ara*f* were correlated with protons in the regions ($\delta_{\text{H-5}}$ 3.77–3.76 and 3.66–3.63) and ($\delta_{\text{H-5}}$ 3.72–3.70 and 3.62–3.60), respectively.

COSY.—From the COSY spectrum (Fig. 4), it was possible to correlate H-1 of linkage *a* ($\delta_{\text{H-1}}$ 4.99) with H-2 ($\delta_{\text{H-2}}$ 4.18) and H-2 to H-3 ($\delta_{\text{H-3}}$ 3.79). These results and the analysis of the HSQC spectrum allowed to assign C-2 and C-3 of residue (1→3)-α-L-Ara*f* to $\delta_{\text{C-2}}$ 80.6 and $\delta_{\text{C-3}}$ 84.5, respectively. The H-1 of linkage *b* ($\delta_{\text{H-1}}$ 4.96) was correlated, in the COSY spectrum, with H-2 and H-3 ($\delta_{\text{H-2}}$ 3.94 and $\delta_{\text{H-3}}$ 3.77–3.76). From the HSQC spectrum, one could then assign C-2 and C-3 of residue T-α-L-Ara*f* at $\delta_{\text{C-2}}$ 82.1 and $\delta_{\text{C-3}}$ 77.4, respectively. H-2, H-3, C-2 and C-3 of the other residues were assigned by a similar procedure. H-1 of linkage *c* ($\delta_{\text{H-1}}$ 4.93) was correlated with H-2 and H-3 ($\delta_{\text{H-2}}$ 4.11 and $\delta_{\text{H-3}}$ 3.90–3.91) and C-2 and C-3 of residue (1→3,5)-α-L-Ara*f* were correlated to $\delta_{\text{C-2}}$ 80.0 and $\delta_{\text{C-3}}$ 83.1, respectively. H-1 of linkage *d* ($\delta_{\text{H-1}}$ 4.90) was correlated to H-2 and H-3 ($\delta_{\text{H-2}}$ 3.94 and $\delta_{\text{H-3}}$ 3.85–3.83) and C-2 and C-3 of residue (1→5)-α-L-Ara*f* were assigned to $\delta_{\text{C-2}}$ 83.2 and $\delta_{\text{C-3}}$ 77.6, respectively, and C-2 and C-3 of residue T-β-L-Ara*f* were assigned to $\delta_{\text{C-2}}$ 77.2 and $\delta_{\text{C-3}}$ 75.1, respectively.

HMBC.—To confirm the assignments made from HSQC and COSY spectra and to deduce more information about the structure of the arabinan, a 2D HMBC spectrum was recorded (Fig. 5). The HMBC spectrum cross peaks H-3–C-5 (linkages *a*, *b* and linkage *d* of T-β-Ara*f*), H-5–C-3 (linkages *b*, *c* and *d*), H-1–C-3 and H-3–C-1 (linkages *b*, *c* and *d*), and similarly, between H-1 with C-5 of other linked residue (linkages *c* and *d*, excepted for T-β-Ara*f*), and H-5–C-1 of two linked residues (linkages *c* and *d*). The HMBC spectrum cross peaks of H-1 and the ^{13}C resonances at δ 82.5–84.8 allowed its identification as C-4 of (1→3)-α-L-Ara*f* ($\delta_{\text{C-4}}$ 83.4), (1→3,5)-α-L-Ara*f* ($\delta_{\text{C-4}}$ 82.5), and T-α-L-Ara*f* ($\delta_{\text{C-4}}$ 84.8) residues. From the results and analysis of the HSQC spectrum, it was possible to ascribe H-4 of (1→3)-α-L-Ara*f* and T-α-L-Ara*f* to the range ($\delta_{\text{H-4}}$ 3.85–3.83) and of (1→3,5)-α-L-Ara*f* to ($\delta_{\text{H-4}}$ 4.14-4.13). The remaining C-3 cross peaks in the HMBC spectrum allowed the assignments of H-4 of residue (1→5)-α-L-Ara*f* ($\delta_{\text{H-4}}$ 4.04–4.03) and T-β-L-Ara*f* ($\delta_{\text{H-4}}$ 3.72–3.70). The C-4 resonances of these residues were obtained from the HSQC spectrum and assigned to $\delta_{\text{C-4}}$ 83.1 and $\delta_{\text{C-4}}$ 83.2, respectively. As C-5 of (1→3,5)-α-L-Ara*f* residues were correlated only with H-1 of linkage *d*, these results do not support the occurrence of contiguous branched residues, but interspersed (1→3,5)-α-L-Ara*f* and (1→5)-α-L-Ara*f* residues in a (1→5)-linked backbone. The cross-link of the C-5 of (1→5)-α-L-Ara*f* with the anomeric proton of linkages *c* and *d* showed the occurrence of contiguous residues of (1→5)-α-L-Ara*f*. Also, the connectivity of C-1 of T-β-L-Ara*f* and the protons at 3.77–3.76 ppm, attributed by HSQC to the H-5 of linkage *d*, support the direct linkage of T-β-L-Ara*f* to a (1→5)-α-L-Ara*f* residue, excluding the direct linkage to a (1→3,5)-α-L-Ara*f* residue (Scheme 1).

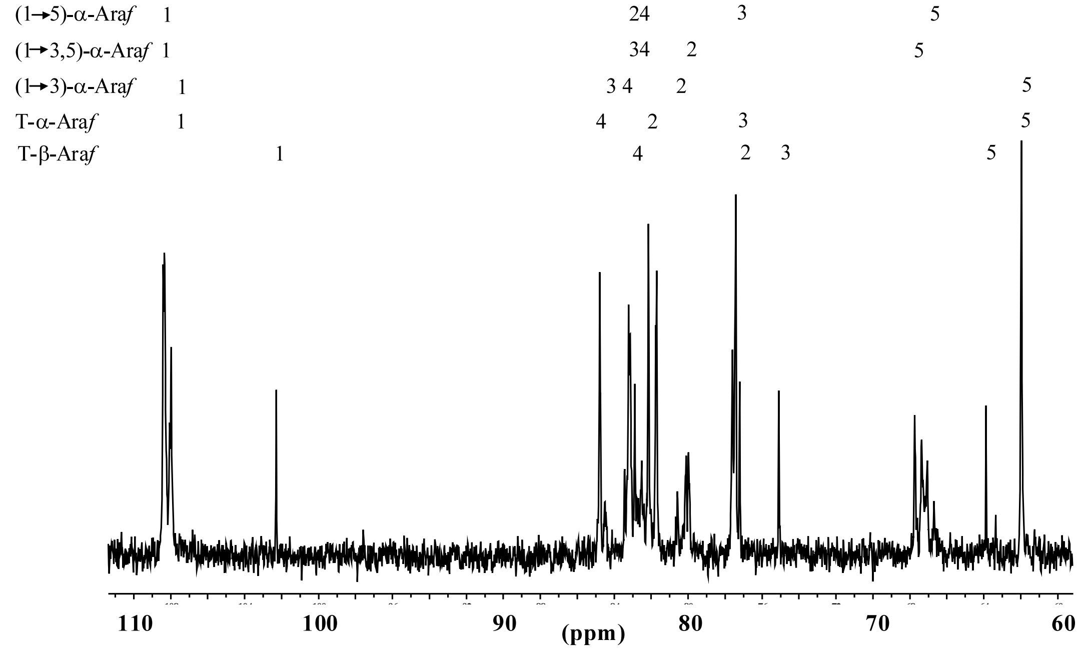


Fig. 2. 126 MHz ^{13}C NMR spectrum of A3 arabinan. The numbers above each peak refer to the carbon atoms positioned across the Ara residues listed in the top left.

NOESY.—The analysis of the NOESY spectrum showed NOE cross peaks between H-1 of linkage *a* [(1 → 3)-α-L-Ara*f*] with H-2 of (1 → 3,5)-α-L-Ara*f* and H-1 of linkage *b* (T-α-L-Ara*f*) with H-2 and H-3 of (1 → 3,5)-α-L-Ara*f*. H-1 of (1 → 3,5)-α-L-Ara*f* residues presented NOE cross peaks with H-2 of (1 → 3,5)-α-L-Ara*f* residues, and H-1 of (1 → 5)-α-L-Ara*f* with H-2 of (1 → 5)-α-L-Ara*f* or of the T-α-L-Ara*f* unit. These observations support the occurrence, for this arabinan, of adjacent (1 → 5)-α-L-Ara*f* and (1 → 3,5)-α-L-Ara*f* residues, in an helicoidal (1 → 5)-α-L-Ara*f* backbone, as previously reported to occur by X-ray conformational analysis.[31] This conformation should allow the orientation of the 3-linked side chains outside the helix.

Table 3
Peak assignments of ^{13}C NMR spectrum of Fraction A3

Residue	Chemical shift (δ)				
	C-1	C-2	C-3	C-4	C-5
(1 → 3)-α-L-Ara*f*	108.0 [a]	80.6 [a]	84.5 [a]	83.4 [b]	62.0 [b]
T-α-L-Ara*f*	108.1 [a]	82.1 [a]	77.4 [a]	84.8 [b]	62.0 [b]
(1 → 3,5)-α-L-Ara*f*	108.3 [a]	80.0 [a]	80.0 [a]	82.5 [b]	67.7 [b]
(1 → 5)-α-L-Ara*f*	108.4 [a]	83.2 [a]	77.6 [a]	83.1 [a]	67.1 [b]
T-β-L-Ara*f*	102.3 [a]	77.2 [a]	75.1 [a]	83.2 [a]	63.9 [b]

[a] Assignment from HSQC.
[b] Assignment from HMBC.

4. Conclusion

An arabinan was isolated from olive pomace by fractionation of a pectic polysaccharide-rich fraction obtained by a hot dilute acid treatment. By methylation analysis and NMR spectroscopy, it was possible to propose the structure in Scheme 1. The linkage compo-

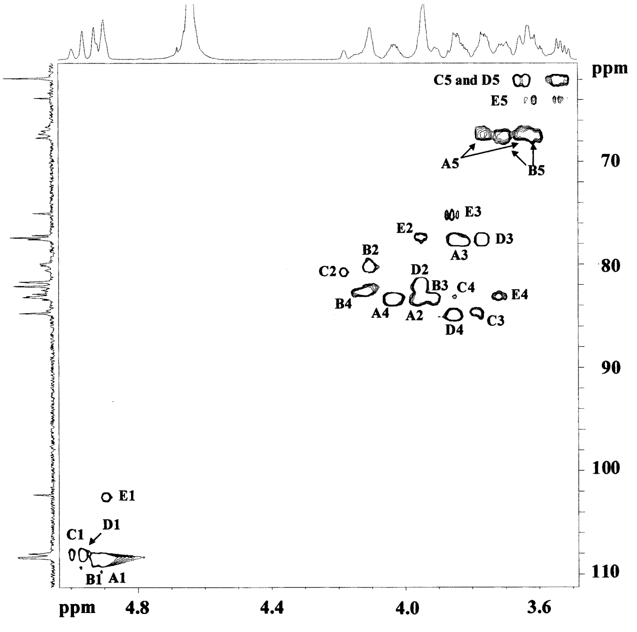


Fig. 3. (^{1}H,^{13}C) gHSQC spectrum of A3 arabinan. Capital letters represent the Ara residues according to Scheme 1. Each number stands for the carbon position giving rise to the cross peak.

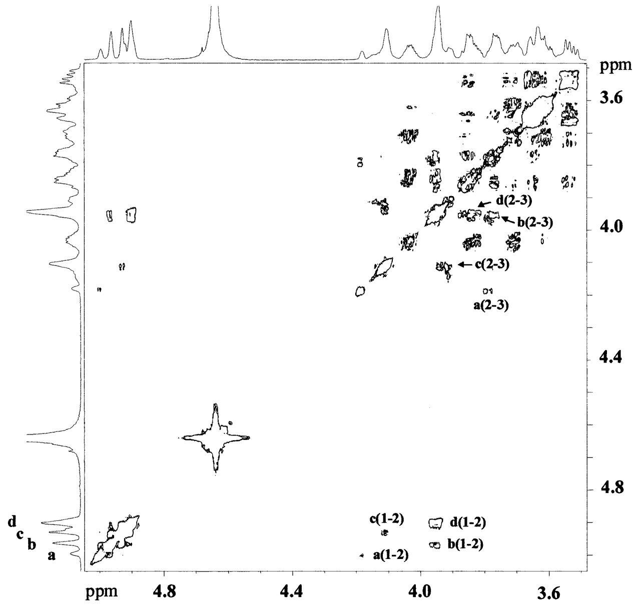


Fig. 4. 2D COSYPR spectrum of A3 arabinan. Letters represent the linkages according to Scheme 1. The numbers in parentheses denote the hydrogen atoms attached to the given carbon atoms.

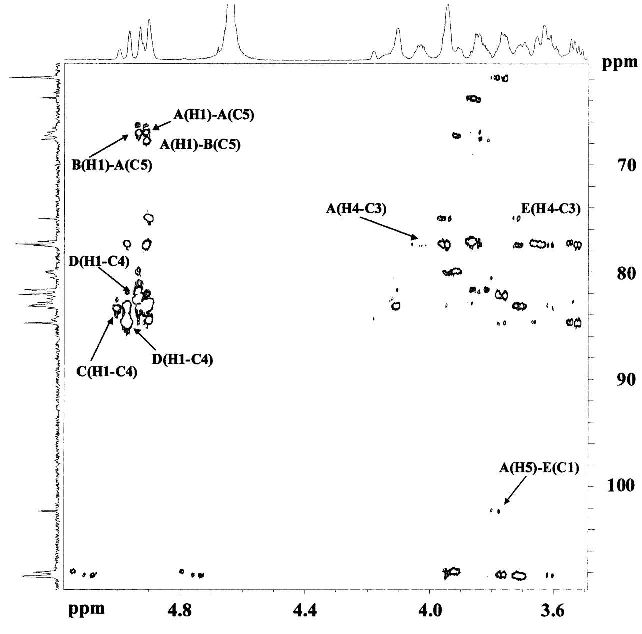


Fig. 5. (^{1}H,^{13}C) gHMBC spectrum of A3 arabinan. Capital letters represent the Ara residues according to Scheme 1.

sition was established as 5:4:3:1 for (1 → 5)-Ara*f*, T-Ara*f*, (1 → 3,5)-Ara*f* and (1 → 3)-Ara*f*, respectively. The T-Ara*f* residues were shown to occur in both α and β anomeric configurations, in proportion 3:1. T-β-Ara*f* was (1 → 5)-linked to a (1 → 5)-Ara*f* residue. In the arabinan (1 → 5)-Ara*f* backbone, the branched (1 → 3,5)-Ara*f* residues were always adjacent to linear (1 → 5)-Ara*f* residues. According to the estimated molecular weight (8.4 kDa) for Fraction A3, it is possible to assume that it contains four arabinan structures of 13 Ara residues, occurring as side chains of a degraded pectic polysaccharide backbone.

Acknowledgements

The authors acknowledge FCT, Portugal, and the University of Aveiro for funding the Research Unit 62/94 "Química Orgânica, Produtos Naturais e Agro-Alimentares". Susana Cardoso was supported by a PhD grant PRODEP III 5.3/N/199.006/00.

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