Carbohydrate Polymers 76 (2009) 485–487

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

Polysaccharide of nectarine gum exudate: Comparison with that of peach gum

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ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 10 November 2008
Accepted 10 November 2008
Available online 20 November 2008

Keywords:
Nectarine
Prunus persica var. *nucipersica*
Gum exudate
Polysaccharide comparison

ABSTRACT

The gum exudate polysaccharide from the trunk of nectarine (PPNEC) was compared with that of peach, being composed of Ara, Xyl, Man, Gal, and uronic acids in 37:13:2:42:6 molar ratio and had M_w 3.93×10^6 g mol^{-1}, compared with 5.61×10^6 g mol^{-1} for peach gum polysaccharide. Methylation analysis of PPNEC indicated a highly branched structure with relatively high amounts of di- (16%) and tri-*O*-substituted (9%) Gal*p* units and nonreducing end-units of Ara*f* (26%) and Xyl*p* (17%). Combination with ^{13}C NMR data, showed the presence of α-L-Ara*f* (nonreducing end, 3-*O*-, 5-*O*-, and 2,5-di-*O*-subst.), β-L-Ara*p* (4-*O*- and 2,4-di-*O*-subst.), β-D-Gal*p* (3-*O*-, 2,3-di-*O*-, 3,6-di-*O*-, and 3,4,6-tri-*O*-subst.), and α- and/or β-D-Xyl*p* nonreducing end-units. A signal appeared from 4-*O*-Me-α-D-Glc*p*A units. PPNEC had structures similar to those of polysaccharide from peach tree gum, although in different proportions and with a lower M_w.

1. Introduction

Gummosis is induced by biotic and abiotic stressors, such as infection, insect attack, and mechanical or chemical injury. In stone fruit spp., as peach and nectarine, gummosis is also caused by climatic factors and by pruning cuts and slits in the bark (Sharma & Gautam, 1999).

Nectarine (*Prunus persica* var. *nucipersica* Schneid.) is a botanical variety of the peach (*P. persica* L. Batsch.), with a fruit known popularly as "a peach with plum skin", having a smooth and fuzzless skin, firm flesh, and being usually smaller than that of peach (Joshi & Bhutani, 1995). As well as the peach, nectarine trees are widely cultivated in the south of Brazil, and produce a copious gum exudate, especially on their trunk. The polysaccharide from peach gum exudate has been characterized as an acidic arabinogalactan with main-chain composed mainly of (1 → 6)-linked β-Gal*p* units (Rosík, Brutenicová-Sósková, Zitko, & Kubala, 1966; Simas et al., 2008).

We now analyze the polysaccharide of the gum exudate of the nectarine, a variety of *P. persica*, and compare its structure with that of the peach tree.

2. Materials and methods

2.1. Collection of nectarine gum exudate and isolation of its polysaccharide

The gum exudate from the trunk and branches of the nectarine (*P. persica* var. *nucipersica* cv. 'Sunred') was collected from trees growing in Lapa (State of Parana, Brazil) at FRUTALAPA Agrocomercial Ltda. Nectarine gum was submitted to the same procedure previously used for peach gum (Simas et al., 2008), giving two polysaccharide fractions, one via aqueous extraction (PPNEC; 52% yield) and the other with 1% aq. KOH extraction (PPNECA; 4% yield), both at 25 °C. ^{13}C NMR examination of PPNEC and PPNECA (not shown together) showed almost identical structures. Accordingly, only the product with better yield was further analyzed.

2.2. Determination of homogeneity and molar mass (M_w)

Determination of homogeneity and molar mass (M_w) was performed on a Waters high-performance size-exclusion chromatography (HPSEC) apparatus coupled to a differential refractometer (RI) (Waters 2410) and a Wyatt Technology Dawn-F Multi-Angle Laser Light Scattering detector (MALLS). Waters Ultrahydrogel columns (2000, 500, 250 and 120) were connected in series and coupled to a multidetection system. $NaNO_2$ (0.1 M) containing NaN_3 (0.5 g l^{-1}) was used as eluent. Fraction PPNEC (1 mg ml^{-1}) was dissolved in this solvent and filtered (0.22 μm) before analysis. The *dn/dc* of PPNEC was 0.151. Data were analyzed using ASTRA 4.70.07 software.

2.3. Monosaccharide composition analysis

Fraction PPNEC (2 mg) was hydrolyzed with 1 M TFA (1 ml) for 8 h at 100 °C. The product was successively reduced with $NaBH_4$, acetylated with Ac_2O–pyridine (1:1, v/v), and the resulting alditol acetates were examined by GC–MS. This was performed with a Varian model 3800 gas chromatograph coupled to a Saturn

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0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.11.013

2000R mass spectrometer using a DB-225 capillary column (25 m × 0.25 mm i.d.): 50 °C during injection, then programmed at 40 °C min^{-1} to 220 °C (constant) with He as carrier gas. The uronic acid content of PPNEC was determined by the method of Filisetti-Cozzi and Carpita (1991).

2.4. Methylation analysis

Fraction PPNEC (10 mg) was per-*O*-methylated (Ciucanu & Kerek, 1984) and the product hydrolyzed (Simas et al., 2008). The mixture of *O*-methylaldoses was successively neutralized ($BaCO_3$), reduced with $NaBD_4$, acetylated and resulting partially *O*-methylated alditol acetates were analyzed by GC–MS (Sassaki et al., 2005).

2.5. ^{13}C nuclear magnetic resonance spectroscopy

The ^{13}C NMR spectra of PPNEC and PPNECA were obtained at 50 °C in D_2O using a 400 MHz Bruker DRX Avance spectrometer equipped with a 5 mm inverse probe. Chemical shifts are expressed in δ PPM relative to an external standard of acetone (δ 30.2). DEPT spectra were obtained according to the Bruker manual.

3. Results and discussion

The polysaccharide from the gum exudate of nectarine (PPNEC) was homogeneous on HPSEC, using r.i. and l.s. detectors (Fig. 1) with M_w 3.93×10^6 g mol^{-1}, compared with 5.61×10^6 g mol^{-1} for peach gum polysaccharide (Simas et al., 2008). However, the M_w of PPNEC is greater than those of other arabinogalactan gum exudates, such as from *Acacia tortuosa* (4.1×10^5 g mol^{-1}; Beltrán et al., 2005) and *Acacia macracantha* (9.2×10^5 g mol^{-1}; Beltrán et al., 2008).

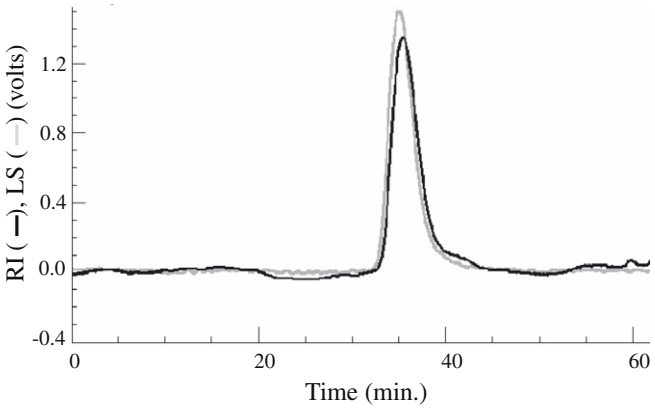

Fig. 1. Elution profile of PPNEC on HPSEC.

PPNEC consisted of Ara, Xyl, Man, Gal, and uronic acids in a 37:13:2:42:6 molar ratio, with traces of Rha (Table 1), suggesting an arabinogalactan structure. Its monosaccharide components are similar to those of peach gum polysaccharide (PPNA; Table 1), although PPNEC had greater Xyl and lower uronic acid contents. Methylation analysis of PPNEC (Table 2) showed a large proportion of di-*O*- (16%) and tri-*O*-substituted (9%) Gal*p* units, indicating a high degree of branching. Only 4% of Gal units (3-*O*-linked) were not substituted by side chains. Accordingly, the relatively high amount of nonreducing end-units (43%) suggested that the most of the branches were terminated by Ara*f* and Xyl*p*. The side chains were composed of 3-*O*-, 5-*O*-, and 2,5-di-*O*-subst. Ara*f*, and 4-*O*- and 2,4-di-*O*-subst. Ara*p* units. These methylation data, when compared with those of peach gum polysaccharide (PPNA; Table 2) indicate that both have the same structural components, with minor quantitative differences.

The ^{13}C NMR spectrum of PPNEC (Fig. 2B) contained five C-1 signals at δ 109.7–107.6 from α-L-Ara*f* units (Delgobo, Gorin, Tischer, & Iacomini, 1999) and a main one at δ 103.2 from β-D-Gal*p* units (Tischer et al., 2002). The signals at δ 102.8, δ 101.5, and δ 100.6 could be from C-1 of β-D-Xyl*p*, β-L-Ara*p*, and α-D-Xyl*p* and/or α-D-Man*p* units, respectively (Bock, Pedersen, & Pedersen, 1984; Simas et al., 2008). Those at δ 99.8 and δ 59.5 (not inverted in ^{13}C NMR-DEPT spectrum) can be attributed to C-1 and C-4 of 4-*O*-Me-α-D-Glc*p*A units, respectively. The group of signals at δ 80.4–84.1 corresponded to C-2 to C-4 of α-L-Ara*f* units (Gorin & Mazurek, 1975).

The inverted signals in the ^{13}C NMR-DEPT spectrum of PPNEC (Fig. 2B – insert) are similar to those from PPNA (Fig. 2A – insert). The signals at δ 68.9 and δ 61.5 were from substituted and non-substituted C-6 of β-D-Gal*p* units, respectively, although the latter could also be from C-5 of α-L-Ara*f* units (Delgobo et al., 1999; Gutiérrez et al., 2005). The signal at δ 65.2 can be assigned to C-5 of nonreducing end-units of β-D-Xyl*p* (Simas et al., 2004) and 5-*O*-subst. α-L-Ara*f* units. The signal at δ 63.0 could be from C-5 of

Table 1
Monosaccharide composition of polysaccharides from peach (PPNA) and nectarine (PPNEC) gum exudates.

Polysaccharide	Monosaccharide composition (%)					
	Rha	Ara	Xyl	Man	Gal	Uronic acid[a]
PPNA[b]	tr.	36	7	2	42	13
PPNEC	tr.	37	13	2	42	6

[a] Determined by the colorimetric method of Filisetti-Cozzi and Carpita (1991).
[b] Data published by Simas et al. (2008).

Table 2
Partially *O*-methylalditol acetates formed on methylation analysis of polysaccharides.

Partially *O*-methylated alditol acetates	Parent linkage	R_t[a]	Polysaccharide fraction (%)	
			PPNA[b]	PPNEC
2,3,5-Me_3-Ara	Ara*f*-(1→	7:42	20	26
2,3,4-Me_2-Xyl	Xyl*p*-(1→	8:07	13	17
2,5-Me_2-Ara	→3)-Ara*f*-(1→	8:57	6	7
2,3-Me_2-Ara	→5)-Ara*f*-(1→, →4)-Ara*p*-(→	9:26	14	19
2,4,6-Me_3-Gal	→3)-Gal*p*-(1→	11:21	7	4
3-Me-Ara	→2,5)-Ara*f*-(1→, →2,4)-Ara*p*-(1→	11:96	–	2
2,3,4-Me_3-Gal	→6)-Gal*p*-(1→	12:46	1	–
4,6-Me_2-Gal	→2,3)-Gal*p*-(1→	12:58	2	1
2,6-Me_2-Gal	→3,4)-Gal*p*-(1→	13:19	4	3
2,4-Me_2-Gal	→3,6)-Gal*p*-(1→	16:27	19	12
2-Me-Gal	→3,4,6)-Gal*p*-(1→	18:29	14	9

[a] Retention times (min) obtained with a DB-225 column at 215 °C.
[b] Data published by Simas et al. (2008).

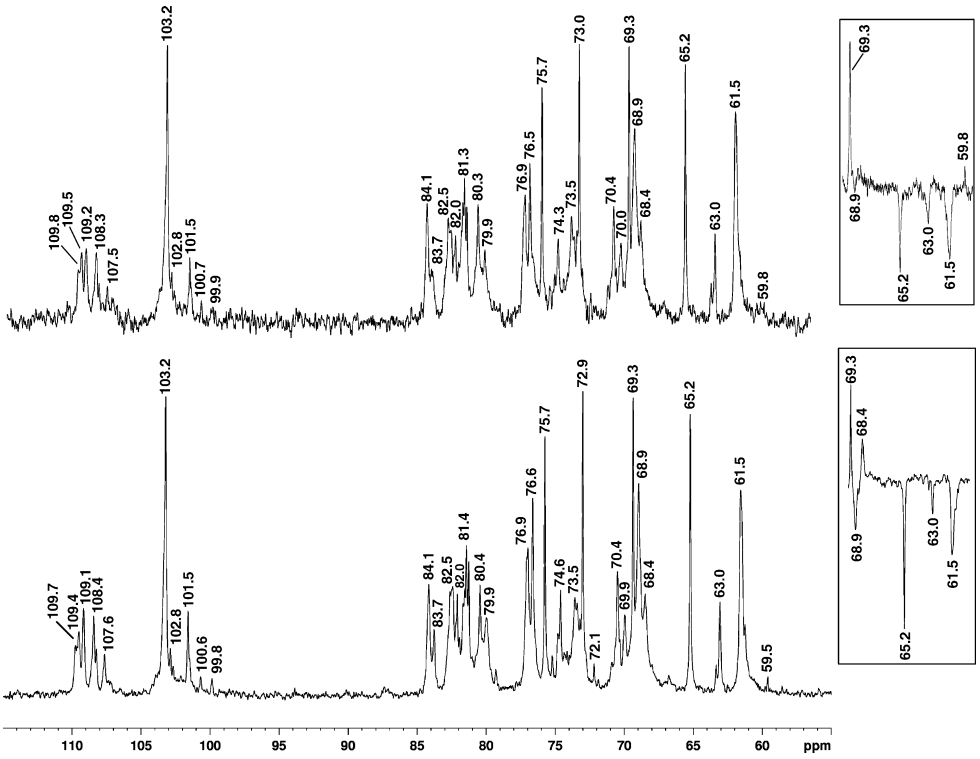

Fig. 2. ^{13}C NMR spectra of PPNA (A) (Simas et al., 2008) and PPNEC (B) from peach and nectarine gum exudate polysaccharides, respectively. Inserts: Inverted signals in ^{13}C NMR-DEPT spectra.

β-L-Ara*p* and nonreducing end-units from α-D-Xyl*p* (Delgobo et al., 1999; Gorin & Mazurek, 1975).

Comparison of the ^{13}C NMR spectra of polysaccharides from nectarine (Fig. 2B) and peach (Fig. 2A) gum exudates indicated structural similarity, although quantitative monosaccharide composition and methylation data showed some differences.

4. Conclusions

The polysaccharide from nectarine gum exudate was characterized as a homogeneous, acidic arabinogalactan, which is highly branched, indicated by methylation data, to have relatively high amounts of di- and tri-*O*-subst. Gal*p* units and nonreducing end-units of Ara*f* and Xyl*p*. These, in combination with ^{13}C NMR data, showed the presence of α-L-Ara*f* (nonreducing end, 3-*O*-, 5-*O*-, and 2,5-di-*O*-subst.), β-L-Ara*p* (4-*O*- and 2,4-di-*O*-subst.), β-D-Gal*p* (3-*O*-, 2,3-di-*O*-, 3,4-di-*O*-, 3,6-di-*O*- and 3,4,6-tri-*O*-subst.), and α- and/or β-D-Xyl*p* nonreducing end-units. A signal appeared from 4-*O*-Me-α-D-Glc*p*A units. The ^{13}C NMR spectra of polysaccharides from nectarine and peach gum exudates showed identical structural components, although monosaccharide composition, methylation, and HPSEC data showed quantitative differences.

Acknowledgments

The authors thank the FRUTALAPA Agrocomercial Ltda. for the gums and the Brazilian agencies, CAPES, CNPq, and Fundação Araucária-PRONEX for financial assistance.

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