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Branched arabinan obtained from sugar beet pulp by quaternization under acidic conditions

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ABSTRACT

Sugar beet pulp was extracted and chemically modified under acidic conditions using glycidyltrimethylammonium chloride in the presence of trifluoroacetic acid (TFA), HCl or H_3PO_4. The goal was to find out how the used acid and quaternization could affect the yield of soluble polysaccharide, its molar mass, monosaccharide composition, and structure. The use of HCl and H_3PO_4 gave smaller yields of eluents when quaternized than in the presence of TFA at ambient pressure (10% of fraction over 10 kDa and 7% of a 1–10 kDa fraction). The monosaccharide composition analysis confirmed that the use of TFA for quaternization under vacuum resulted in fraction with the higher arabinose content (86%) than the use of HCl or H_3PO_4. The molar masses were similar as on analogical extracts obtained under alkaline conditions. The NMR data indicated that the arabinan structure is modified with the quaternary group at terminal C-5 of arabinofuranose unit.

1. Introduction

Sugar beet pulp (SBP) and other agricultural byproducts are important sources of pectin and hemicelluloses which might be used as paper additives (Gigac, Fišerová, & Rosenberg, 2008), components of composite materials (Liu, Fishman, Hicks, & Liu, 2005) or for L-arabinose production (Tanaka, Yoshikawa, Mukai, Nishikawa, Morimota, 2003). Until now the solubilization of noncellulosic polysaccharides was done by a combination of enzymatic and acidic pretreatment (Panoulle, Thibault, & Bonnin, 2006; Phatak, Chang, & Brown, 1988). The structure of the isolated polysaccahrides could differ in relation to used sources (Ishii & Matsunaga, 1996; Keenan, Belton, Matthew, & Howson, 1985; Ridley, O'Neill, & Mohnen, 2001). The results are also affected by the pH used for extraction (Sakamoto & Sakai, 1995; Yapo, 2009).

In our study we tried to use a new approach related to our previous findings on fractionation and quaternization of sugarcane, sugar beet, corn fiber, corn cobs, wheat straw and pea stems (Šimkovic, Alföldi, Auxtová, Lišková, & Lerouge, 1996; Šimkovic, Antal, & Alföldi, 1994; Šimkovic, Mlynár, & Alföldi, 1990; Šimkovic, Mlynár, & Alföldi, 1992; Šimkovic, Nuñez, et al., 2009; Šimkovic, Yadav, Zalibera, & Hicks, 2009). All the above studies were done under alkaline conditions. Their advantage is that by introduction of the ion-exchanging group more material could be solubilized and simultaneously the solubilized product is quaternized which results in new properties. In the past this type of derivatives were used as paper additive to improve paper strength (Antal, Ebringerová, & Micko, 1991). The new idea used in the present study was to quaternize SBP under acidic conditions, which could be more sensitive for pectin extraction. We came to this conclusion because pectin is commercially isolated under acidic conditions with inorganic and organic acids (Yapo, Wathelet, & Paquot, 2007). The only work relating to pectin quaternization was done by the author of the present paper on polygalacturonic acid, or on SBP (Šimkovic, 1997; Šimkovic, Nuñez, et al., 2009), but not under acidic conditions. We expected that the interaction between quaternary group and pectin carboxyls might affect the extraction process. We have used trifluorocateic acid (TFA), which proved to be selective in sugar beet extraction (Dinand & Vignon, 2001) and also as a solvent of polysaccharide in the absence of water (Šimkovic & Alföldi, 1990). Samples were extracted using vacuum evaporator which we use for introducing quaternary group by reaction with glycidyltrimethylammonium chloride (GTMAC). To prevent the epoxy-ring hydrolysis of GTMAC prior to linking to polysaccharide the water was evaporated during modification. For comparison identical procedure at ambient pressure was also performed. Samples were analyzed by SEC-MALLS, NMR, and elemental analysis for molar mass, structure determination and quaternary group linkage approval. The eluents were dialyzed through 1 kDa MWCO dialysis tubing and subsequently membrane filtered with 10 kDa

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MWCO membrane to see how the yields of these two fractions were affected by the conditions used.

2. Materials and methods

2.1. Materials and procedures used

SBP (*Betula vulgaris* L.; P, 1.07%; N, 1.38%; C, 40.94%; H, 6.08%) was obtained from Willamette Valley, Idaho Milling & Grain and milled to 4 mm screen size after drying. Glycidyltrimethylammonium chloride (GTMAC, Fluka) and all other chemicals were used without further purification. The chemical modification experiments were run (in triplicate) on rotary vacuum evaporator under vacuum (3.3–4 kPa) or at ambient pressure by mixing SBP (0.5 g) with water (5 ml), with or without GTMAC (0.5 g), at pH 1.5 (adjusted with concentrated TFA, HCl or H_3PO_4) for 3 h at 60 °C in 500 ml round-bottom flask. To learn how the vacuum affects the treatment, analogous experiments were also run without vacuum. The mixture was separated into a soluble part and a non-soluble residue by filtration on fritted glass funnel (G4, Koch-Light), and washed with excess of water to ensure complete elution of water soluble part. Subsequently all the eluents were neutralized to pH 7 and preconcentrated and dialyzed through 1 kDa MWCO dialysis tubing (Spectra/Por®; reorder # 132640). The retained part was filtered through 10 kDa MWCO membrane (Amicon) at 3.5 kg/cm^2 pressure.

2.2. Sugar analysis

Sugars were analyzed by HPAEC-PAD using methanolysis, combined with TFA hydrolysis (Yadav, Johnston, & Hicks, 2007). The polysaccharide samples to be analyzed were first dissolved in de-ionized water (1 mg/ml). An aliquot of 100 μl from each sample solution along with 100 nmol myo-inositol (internal standard) were dried in a Teflon-lined screw cap glass vial by blowing with filtered nitrogen followed by drying in a vacuum oven at 50 °C overnight. In three separate glass vials were placed 200, 300 and 500 nmol of a mixture of standard sugars containing fucose, arabinose, rhamnose, galactose, glucose, xylose, glucuronic acid and galacuronic acid. Then, 100 nmol of myo-inositol (internal standard) was added to each vial, evaporated and dried as above. The polysaccharide samples and the standard sugars mixture were methanolyzed with 1.5 M methanolic HCl in the presence of 20% (v/v) methyl acetate for 16 h, cooled to room temperature and dried by blowing with filtered N_2 after adding five drops of t-butanol. The methanolyzed samples were hydrolyzed with 0.5 ml 2 M TFA at 121 °C for 1 h, evaporated by blowing with filtered N_2 at 50 °C and the residue was washed by sequential addition and evaporation of three aliquots (0.5 ml) of methanol. Hydrolyzates were analyzed for neutral and acidic sugars by HPAEC-PAD using a Dionex ICS-2500 system that included a CarboPac PA10 column and guard column, a GP 50 gradient pump, an ED50 electrochemical detector utilizing the quadruple potential waveform (gold working electrode and pH reference electrode), an AS50 autosampler with a thermal compartment (30EC column-heater), and a PC10 pneumatic controller post column addition system. The mobile phase consisted of isocratic 25 mM KOH eluent for 30 min followed by 100 mM KOH and 20 mM CH_3COOK for 10 min at a flow rate of 0.5 mL/min at ambient temperature. A 5-min column wash with 500 mM KOH followed by 15-min equilibration with 25 mM KOH at a flow rate of 1 mL/min at ambient temperature was required to yield highly reproducible retention times for the monosaccharides. The total run time was ca. 60 min. In order to minimize baseline distortion due to change in pH of the eluent during monosaccharides detection by PAD, 730 mM KOH was added to the postcolumn effluent via a mixing tee.

2.3. SEC-MALLS analysis

The characterizations were performed using the on-line model H502C differential viscometer (DV) detector from Viscotek Corp. (Houston, TX, USA). The SEC-MALLS system consisted of an Alliance 2690 separation module, a 2414 differential refractometer (DRI) from Waters (Milford, MA, USA), and a MALLS Dawn DSP-F photometer from Wyatt (Santa Barbara, CA, USA). The wavelength of the MALLS laser was 632.8 nm. The light scattering signal was detected simultaneously at 15 scattering angles ranging from 14.5° to 151.3°. The calibration constant was calculated using toluene as standard assuming a Rayleigh factor of 1.406×10^{-5} cm^{-1}. The angular normalization was performed by measuring the scattering intensity of a BSA globular protein in the mobile phase assumed to act as an isotropic scatterer. The RI increment, dn/dc of polymers with respect to the solvent was measured by a KMX-16 differential refractometer from LCD Milton Roy (Riviera Beach, FL, USA). Dry samples (1–5 mg/ml of sample concentration depending on molar mass) were dissolved in the aqueous mobile phase over night and filtered through a 0.20 μm filter before the injection in the SEC-MALLS system. The running SEC conditions were the following: carbonate buffer pH 10 as SEC mobile phase, temperature 35 °C, flow rate 0.8 mL/min, injection volume 100 μl. Two aqueous TSKgel PW columns (G4000 and G3000) from Tosoh Bioscience (Stuttgart, Germany) were used. All the analysis was run in triplicate.

2.4. NMR analysis

NMR measurements were performed in D_2O at 25 °C on VNMRS 600 MHz Varian spectrometer equipped with 5 mm ^{1}H–^{19}F/^{15}N–^{31}P PFG AutoX DB NB probe head. ^{1}H and ^{13}C chemical shifts were referenced to internal acetone (δ 2.225 and 31.07 ppm for ^{1}H and ^{13}C, respectively). The experiments presented in Fig. 1 were run using the salt tolerant CryoProbe with increased ^{13}C sensitivity. The multiplicity edited ^{1}H–^{13}C HSQC spectrum was recorded in phase-sensitive pure absorption mode with optimization on one bond coupling constant $^1J_{CH}$ = 140 Hz. The ^{1}H–^{1}H COSY spectrum with gradient selection and the ^{1}H–^{13}C HMBC spectrum were measured in absolute intensity mode. The ^{1}H–^{13}C HMBC was performed with J_{nCH} = 8 Hz. The ^{1}H–^{1}H TOCSY spectrum was recorded in phase-sensitive mode at mixing time 80 and 120 ms. The spectral widths employed in 2D NMR experiments were typically 5000 Hz (^{1}H) and 30,000 Hz (^{13}C), respectively. The numbers of acquired transients (nt) in the directly detected dimension were 24 or 80 for homonuclear experiments, and were between 128 and 200 for heteronuclear experiments. The numbers of indirectly detected transients (ni) for homonuclear experiments were 400 or 512, and for heteronuclear experiments they were 200. The F1 dimension of all heteronuclear spectra were forward linear predicted up to 2–3 times the number of data points, using either the full data set, or half, as the basis. Spectra were apodized with a sine-bell function and a shift of 90°.

3. Results and discussion

3.1. Extraction/chemical modification procedures

All the extraction and quaternization procedures are listed in Table 1. Under vacuum higher yield of soluble part (VE_1) was obtained when extracted in TFA solution than at ambient pressure. For hydrochloric and phosphoric acid more material was extracted at ambient pressure than under vacuum. It could be explained by the fact that TFA was evaporated before water during the procedure under vacuum which resulted in less hydrolyzed solubilized

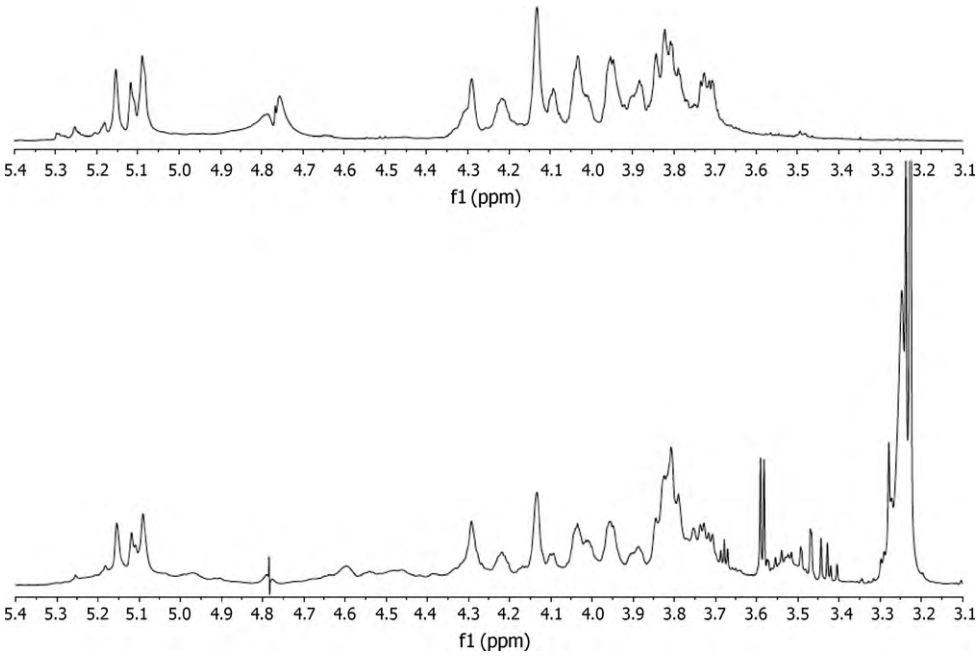

Fig. 1. ^{1}H-NMR spectrum of fraction VE_1 (top) and VE_4 (bottom).

fraction. When SBP was quaternized in the presence of TFA the yield of eluted polysaccharide at ambient pressure (E_4; at 1.00% of nitrogen content) was higher than with analogical procedure under vacuum (VE_4; N = 1.56%). This indicates that quaternization was more effective under vacuum as higher nitrogen content was obtained for the eluent as well as for the residue than under ambient conditions. Also the autocatalytic effect the qauternizing agent should be taken into account under vacuum after TFA evaporation. This was demonstrated before when SBP was quaternized in the absence of any other chemical (Šimkovic, Nuñez, et al., 2009). The same amount of polysaccharide was isolated at ambient pressure when quaternized in the presence of HCl (E_5) as with TFA. The amount obtained under vacuum (VE_5) was smaller than at ambient pressure (E_5). With H_3PO_4 at ambient pressure the yield was the smallest but with the highest content of quaternary group (N = 5.46%). The amounts of obtained residues under vacuum or at ambient pressure with TFA (70/72%) were less different from values obtained with HCl (61/22%) and H_3PO_4 (73/57). As all the experiments were run in triplicate, this could be explained by the more severe conditions applied at ambient pressure with inorganic acids when acid hydrolysis was more effective on cellulose component. The obtained residues were quaternized more effectively than the

Table 1
Yields of individual fractions and their characteristics.

Fraction	Acid	QA	Yield[a]	N[b]	M_w[c]	M_w/M_n[c]	M_p[c]	Yield[d]	N[e]
VE_1	TFA	–	5	–	800	16.7	2290	3	–
VR_1	TFA	–	70	–	–	–	–	–	–
E_1	TFA	–	2	–	175	2.6	72	2	–
R_1	TFA	–	72	–	–	–	–	–	–
VE_2	HCl	–	3	–	1768	1.3	1954	4	–
VR_2	HCl	–	61	–	–	–	–	–	–
E_2	HCl	–	4	–	1760	1.5	2094	2	–
R_2	HCl	–	22	–	–	–	–	–	–
VE_3	H_3PO_4	–	2	–	1202	10.5	55	10	–
VR_3	H_3PO_4	–	73	–	–	–	–	–	–
E_3	H_3PO_4	–	3	–	1297	1.7	3115	4	–
VE_4	TFA	+	6	1.56	43	5.9	15	1	0.15
VR_4	TFA	+	72	1.74	–	–	–	–	–
E_4	TFA	+	10	1.00	49	6.2	12	7	0.43
R_4	TFA	+	57	1.40	–	–	–	–	–
VE_5	HCl	+	3	1.50	787	3.0	2535	3	0.36
VR_5	HCl	+	49	1.95	–	–	–	–	–
E_5	HCl	+	10	1.78	2200	1.4	2800	3	0.21
R_5	HCl	+	71	2.25	–	–	–	–	–
VE_6	H_3PO_4	+	4	1.80	850	2.6	1860	2	0.45
VR_6	H_3PO_4	+	73	2.14	–	–	–	–	–
E_6	H_3PO_4	+	3	1.49	733	2.5	2409	4	0.25
R_6	H_3PO_4	+	59	2.06	–	–	–	–	–

[a] Yield of fraction >10 kDa [%].
[b] Nitrogen content [%].
[c] M_w = weight-average molar mass [kg/mol]; M_w/M_n = polydispersity index (D); M_p = molar mass at a peak; M_n = numeric-average molar mass.
[d] Yield of 1–10 kDa fraction [%].
[e] Nitrogen content of 1–10 kDa fraction [%].

Table 2
Monosaccharide composition [mol%] of isolated fractions[a].

Fraction	Ara	Gal	GalA	Rha	GlcA	Xyl	Fuc	Glc
VE_1	77	6	10	5	0	1	0	1
E_1	47	13	9	7	4	9	3	8
VE_2	6	23	5	6	4	14	12	30
E_2	6	23	9	13	2	17	10	20
VE_3	14	21	23	6	4	13	3	16
E_3	10	20	6	13	2	22	8	19
VE_4	86	7	1	3	1	1	0	1
E_4	51	24	1	10	4	3	2	5
VE_5	23	18	29	11	3	3	0	13
E_5	5	25	7	8	4	15	10	26
VE_6	12	16	42	13	3	2	4	8
E_6	16	22	5	11	0	14	4	28

[a] Monosaccharide abbreviation are as used in the text.

solubilized fractions (Table 1). The yields of 1–10 kDa fractions were usually smaller than of fractions retained by the membrane with an exception for H_3PO_4 treatment under vacuum (10% for VE_6). This indicates that the solubilized fraction was more effectively hydrolyzed by H_3PO_4, which could not be evaporated. It must be also taken into account that most of the hydrolyzed fraction was probably dialyzed through 1 kDa tubing and could not be retained in the 1–10 kDa fraction. But the sum yield of both fractions isolated at ambient pressure with TFA using quaternization procedure was the highest from all experiments. The obtained yields are lower in comparison to corresponding yields of both fractions: >10 kDa as well as 1–10 kDa fractions from SBP or corn fiber obtained when quaternized under alkaline conditions (Šimkovic, Nuñez, et al., 2009; Šimkovic, Yadav, et al., 2009). To explain this phenomenon the monosaccharide composition, molar masses and the structure of individual fractions needs to be considered.

3.2. Monosaccharide composition

The monosaccharide composition of the extracts obtained is listed in Table 2. On the basis from previous studies the monosaccharide composition of the insoluble residues was not performed due to the fact that complete hydrolysis of cellulose component could not be guarantied. This was concluded on the basis of low glucose (Glc) contents in the monosaccharide composition of insoluble residues (Šimkovic, Yadav, et al., 2009). The use of TFA under vacuum (VE_1) or at ambient pressure (E_1) resulted in high arabinose (Ara) content in comparison to the use of HCl or H_3PO_4. Under vacuum the Ara content was much higher (77%) than for experiment run at ambient pressure (47%). The use of HCl under both conditions resulted in a dramatic decrease of Ara content (6%). With H_3PO_4 the situation was similar. Generally, the use of inorganic acids resulted in increased Glc content, probably due to cellulose hydrolysis, as these acids were not evaporated by the treatment in contrary to TFA. When the SBP was quaternized in the presence of TFA under vacuum (VE_4), the highest Ara content from all samples was observed. At ambient pressure (E_4) the content of Ara dropped to the second highest and the galactose (Gal) content simultaneously increased. This could be explained by the sensitivity of arabinofuranose glycosidic bonds to acid hydrolysis due to the fact that TFA was not evaporated when ambient pressure experiment was run. That is why also with HCl and H_3PO_4 lower contents of Ara were obtained. In general, it indicates that quaternization of SBP in the presence of TFA is useful for isolation of fractions with high Ara content, while in the presence of HCl and H_3PO_4 under vacuum D-galacturonic acid (GalA) is the predominant monosaccharide component. At ambient pressure Glc and Gal are predominant in E_5 and E_6 fractions. The results also show that direct extraction with TFA does not extract (4-O-methyl-D-glucurono)-D-xylan as in case of NaOH and $NaClO_2$ pretreatment and subsequent TFA extraction (Dinand & Vignon, 2001). It is evident from the low D-xylose (Xyl) content of E_1 and VE_1 fractions. More coherent description of the data is not possible because the isolated fractions are mixtures of arabinogalactans and rhamnogalacturonans. The separation of these types of mixtures probably requires enzymatic pretreatment which could hydrolyze one of the components as mentioned above (Panoulle et al., 2006; Phatak et al., 1988).

3.3. SEC-MALLS analysis

The fraction obtained under vacuum when treated only with TFA (VE_1) exhibited higher weight-average molar mass (M_w) but the highest polydispersity (D) from all studied samples (Table 1). The difference between the M_w of VE_1 and E_1 could be explained by the different TFA concentration during the treatment due to its evaporation under vacuum before water. Higher D value indicates hydrolysis resulting in fractions with lower molar mass than average. It might also indicate that some of the polysaccharide fractions have branched structure. When HCl was used the difference between molar masses of VE_2 and E_2 was minimal due to the fact that an inorganic acid could not be evaporated from the mixture. The treatment with H_3PO_4 resulted in slightly smaller M_w for VE_3 than for VE_2 and the second biggest D value. The E_3 sample had similar molar mass as the VE_3, but much smaller D value. This indicates that the treatment with H_3PO_4 under vacuum resulted in more dramatic hydrolysis due to the increased acid concentration due to water evaporation. When quaternization was performed in the presence of TFA, the M_w and D values were similar for both VE_4 and E_4 experiments, although both the M_w values were the smallest from all the observed data. This could be explained by the synergic action of TFA and quaternizing agent which seams to be supporting the hydrolysis of solubilized polysaccharides. It also affected the D values which were bigger than all the rest except the values for VE_3 and VE_1. Identical treatment with HCl resulted in higher M_w for VE_5 and E_5 in comparison with VE_4 and E_4, and also the D values decreased. Both inorganic acids produced larger masses of quaternized material than for analogical TFA data. The biggest M_w value was obtained for E_5, accompanied with the $D = 1.4$. On the other side, the smallest M_w values resulted form VE_4 and E_4 samples, while the smallest D value was observed when extracted in the presence of HCl (VE_2). The main explanation for the differences is due to the fact of TFA evaporation phenomenon during the vacuum treatment. It might be also a certain interaction between quaternary ion-exchanging groups and the anion of dissociated acid used which might contribute to the results. The molar masses of the quaternized samples were similar to analogical fractions obtained under alkaline condition. Similar was also the molar mass value obtained by the TFA extract and water extract obtained previously (Šimkovic, Nuñez, et al., 2009). The difference was the high D values for the TFA samples and VE_3 sample in comparison to other samples.

3.4. NMR characterization of fractions

The fraction isolated by TFA extraction (Table 1, VE_1) produced five signals in the anomeric region of both the ^{1}H-NMR (Fig. 1, top) and HSQC (Fig. 2, left) spectra (Table 3). They were at 108.37/5.09, 108.33/5.12, 108.01/5.16, 107.92/5.19, and 107.23/5.26 ppm and could be assigned to Ara units (Dourado, Cardoso, Silva, Gama, & Coimbra, 2006). Additionally there were three less intense anomeric signals which according to monosaccharide composition (Table 1) could be related to GalA (102.44/5.10 ppm), Gal (105.35/4.64 ppm) and Rha (96.23/5.30 ppm). The rest of the signals listed in Table 3 were assigned according to COSY, TOCSY, HSQC (Fig. 2, left window) and HMBC experiments. The HMBC

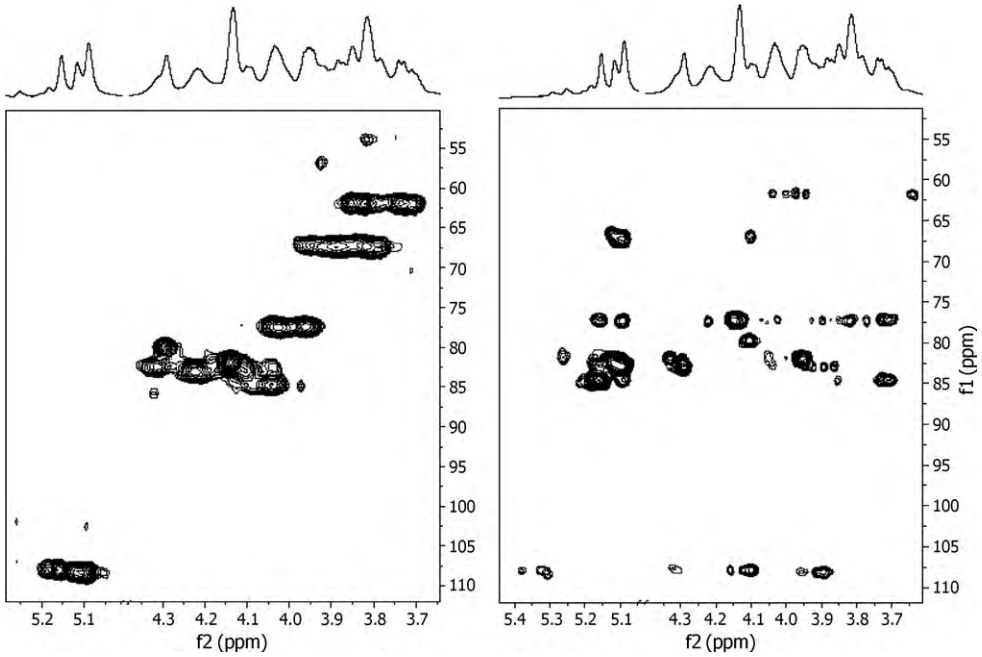

Fig. 2. HSQC (left window) and HMBC (right window) spectra of VE_1 fraction.

(Fig. 2, right) experiment indicates that there are except (1 → 5)-linked Ara also additional Ara units linked at positions 2, 3, and 5 to other units. Additionally there are two different terminal Ara units. This indicates that the arabinan in VE_1 fraction is branched. The less intense ring signals of Gal, GalA, and Rha could not be completely assigned. Five different acetyl groups were observed at 21.23/2.21, 21.27/2.14, 21.27/2.12, 21.32/2.07, and 21.45/2.18 ppm. The presence of acetyl groups in sugar beet pectin is known (Keenan et al., 1985). Also a methylester signal of GalA was observed at 53.79/3.81 ppm, which is in agreement with data observed on fully esterified polygalacturonic acid (Šimkovic, Synytsya, Uhliariková, & Čopíková, 2009). We assume that the second uronic acid unit (GalA′) was not esterified, as the similar GalA unit chemical shifts were observed under alkaline conditions (Šimkovic, Yadav, et al., 2009). The above branching of arabinan was not observed under alkaline conditions mentioned above. This branching is also supported by the high *D* values of TFA treated samples.

The fraction VE_4 which was isolated after quaternization in the presence of TFA under vacuum gave almost identical ^{1}H NMR spectrum at the anomeric region with some new signals related to quaternary group (Fig. 1, bottom). The 2D spectra when analyzed with the equipped CryoProbe resulted also in similar data as observed on VE_1 (Table 3). The other anomeric signals present in the HSQC spectrum (Fig. 3, left) confirmed the presence of Gal (105.32/4.64 ppm), GalA (100.44–101.19/4.97–5.11 ppm) and Rha (93.10/5.31 ppm). The rest of the signals were assigned tentatively using the two-dimensional experiments and literature data. New signals of sugar ring substituents were at 55.05/3.23 (CH_3 groups), 67.25/4.29 (CHOH group), 69.05/3.47, 3.43 and 64.47/3.59 ppm (CH_2 groups). According to the known data we suppose that the

Table 3
NMR data of VE_1 and VE_4 samples (ppm).

Sample/unit	H_1/C_1	H_2/C_2	H_3/C_3	H_4/C_4	H_5/C_5	H'_5	H_6/C_6
VE_1/Ara[a]	5.09/108.37	4.14/81.94	4.02/77.54	4.22/83.11	3.80/67.69	3.89	–
VE_1/Ara[b]	5.12/108.33	4.29/80.03	4.10/83.07	4.32/82.42	3.84/67.31	3.94	–
VE_1/Ara[c]	5.16/108.01	4.14/81.94	3.95/77.42	4.05/84.78	3.72/61.96	3.83	–
VE_1/Ara[d]	5.19/107.92	4.13/81.94	3.96/77.42	4.05/84.78	3.72/61.96	3.83	–
VE_1/Ara[e]	5.26/107.23	4.31/85.79	4.09/84.80	4.26/ni	3.84/66.91	3.92	–
VE_1/Gal	4.64/105.35	3.69/73.30	ni	ni	ni	–	ni
VE_1/GalA	5.10/102.44	3.78/ni	ni	ni	ni	–	–/171.76
VE_1/GalA′	5.26/102.20	ni	ni	ni	ni	–	ni
VE_1/Rha	5.30/96.23	4.09/76.90	3.93/69.30	ni	3.79/ni	–	1.30/17.80
VE_4/Ara[a]	5.09/108.38	4.13/81.96	4.02/77.55	4.22/83.16	3.80/67.70	3.89	–
VE_4/Ara[b]	5.11/108.34	4.29/80.04	4.10/83.15	4.31/82.48	3.83/67.35	3.95	–
VE_4/Ara[c]	5.15/108.01	4.14/82.15	3.96/77.42	4.04/84.79	3.72/61.95	3.83	–
VE_4/Ara[d]	5.18/107.90	4.14/82.15	3.96/77.42	4.04/84.79	3.72/61.95	3.83	–
VE_4/Ara[e]	5.26/107.30	4.32/85.79	4.08/84.92	4.26/81.04	3.83/66.76	3.95	–
VE_4/GalA	4.97–5.11/100.44–101.19	3.73–3.76/68.75	3.98–4.00/68.88	4.40–4.46/79.74–79.88	4.69–4.74/72.26	–	–/171.36
VE_4/Gal	4.64/105.32	3.68/72.67	3.78/74.18	4.17/78.54	3.69/76.10	–	ni
VE_4/Rha	5.31/93.10	4.10/77.36	3.92/69.42	3.48/72.17	3.79/ni	–	1.26/17.50

ni – not identified.
[a] (1–5)-linked unit.
[b] (1,3,5)-linked unit.
[c] Terminal unit.
[d] Different terminal unit.
[e] (1,2,3,5)-linked unit.

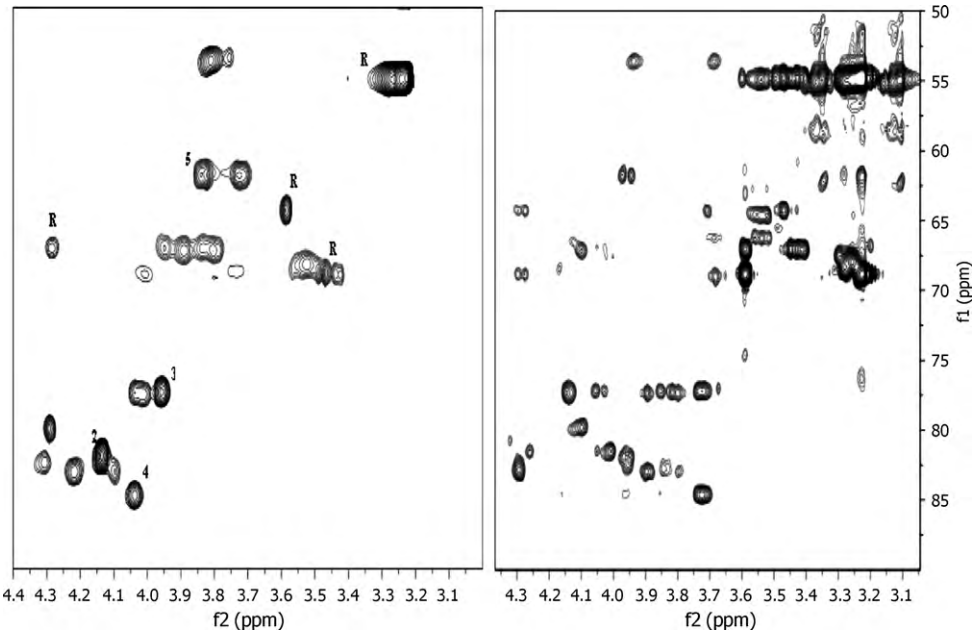

Fig. 3. HSQC (left window) and HMBC (right window) spectra of VE_4. The numbers 2, 3, 4, 5, and R are related to signals of Ara unit and substituted group indicating the correlation between *H*-5 and *C*H_2 of the substituent linked through ether bond.

signal at 69.05 ppm belongs to CH_2 linked to quaternary nitrogen (Šimkovic, Yadav, et al., 2009). From the HMBC (Fig. 3, right) spectrum it could be concluded that there is a correlation between *H*-5 of the Ara at 3.72 ppm and *C*H_2 signal at 64.47 ppm. This confirms the linkage of the quaternary group at the terminal arabinose unit. The rest of the correlations are the same as on unmodified VE_1 eluent. There are five acetyl CH_3 groups present according to the HSQC spectrum at 20.95/2.01, 21.93/2.08, 21.03/2.12, 21.02/2.14, and 21.31/2.18 ppm. The new result obtained from the NMR data is the confirmation of linkage to the substituent at C-5 of terminal arabinofuranose. As there is no other cross peak in correlation between peaks assigned to the substituent, signed as R in the HSQC window (Fig. 3, left) and signals in the HMBC spectrum (Fig. 3, right) we can conclude that the substituent is linked exclusively at C-5 of nonreducing arabinose unit. The higher nitrogen content in VE_5 sample could be interpreted as due to formation of the ionic bonds between pectin carboxyls and quaternary nitrogen of the hydrolyzed quaternary agent. This linkage could not be proved by HMBC due to four-bound linkage distance of possible qauternizing agents methyl hydrogen and pectin carboxyl carbon interaction. Under alkaline conditions the substitution was observed at both C-2 and C-3 of arabinose units (Šimkovic, Yadav, et al., 2009).

4. Conclusions

Quaternized polysaccharides could be obtained by chemical modification under acidic conditions. Up to 10% of material with molar mass over 10 kDa could be isolated as well as 7% of 1–10 kDa fraction in the presence of TFA. The linkage of TMAHP-group through terminal C-5 of arabinose unit as well as the branching of arabinan at C-2 and C-3 was confirmed by NMR spectroscopy. The isolated polysaccharides represented a mixture of arabinogalactans and rhamnogalacturonans with high content of arabinose rich arabinogalactan when the extraction was run in the presence of TFA. The treatment with TFA and absence of quaternary agent under vacuum resulted in the highest $D = 16.7$ and $M_w = 800$ kg/mol, which is bigger than the value measured at ambient pressure (175 kg/mol and $D = 2.6$). When the extraction was conducted in the presence of HCl or H_3PO_4 part of the cellulose was hydrolyzed and contaminated the arabinan fraction. Quaternization run in the presence of TFA resulted in the highest content of Ara in the polysaccharide although the molar mass dropped in comparison to the value obtained under analogical conditions without the use of the quaternary agent. The presented method might find application for preparation of paper additives with the use of HCl or H_3PO_4 or for some other applications as the differences in yields are not that dramatical.

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