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Combined HILIC-ELSD/ESI-MS^n enables the separation, identification and quantification of sugar beet pectin derived oligomers

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ABSTRACT

The combined action of endo-polygalacturonase (endo-PGII), pectin lyase (PL), pectin methyl esterase (fungal PME) and RG-I degrading enzymes enabled the extended degradation of methylesterified and acetylated sugar beet pectins (SBPs). The released oligomers were separated, identified and quantified using hydrophilic interaction liquid chromatography (HILIC) with online electrospray ionization ion trap mass spectrometry (ESI-IT-MS^n) and evaporative light scattering detection (ELSD). By MS^n, the structures of galacturonic acid (GalA) oligomers having an acetyl group in the *O*-2 and/or *O*-3 positions eluting from the HILIC column were elucidated. The presence of methylesterified and/or acetylated galacturonic acid units within an oligomer reduced the interaction with the HILIC column significantly compared to the unsubstituted GalA oligomers. The HILIC column enables a good separation of most oligomers present in the digest. The use of ELSD to quantify oligogalacturonides was validated using pure GalA standards and the signal was found to be independent of the chemical structure of the oligomer being detected. The combination of chromatographic and enzymatic strategies enables to distinguish SBPs having different methylesters and acetyl group distribution.

1. Introduction

Commercially extracted pectin is commonly used as viscosifying or gelling agent in the food industry. Pectic substances are present in high proportions in sugar beet pulp (Oosterveld, Beldman, Schols, & Voragen, 1996), which is seen as potential raw material for the pectin industry. The primary building units of sugar beet pectin (SBP) are the homogalacturonan (HG) and rhamnogalacturonan (RG-I) regions (Guillon, Thibault, Rombouts, Voragen, & Pilnik, 1989). The HG consists of a backbone of galacturonic acid moieties, which can be methylesterified at the carboxyl group. The RG-I subunit has alternating units of α-1,4-linked D-galacturonosyl-α-1,2-L-rhamnose and 20–80% of the rhamnose units are substituted with neutral sugar side chains, mainly arabinans (Voragen, Coenen, Verhoef, & Schols, 2009). The galacturonic acid unit in both RG-I and HG can be acetylated at positions *O*-2 and/or *O*-3 (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

The suitability of a pectin as gelling agent or stabilizer is determined by its structural features i.e. the amount and distribution of methylesters and acetyl groups over the pectin backbone. Although SBP has an essential property as emulsifier (Williams et al., 2005), the acetyl group inhibits the ability of SBP to form good gels. Hence, it cannot compete commercially with apple and lemon pectins (Thakur, Singh, Handa, & Rao, 1997). Previous methods to study the pattern of pectin's methylesterification and amidation included enzymatic fingerprinting using endo-polygalacturonase (endo-PG) from *Kluyveromyces fragilis*, high performance anion exchange chromatography (HPAEC) at pH 5 and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Daas, Arisz, Schols, De Ruiter, & Voragen, 1998; Guillotin, Van Kampen, Boulenguer, Schols, & Voragen, 2006). These methods have been developed and proven to be effective. However, they cannot be applied on SBP. Due to its high degree of complexity and heterogeneity, it has been demonstrated that SBP hydrolysis by endo-PG alone is not able to generate sufficient amounts of GalA oligomers (Buchholt, Christensen, Fallesen, Ralet, & Thibault, 2004). In addition, the use of HPAEC pH 5 with online MS identification is not easy since an online desalting step would be necessary due to the high salt content of the mobile phase (Westphal, Schols, Voragen, & Gruppen, 2010). Also, the absence of standards makes the quantification of methylesterified and acetylated GalA residues using pulsed amperometric detection (PAD) difficult.

A few years back, several methods specific for SBP have been developed using preparative anion exchange and size exclusion chromatography (Bonnin, Dolo, Le Goff, & Thibault, 2002) and the purified oligomers were analyzed by offline ESI-MS^n (Ralet et al., 2005). However, these chromatographic methods require relatively high amounts of sample material and the method is quite

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Table 1
Sugar composition of beet pectin samples.

Pectin	GalA	Rha	Ara	Xyl	Gal	Glc	DM (%)[c]	DA (%)[c]
	mg/g of dry matter							
P5328[a]	582	55	109	2	99	4	53	28
F5129[a]	567	51	116	3	94	2	51	29
SBP5317[b]	560	30	40	n.d.	80	1	53	17

[a] Sugar composition (Buchholt et al., 2004).
[b] Sugar composition determined in this study.
[c] Moles acetyl or methanol per 100 mol of galacturonic acid.
n.d., not detected.

laborious and time consuming. Recently, hydrophilic interaction liquid chromatography (HILIC) using a BEH amide column coupled to electrospray ionization ion trap mass spectrometry (ESI-IT-MS^n) with evaporative light scattering detection (ELSD) was described for the analysis of acidic oligosaccharides (Leijdekkers, Sanders, Schols, & Gruppen, 2011). Combined HILIC–MS method enables the separation and online peak annotation of various GalA oligomers by their m/z values. This method was demonstrated to be a valuable tool to characterize methylesterified and acetylated pectins providing a much better separation and retention compared to other techniques. However, Leijdekkers et al. (2011) did not demonstrate the complete identification and quantification of all oligomeric degradation products in extensively digested SBP. Hence, in this paper, the HILIC-ELSD-ESI-IT-MS^n method was further optimized and applied to separate, identify and quantify the complex mixture of SBP oligomers generated by the combined action of pectolytic enzymes.

2. Experimental

2.1. Pectin samples

Experimental sugar beet pectins (SBPs), modified by plant PME (P5328) and fungal PME (F5129); and the commercial SBP5317 were obtained from Danisco (Brabrand, Denmark) (Table 1). Determination of the neutral sugar composition of SBP5317 by gas chromatography of alditol acetates was achieved after subsequent hydrolysis by 72% (w/w) sulfuric acid and 1 M sulfuric acid (Selvendran, March, & Ring, 1979). The uronic acid was determined by an automated colorimetric m-hydroxydiphenyl method as described previously (Kuhnel, Schols, & Gruppen, 2011). Pectin samples (≈1 mg) were saponified by 1 M NaOH to determine the degree of methylesterification (DM) using colorimetric method (Guillotin, Van Loey, Boulenguer, Schols, & Voragen, 2007) while the degree of acetylation (DA) was analyzed using Megazyme acetic acid kit (Megazyme, Wicklow, Ireland).

2.2. Enzymes

Pure and well characterized RG-I and HG degrading enzymes were used to hydrolyse sugar beet pectins. The enzymes used in this study were *Aspergillus aculeatus* endo-galactanase (EC 3.2.1.89) (Schols, Posthumus, & Voragen, 1990), endo-arabinanase (EC 3.2.1.99) (Beldman, Searle-van Leeuwen, De Ruiter, Siliha, & Voragen, 1993), RG-hydrolase (EC 3.2.1.B9) (Mutter, Renard, Beldman, Schols, & Voragen, 1998), *Chrysosporium lucknowense* (C1) exo-arabinase (EC 3.2.1.1) (Kühnel et al., 2010), *Aspergillus niger* fungal pectin methyl esterase (fungal PME) (EC 3.1.1.11) (Van Alebeek, Van Scherpenzeel, Beldman, Schols, & Voragen, 2003), pectin lyase (EC 4.2.2.10) (Harmsen, Kusters-van Someren, & Visser, 1990) and endo-polygalacturonase II (EC 3.2.1.15) (Limberg et al., 2000).

2.3. Enzymatic hydrolysis

Sugar beet pectin (SBP5317) in 50 mM sodium citrate buffer pH 5 (5 mg/ml) was digested at 40 °C by RG-I (endo-galactanase + endo/exo arabinase + RG hydrolase) and HG (PL + endo-PGII) degrading enzymes to hydrolyse the SBP samples as far as possible. The hydrolysis was done by incubating the pectin solution with RG-I degrading enzymes and PL for 6 h followed by the addition of endo-PGII and fungal PME followed by the subsequent incubation for another 18 h. Sugar beet pectins (P5328 and F5129) were digested in the same way although PME addition was omitted during digestion. Enzyme doses were sufficient to degrade theoretically their corresponding substrates within 6 h into monomers. Inactivation of enzymes was performed at 100 °C for 6 min and the reaction products were analyzed by high performance size exclusion chromatography (HPSEC) and UHPLC–HILIC coupled to ESI-IT-MS^n and ELSD detectors.

2.4. HPSEC

Sugar beet pectin digests were analyzed using HPSEC on an Ultimate 3000 system (Dionex, Sunnyvale, CA, USA). A set of four TSK-Gel super AW columns (Tosoh Bioscience, Tokyo, Japan) was used in series: one guard column (6 mm ID × 40 mm) and the three separation columns 4000, 3000 and 2500 (6 mm × 150 mm). The column temperature was set to 55 °C. Samples (20 μl, 2.5 mg/ml) were eluted with filtered 0.2 M $NaNO_3$ at a flow rate of 0.6 ml/min and the elution was monitored by refractive index detection (Shodex RI 101; Showa Denko K.K., Kawasaki, Japan) and UV_{235} detection (Dionex Variable Wavelength Detector, Sunnyvale, CA, USA).

2.5. UHPLC-ELSD–ESI-IT-MS^n

Digests were analyzed using UHPLC in combination with ESI-IT-MS^n and ELSD on a HILIC BEH amide column as described previously (Leijdekkers et al., 2011). The composition of the two mobile phases was (A) 80:20 (v/v) acetonitrile (ACN)/water, and (B) 20:80 (v/v) ACN/water, both containing 0.01 M ammonium formate and 0.05 M formic acid. The following elution profile was used: 0–1 min, isocratic 100% A; 1–60 min, linear from 30% to 80% B; followed by column re-equilibration: 61–67 min, linear from 20% to 100% A; 68–75 min, isocratic 100% A. The eluent was split into 1:1 using an ASI flow splitter (Analytical Scientific Instruments, CA, USA) before leading to the ELSD and the ESI-IT-MS detector. Mass spectra were acquired over the scan range m/z 150–2000. Xcalibur software was used to process the data (Thermo). Commercial GalAs degree of polymerization (DP) 1–3 (Sigma-Aldrich, Steinheim, Germany), unsaturated (DP 2–6) and saturated (DP 4–5) galacturonic acid standards were used as purified in our laboratory as described (Van Alebeek, Christensen, Schols, Mikkelsen, & Voragen, 2002). To estimate the amount of oligomers by ELSD, curve fitting of each GalA standard using a power function of $f(x) = ax^b$ was used, where $f(x)$

is the peak area, x is the sample amount, a is the response factor and b is the slope (Decroos et al., 2005; Mengerink, De Man, & Van der Wal, 1991). The average responses of DP 1–3 were plotted and the mathematical equation was derived (Eq. (1)).

$$\text{concentration [ppm]} = \exp\left(\frac{\ln(\text{ELSD peak area}/0.4921)}{1.7014}\right) \quad (1)$$

3. Results and discussion

3.1. Enzymatic hydrolysis of SBP

In this study, RG-I degrading enzymes and PL were added to degrade the pectin side chain and methylesterified HG, respectively. The mixture was then digested with endo-PGII and fungal PME. As endo-PGII and PL cannot sufficiently degrade highly substituted SBPs (Buchholt et al., 2004), fungal PME enabled to generate a complete as possible mixture of different GalA oligomers.

In order to monitor the degradation of SBP by pectolytic enzymes, HPSEC with online UV and RI detector was used. Fig. 1 illustrates the enzyme-treated SBP resulting in a shift in molecular weight (M_w) to yield low M_w oligomers. The UV_{235} signal indicates that next to the release of saturated GalA oligomers by PG, also unsaturated GalA oligomers resulting from PL action were released. HPSEC analysis indicates clearly that PG and PL together sufficiently degrade SBP to a broad range of diagnostic oligomers (<10 kDa) eluting at retention time >10.5 min.

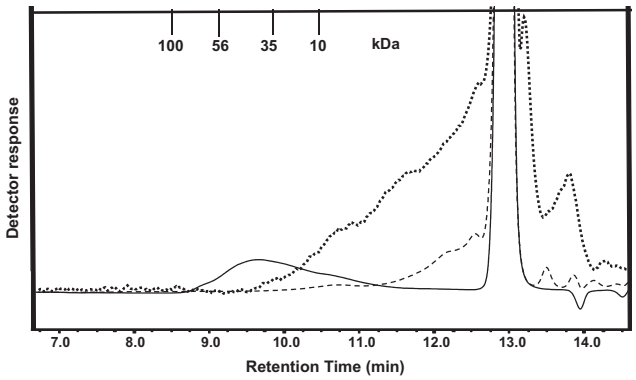

Fig. 1. HPSEC elution pattern of SBP5317 (DM 53, DA 17) before (—, RI) and after (UV_{235} ····; ---- RI) digestion with RG-I and HG degrading enzymes. Molecular masses of pectin standards (in kDa) are indicated.

3.2. Separation and annotation of the reaction products by HILIC–MS

The HILIC elution pattern of SBP5317 (Fig. 2A) illustrates that besides the unsubstituted dimer (2^{00}) and trimer (3^{00}), partially

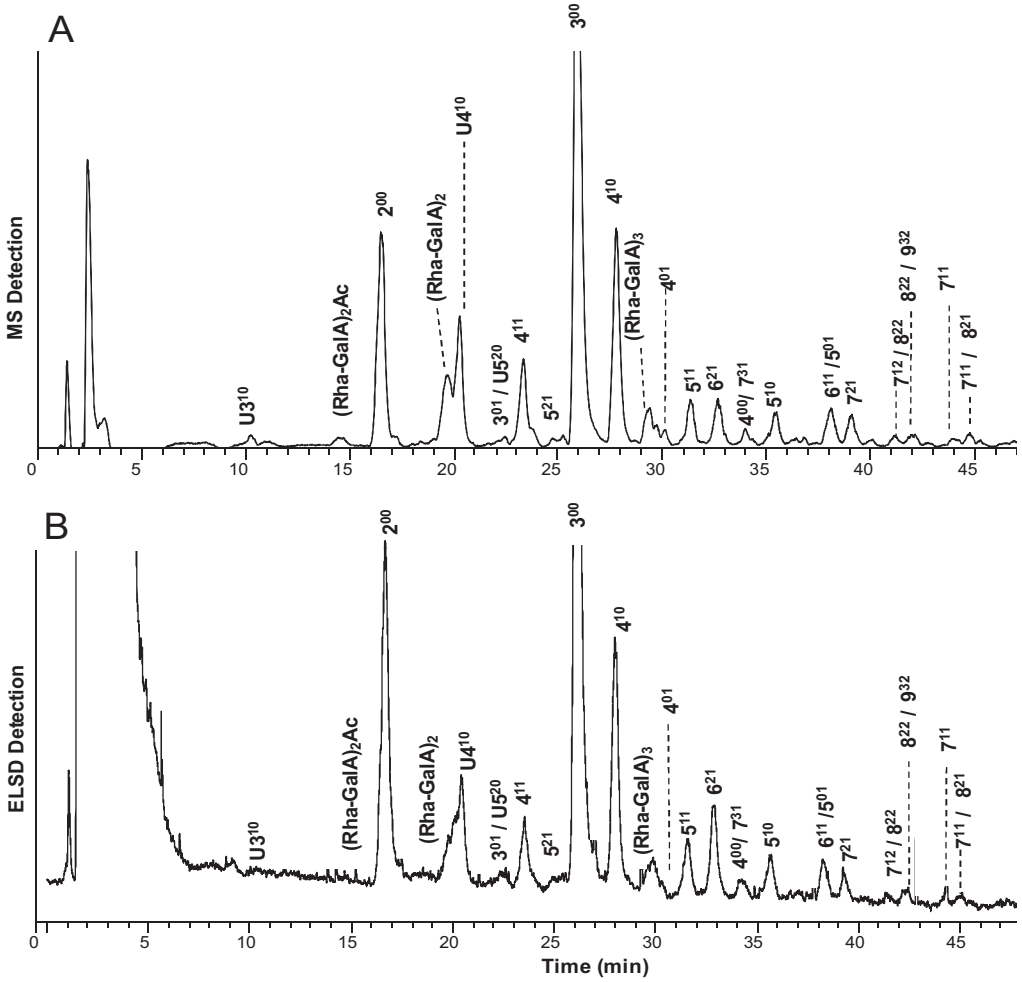

Fig. 2. HILIC elution pattern of SBP5317 digested by RG-I and HG degrading enzymes using (A) ESI-IT-MS^n (B) ELS detection. Peak annotation: 5^{11}, DP 5; one *O*-methylester and one *O*-acetyl group. U: unsaturated GalA. Rha: rhamnose, GalA, galacturonic acid, Ac: acetyl group.

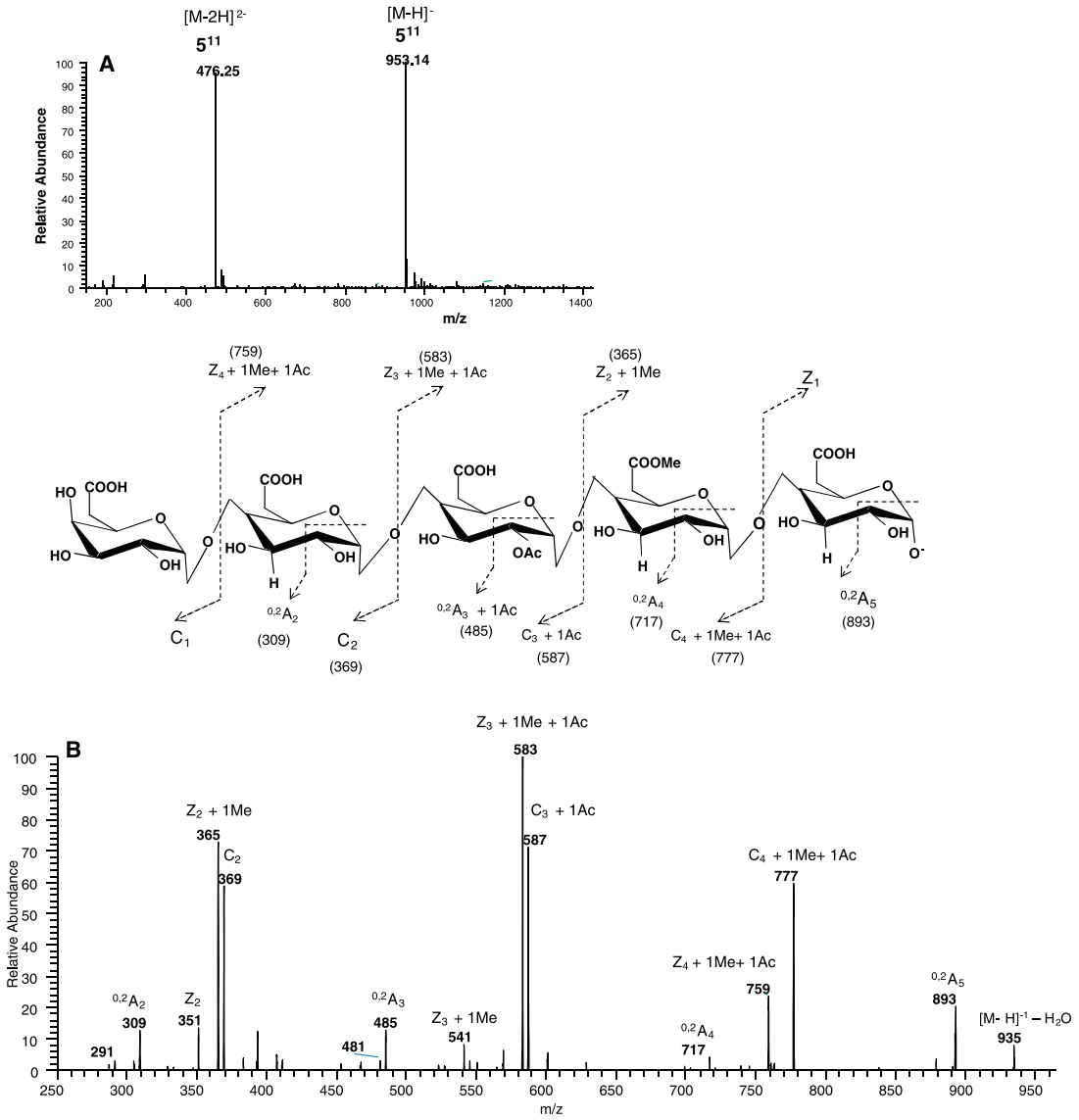

Fig. 3. Full MS pattern (A) and MS^2 fragmentation pattern (B) of $GalA_5$ (5^{11}) oligomer (*m*/*z* 953) DP 5 with *O*-methylester and *O*-acetyl group eluting at 31.3 min in Fig. 2A using HILIC with online MS.

methylesterified and/or acetylated saturated and unsaturated GalA oligomers of different DP were present as main degradation products. Partially acetylated RG-I oligomers i.e. $(Rha\text{-}GalA)_2Ac$ were identified as well as unsaturated GalA oligomers containing methylesters ($U3^{10}$, $U4^{10}$, $U5^{20}$). The elution behavior of GalA oligomers of the same charge and DP was also influenced by the presence of an acetyl group. As an example: tetramer 4^{11} (*m*/*z* 777) with a methylester and an acetyl group, eluted before tetramer 4^{10} (*m*/*z* 735) with a methylester but without acetyl group.

The efficient separation and rapid identification of a complex SBP digest with HILIC exemplify the advantage of the technique for screening SBP digests compared to the conventional preparative separation (Bonnin et al., 2002; Ralet et al., 2005). It is evident that with HILIC analysis, oligosaccharides in pectin digests containing unsubstituted GalAs can be completely differentiated from the oligosaccharides containing methylesterified and acetylated GalA units. In this way, fingerprinting of pectins and determination of the degree of blockiness can be achieved with higher accuracy compared to HPAEC pH 5 separation method (Daas, Meyer-Hansen, Schols, De Ruiter, & Voragen, 1999). The good alignment between MS and ELSD chromatograms (Fig. 2B) also allows the possibility of peak identification in the ELSD elution profile, the latter being used for quantification (Section 3.4).

3.3. Structure elucidation of acetylated GalA oligomers by HILIC–MS^n

To verify the structural information provided by HILIC–MS^1 and to elucidate the precise position of the methylesters and acetyl group within the pectic oligosaccharides, MS^n fragmentation analysis was performed. Using the negative mode detection, it was assumed that C and Z ions are more abundantly produced in the MS than Y and B ions (Körner, Limberg, Christensen, Mikkelsen, & Roepstorff, 1999). The fragmentation patterns were annotated according to Domon and Costello (1988). To demonstrate the effectiveness of the MS, the small peak eluting at 31.33 min (*m*/*z* 953.14) indicated in Fig. 2A, is further characterized by MS^n. The full MS spectrum (MS^1) of this small peak shows two abundant signals present, *m*/*z* 953.14 and *m*/*z* 476.25 (Fig. 3A). These signals are

Table 2
Retention times, *m*/*z* values, concentrations and proposed structures of oligomers present in SBP5317 digest as determined by HILIC–MS^n (Fig. 2).

RT (min)	*m*/*z*	C (ppm)	Proposed structure
10.25	541	43	$U3^{10}$
14.42	703.2	54	$(Rha\text{-}GalA)_2Ac$
16.52	369	360	2^{00}
19.62	661.1	157	$(Rha\text{-}GalA)_2$
20.28	717.1	188	$U4^{10}$
22.41	587.0/907.1	63	$3^{01}/U5^{20}$
23.35	777.1	184	4^{11}
25.26	967.3	29	5^{21}
25.93	545.1	652	3^{00}
27.73	735.0	279	4^{10}
29.27	983.2	91	$(Rha\text{-}GalA)_3$
29.68	925.3	118	5^{20}
30.18	763.0	114	$5^{20}/4^{01}$
31.33	953.1	140	5^{11}
32.64	1143.2	174	6^{21}
34.02	721.1/666.4	33	$4^{00}/7^{31}$
35.41	911.2	168	5^{10}
38.09	564.3/469.01	134	$6^{11}/5^{01}$
39.09	659.3	94	7^{21}
41.22	673.3/768.3	49	$7^{12}/8^{22}$
42.04	768.4/863.3	83	$8^{22}/9^{32}$
44.75	652.4	45	7^{11}
44.82	652.3/747.3	41	$7^{11}/8^{21}$

(●) unsubstituted GalA; (○) methylesterified GalA; (◫) acetylated GalA; (◉) unsaturated GalA; (■) rhamnose.

derived from the same component but are caused by the different charge status of the oligomers. Glycosidic cleavage fragmentation was performed to determine the position and correct assignment of the methylester and acetyl group in $GalA_5$ oligomer. Fig. 3B shows the MS^2 of the singly deprotonated pseudomolecular ion (*m*/*z* 953.14). The most abundant peak *m*/*z* 583.17 with the corresponding ion (Z_3 + Me + Ac) was annotated as a GalA oligomer having three adjacent galacturonic acid units with a methylesterified GalA next to an acetylated GalA unit. Based on the fragmentation pattern analysis, the structure of DP 5 oligomer is proposed to be GalA-GalA-GalA(OAc)-GalA(OMe)-GalA. Furthermore, the presence of the specific cross-ring cleavage fragments is useful for the annotation of the acetyl group in *O*-2 and/or *O*-3 positions within the GAlA unit (Quéméner, Cabrera Pino, Ralet, Bonnin, & Thibault, 2003). The C_3 fragment (*m*/*z* 587), which contained the acetyl group, was further fragmented and resulted to a cross-ring cleavage $^{0,2}A_3$ fragment (*m*/*z* 485.17). The MS^2 analysis (Fig. 3B) showed the neutral loss of 102 Da (60 + 42) on [C_3 + 1 Ac] ion to produce $^{0,2}A_3$ fragment. The elimination of 60 Da ($C_2H_4O_2$) and 42 Da (1 Ac) by specific cross-ring cleavage ($^{0,2}A_3$ + 1 Ac) allows the correct assignment of the acetyl group at the *O*-2 position in GalA unit within 5^{11} oligomer. When an acetyl group is in *O*-3 position the $^{0,2}A_3$ fragment would have resulted to the elimination of only $C_2H_4O_2$ species (60 Da) and the $^{0,2}A_3$ fragment must be *m*/*z* 527.Using the MS fragmentation technique, all abundantly present oligosaccharides in the mixture have been structurally determined by HILIC–MS^n to locate the methoxylation and acetylation sites within the pectin oligomer. A wide range of oligomers was identified carrying methylesters and acetyl groups, saturated and unsaturated as well as acetylated rhamnogalacturonans. Table 2 shows an overview of retention times (min), *m*/*z* values, and proposed structures of various acidic oligosaccharides. Saturated GalAs were identified and originated from the endo-PGII digestion while PL digestion generated the unsaturated GalA oligomers through

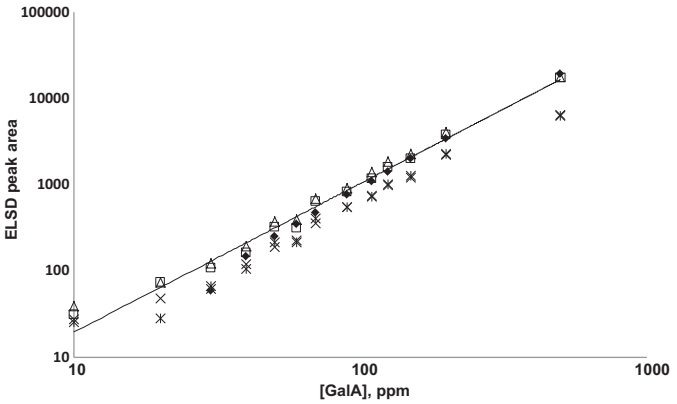

Fig. 4. Calibration curves of saturated and unsaturated GalA standards by ELSD analysis. The mean of the peak areas (DP 1–3) is used to calculate the response factor function $f(x) = 0.4921x^{1.7014}$ with R^2 of 0.99 (—).

the β-eliminative action. The MS/MS fragmentation analysis of unsaturated DP 4 ($U4^{10}$) oligomer with one methylester resulted in the proposed structure UGalA-GalA-GalA(OMe)-GalA. The common structural feature of the saturated and unsaturated products showed that GalA units at the reducing and the non-reducing end were unsubstituted, probably caused by the action of fungal PME present in the enzyme mixture. Also, most of the GalA oligosaccharides recovered are saturated GalAs (methylesterified and/or acetylated), which indicated that PL had difficulties to extensively degrade 53% methylesterfied pectin, especially in the presence of high levels of acetyl groups (DA ~ 17%). Buchholt et al. (2004) reported the difficulty to degrade the highly acetylated SBPs by endo-PGII and the absence of acetylated unsaturated oligosaccharides can be explained by the fact that the acetyl groups hinder pectin depolymerizing enzymes. Saturated and acetylated GalA oligomers having the acetyl group (*O*-2 position) next to non- and/or methylesterified GalA units are in agreement with the findings of Needs, Rigby, Colquhoun, & Ring (1998) and Perrone et al. (2002). Trimer and tetramer GalA oligomers having an acetyl group at the *O*-3 position were also detected (Ralet, Crepeau, & Bonnin,

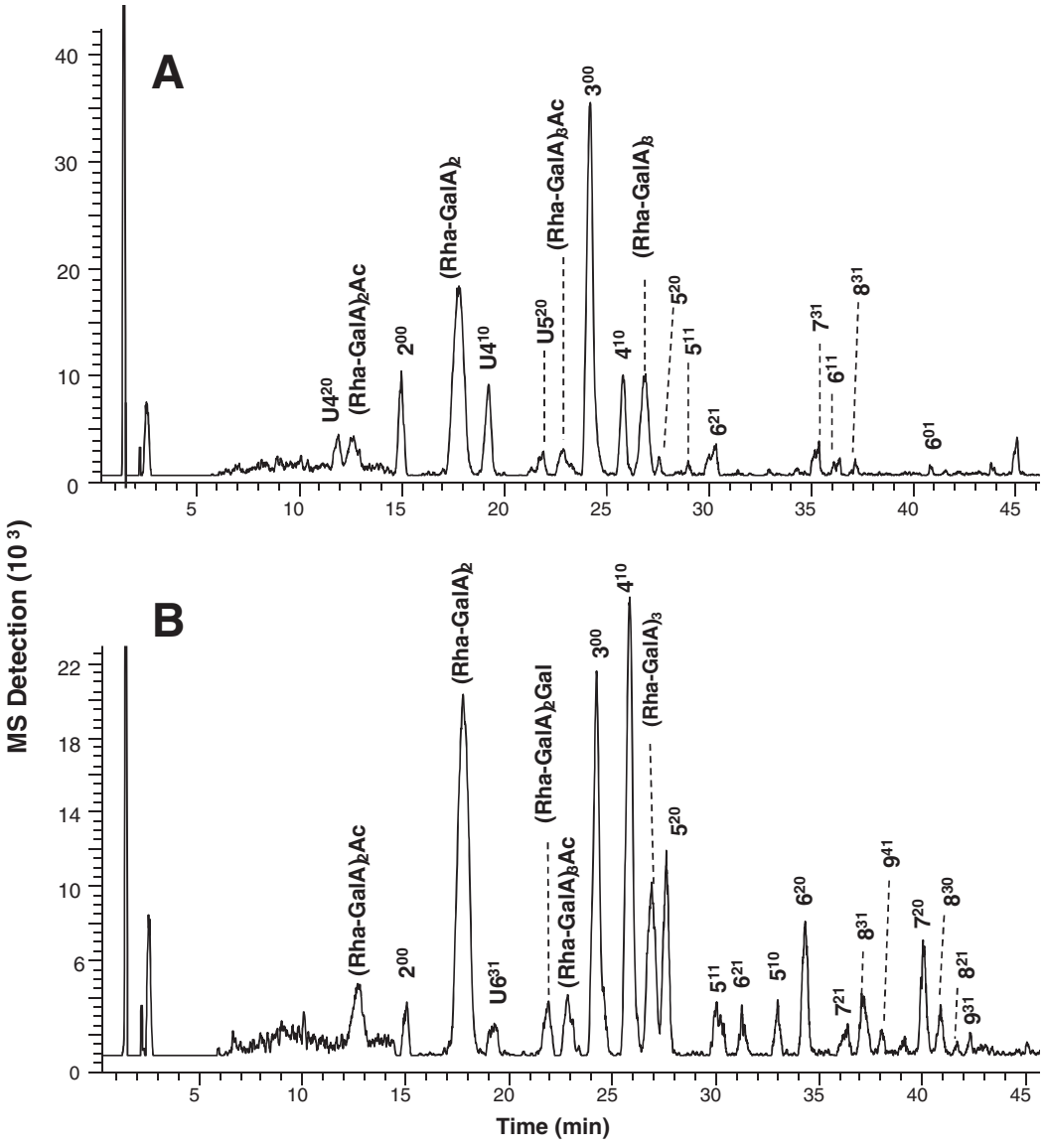

Fig. 5. HILIC elution profile of modified SBPs (A) P5328, (B) F5129 after hydrolysis by RG-I degrading enzymes with endo-PGII + PL using MS detector. Peak annotation: GalA oligomer 5^{11} (m/z 953) DP 5 in bold number with *O*-methylester and *O*-acetyl group. U, unsaturated GalA; Rha, rhamnose; GalA, galacturonic acid; Ac, acetyl group.

2008). These pectic oligomers appeared to be the main degradation products of SBP hydrolysis. A single GalA unit within pectin oligomer having both a methylester and an acetyl group was not detected and such a combination of substitution seems indeed to be rare in native pectin as reported by Ralet et al. (2005).

3.4. Calibration curves and quantification by ELSD

Quantification of the reaction products present in SBP digest is necessary in order to model the native pectin's structure. Previous studies have shown that ELSD enables the quantification of the compounds by correlating the peak area of the analyte versus the concentration by using a double logarithmic scale (Decroos et al., 2005; Mengerink et al., 1991) and the same method was applied in the present study. Fig. 4 shows that the observed ELSD response of commercial and laboratory made GalA standards at different concentrations was linear with a minimum detection limit of ≈20 ppm. Similar slopes were observed for the monogalacturonic, di- and trigalacturonic acid standards with linear correlation coefficients ranging from 0.988 to 0.999. The mean of DP 1–3 was calculated as $f(x) = 0.4921x^{1.7014}$ ($R^2 = 0.99$) (Eq. (1)). Besides the quantification of saturated GalA oligomers, an attempt to quantify the amounts of higher saturated and unsaturated oligomers was performed by using the available saturated and unsaturated GalA oligomers (purity ≥ 85%) as prepared and described by Van Alebeek et al. (2002). The ELSD response of unsaturated tetragalacturonic acid representing other unsaturated oligomers, and of GalA saturated tetramer and pentamer (Fig. 4) was found quite similar. The observed deviation among the slopes of different GalA standards was due to the purity of the compounds. The results indicate that the method is independent of the molecular structure of the oligomers tested as previously proven by Decroos et al. (2005) for the quantification of soy saponins. The same type of mathematical equation can be used to quantify saturated, unsaturated, methyl esterified and acetylated GalA oligomers without the need of specific standards as would be required in the MS analysis. The oligomers released after the hydrolysis of an enzyme-treated SBP (Fig. 2) as quantified by ELSD, represented 90 ± 5% of the GalA residues present in the pectin. The calculated amount of GalAs allows a valid reconstruction of the original pectin molecules. Furthermore, quantification by HPAEC-PAD (pH 12) confirmed that 90% of the total GalA oligosaccharides are recovered in the digest.

3.5. Analysis of differently PME modified SBPs

To test the effectiveness of the method, two different sugar beet pectins, modified enzymatically by plant and fungal PME, having an almost identical level of methylesterification and acetylation were analyzed. The modified SBPs (P5328 and F5129) both having a DM ≈ 50 and a DA ≈ 30 were hydrolyzed by a cocktail of enzymes containing RG-I and HG degrading enzymes. Partially esterified saturated and unsaturated GalAs; and considerable amounts of $(Rha\text{-}GalA)_n$ oligomers were released, separated and annotated (Fig. 5). The P5328 digest (Fig. 5A) comprises predominantly non- and partially methylesterified GalA oligomers of various DP. Due to the blockwise distribution of methylesters in P5328 as caused by plant PME treatment (Buchholt et al., 2004), endo-PGII has degraded blocks of nonesterified GalA residues releasing unsubstituted dimers and trimers as main reaction products. Unsaturated GalA oligomers were also detected due to PL digestion but in relatively minor amounts. Besides partially esterified saturated and unsaturated GalA residues of DP 4–8, non- and acetylated $(Rha\text{-}GalA)_n$ oligomers were identified.

Using fungal PME treated beet pectin as a substrate (F5129), representing a more regular substituted pectin, only limited amounts of non-methylesterified GalA oligomers were identified in the digest as reflected by their low MS intensities (Fig. 5B). Unsaturated GalA was hardly present in this digest confirming the absence of highly methylesterified GalA sequences. Moreover, methylesterified tetramer was the most dominant oligomer present in F5129.

The HILIC chromatograms of P5328 and F5129 digests showed slightly different intensities for the saturated di- and trigalacturonic acids and the amounts of di- and trigalacturonic acid oligomers was twice as high in P5328 than in F5129. Relatively similar levels of acetylated and non-acetylated $(Rha\text{-}GalA)_n$ oligomers were present in both SBP digests. Obviously, a wider range of products was released after the digestion of F5129 compared to P5328. Their separation and identification by HILIC–MS clearly allow quantification of the individual oligomers present in the digest.

4. Conclusions

A novel method has been developed and applied to analyze and quantify the partially methylesterified and acetylated oligogalacturonides after degradation by pectic enzymes. The results demonstrated the remarkably easy to interpret HILIC chromatograms. It implies that the use of the HILIC–MS approach provides essential and detailed information on the composition of pectin structure. ELSD has proven to be a versatile method to quantify the absolute amount of GalA oligomers present in the pectin digest. This method can now be used to accurately determine the degree of blockiness and to predict the functional properties of sugar beet pectins.

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