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Extraction, characterisation, and enzymatic degradation of lemon peel pectins

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Abstract

The albedo of Spanish lemons (16.0% w/w of lemon fresh weight) was extracted to obtain a chelating agent soluble pectin fraction, a diluted sodium hydroxide soluble pectin fraction and a residue (4.2, 1.8, and 5.0% w/w of fresh albedo, respectively). These fractions represented 61.3, 12.4, and 10.4% of the galacturonic acid present in the albedo, respectively. Fractions were studied and their characteristics were compared with those of lemon albedo modified hairy regions (MHR) (1.4% w/w of fresh albedo), solubilised from the albedo after treatment by a technical enzyme preparation. All extracted pectins were rich in galacturonic acid, next to varying amounts of arabinose, galactose, and glucose residues. Based on molecular size, albedo-MHR consisted of at least five populations. From the rhamnose:galacturonic acid ratio (0.21–0.28), the high acetyl content, and the degradability by rhamnogalacturonase, it was concluded that the four major fractions represent rhamnogalacturonans having only small differences within their fine structure. The fifth fraction contained more galacturonic acid and was only degraded by polygalacturonase.

Keywords: Lemon; Albedo; Pectin; Extraction; Enzymic degradation

1. Introduction

For many years pectins have been the subject of extensive research. There has been particular interest in their structural features [1], their functions within the plant cell wall [2] and their characterisation as food additives [3]. In recent publications the presence and location of arabinan and galactan side chains in pectins from different sources have

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been reported [4–7]. In 1965, Zitko and Bishop [8] suggested that pectic acids extracted from citrus fruits contained two acidic components, one being a homogalacturonan free of neutral sugars, the other a galacturonan to which neutral sugars were attached, probably as side chains. Aspinall et al. [9] observed that the rhamnose residues in pectin from lemon peel are unevenly distributed over the pectin backbone and are probably concentrated in certain regions, where they may alternate with galacturonic acid residues. A model was suggested by De Vries et al. [10] for apple pectin in which the neutral sugar side chains are concentrated on relatively short segments of the galacturonan backbone as blocks giving hairy regions.

Thibault et al. [11] obtained different pectin fractions from citrus pulp by sequential extraction using a 30 m*M* sodium acetate buffer pH 5.0 and chelating agents, representing 4.9 and 12.9% of the dry starting material, respectively. Ralet and Thibault [12] solubilised a pectin fraction from commercial lemon dietary fibre using water, representing 4.0% of the dry starting material. An acid extraction (85 °C) from the same lemon fibre solubilised 34.1% of the starting material [12]. The larger parts of the pectin molecules are present as smooth regions (homogalacturonans), while also the earlier described findings of De Vries et al. [13] on the presence of hairy regions in pectin from lemon peel were confirmed.

Since pectin is an important constituent of citrus peel being the main raw material for pectin production and may play also a role in the storage and processing of citrus fruits and citrus-derived products, structural information on citrus pectin is essential. The availability of pure enzymes such as polygalacturonase (PG), pectin esterase (PE), and rhamnogalacturonase (RGase) enabled us to isolate and degrade the pectic hairy regions of various fruit and vegetables in a defined way [14]. In this study, we have characterised pectins from lemon albedo using this approach.

2. Experimental

Preparation of pectin fractions.—The flavedo was removed from lemons (*Citrus limon*; Murcia, Spain) and the remaining albedo was frozen directly in liquid nitrogen. Part of the material obtained was used to prepare albedo-AIS [15]. AIS (10 g) was sequentially extracted using a 50 mM EDTA/50 mM ammonium oxalate in a 50 mM sodium acetate buffer pH 5.2 (70 °C) [15], and a 50 mM sodium hydroxide (3 × 30 min, 0 °C). Fractions obtained were dialysed and lyophilised to yield pectin soluble in chelating agents (ChSS), pectin soluble in diluted alkali (ASS) and a residue. The albedo-MHR were isolated by the liquefaction process from 900 g material of the same batch of mashed albedo suspension used for AIS preparation using the technical enzyme preparation Rapidase C600 [7]. The isolation of the various MHR populations was performed by size-exclusion chromatography (SEC) using a Sephacryl S300 HR (Pharmacia, Uppsala, Sweden) column [6] installed in a Pharmacia HiLoad system including a P-50 pump set to 2.5 mL/min.

Enzymatic hydrolysis.—Prior to incubation with enzymes, the substrates were saponified to remove methyl esters and acetyl groups [6] to facilitate the action of a pure PG from *Kluyveromyces fragilis* [16] or a pure RGase from *Aspergillus aculeatus* [16].

Solutions of saponified substrate (0.2% w/v) in a 50 mM sodium acetate buffer pH 5.0 were incubated with the PG and RGase.

Analytical methods.—Neutral sugars were determined by GLC after pre-treatment (30 °C, 1 h) with aq 72% sulfuric acid followed by hydrolysis with 1 M sulfuric acid (100 °C, 3 h) and conversion of the products into alditol acetates [17]. Hydrolysis using 2 M trifluoracetic acid (TFA, 120 °C, 1 h) was used to determine non-cellulosic glucose. Uronic acids were determined in the sulfuric acid hydrolysate by the automated [18] colorimetric *m*-hydroxybiphenyl assay [19]. The degree of methylation (DM) and degree of acetylation (DA) were determined by HPLC [20]. Linkage composition was established by methylation analysis before and after carboxyl reduction as described [6]. All enzyme digests were analysed by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC) [6,21].

3. Results and discussion

Isolation and fractionation of AIS.—16% of the lemon fresh weight was recovered in the albedo fraction. AIS derived from the albedo fraction represented 2.0% of the fresh lemon and 12.6% of fresh albedo. As shown in Table 1, galacturonic acid and glucose were the main components (44 and 29 mol%, respectively). Arabinose and galactose were present in reasonable amounts, while rhamnose, xylose, and mannose residues were present as minor components. The presence of fucose was very low (< 1 mol%). The carbohydrates determined only accounted for 59% of the AIS. The remainder may consist of, e.g., protein and some moisture. In this study, only the carbohydrate part was studied in detail. It should also be stated that the amount of galacturonic acid depended

Table 1
Sugar composition (mol%) of lemon albedo fractions

	AIS	ChSS	ASS	Residue
Yield [a]		33.4	13.9	40
Rha	3 (1.47) [b]	1 (0.28)	1 (0.79)	1 (0.17)
Fuc	< 1 (0.22)	nd [c]	nd	1 (0.19)
Ara	10 (4.67)	4 (0.66)	20 (1.26)	11 (2.67)
Xyl	4 (2.07)	< 1 (0.03)	2 (0.12)	8 (1.91)
Man	3 (2.04)	1 (0.15)	< 1 (0.02)	6 (1.80)
Gal	7 (3.81)	2 (0.49)	5 (0.40)	10 (2.89)
Glc	29 (16.8)	2 (0.56)	11 (0.82)	52 (15.2)
GalA	44 (28.3)	90 (21.5)	51 (4.35)	11 (3.63)
OMe	63 (1.76)	79 (1.35)	14 (0.05)	0
OAc	12 (1.04)	2 (0.08)	1 (0.01)	8 (0.07)
Total sugars	59%	71%	56%	72%

[a] Gram quantities extracted per 100 g of AIS.
[b] Gram quantities per sugar residue in the polysaccharides originating from 100 g of AIS.
[c] Not detected.

on the method of analysis. Data reported in Table 1 were obtained after pre-hydrolysis with 72% sulfuric acid (30 °C, 1 h) followed by dilution to 1 M sulfuric acid and hydrolysis (100 °C, 3 h) [22] and the colorimetric *m*-hdp assay. Solubilisation of the pectin-rich material in 98% sulfuric acid, followed by addition of water [19] resulted in a somewhat higher uronic acid content. The reason for this discrepancy is not yet clear. The galacturonic acid content of albedo-AIS reported here is higher than reported before by De Vries et al. [13] for the albedo-AIS of lemons from Italy (28.3 versus 20.7% w/w), while we also recovered a higher amount of AIS (12.6%) than reported by De Vries et al. (7.3%) [13]. The sugar composition of albedo-AIS is rather similar to the composition of lemon pulp [23] (0.8% Rha, 0.5% Fuc, 7.5% Ara, 4.7% Xyl, 2.6% Man, 5.8% Gal, 25.7% Glc, 22.3% GalA) and to the composition of commercially available lemon dietary fibre [12] (1.4% Rha, 0.4% Fuc, 6.8% Ara, 2.6% Xyl, 3.2% Man, 3.5% Gal, 23.5% Glc, 26.0% GalA).

Albedo-AIS was fractionated with non-destructive extractants basically using the method of De Vries et al. [13,15]. All buffer soluble pectins and Ca^{2+}-complexed pectins were isolated in one single fraction (ChSS), representing 33.4% of the AIS (4.2% of the fresh albedo). Extraction with diluted sodium hydroxide at 0 °C (saponification of esters, detachment of hydrogen bonds) resulted in the solubilisation of 13.9% of the AIS (ASS, 1.8% of the fresh albedo). Almost 40% of the AIS (5.0% of the fresh albedo) remained insoluble representing a mixture of unextractable pectin, hemicelluloses, and cellulosic material. The sugar composition of the fractions is given in Table 1 and the sugar content of 56–72% revealed that still some non-carbohydrate material like, e.g., salts and proteins was present.

The ChSS fraction was rich in galacturonic acid residues, which were highly methyl esterified. Only small amounts of Ara, Gal, and Glc were found to be present. The composition of this pectin fraction suggests that ChSS consists mainly of homogalacturonans. De Vries et al. [13] reported that 56.0% of all uronides present in the lemon albedo was solubilised in buffer and EDTA, while we recovered 61.3% of all the uronic acids in the ChSS fraction. The sugar composition of the ASS pectin was more heterogeneous than the ChSS pectin. The ASS fraction was relatively enriched in Rha, Ara, and Gal, which suggests higher proportions of hairy regions. Also Glc was present in somewhat higher amounts in this fraction. Although the fraction has been treated by NaOH, surprisingly methyl esters and acetyl groups were still present (DM = 14% and DA = 1%). Also in experiments with pectins from other sources, the extraction conditions used (50 mM NaOH, 3 × 30 min, 0 °C) did not result in a complete removal of all esters (results not shown).

The most important sugars in the residue were Glc next to Ara, Gal, and GalA. To be able to distinguish between non-cellulosic and cellulosic Glc, a sulfuric acid hydrolysis as well as a TFA hydrolysis step was carried out. The latter method is unable to hydrolyse cellulose polymers [24]. It could be concluded that 95.2% of all glucose originated from cellulosic Glc. The non-cellulosic Glc and some Xyl might originate from xyloglucan.

The composition of the ChSS and ASS pectin was quite similar to the composition as described for the extracted pectins from citrus pulp [11], although the yield was quite higher in our experiments. The water-extract of a commercial lemon fibre [12] differed

Table 2
Sugar composition (mol%) of lemon albedo-MHR and populations

	Albedo-MHR	Population				
		I	II	III	IV	V
Rha	5	5	5	5	6	2
Fuc	< 1	< 1	< 1	nd [a]	1	1
Ara	56	59	63	70	58	14
Xyl	1	1	1	< 1	< 1	4
Man	1	< 1	1	1	1	5
Gal	8	14	13	6	5	5
Glc	1	1	1	< 1	1	9
GalA	28	20	18	18	28	60
OMe	17	5	7	11	22	44
OAc	25	69	56	23	14	10
Total sugars	65%	75%	73%	69%	71%	68%
Rha:GalA	0.18	0.25	0.28	0.28	0.21	0.03

[a] Not detected.

significantly from our fractions, where we also found lower degrees of esterification. The neutral sugar composition of the ASS pectin was relatively similar to that of an acid extract (85 °C) of lemon fibre (yield 34.1%) [12], although the GalA content of this acid extracted pectin was higher [12]. As can be calculated from Table 1, over 91% of the GalA residues present in the AIS were recovered in both ASS and ChSS extracts. Most of the neutral sugars (80%) appeared in the residue, although the recovery varied for the individual sugar residues.

Isolation and fractionation of albedo-MHR.—To obtain MHR, lemon albedo was treated with Rapidase C600 and the solubilised polymeric material was isolated by ultrafiltration [7]. The albedo-MHR constituted polymeric material resistant to the enzymes present in this enzyme preparation. It represented 0.2% of the fresh lemon and 1.4% of the fresh albedo. The constituent sugar residues were Ara and GalA (Table 2), next to lower amounts of Gal and Rha. This pectin fraction still carried methyl esters (DM = 17%) in addition to relatively high amounts of acetyl groups (DA = 25%). Ralet and Thibault [12] reported rather similar values for the composition of hairy region fractions obtained from purified pectins from commercial lemon dietary fibre after treatment by a pure endo-PG and isolation by SEC (7% Rha, 57% Ara, 7% Xyl, 1% Man, 13% Gal, 3% Glc, 12% GalA). The GalA content of lemon fibre-HR was substantially lower than the content established for albedo-MHR; the Rha content was slightly lower, resulting in a higher Rha:GalA ratio (0.58 versus 0.18 found for lemon albedo-MHR). Albedo-MHR has been isolated using a technical mixture of enzymes, and therefore should be considered as being resistant to further degradation by pectic enzymes like PG, PE, and PL. Since it is obvious that more GalA residues are present in this enzyme resistant MHR fraction than can be accounted for by a alternating sequence of Rha and GalA, it is suggested that these GalA residues might be present as short sequences: too short to be accessible for PG. Another characteristic of albedo-MHR is

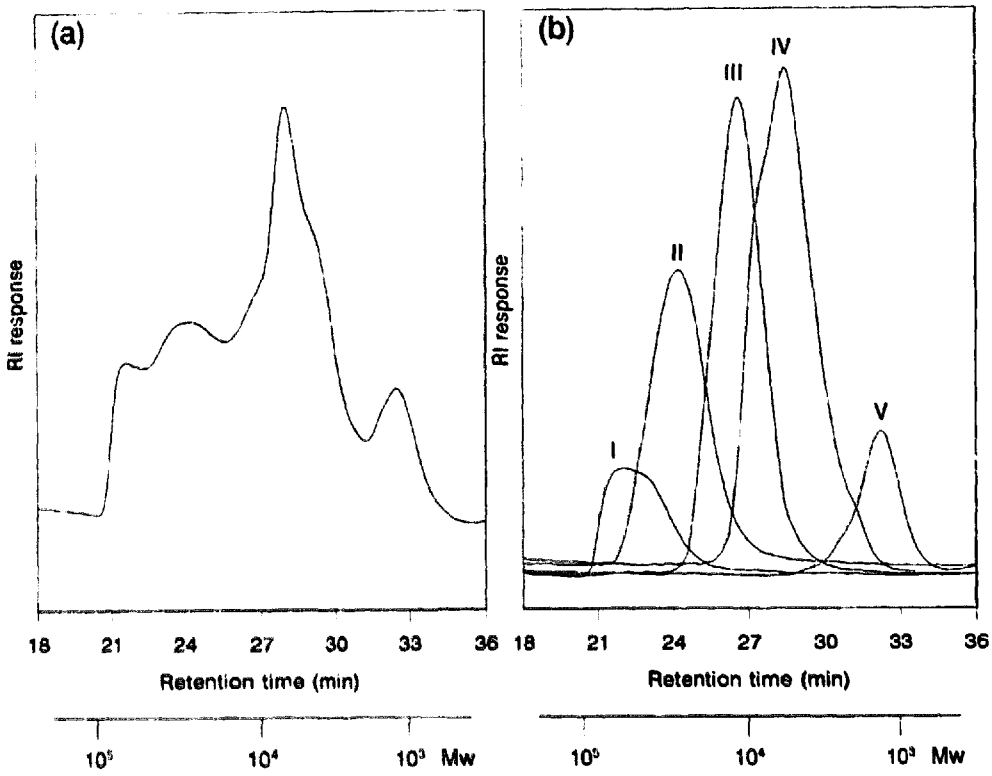

Fig. 1. High-performance size-exclusion chromatography elution profile of (a) albedo-MHR, and (b) the fractions of MHR as isolated by chromatography over Sephacryl S300.

its low Xyl content which is in contrast with the Xyl content of the hairy regions from lemon fibre [12]. Small Xyl contents were also found in MHR from carrot and leek, while apple and pear MHR are relatively rich in Xyl [7]. The differences in Rha:GalA ratio and the difference in the Xyl content when lemon MHR and lemon fibre are compared is not clear, but might arise from the source and procedure of isolation. In general, the characteristics of lemon albedo-MHR resemble those of MHR fractions isolated from various fruit and vegetable tissues [7].

Analysis of the albedo-MHR fraction by HPSEC showed that this fraction was constituted of different populations (Fig. 1a) having M_w values in the range of 2 to 60 kDa based on calibration with pectins [6]. Preparative fractionation of albedo-MHR using Sephacryl S300 yielded five different populations (I–V), representing 6, 23, 15, 44, and 12% of the albedo-MHR, respectively (Fig. 1b). Although all MHR populations were constituted mainly of Ara and GalA acid residues, next to small amounts of Gal and Rha, their relative amounts in populations I–IV and V were different. Population V was richer in GalA, with almost three times the content of fraction I–IV, and also in Xyl, Man, and Glc. The sugar composition of fractions I–IV was in agreement with those previously reported for hairy regions having similar Rha:GalA ratios. They differed by their M_w and Ara and Gal content, with decreasing amounts of Gal from fractions I–IV. Fuc, Xyl, Man, and Glc were only present in minor quantities in all populations. The DM increased and the DA decreased as the molecular weight decreased (Table 2). This trend has been reported before [7].

The relatively large proportion of MHR isolated from fresh albedo suggests that it may originate from the soluble pectins from the albedo cell wall as well as from the insoluble "proto-pectin" fraction. This can also be concluded from the fact that Ara in albedo-MHR represent 73% of the total Ara in the original albedo, while only 41% was recovered in the pectin fractions ChSS and ASS. Increasing Ara:GalA ratios were found for the fractions AIS (0.23), ASS (0.39), residue (1.00), and albedo-MHR (2.00). Pectic hairy regions with side chains of Ara, isolated after enzymatic treatment of the cell walls with pectic enzymes, have been reported from lemon [12] (57 mol% of Ara) and several other sources [5,7,25,26].

Linkage composition.—The linkage composition was determined by methylation analysis, which resulted in qualitative rather than quantitative information. ChSS pectin, as well as the saponified-ChSS fraction were insoluble in M_2SO, also after extended sonification. For this reason methylation analysis was carried out using ChSS after carboxyl reduction. Complete carboxyl reduction of the ChSS fraction could not be obtained, since the colorimetric *m*-hdp assay showed that 10% of the uronides remained when carboxyl-reduction was carried out three times. Reliable data on the sugar linkage composition of ASS pectin and albedo-MHR could only be obtained for the non-reduced sample. This does not form a serious drawback; for pectins from citrus origin, Xyl is reported to be the only sugar residue to be connected directly to GalA [9,27], whereas almost no Xyl was found in our fractions. In general, the same types of sugar linkages were found in the fractions studied (Table 3), although the sugar composition as determined before and after methylation analysis differed in some cases; especially the Ara content was relatively high after methylation. As expected the reduced-ChSS fraction contained a very high amount of (1 → 4)-linked Gal, which included more than 90% of the GalA residues present in the starting material. More than 50% of Ara residues were (1 → 5)-linked indicating a rather linear arabinan. More than half of the Rha residues were singly or doubly branched, while an unexpected high amount of the Rha residues appeared as terminally linked. No explanation for this phenomenon can be given. As was found for ChSS, the Ara residues in the ASS fraction were also mainly (1 → 5)-linked, while the high amount of terminally linked Ara residues could not be explained by the number of branched Ara residues. So, terminally linked Ara residues were probably also present in the highly branched galactan side chains. Similar results were also obtained for the albedo-MHR fraction in which 66% of the Ara was 1,5-linked and 20% was terminal linked. The low proportion of 1,2,5- and 1,3,5-linked Ara in all lemon fractions indicated a relatively low degree of branching compared with pectin arabinans from sugar beet [28] and apple [29], but were in good agreement with earlier findings for the arabinan in lemon hairy regions [12]. Rha residues were terminally, (1 → 2)- or (1 → 2,4)-linked, while the Gal residues were involved in almost all of the linkages possible. Fuc and Xyl, which were only present in low amount, occurred mainly terminally linked. The types of sugar linkages present in albedo-MHR and the relative amounts of each linkage are in agreement with the methylation analysis results reported for MHR from various sources [6,7], and for HR from lemon fibre [12].

Structural studies of the various pectins using enzymes.—The structure of the pectins present in the various fractions was further studied by degrading them with pure PG and RGase and the reaction products released were monitored by HPSEC and HPAEC. Since

Table 3
Glycosidic linkage composition of the neutral sugar fraction of ChSS, ASS, and MHR isolated from lemon albedo

Sugar residue		Glycosidic linkage composition [a]		
		ChSS [b]	ASS	Albedo-MHR
Rhamnose	T-Rha*p* [c]	0.6 (26)	2.6 (19)	1.1 (15)
	1,2-Rha*p*	1.3 (57)	3.1 (23)	1.6 (22)
	1,2,4-Rha*p*	nd [d]	2.6 (19)	3.6 (50)
	1,2,3,4-Rha*p*	0.4 (17)	5.1 (38)	0.9 (13)
	Total	2.3	13.4	7.2
Fucose	T-Fuc*p*	0.2 (100)	1.0 (100)	0.6 (100)
	Total	0.2	1.0	0.6
Arabinose	T-Ara*f*	3.2 (24)	13.2 (26)	16.0 (20)
	1,5-Ara*f*	7.1 (53)	31.0 (60)	53.9 (66)
	1,3,5-Ara*f*	1.7 (13)	7.2 (14)	4.0 (5)
	1,2,5-Ara*f*	0.5 (4)	nd	2.9 (4)
	1,2,3,5-Ara*f*	1.0 (7)	nd	4.5 (6)
	Total	13.5	51.4	81.3
Xylose	T-Xyl*p*	0.4 (100)	1.9 (100)	1.4 (67)
	1,3-Xyl*p*	nd	nd	0.7 (33)
	Total	0.4	1.9	2.1
Galactose	T-Gal*p*	2.7 (3)	3.8 (17)	3.2 (46)
	1,6-Gal*p*	0.7 (1)	4.6 (20)	0.7 (10)
	1,4-Gal*p*	74.1 (92)	5.1 (23)	1.8 (26)
	1,3-Gal*p*	nd	3.5 (15)	0.4 (6)
	1,2,4-Gal*p*	0.8 (1)	2.1 (9)	nd
	1,3,4-Gal*p*	1.3 (2)	nd	nd
	1,3,6-Gal*p*	0.7 (1)	3.6 (16)	0.9 (13)
	Total	80.3	22.7	7.0
Glucose	T-Glc*p*	1.4 (42)	1.7 (18)	0.4 (24)
	1,4-Glc*p*	1.9 (58)	7.9 (82)	1.3 (77)
	Total	3.3	9.6	1.7
Ratio terminal/branching		1.09	0.95	1.01

[a] Linkage types in mol%, numbers in brackets indicate percentage of each linkage in a sugar residue.
[b] After carboxyl reduction.
[c] 1,2-linked Rha, etc.; T connotes a terminal residue.
[d] Not detected.

PG hydrolyses preferentially low methoxyl pectins and polyGalA [4,30,31] and RGase is hindered by acetyl groups [16], the substrates were saponified prior to incubation. HPSEC analysis of the non-treated fractions revealed that the pectin molecules in the

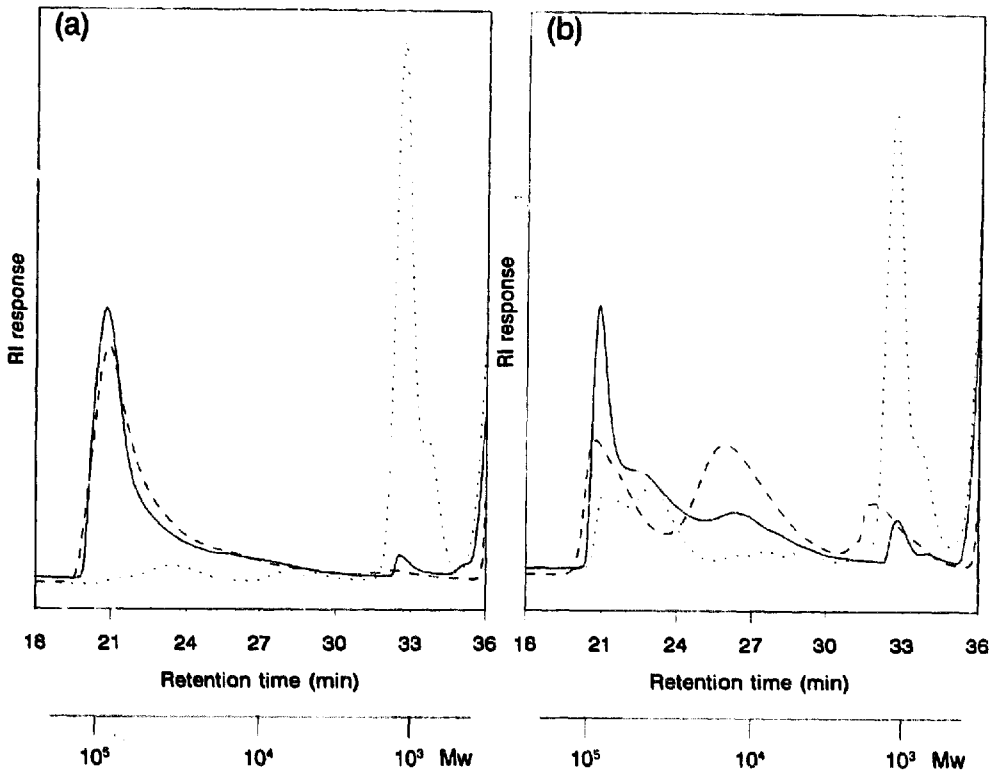

Fig. 2. High-performance size-exclusion chromatography elution profile of (a) saponified-ChSS and (b) saponified-ASS, without enzyme treatment (—) and after degradation by PG (---) or RGase (– – –) at 40 °C and pH 5.0 for 24 h.

ASS fraction covered a rather broad M_w range as compared to those in the CHSS fraction. Analysis of the enzyme digests showed that PG degraded the saponified-ChSS almost completely; saponified-ASS was degraded only partly (Fig. 2). The main reaction products were mono-, di-, tri-, and tetra-GalA, in addition to a high M_w fraction resistant to further degradation. PG also solubilised high molecular weight material from the residue, together with oligo-galacturonides (results not shown). This material probably originates from "matrix-bound", insoluble, pectin containing homogalacturonan regions. Degradation of these homogalacturonan regions resulted in the solubilisation of (small) uronide fragments and ramified regions which were not anchored to the hemicellulosic and cellulosic part of the cell wall. The amounts of oligomers, were determined by HPAEC analysis and using the detector response of monoGalA, it was calculated that ca. 20–30% of the GalA in the ChSS and ASS fractions were released as monomer or oligomer. This is only an estimation, since higher oligo-galacturonides are quantified using the response factor of monoGalA [32]. The fact that PG treatment of ChSS and ASS pectin resulted in equal amounts of oligo-uronides, while the ratio between GalA and neutral sugars (Table 1) is quite different, may suggest that the structure of the pectins differed. From the pectins present in the residue, ca. 20% of the GalA residues are released as oligomer, indicating a more complex structure or a poor physical accessibility. The degradability of saponified-ChSS and saponified-ASS fractions by PG from *Kluyveromyces fragilis* agrees with the degradability of saponified

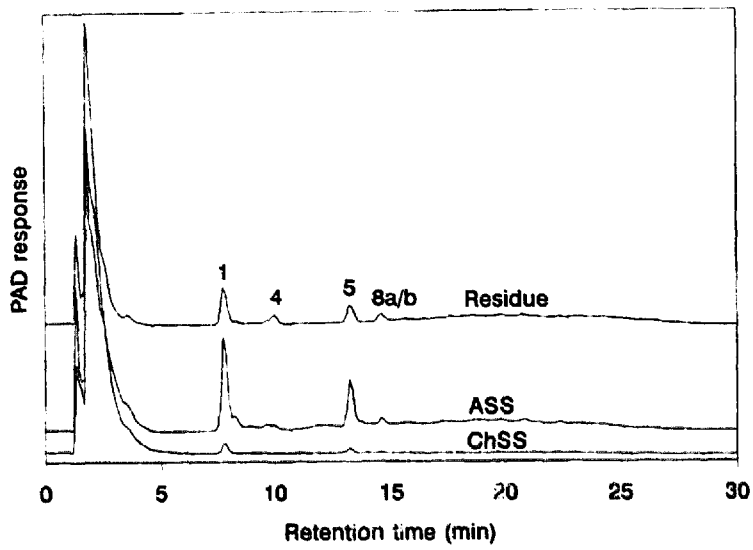

Fig. 3. High-performance anion-exchange chromatography elution profile of saponified-ChSS, ASS, and residue after treatment with RGase at 40 °C and pH 5.0 for 24 h. The structures of the oligomers are described in ref. [19]: **1**, Rha_2GalA_2; **4**, $Rha_2GalA_2Gal_2$; **5**, Rha_3GalA_3; **8a/b**, $Rha_3GalA_3Gal_2$.

purified pectins from commercial lemon dietary fibre by PG from *Aspergillus niger* as reported before [12].

RGase is known to hydrolyse only linkages between Rha and GalA, resulting in the liberation of typical RGase oligomers [21]. As expected from the sugar composition and PG degradation studies, saponified-ChSS pectin was not a very good substrate for RGase. Incubation with RGase did not result in a shift in molecular weight (Fig. 2a), although some oligomers were formed (Fig. 3). RGase was more active towards saponified-ASS (Fig. 2b) and the action of the enzyme resulted clearly in a partial shift to lower M_w values. RGase also proved to be able to hydrolyse some linkages within the pectic material in the residue as indicated by the solubilisation of polymeric material after incubation (results not shown). Oligomers formed were determined by HPAEC and identified against known standards produced by RGase [21]. The only products formed from saponified-ChSS were the linear Rha-GalA tetramer Rha_2GalA_2 and hexamer Rha_3GalA_3 [21], which were present in a ratio of 1:2 at relatively low concentrations. The HPAEC elution pattern of the digest of saponified-ASS showed the same oligomers in higher concentrations. The products released from the residue were linear oligomers consisting of alternating Rha and GalA next to small amounts of the same oligomers with one or two Gal residues (oligomers **4** and **8a/b** in ref. [21]). Alternating sequences of GalA and Rha in the pectic backbone, mostly without Gal substitution, are present in all lemon albedo pectic fractions investigated. However, since only a small percentage of all glycosidic linkages within the large molecules were hydrolysed, no information is available yet on the distribution of the RGase oligomers over the hairy regions.

Enzymatic degradation of albedo-MHR and albedo-MHR populations.—The structure of the albedo-MHR and albedo-MHR populations was also further studied by degrading them with pure PG and RGase before and after saponification. The HPSEC

elution pattern of the PG-digest of saponified-albedo-MHR was similar to that of the non-treated material indicating that albedo-MHR were almost completely resistant to PG action (data not shown) as was already suggested from the isolation procedure. Saponified-albedo-MHR was a slightly better substrate for RGase than the initial MHR, due to hindrance of this enzyme by methoxyl esters and acetyl groups. Using commercial GPC-software [6], it was calculated that without chemical saponification prior to incubation, RGase was able to hydrolyse 2.4% of all linkages. After saponification this value was 2.7%. The populations of albedo-MHR were degraded differently (Fig. 4): the populations having a high M_w were more sensitive to degradation by RGase and the degradability was enhanced by saponification. PG is almost unable to degrade populations I–IV (Fig. 4b–e) and only limited amounts of GalA oligomers were released (ca. 1–10 μg/mg) without changing the molecular weight distribution notably. In contrast, population V was degraded more readily by PG (> 100 μg oligomers per mg), while it was almost impervious to RGase. The M_w distribution of populations I, II, and III almost completely shifted to lower molecular weights, while only 3.4, 3.5, and 4.9% of all linkages were hydrolysed, respectively. For population IV 2.3% of all linkages were hydrolysed. In all digests, intermediate molecular weight fragments were formed, resistant to further action. Although the degree of hydrolysis is of the same order of magnitude for populations I–IV, the HPSEC elution profiles showed a more spectacular shift to lower M_w values for the populations having the highest M_w value. HPAEC analysis of the RGase digests of saponified-albedo-MHR and saponified-albedo-MHR populations (Fig. 5) indicated that reaction products were the same galactosylated RGase oligomers as discussed before. The amount of RGase oligomers containing Gal side chains decreased as molecular weight of the substrate decreased and they were absent in the digest of populations IV and V. The proportion of linear RGase oligomers without Gal side chains increased in the digests of populations I (ca. 10 μg/mg substrate) to III by a factor 3. The elution pattern of the RGase digests of saponified-albedo-MHR and saponified-albedo-MHR populations also indicated the presence of polymeric material. The polymeric material can be considered to be resistant to further degradation by RGase. Both the differences in relative and absolute amount of the various RGase oligomers released suggest differences within the backbone of the various populations. The observation that removal of methoxyl and acetyl groups increased RGase activity within albedo-MHR is only partly in agreement with Schols et al. [16], who reported that RGase was not active at all against non-saponified-apple-MHR. This can be explained by the lower acetyl content in albedo-MHR, although also the distribution of the substituents might have a significant effect. The degradability of lemon MHR by RGase as monitored both by HPSEC and HPAEC is in good agreement with that of other MHR material [7]. These results further substantiate the findings that MHR from various sources is rather similar and is more regular in structure than generally assumed.

This study illustrated that pectins from lemon albedo have many characteristics in common with pectins of other sources: smooth and hairy regions and alternating Rha/GalA sequences, mainly Ara and Gal residues in the side chains and methoxyl esters and acetyl groups within the hairy regions. Differences deal with the relative amounts of sugar residues and ester groups and type and amount of oligomers released by RGase or PG treatment. The characteristics of the lemon albedo modified hairy

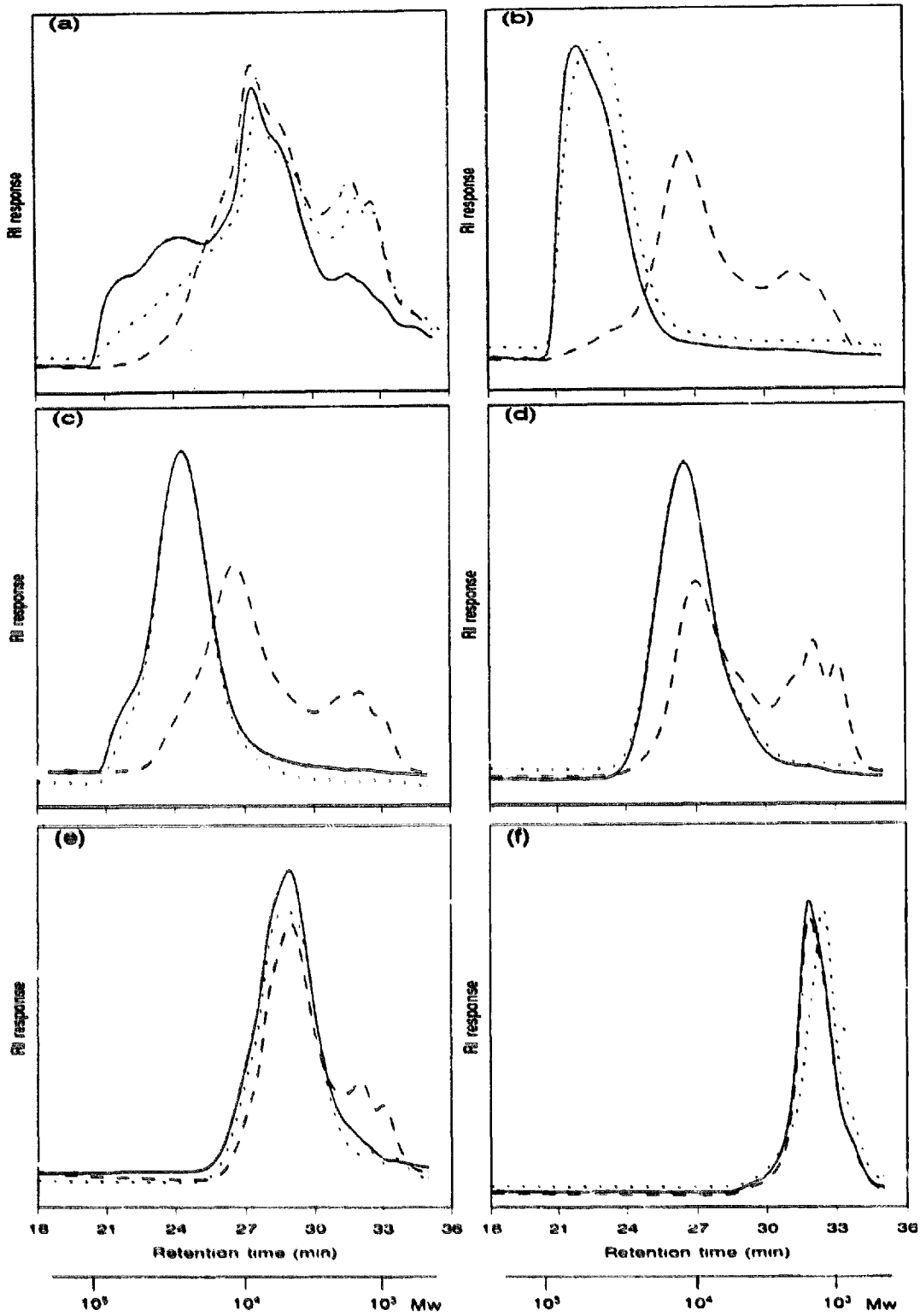

Fig. 4. High-performance size-exclusion chromatography elution profile of (a) albedo-MHR without enzyme treatment (—) and after degradation by RGase without (- - -) or with (– – –) saponification prior to incubation, and HPSEC elution profile of saponified-albedo-MHR population (b) I, (c) II, (d) III, (e) IV, and (f) V without enzyme treatment (—) and after degradation by PG (- - -) or RGase (– – –) at 40 °C and pH 5.0 for 24 h.

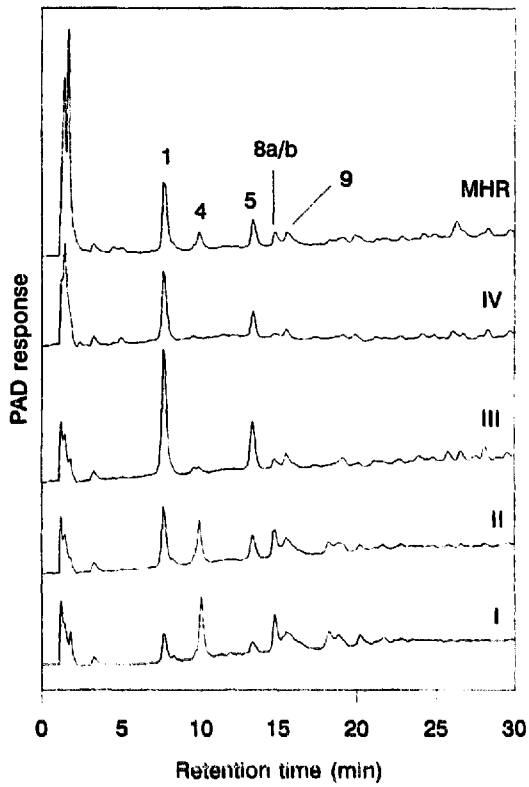

Fig. 5. High-performance anion-exchange chromatography elution profile of saponified-albedo-MHR and albedo-MHR populations I, II, III, and IV, after treatment with RGase at 40 °C and pH 5.0 for 24 h. The structures of the oligomers as given in Fig. 3; peak 9 represents $Rha_3GalA_3Gal_3$.

regions were very similar, the most evident different being the degree of acetylation while the amount of RGase oligomers increased significantly with decreasing molecular weight.

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