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Lemon albedo cell walls contain distinct populations of pectic hairy regions

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Abstract

Alcohol insoluble solids from lemon albedo were extracted to obtain a chelating agent soluble pectin fraction (ChSS), a diluted sodium hydroxide soluble pectin fraction (ASS) which was separated into a water soluble and water insoluble part (ASSws and ASSwi), and an insoluble residue (Residue). ChSS, ASSws, ASSwi and Residue represented 64.3%, 10.4%, 0.4% and 8.1% of the galacturonic acid residues present in the lemon albedo, respectively. The pectin fractions and the residue were characterised by their sugar composition, degrees of methylation and acetylation, and molecular weight distribution. After extensive digestion with polygalacturonase and pectinesterase, the remaining high molecular weight fragments, representing the so-called pectic hairy regions (HR), were isolated and further characterised. After cold alkaline treatment (de-esterification), HR shown to be accessible to a further degradation by rhamnogalacturonan hydrolase (RGase) and the digests were analysed by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC). The various HR fractions differed in their degradation pattern: typically 2–4% of all glycosidic linkages were hydrolysed. The amount of RGase oligomers formed from the various fractions decreased in the order these were extracted while the degree of galactose substitution of the oligomers increased. Indications were found for the presence of small quantities of xylogalacturonan fragments within the pectic hairy regions of lemon albedo. On the basis of these results the structure of lemon pectin is compared with the structure of pectins extracted from other sources.

Keywords: Lemon albedo; Pectin; Pectic hairy regions; Rhamnogalacturonan

1. Introduction

Pectins are important structural polysaccharides of the plant cell wall and are considered to consist of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. In addition to these pectic polymers, arabinoxylan, xyloglucan and cellulose are considered to make up the six polysaccharides of which all plant cell walls consist (Albersheim et al., 1996). These authors further argue that, depending on the type and function of the cell wall, other types of polymers may be present having (slightly) different (pectic) structures, such as xylogalacturonan and apiogalacturonan.

Pectins are used in the food industry as gelling and thickening agents, while they also may have pharmaceutical activities (Voragen et al., 1995). The homogalacturonan segments or 'smooth regions', as predominantly present in acid extracted commercial pectins, have been the subject of numerous studies. Many of these studies were directed towards the length of the homogalacturonan segments (Thibault et al., 1993) and the distribution of the methyl ester groups over the molecules (De Vries et al., 1986; Kravtchenko et al., 1993) because these parameters are thought to determine the functional properties of pectins. The RG-I segments of pectins have been investigated thoroughly, for example by O'Neill et al. (1990). They consist of alternating sequences of Rha and GalA, while the Rha moieties may carry side chains of varying length consisting of Ara and/or Gal residues (O'Neill et al., 1990; De Vries et al., 1982). It is still an open question whether the pectins present in the edible parts of fruit and vegetables only consist of homogalacturonan regions and RG-I-like segments with strictly alternating Rha–GalA sequences (Schols and Voragen, 1996; Albersheim et al., 1996). Pectin fragments have been isolated from many different sources using pectic enzymes such as polygalacturonase and pectin

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esterase (Schols and Voragen, 1994, 1996; and references therein), and it was found that the Rha to GalA ratio was usually different from 1 (as found for RG-I) and ranged from 0.2 to 0.9. The term 'hairy regions' (HR) was suggested for these complex segments (De Vries et al., 1982; Schols et al., 1990a). Using rhamnogalacturonan hydrolase (RGase), it was found that HR from apple pectin could be degraded into xylogalacturonan segments, galactose-substituted rhamnogalacturonan oligomers and segments representing residual stubs of the rhamnogalacturonan backbone, rich in highly-branched arabinan side chains (Schols et al., 1995b; Schols and Voragen, 1996).

Because by-products from the citrus juice industry are traditionally used for the isolation of pectins to be used as food ingredients, studies have also been directed towards both native and industrially extracted pectin fractions from orange, lemon, etc. (Zitko and Bishop, 1965; Aspinall et al., 1968; De Vries et al., 1984; Kravtchenko et al., 1993; Ralet and Thibault, 1994; Ralet et al., 1994). Recently, the isolation of alcohol insoluble solids (AIS) from lemon albedo and its fractionation into various pectin fractions was described in detail (Ros et al., 1996). Modified HR (MHR), obtained after extensive degradation of the albedo by a technical enzyme preparation was also characterised in the same study. They also studied the degradability of the intact pectins and the MHR fraction by RGase and the formation of the typical RGase oligomers (Schols et al., 1994) was monitored.

In the present study, HR fractions mildly extracted from various albedo pectin fractions obtained from lemon peel were obtained using purified pectic enzymes. These HR fractions were also degraded by RGase and both the high and low molecular weight degradation products were characterised in detail.

2. Experimental

2.1. Preparation of pectin fractions from the alcohol insoluble solids from lemon albedo

Lemon albedo alcohol insoluble solids (AIS) were obtained as described by De Vries et al. (1981). AIS (20 g) was sequentially extracted using chelating agents and diluted sodium hydroxide (De Vries et al., 1981; Ros et al., 1996). Fractions obtained were dialysed and lyophilised to yield a chelating agent soluble pectin (ChSS), a water soluble and a water insoluble fraction obtained after dialysis from the diluted alkali extract (ASSws and ASSwi respectively), and a residue (Residue), which is a mixture of cellulose, various hemicelluloses and residual pectins.

2.2. Isolation of pectic hairy regions

Pectin fractions from albedo AIS were incubated with a combination of PG and PE according to Schols et al. (1995a) in order to degrade the homogalacturonic segments and to release the HR populations. The isolation of the HR populations was performed by size-exclusion chromatography (SEC) of the digests using a Sephacryl S300 HR (Pharmacia, Uppsala, Sweden) column installed in a Pharmacia HiLoad system including a P-50 pump set to 2.5 mL/min as described by Schols et al. (1990a).

2.3. Enzymatic hydrolysis

Prior to a further incubation with enzymes, the isolated hairy regions were saponified to remove methyl esters and acetyl groups to facilitate the action of PG and Rgase (Schols et al., 1990a). Solutions of saponified substrate (0.2% w/v) in 50 mM sodium acetate buffer pH 5.0 were incubated with a pure PG from *Kluyveromyces fragilis* or with a pure RGase from *Aspergillus aculeatus* (Schols et al., 1990b). Digests were analysed by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC).

2.4. Analytical methods

Uronic acids were determined by the automated (Thibault, 1979) colorimetric *m*-hydroxybiphenyl assay (Ahmed and Labavitch, 1977). Neutral sugars were determined by GLC after pre-treatment (30°C, 1 h) with aqueous 72% sulphuric acid (Saeman hydrolysis) followed by hydrolysis with 1 M sulphuric acid (100°C, 3 h) and conversion of the products into alditol acetates (Blakeney et al., 1983). The degree of methylation (DM) and degree of acetylation (DA) were determined by HPLC (Voragen et al., 1986). HPSEC and HPAEC were performed as described Schols et al. (1990a, 1994).

3. Results

3.1. Extraction and characterisation of the various pectin fractions

As relatively high amounts of pectin fractions were required to obtain sufficient amounts of HR for our studies, the fractionation as carried out by Ros et al. (1996) was repeated on a larger scale. It was shown (Table 1) that the sugar composition of albedo AIS fractions was essentially the same as those reported previously. As we had previously found that part of the alkali extracted pectic molecules became insoluble during dialysis, we decided to distinguish between these fractions. So, the mildly extracted polysaccharides from albedo were recovered into four fractions: ChSS, ASSws (water soluble), ASSwi (water insoluble) and Residue. The fraction ChSS represented 39% of the AIS (4.9% of the fresh albedo), while the ASSws and ASSwi fractions represented 17% and 5% respectively of the AIS (2.2% and 0.6% of the fresh albedo). The Residue

Table 1
Sugar composition (mol%) of pectins, extracted from lemon albedo

	AIS		ChSS		ASSws		ASSwi		Residue	
Yield[a]			38.5		17.4		4.9		34	
Rha	3	(1.47)[b]	1	(0.25)	2	(0.16)	37	(0.79)	1	(0.11)
Fuc	<1	(0.22)	nd[c]		nd		nd		1	(0.22)
Ara	10	(4.67)	6	(1.16)	39	(2.75)	4	(0.09)	10	(1.96)
Xyl	4	(2.07)	<1	(0.12)	2	(0.17)	2	(0.04)	10	(1.89)
Man	3	(2.04)	2	(0.50)	<1	(0.03)	2	(0.04)	6	(1.42)
Gal	7	(3.81)	3	(0.86)	8	(0.64)	3	(0.08)	8	(1.91)
Glc	29	(16.8)	2	(0.43)	1	(0.10)	46	(1.11)	53	(12.6)
GalA	44	(28.3)	86	(23.4)	48	(4.45)	6	(0.14)	11	(2.83)
OMe	63	(1.76)	79	(1.68)	1	(0.03)	25	(0.12)	0	
OAc	12	(1.04)	2	(0.29)	0		9	(0.13)	7	(0.30)
Total sugars	59%		69%		48%		47%		67%	

[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.

fraction represented all material not extracted by the diluted alkali treatment and represented 34% of the AIS (4.3% of the fresh albedo).

The ChSS fraction had a GalA content of 86 mol% (Table 1) and was highly esterified (degree of methylation of 79%) while almost no O-acetyl groups were found in this fraction. Next to galacturonic acid residues, this fraction consisted of small amounts of other sugars. The ASSws was more heterogeneous in composition than ChSS; main sugar residues were GalA (48 mol%) and Ara (39 mol%), in addition to Gal (8 mol%). Although some methoxyl and O-acetyl groups were still present in this fraction, most of these groups were probably removed during alkaline extraction conditions. Although they were extracted together, the sugar compositions of ASSwi and ASSws were rather different. The major sugars found were Glc and Rha (46 mol% and 37 mol% respectively), while the content of GalA was only 6 mol%. Especially in the ASSwi fraction, some methyl esters and O-acetyl groups were still found; DM = 25 and DA = 9. The most important sugar moiety in the Residue fraction was Glc (53 mol%) in addition to other sugar residues such as Ara, Xyl, Man, Gal and GalA as was reported previously (Ros et al., 1996).

3.2. Isolation and characterisation of pectic hairy regions

The degradability of intact pectins from lemon albedo and albedo MHR by RGase has been described before (Ros et al., 1996). However, it should be realised that the MHR fraction, as obtained from digestion of the AIS with technical enzyme preparations, originates from pectic materials located in different sites of the cell wall. Based on studies of the structure of pectins isolated from apple pectins, it was found by Schols et al. (1995a) that hairy regions obtained from pectins of different origin (based on their extractability) showed structural differences. In the

Table 2
Sugar composition (mol%) of pectic hairy region population I and II, obtained after treatment of different pectin fractions of lemon albedo AIS with pectin esterase and polygalacturonase, followed by size-exclusion chromatography

	ChSS HR		ASSws HR		ASSwi HR-I	Residue HR-I
	I	II	I	II		
Rha	4	5	3	5	2	2
Fuc	<1	1	<1	1	nd[a]	<1
Ara	59	23	58	28	54	53
Xyl	1	7	5	28	2	2
Man	1	13	1	1	1	<1
Gal	21	11	19	8	29	33
Glc	1	9	<1	1	5	1
GalA	15	31	16	29	8	10
OMe	25	55				
OAc	22	41				
Total sugars	70%	66%	58%	69%	86%	75%
Rha:GalA	0.27	0.16	0.19	0.17	0.25	0.21
Ara:Gal	2.81	2.09	3.05	3.50	1.86	1.61

[a] Not detected.

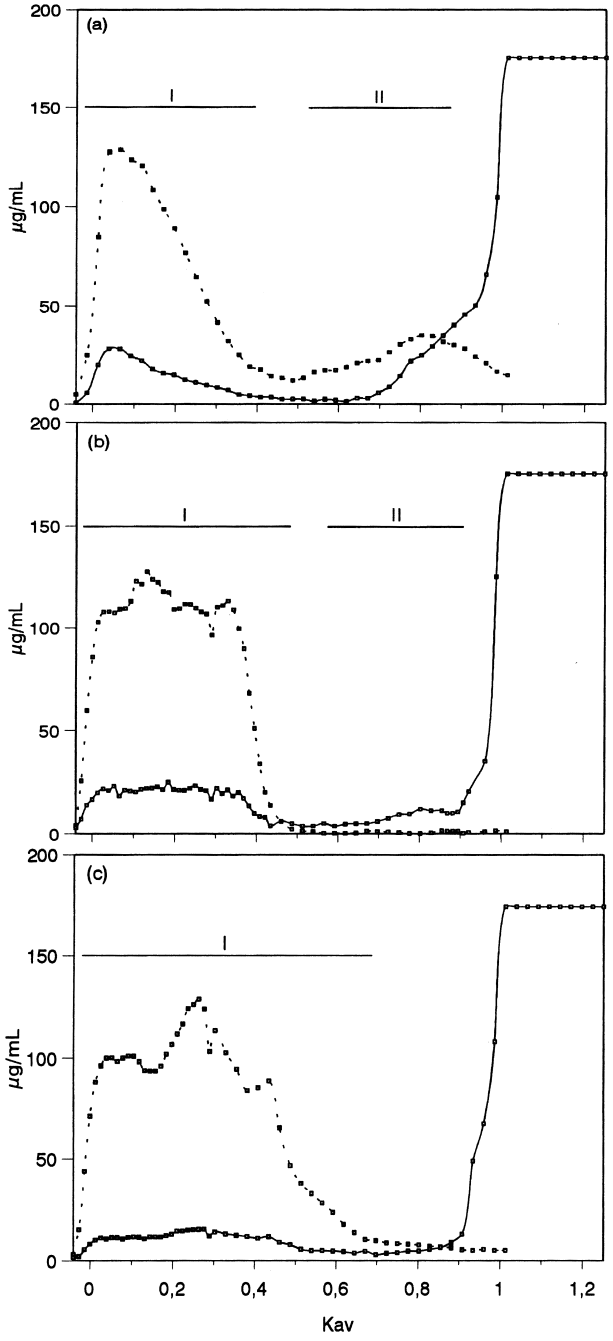

Fig. 1. Size-exclusion chromatography on Sephacryl S300 of the PE/PG digest of (a) ChSS, (b) ASSws and (c) Residue. (· · ·) neutral sugars, (———) uronic acids.

present work the isolation of HR from ChSS, ASSws, ASSwi and Residue fractions was performed using *purified* enzymes (PE and PG) followed by size-exclusion chromatography on a preparative scale to enable a more detailed study of the HR of the individual pectins. Fig. 1 shows the neutral sugar and uronic acid elution profiles of the digests of ChSS, ASSws and Residue by PE and PG on Sephacryl S300. The elution profile of both the neutral sugars and the uronides of the digest of ASSwi (not shown) was similar to that of ASSws. Most digests consisted of a high molecular weight fraction (hairy regions fraction I; HR-I) in addition to galacturonic acid oligomers eluting in the included volume of the column. Relatively low amounts of material eluted in between these distinct populations which were recovered as the HR-II fraction.

HR-I and HR-II were considered to be resistant towards the action of PG, which was confirmed by a re-incubation with PG after alkaline treatment to remove ester groups: no degradation was observed.

As judged by the sugar composition estimated for some fractions within the peak of HR-I from ASSws and the Residue (not shown), this polymeric material was found to be quite homogeneous. The oligomers eluting at ≈Kav 1 were galacturonic acid oligomers as confirmed by HPAEC (not shown). ASSwi HR-II and Residue HR-II were obtained in low amounts (<1 mg) which prevented a further study of these fractions. The various HR-I fractions differed only slightly in their sugar composition (Table 2). Neutral sugar residues were present in higher proportions than the uronic acid residues and the Rha to GalA ratio varied between 0.16 and 0.27. The most important neutral sugar residue was Ara, in addition to Gal and minor amounts of Rha. The HR-II fractions were shown to be more heterogeneous due to higher amounts of xylose and glucose. Also the galacturonic acid contents were high compared to those of the HR-I fractions. The galactose and arabinose contents in the HR-II fractions were both much lower compared to the HR-I fractions. The methyl esterification of ChSS HR-I (DM = 25) is much lower for the ChSS HR-II fraction (DM = 55). The same is true for the acetyl contents in these HR-I and HR-II fractions: 22% and 41% respectively. Notwithstanding the alkaline extraction conditions, the ASS and Residue pectins still contained some methyl esters and O-acetyl groups. However, the DM and DA values were not determined because they did not represent any native situation.

3.3. Enzymatic degradation of pectic hairy regions

Incubation of the saponified HR-I fractions with RGase resulted in a significant shift of the molecular weights (HPSEC; Fig. 2). The digests of all HR-I fractions contained oligomeric fragments (32–33 min; ~1 kDa), in addition to higher molecular weight material eluting at ~26 min. Based on the change in Mw and Mn calculated using GPC software (Schols et al., 1990b), it was calculated that RGase was able to hydrolyse 1.7–3.8% of all glycosidic linkages present in the HR-I fractions (Table 3). Although almost no shift towards lower Mw values for the HR-II fractions was observed, it could be calculated that about 1% (0.7–0.9%) of all linkages were split by RGase (Table 3). The digests were analysed by HPAEC and the relative amount of each oligomer was estimated using GalA as standard. Because PAD response depends on size, sugar residues and linkage types present (Schols et al., 1994), the concentrations of the

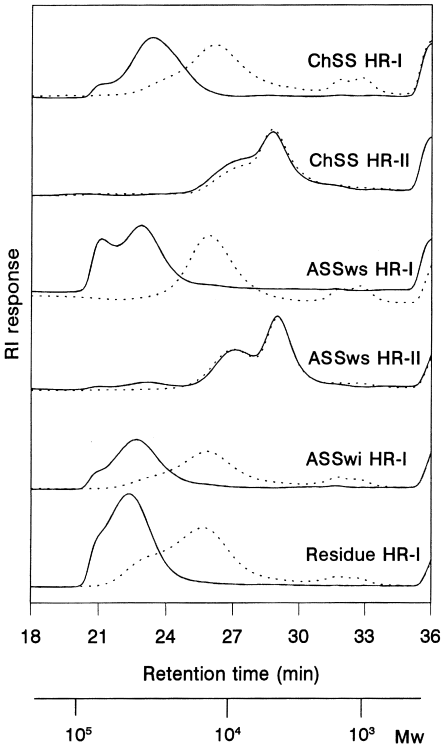

Fig. 2. High-performance size-exclusion chromatography elution profile of saponified HR fractions before (———) and after (· · ·) degradation by RGase at 40°C and pH 5.0 for 24 h.

higher oligomers especially might be underestimated. The oligomers found (Fig. 3) were the same as the ones found in the RGase digest of lemon albedo MHR (Ros et al., 1996). However, as can be seen in Table 3, the amounts of the individual oligomers differed for the various HR fractions. The amount of oligomers without galactose side chains (**1**, Rha_2GalA_2 and **5**, Rha_3GalA_3) was lower in the HR fractions from pectins extracted under increasingly severe conditions. In contrast, the oligomers **4** ($Rha_2GalA_2Gal_2$) and **8a/b** ($Rha_3GalA_3Gal_2$) having single-unit Gal branches were liberated in higher amounts as stronger extraction conditions were used. Other oligomers (**2** and **3**, Rha_2GalA_2Gal and **9**, $Rha_3GalA_3Gal_3$) were released in almost constant amounts (1 μg/mg substrate).

Individual amounts of oligomers released by RGase from all saponified HR-II fractions were quite low and no relationship with extraction conditions could be established.

3.4. Isolation and characterisation of polymeric degradation products

The polymeric material, present in the RGase digest of the saponified HR-I fractions of ChSS, ASSws and Residue, was isolated on a preparative scale using SEC. HPSEC analysis showed the presence of material eluting at 26 min in all isolated HR-I fractions, representing the high molecular weight degradation products (data not shown). Based on the sugar composition, it was not possible to recognise individual segments of the HR backbone released by RGase as was the case for apple HR (Schols and Voragen, 1996). Due to the fact that the oligomers liberated by RGase and removed by SEC accounted only for a small percentage of the total HR, the sugar composition of the RGase resistant part was very similar to that of the fractions from which they originated (Table 2).

4. Discussion

4.1. Pectin fractions

The extracted amounts and sugar compositions of ChSS, ASSws, ASSwi and Residue from lemon albedo AIS were in full agreement with those previously reported by us (Ros et al., 1996). More than 90% of all GalA residues present in the AIS (75% of the GalA moieties present in the lemon albedo) were recovered in the ChSS and the ASS fractions. Using chelating agents, 64% of all GalA residues present in the AIS was solubilised, which is in fair agreement with the findings of De Vries et al. (1984) who reported a value of 56%. Most of the neutral sugars (68%) were recovered in

Table 3
Oligomers released by RGase from saponified lemon albedo hairy region populations[a]

	ChSS HR-I	ChSS HR-II	ASSws HR-I	ASSws HR-II	ASSwi HR-I	Res HR-I
1[b]	17	1	14	2	6	5
2	1	1	1	1	1	1
3	1	nd[c]	1	1	1	1
4	1	<1	2	1	4	4
5	5	<1	5	1	2	2
8a/b	1	nd	2	1	2	3
9	1	nd	1	<1	<1	<1
Total	27	2	26	7	16	16
% hydrolysis[d]	3.3	0.9	3.8	0.7	1.9	1.7

[a] Quantities calculated on the response of anhydrous monogalacturonic acid (in micrograms of oligomer per milligram of substrate).
[b] According to the structure of the oligomer as reported by Schols et al. (1994).
[c] Not detected.
[d] Percentage of linked hydrolysed by the enzyme.

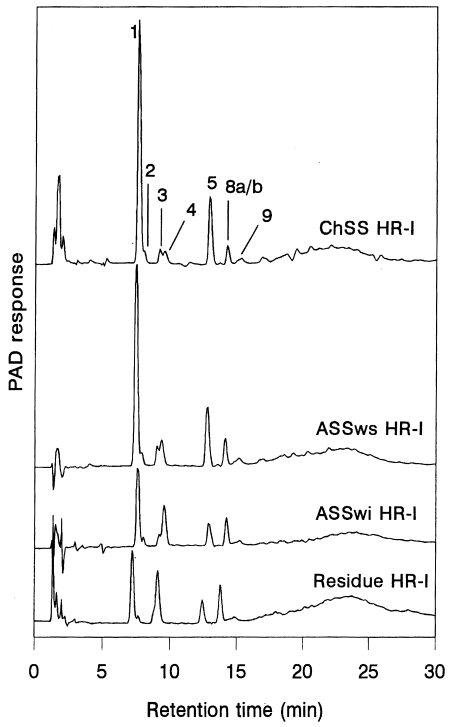

Fig. 3. High-performance anion-exchange chromatography elution profile of saponified ChSS HR-I, ASSws HR-I, ASSwi HR-I, and Residue HR-I after treatment with RGase at 40°C and pH 5.0 for 24 h. The structures of the oligomers are described by Schols et al. (1994): **1**, Rha_2GalA_2; **2** and **3**, Rha_2GalA_2Gal; **4**, $Rha_2GalA_2Gal_2$; **5**, Rha_3GalA_3; **8a/b**, $Rha_3GalA_3Gal_2$; **9**, $Rha_3GalA_3Gal_3$.

the Residue. In the extracts obtained from the albedo AIS, the carbohydrate part only represents 50–70% of the total weight of the fractions. The other 50–30% may be accounted for by, for example, protein, moisture and salts (e.g. EDTA) remaining from insufficient dialysis.

The characteristics of the ChSS fraction (high molecular weight, high GalA content and high DM, together with a low neutral sugar content), accounting for about 40% of the albedo AIS, are in full agreement with earlier findings on lemon (Aspinall and Cotrell, 1970; Ralet et al., 1994; Ros et al., 1996), and also quite similar to those reported for grapefruit (Nisperos and Robertson, 1982). Due to different solubility behaviour, it was decided to separate the material extracted by alkaline conditions into a water soluble and a water insoluble fraction. The characteristics of the ASSws fraction indicated the presence of pectic material, although these pectic molecules contained more neutral sugars compared to the ChSS fraction. This observation was also made by Schols et al. (1995a), who reported that the neutral sugar content of apple pectins increased as more severe conditions were used during extraction. For the ASSwi fraction, a rather different sugar composition was found: the Glc content was quite high (46%), while only 6% of the sugars was found to be GalA. Since only trace amounts of Xyl is present in this fraction, it is not believed that the Glc residues originate from xyloglucans, while under the extraction conditions used, cellulose would not be soluble. It would be of interest to study the nature of this fraction in more detail to examine whether the GalA residues are present as separate molecules or covalently linked to glucose-rich molecules. This small fraction, which is easily overlooked during extraction, might hold important information on the structure of cell wall polysaccharides.

The Residue fraction was composed mainly of Glc (53 mol%), although substantial amounts (6–11%) of GalA, Ara, Xyl, Man and Gal were present. It has been reported before (Ros et al., 1996) that 95% of the Glc in the Residue after mild alkaline extraction of lemon AIS originates from cellulose.

4.2. Pectic hairy regions

Pectin HR were obtained using purified PG and PE to avoid possible modification of the neutral sugar side chains. Although HPSEC analysis of the PG/PE digest of the pectin extracts suggested the possibility of two distinct populations in the high molecular weight area (20–24 min), sugar composition analysis showed that these high molecular weight populations were quite homogeneous and the isolated polymeric fragments were pooled into one fraction. It was calculated that the HR-I fractions had an average molecular weight of about 50 kDa. Also a population of PG/PE resistant material having a molecular weight of 10 kDa (Kav = 0.7–0.8) was recovered in some cases as HR-II. A certain regularity in the relative amounts of the sugars present in HR-I and HR-II was recognised: the Rha to GalA ratios for the HR-I fractions were fairly constant (0.21–0.27) and were slightly higher than the values for the HR-II fractions (~0.18). The ratio of Ara to Gal residues varied for the various HR fractions and fluctuated from 1.61 to 3.05. Xyl was present in higher concentrations in the HR-II fractions, especially the HR-II fraction from ASSws material (28 mol%). This fraction, probably representing a xylogalacturonan, is not as pure as the one isolated from apple pectin (28 mol% arabinose present). The presence of such a xylogalacturonan segment could not be predicted to be present from the xylose content of lemon AIS pectins: ChSS pectin (<1% xylose) and ASSws (2% xylose). From the elution behaviour on HPSEC of the HR-II fractions, a first indication can be obtained of the molecular weight (7–10 kDa), which is significantly lower than the value of 20–30 kDa reported for the apple xylogalacturonan (Schols et al., 1995b). More research should be directed to a further characterisation of these xylose-rich molecules from lemon.

In general, the sugar compositions are in the same range as previously reported for HR from commercial lemon dietary fibre (Ralet and Thibault, 1994) and lemon MHR (Ros et al., 1996). GalA in both ChSS-HR fractions represented 2% of the total GalA present in ChSS, which indicates that 98% of the GalA in ChSS is involved in the smooth regions. When the neutral sugars were integrated in the calculation, ~8% of the ChSS was recovered as hairy regions. ASSws HR fractions represented 20.1% of

the total ASSws fraction, showing that ~80% of ASSws was present within homogalacturonan segments. ASSwi-HR represented 0.8% of ASSwi, while only 5.6% of the Residue fraction was recovered as HR. However, it should be mentioned that during the isolation step, only HR fractions were solubilised which were not physically or covalently embedded within the cell wall matrix, while it cannot be ruled out that PG was not able to degrade all homogalacturonan segments present.

4.3. Enzymatic degradation of pectic hairy regions

The fact that the isolated HR populations (after removal of any ester groups present) were resistant to the action of PG indicates that the degradation by PG and PE was indeed complete. As found before for the (modified) hairy regions from apple and other fruit and vegetable tissues (Schols et al., 1990a, 1995a; Schols and Voragen, 1994), the ratio of rhamnose to galacturonic acid was much less than 1, as found for rhamnogalacturonan I, suggesting an even more complex structure.

The degradation by RGase was different for HR-I compared to the HR-II fractions. All saponified HR-I fractions shifted completely to smaller molecular weight material in addition to the liberation of typical RGase oligomers. The relative amounts of these oligomers differed. Incubation of saponified HR-II populations with RGase only resulted in the release of very small amounts of oligomers, while the remaining polysaccharide was eluted on HPSEC in exactly the same fashion as the starting material. This result suggests that the degradable parts of the RG backbone were located at different sites of the molecule for the HR-I and HR-II fractions.

Structural differences in the various HR fractions was also observed by the differences in HPAEC patterns of the RGase digests. The identity of the oligomers released by RGase indicates that the single-unit side chains of Gal on the alternating Rha–GalA sequences were present in native pectins and are not a result of the isolation protocols used to isolate lemon MHR (Ros et al., 1996). Both the absolute as well as the relative amounts of the various oligomers strongly depend on the type of pectin from which the HR originated. The ratio of oligomers having a tetrameric rhamnogalacturonan unit to oligomers having a hexameric unit is similar for all HR digests, while dissimilarities were observed for the ratio of single branched tetrameric rhamnogalacturonan units to doubly branched tetramer units. Because only relatively small amounts of oligomers were liberated from the HR fractions, the RGase resistant material did not differ significantly from the starting material.

In conclusion, it can be stated that differences could indeed be observed for the individual pectin fractions obtained after sequential extraction of lemon peel, especially within the ramified regions (e.g. amount and type of RGase oligomers released). However, the distribution of the HR over the pectin molecules and the sequence of the RGase oligomers over the hairy regions is still unknown. Also, the effect of the differences in distribution of various subunits on functional properties of the pectins in vivo or on the use of extracted pectins as food additives remains to be established.

The overall model as proposed by Voragen et al. (1993) and Schols and Voragen (1996) suggests that pectins from different sources have the same subunits (e.g. homogalacturonan, xylogalacturonan, RG-I, RG-II and rhamnogalacturonan segments with a ratio of Rha to GalA different from 1). This model also appears to be valid for pectins extracted from lemon pectins. The proportion of the various subunits appears to depend on the type of tissue (variety, part of the plant and/or fruit). Future research will focus on the distribution of the various substituents (including methyl esters) and subunits over the pectin molecule.

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