Structural Features of Cell Walls from Potato (*Solanum tuberosum* L.) Cultivars Irene and Nicola

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Cell wall material (CWM) isolated from non-cooked potato tissue of the cultivars Irene (mealy cooking) and Nicola (non-mealy cooking) was successively extracted with buffer, CDTA, Na_2CO_3 at 4 and 20 °C and 0.5 M KOH. The sugar composition of fractions and the remaining residues was determined. Information about the structure of the pectic polysaccharides was obtained by studying the degradation of the fractions and residues with purified polygalacturonase (PG) and rhamnogalacturonase (RGase). The results indicated that the pectic polysaccharides extracted by buffer, CDTA, and cold Na_2CO_3 contain more and/or longer side chains for cv. Irene than for cv. Nicola. From the CWM of cv. Nicola more branched and a higher amount of branched pectic polysaccharides were extracted with Na_2CO_3 at 20 °C and 0.5 M KOH, respectively, while the remaining CWM of cv. Irene contained 24% more residue.

Keywords: *Potato; Solanum tuberosum L.; texture; cell wall; middle lamella; polygalacturonase (PG); rhamnogalacturonase (RGase); pectic polysaccharides*

INTRODUCTION

Structure and composition of potato cell walls, and in particular the pectic polysaccharides, have been studied for many years (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990).

Pectin is the main component of the potato cell wall (54%) (Jarvis et al., 1981; Ryden and Selvendran, 1990). Three types of pectic polysaccharides are present in all primary plant cell walls, namely, homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II (Albersheim et al., 1996). Pectin determines cell wall porosity and thickness (Bacic et al., 1988; Carpita and Gibeaut, 1993), and it plays an important role in maintaining tissue integrity. Upon processing of potatoes, pectic material is degraded and partly solubilized into the cooking media (Hughes et al., 1975a,b; van Marle et al., 1994). This degradation greatly influences intercellular adhesion and the structure of the remaining cell walls, which are both important texture parameters (van Marle et al., 1992). However, little is known about differences in structure and composition of pectic polysaccharides with respect to texture development during processing. Recently, Quinn and Schafer (1994) characterized the pectic substances of two potato cultivars with different sensitivities to prewarming. They reported differences in yield and composition of the pectic material extractable by sodium hydroxide. Our previous study revealed differences in total amount of cell wall material and amount and composition of solubilized pectic material for the potato cultivars Irene (mealy cooking) and Nicola (non-mealy cooking) (van Marle et al., 1997).

Using either purified enzymes (Ishii, 1981) or chemical extractants (Jarvis et al., 1981; Ryden and Selvendran, 1990) several fractions of pectic polysaccharides were isolated which originated from different parts of the potato cell walls and which were different in composition and structure. For instance, pectic polysaccharides from the middle lamellae were more easily solubilized than those present in the primary cell walls (Ishii, 1981; Ryden and Selvendran, 1990). Furthermore, the pectic polysaccharides solubilized with more severe extractants had a higher ratio of neutral sugars to galacturonic acid, indicating the presence of more hairy regions (Ryden and Selvendran, 1990).

More knowledge about the relative abundance of homogalacturonan and/or branched rhamnogalacturonan regions is the various fractions can be obtained by enzymatic degradation of those fractions with purified endopolygalacturonase (endoPG) and rhamnogalacturonase (RGase) in combination with high-performance size-exclusion chromatography (HPSEC) and examination of the structure and composition of the oligomers formed (Kravtchenko, 1992; Schols, 1995).

In this study, the structure of the pectic polysaccharides present in cell walls of the cultivars Irene and Nicola is examined. Cell wall material was fractionated using chemical extractants. The composition of the fractions was analyzed, and the structure of the pectic polysaccharides was studied by degradation with purified endoPG and RGase.

MATERIALS AND METHODS

Potatoes. The isolation procedure and the characterization of cell wall material (CWM) from the potato cultivars Nicola and Irene, non-mealy and mealy cooking, respectively, were described in a previous paper (van Marle et al., 1997). For this study, the CWM isolated in duplicate from non-cooked tissue was used.

Fractionation of CWM. For the sequential extraction of CWM the method described by Selvendran et al. (1985) was used, which was slightly adapted.

A suspension (100 g; 2% w/w CWM in 70% ethanol) was centrifuged (15 min; 12000*g*). Ammonium acetate buffer (150 mL of 0.05 M solution), pH 4.7, was added to the residue, and

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the suspension was stirred for 16 h at 20 °C and centrifuged. Subsequently, the residue was washed with 50 mL of the same buffer and 50 mL of demineralized water, respectively. Supernatant and washings were combined, and this "buffer" fraction was dialyzed. The residue was suspended in 150 mL of 0.05 M CDTA, pH 6.5, and stirred for 16 h at 20 °C. After centrifugation, the residue was washed twice with 50 mL of CDTA and once with 50 mL of demineralized water. Supernatant and washings formed the CDTA fraction. This fraction was dialyzed three times against NH_4OAc buffer, pH 4.7 (48 h), and demineralized water, respectively (Mort et al., 1991). The residue was suspended in 150 mL of cold (4 °C) 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$ and stirred 16 h at 4 °C. After centrifugation, the supernatant was filtered through a GF/C glass fiber filter (Whatman) and the filtrate was acidified to pH 5 with HAc and dialyzed [Na_2CO_3 (4 °C) fraction]. The residue was suspended in 150 mL of 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$, stirred for 3 h at 20 °C, and centrifuged. The residue was washed twice with 50 mL of Na_2CO_3 and once with demineralized water. Supernatant and washings were combined and filtered through a GF/C glass fiber filter, and subsequently the filtrate was acidified to pH 5 and dialyzed [Na_2CO_3 (20 °C) fraction]. Potassium hydroxide (100 mL of a 0.5 M solution) containing 10 mM $NaBH_4$ was added to the residue, and the resulting solution was stirred for 16 h at 20 °C and centrifuged. The residue was washed with 50 mL of KOH. Supernatant and washing were combined and filtered through a GF/C glass fiber filter, and the filtrate was acidified to pH 5 and dialyzed (KOH fraction). The residue was suspended in 75 mL of demineralized water, acidified to pH 5, and dialyzed (residue). After dialysis all fractions and the residue were freeze-dried and milled with a ball-mill. For each cultivar the fractionation was performed in duplicate.

Saponification. Buffer and CDTA fractions (25 mg) were stirred with 2 mL of 0.1 N NaOH (16 h; 4 °C) to remove the methyl esters and acetyl groups. The solutions were neutralized with 2 mL of 0.1 N HAc, and 0.05 N NaOAc buffer, pH 5.0, was used to make the final concentration of the saponified fractions 3 mg/mL.

Enzymic Hydrolysis. The different fractions and residues (3 mg/mL of 0.05 M NaOAc, pH 5.0) and the saponified samples were incubated with either PG (10 nkat/mL) or RGase for 16 h at 40 °C as described by Schols et al. (1995). PG was purified from *Kluveromyces fragilis*, and RGase was purified from *Aspergillus aculeatus*. Both enzymes were provided by the Department of Food Science, Agricultural University, Wageningen (Schols et al., 1995). After incubation, the enzymes were inactivated (10 min; 100 °C). The digests were centrifuged (12000*g* for 10 min) to remove the insoluble cell wall material, and the supernatants were analyzed by HPSEC and HPAEC.

Chromatography. All fractions and residues, both before enzymic degradation and after treatment with PG and RGase, were analyzed by high-performance size-exclusion chromatography (HPSEC), carried out as described by Schols et al. (1990).

The mono- and oligomers in the PG and Rgase digests were analyzed by high-performance anion-exchange chromatography (HPAEC), performed on an HPLC (Pharmacia LKB low-pressure mixer, Waters 625 LC pump, and 2157 autosampler) equipped with a Carbopack PA1 column (250 × 4 mm, Dionex) as described by Schols et al. (1994). For analysis of the RGase digests the method described by Schols et al. (1994) was slightly adapted. After sample injection a linear gradient was started from 150 mM NaOAc in 100 mM NaOH to 250 mM NaOAc in 100 mM NaOH within 40 min. For analysis of the PG digests the method described by Kravtchenko et al. (1993) was used, which was slightly adapted. After sample injection two successive linear gradients were used, 400−670 mM NaOAc in 100 mM NaOH within 35 min and 670 mM to 1 M NaOAc in 100 mM NaOH within 5 min. The column was washed with 1 M NaOAc in 150 mM for 5 min and equilibrated with 400 mM NaOAc in 100 mM NaOH for 15 min.

Analytical Methods. *Starch Content.* Starch content was determined enzymatically using the Boehringer test kit (Mannheim GmbH) on two replicates (if enough material was available). For sample preparation, amounts of sample and reagents were adapted to obtain a final solution with a starch concentration between 15 and 200 μg/mL in a total volume of 1 mL. This solution (0.10 mL) was used for the starch assay, which was adapted by using half the amounts of reagents resulting in a final volume of 1.16 mL in the cuvette.

Neutral Sugar and Uronic Acid Content. All fractions were hydrolyzed with 1 M H_2SO_4 at 100 °C. Only the residues were pretreated with 72% H_2SO_4. After neutralization with $BaCO_3$ the hydrolysates were analyzed for neutral sugars by HPLC (Pharmacia LKB low-pressure mixer, Waters 625 LC pump, and 2157 autosampler equipped with a Carbopack PA1 column (250 × 4 mm, Dionex). The eluents, consisting of Milli Q water and 150 mM NaOH, were sparged and pressurized with helium. Prior to injection, the system was equilibrated with 30 mM NaOH for 13 min at a flow rate of 1.0 mL/min at ambient temperature. At 0.1 min after injection, the eluent was switched from 30 mM NaOH to Milli Q water. After each run, the column was regenerated with 150 mM NaOH for 10 min. Sugars were detected using a pulsed amperometric detector (Dionex) fitted with a gold working electrode as described by Stolle-Smits et al. (1995). The data system used was a Millenium 2.0 Chromatography Manager (Waters Corporation). Each sample was analyzed in duplicate.

Non-starch glucose content was determined as the difference between glucose content as measured with the HPLC and with the Boehringer test kit.

Uronic acid content was determined in the filtrate (0.2 mL) using the *m*-hydroxydiphenyl method of Ahmed and Labavitch (1977). Determinations were made on three replicates.

Number of Methyl Esters and Acetyl Groups. Methyl esters and acetyl groups were determined by HPLC according to Voragen et al. (1986).

RESULTS

Yield and Composition of Fractions. The yields (mg/g CWM) of buffer, CDTA, and both Na_2CO_3 fractions were comparable for the cultivars Irene and Nicola (Table 1). However, on basis of fresh weight the yield of these fractions was higher for cv. Irene than for cv. Nicola, since cv. Irene contained 0.95 g of CWM per 100 g fresh weight and cv. Nicola contained 0.79 g of CWM per 100 g fresh weight (Marle et al., 1996). Furthermore, cv. Nicola had a higher yield of the KOH fraction, resulting in more residue for cv. Irene, both on the basis of CWM and of fresh weight (Table 1). Comparing fractions and residues of both cultivars on basis of total non-starch polysaccharides (NSP) gave similar results as the comparison made on basis of yield, except for the buffer fraction. The total NSP content of the buffer fraction was slightly higher for cv. Irene than for cv. Nicola. The sugar composition of CWM and the respective fractions and residues is also shown in Table 1. The isolated CWM contained 3.4%−7.8% starch, despite several washing and sieving (mesh: 90 μm) steps (van Marle et al., 1996). Observations with a light microscope revealed that large starch granules (o.d. > 90 μm) were left in the CWM together with starch granules in some small cells, which were not broken during ball-milling. During fractionation, a large amount of the residual starch present in the CWM was found in the buffer fractions (11% and 19% of the fractions for cv. Irene and cv. Nicola, respectively). Furthermore, the CDTA and KOH fractions also contained ca. 5% starch. The presence of starch in these first two fractions was not expected, but particularly decanting of the buffer solution after centrifugation was difficult due to a quite instable residue.

The total sugar content (NSP) of the buffer fractions accounted for 47% and 37% of the isolated material for cv. Irene and cv. Nicola, respectively (Table 1). This

Table 1. Yield and Sugar Composition of CWM, Fractions, and Residues for the Cultivars Irene and Nicola (mg/g CWM)[a]

		fraction						
				Na_2CO_3				
	CWM	buffer	CDTA	4 °C	20 °C	KOH	residue	total
				cv. Irene (0.95 g of CWM/100 g fresh weight)				
yield	1000	64 (11)	54 (7)	95 (5)	107 (6)	222 (2)	497 (9)	1039 (40)
UA	167 (10.5)	5.5 (0.7)	19.4 (4.3)	41.6 (2.7)	26.6 (1.5)	28.3 (1.8)	66.9 (1.0)	188 (12.1)
ARA	39.9 (0.8)	2.1 (0.3)	1.3 (0.4)	3.4 (0.1)	5.5 (0.4)	9.6 (0.5)	16.4 (0.6)	38.4 (2.2)
GAL	312 (2.7)	19.2 (2.3)	15.4 (4.9)	30.3 (0.3)	48.8 (2.7)	93.8 (4.5)	89.4 (2.8)	297 (17.5)
RHA	9.1 (0.1)	0.3 (0.0)	0.3 (0.1)	0.7 (0.1)	0.7 (0.1)	1.3 (0.1)	0.8 (0.2)	4.0 (0.5)
MAN	14.8 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.1)	0.1 (0.0)	0.0 (0.0)	6.7 (0.6)	7.1 (0.7)
XYL	9.1 (0.8)	0.1 (0.0)	0.1 (0.0)	0.2 (0.0)	0.2 (0.0)	1.6 (0.1)	13.3 (0.4)	15.5 (0.6)
GLU[b]	243 (3.2)	2.3 (1.3)	0.8 (0.0)	1.1 (0.4)	0.9 (0.2)	29.9 (0.9)	146 (11.6)	181.2 (14.5)
NSP[c]	795 (18)	30 (5)	37 (10)	77 (3)	83 (5)	165 (6)	340 (17)	731 (44)
starch	51 (17)	11 (7)	1.7 (0.6)	0.5 (0.2)	0.5 (0.3)	11 (2)	0.6 (0.0)	25 (10)
OMe	14.4 (0.6)	0.44 (0.03)	0.34 (0.00)					
OAc	24.4 (0.6)	0.82 (0.11)	0.71 (0.12)					
				cv. Nicola (0.79 g of CWM/100 g fresh weight)				
yield	1000	60 (5)	55 (6)	84 (8)	114 (2)	299 (26)	379 (19)	991 (66)
UA	157 (5.7)	5.2 (0.6)	22.2 (2.9)	37.3 (5.3)	27.1 (1.1)	37.5 (6.1)	44.3 (1.9)	174 (18.0)
ARA	41.3 (0.1)	1.7 (0.1)	1.1 (0.1)	3.0 (0.4)	6.0 (0.2)	14.4 (2.2)	11.5 (0.3)	37.7 (3.4)
GAL	316 (5.3)	12.6 (1.4)	10.0 (0.8)	25.5 (3.7)	56.4 (2.4)	133 (15.1)	54.5 (6.5)	292 (29.8)
RHA	8.5 (0.0)	0.2 (0.0)	0.3 (0.0)	0.6 (0.1)	0.8 (0.1)	2.0 (0.1)	0.8 (0.1)	4.9 (0.4)
MAN	14.2 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	5.5 (0.6)	5.8 (0.7)
XYL	7.1 (0.4)	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.2 (0.0)	1.5 (0.2)	11.6 (1.2)	13.6 (1.5)
GLU[b]	235 (15.8)	2.3 (1.1)	1.5 (0.1)	1.5 (0.0)	1.4 (0.0)	48.4 (2.7)	132 (10.7)	187 (14.6)
NSP[c]	779 (27)	22 (1)	35 (4)	68 (10)	92 (4)	237 (21)	260 (4)	715 (43)
starch	72 (6)	19 (0.4)	4.0 (0.1)	0.8 (0.1)	0.8 (0.0)	10 (3)	1.0 (0.2)	36 (4)
OMe	14.7 (0.8)	0.38 (0.12)	0.51 (0.07)					
OAc	22.7 (0.2)	0.68 (0.09)	0.38 (0.01)					

[a] SD given in parentheses. [b] Non-starch glucose. [c] NSP, non-starch polysaccharides.

low yield is mainly due to the large amount of starch present. For the CDTA, Na_2CO_3, KOH fractions, and the residues, the total sugar content (NSP) accounted for 70%−80% of the isolated material (Table 1). Ryden and Selvendran (1990) found a total sugar content of ca. 60% for the CDTA fractions. In our CDTA fractions of the cultivars Irene and Nicola, CDTA was probably removed to a larger extent due to dialysis against buffer (instead of demineralized water) as was also reported by Mort et al. (1991).

The sugar composition of the CWM from the two cultivars was discussed previously (van Marle et al., 1997). The amounts of arabinose, rhamnose, mannose, and xylose were slightly different in the CWM of the two cultivars. However, the molar composition of the CWM was comparable for both cultivars. The compositions of the pectic polysaccharides in the buffer, CDTA, and cold Na_2CO_3 fractions were quite comparable for both cultivars, with the exceptions that for cv. Irene more rhamnose was found in the buffer fraction and more galactose in the buffer and cold Na_2CO_3 fractions compared with cv. Nicola.

Sequential extraction of the CWM with buffer, CDTA, Na_2CO_3 (at 4 and 20 °C), and 0.5 M KOH yielded different fractions of pectic polysaccharides and still a considerable fraction of pectic material (ca. 30% uronic acid, ca. 35% arabinose, ca. 25% galactose from initial CWM) remained in the residues. Galactose was the most abundant neutral sugar in the CWM, the fractions (except the CDTA and cold Na_2CO_3 fractions), and the residues. The following differences between fractions and residues of both cultivars were observed. At room temperature, Na_2CO_3 extracted more galactose and glucose for cv. Nicola than for cv. Irene. With KOH more rhamnose, arabinose, galactose, uronic acid, and glucose were extracted for cv. Nicola than for cv. Irene. Finally, in the residue of cv. Irene more arabinose, galactose, uronic acid, and xylose were found than in the residue of cv. Nicola.

Table 2. Molar Ratio of Arabinose + Galactose to Uronic Acid in CWM, Fractions, and Residues of the Cultivars Irene and Nicola[a]

cultivar	sample	molar ratio ARA+GAL:UA
Irene	CWM	2.3 (0.2):1.0
	buffer	4.3 (0.3):1.0
	CDTA	0.9 (0.2):1.0
	Na_2CO_3 (4 °C)	0.9 (0.1):1.0
	Na_2CO_3 (20 °C)	2.3 (0.0):1.0
	KOH	4.1 (0.3):1.0
Nicola	CWM	2.6(0.1):1.0
	buffer	3.1 (0.2):1.0
	CDTA	0.6 (0.0):1.0
	Na_2CO_3 (4 °C)	0.8 (0.1):1.0
	Na_2CO_3 (20 °C)	2.6 (0.1):1.0
	KOH	4.4 (0.4):1.0

[a] SD given in parentheses.

The molar ratio of ARA+GAL to uronic acid can give information about the branching of the pectic polysaccharides, assuming that all neutral sugars are present as side chains. Ryden and Selvendran (1990) fractionated their pectic extracts by anion-exchange chromatography. They reported that uronic acid was the main constituent of all obtained fractions, except for one small neutral fraction (rich in arabinose and galactose) obtained from the CDTA extract. However, this small fraction accounted for only 1.7% of the total potato cell wall material. Therefore, the molar ratio of ARA+GAL to uronic acid was calculated for the two cultivars (Table 2). A higher ratio implicates the presence of more and/or longer neutral sugar side chains. The consecutive

fractions from the CDTA fraction up to and including the KOH fraction showed an increase in the molar ratio from ca. 1 to ca. 4.5. Buffer extracted pectic polysaccharides with a molar ratio between 3 and 4.5. When both cultivars were compared, the ratio was higher in the buffer and CDTA fractions of cv. Irene and in the CWM and Na_2CO_3 (20 °C) fractions of cv. Nicola.

The molar ratio of uronic acid to rhamnose can be used as an indication for the number of side chains. It should be kept in mind, however, that this ratio may not be very reliable, since only part of the rhamnose residues are substituted (Bacic et al., 1988; Carpita and Gibeaut, 1993) and, furthermore, the rhamnose content of the cell wall is relatively low. For cv. Nicola a higher ratio was found in the buffer fraction (21 for cv. Nicola and 18 for cv. Irene), and for cv. Irene the ratio was higher in the Na_2CO_3 (20 °C) fraction (38 for cv. Irene and 30 for cv. Nicola).

For both cultivars, buffer and CDTA extracted about 6% of the methyl esters and acetyl groups, while 16% of the uronic acid present in the CWM was extracted in these fractions. For both cultivars a similar degree of methylation (DM = mol of methyl esters per 100 mol of uronic acid) was established for the CWM, buffer fractions, and CDTA fractions. Furthermore, the DM of the buffer fractions (43%) was comparable with the DM of the initial CWM (51%), while the DM was lower for the CDTA fractions (about 12%). This low DM of the CDTA fractions was expected, since CDTA was used with the aim to extract calcium-bound pectate. The degree of acetylation (DA = mol of acetyl groups per 100 mol of uronic acid) was comparable for the CWM of cv. Irene and cv. Nicola (ca. 44%) and for the buffer fractions of cv. Irene and cv. Nicola (ca. 42%). The CDTA fractions had a lower DA, and moreover there was a difference between cv. Irene (11%) and cv. Nicola (5%).

Enzymic Degradation of Fractions. To obtain additional information about the relative abundance of homogalacturonan and branched rhamnogalacturonan regions in the pectic polysaccharides, the fractions and residues of both cultivars were degraded by PG and RGase. The buffer and CDTA fractions were saponified to remove the methyl ester and acetyl groups, which block degradation by PG and RGase. Fractions and residues were not completely soluble in the buffer solution used for the enzymic degradation studies. Only the buffer-soluble material, before and after enzymic treatment, was analyzed by HPSEC and HPAEC. The HPSEC elution patterns of the saponified buffer and CDTA fractions and the Na_2CO_3 and KOH fractions and residues before and after enzymic degradation are shown in Figure 1. The HPAEC elution patterns are not shown, since very low amounts of oligomers were formed during incubation.

For the buffer and to lesser extent for the CDTA fractions broad molecular weight distributions were found. This is probably due to the presence of relatively low molecular weight starch in these fractions, which was also detected by refractive index. Low molecular weight starch may be a result of the combination of mechanically damaged starch granules (due to ball-milling of fractions and residues) and subsequent heating to 100 °C used to inactivate enzymes after enzymic hydrolysis. The HPSEC elution patterns of the CDTA fractions in particular showed for cv. Nicola more material with retention times between 20 and 26 min than was found for cv. Irene. Since about 80% of the uronic acid present in the CDTA fractions was buffer-soluble (data not shown), the low amount of residual starch in the CDTA fractions (Table 1) (from which only a part will be soluble) will contribute only a few percent to the total refractive index (RI). Therefore the observed difference between the elution patterns was not (only) due to the difference in starch content between the CDTA fractions of both cultivars. Undigested non-saponified buffer and CDTA fractions gave comparable HPSEC elution patterns as the saponified fractions.

The HPSEC elution patterns of the Na_2CO_3 (4 °C), Na_2CO_3 (20 °C), KOH fractions, and residues showed the presence of high amounts of high molecular weight material with retention times between 18 and 21 min. In these fractions starch was not interfering, since it was almost absent. Differences between the cultivars were found in the KOH fraction, where material of cv. Nicola was more (34%) soluble and in the residues, where material of cv. Irene was more (23%) soluble as measured from the RI signal from the respective HPSEC elution patterns.

After incubation of the various fractions and residues with PG, material with retention times between 21 and 28 min and oligo- and monomers, degree of polymerization (DP) 1−4, with retention times between 30 and 36 min (HPAEC, results not shown) were formed at the expense of high molecular weight material. Comparing both Na_2CO_3 fractions and the KOH fraction with each other revealed that less oligomers were released resulting in relative more material with retention times between 21 and 28 min in the consecutive fractions. Also in the NaOH fraction and residue of apple cell walls molecular weight material with a retention time of 26 min was formed after incubation with PG (Schols et al., 1995). Remarkable differences between cultivars were found in the following fractions. After treatment of the saponified CDTA fractions with PG, more material with retention times between 20 and 26 min was present for cv. Irene than for cv. Nicola. This observation contrasted with the elution patterns of the undigested CDTA fractions. PG degraded relatively more material for cv. Nicola than for cv. Irene. The solubility of material in the residues increased after incubation with PG and comparable amounts were solubilized for both cultivars.

The respective fractions and residues were also incubated with RGase. Non-saponified buffer and CDTA fractions showed comparable HPSEC elution patterns before and after incubation with RGase. A similar observation was found for the cold buffer fraction from apple cell wall material (Schols et al., 1995). Saponification of buffer and CDTA fractions and subsequent incubation with RGase resulted for both cultivars in the formation of material with retention times between 22 and 26 min at the expense of initial higher molecular weight material (Figure 1). No oligomers could be detected. Incubation of the Na_2CO_3, KOH fractions, and residues with RGase resulted in the formation of material, with an increasing average retention (from 22 to 25 min) for the consecutive fractions. For the Na_2CO_3 (20 °C), KOH fractions, and residues, the elution patterns obtained by HPAEC showed the presence of typical RGase oligomers. These patterns were comparable with those obtained by Schols et al. (1994). Schols et al. also identified the structures of the RGase oligomers. On the basis of these results it was concluded that for both potato cultivars mainly the hexamer and octamer (of alternating galacturonic acid and rhamnose

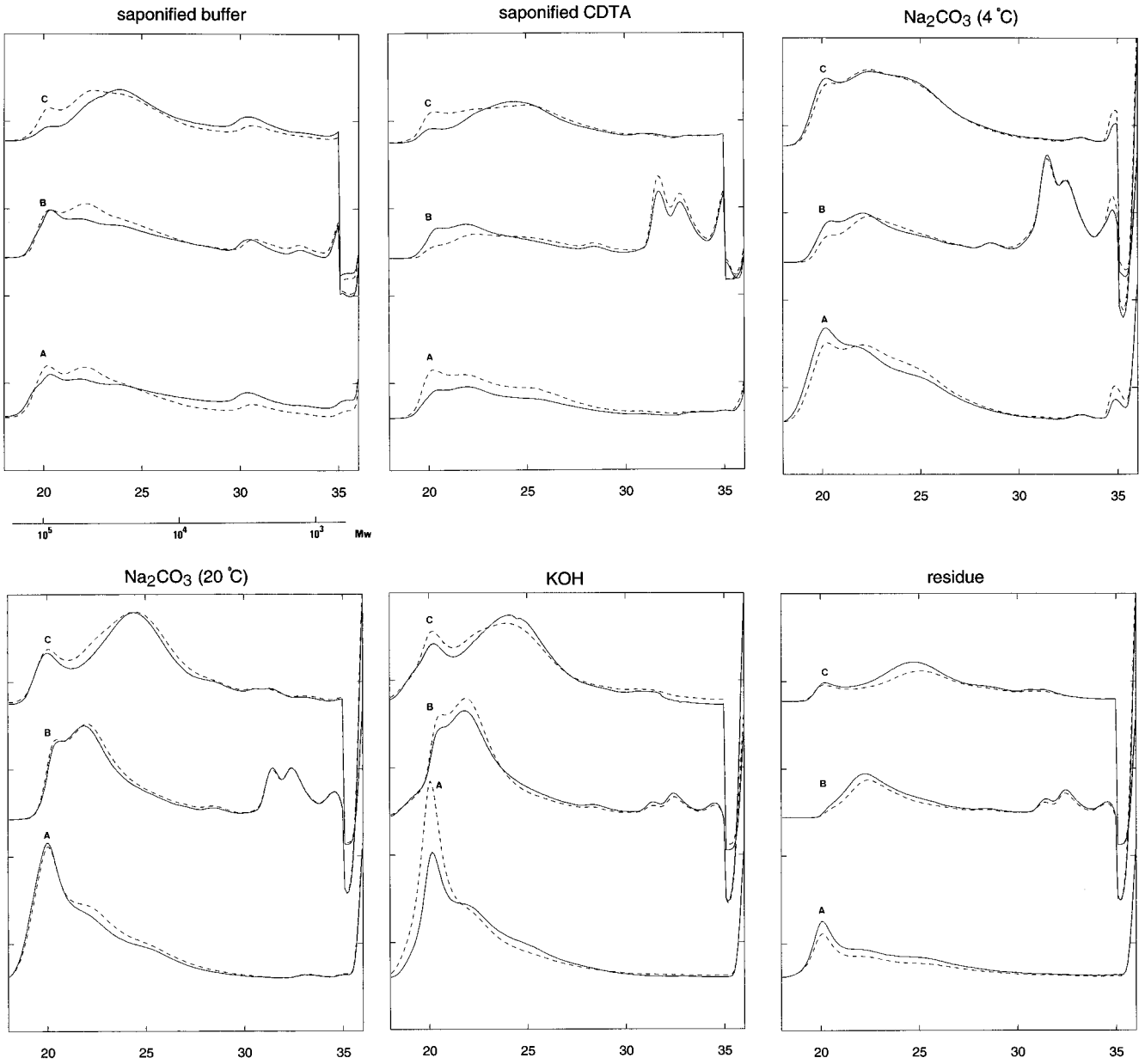

Figure 1. HPSEC elution patterns of fractions and residues from the cultivars Irene (solid line) and Nicola (dashed line). (A) Before enzymic degradation and after treatment with (B) PG and (C) RGase. *Y* axis is the refractive index; *X* axis is the retention time (in min).

residues) were found, which contained two rhamnose residues substituted with a galactose side chain.

DISCUSSION

Previously it was reported that upon cooking different types and amounts of pectic material were solubilized for potatoes of the cultivars Irene and Nicola (van Marle et al., 1994, 1997). These results can be explained by differences in structure of the non-cooked cell wall material for these cultivars. To validate this hypothesis, non-cooked cell wall material from both cultivars was fractionated by sequential extraction with various solvents to obtain information about the composition, structure and amounts of the different types of pectin present in the cell wall.

The pectic material extracted by buffer, CDTA and cold Na_2CO_3 originated at least partly from the middle lamellae (Ishii, 1981; Jarvis et al., 1981; Ryden and Selvendran, 1990; Selvendran et al., 1985). This material constituted 20% of the CWM for the cultivars Irene and Nicola. Ryden and Selvendran (1990) solubilized 36% of their CWM after extraction with CDTA and cold Na_2CO_3, while Jarvis et al. (1981) reported that 55% of the CWM was extracted with hot oxalate-citrate buffer and cold Na_2CO_3.

The branched pectin extracted with Na_2CO_3 at 20 °C probably originated from the primary cell wall (Ryden and Selvendran, 1990) and contained more neutral sugars than galacturonic acid (Table 2). Using 0.5 M KOH, an additional amount of more branched pectic material and a small part of the xyloglucans were extracted. Vincken (1996) reported that stronger alkali (1 and 4 M KOH) extracted most of the xyloglucan from blanched potato tissue. After sequential extraction including 0.5 M KOH, 70% of the galactose and 64% of the uronic acid present for cv. Irene and 81% of the galactose and 75% of the uronic acid present for cv. Nicola in CWM were solubilized. Ryden and Selvendran (1990) extracted 40% of the galactose and 60% of the uronic acid present in the CWM after the extraction

with 0.5 M KOH, but Jarvis et al. (1981) found the main part of galactose (about 90%) and uronic acid (about 95%) in the oxalate-citrate buffer and cold Na_2CO_3 fractions. As appeared from the above results the extractability of potato pectic polysaccharides is strongly cultivar and/or method dependent. In the residues of both cultivars considerable amounts of pectic material were found, which are not extracted probably due to alkali-resistant cross-linking and/or entanglement with other cell wall material (Ryden and Selvendran, 1990; Schols et al., 1995).

Galactose is the most abundant neutral sugar in potato cell walls (Jarvis et al., 1981) and also in the respective fractions and residue (Table 1). The relative high amount of galactose (and also arabinose) and the allied high ratio of ARA+GAL to uronic acid found in the buffer fractions was unexpected, since during the isolation procedure no elevated temperatures were used, which could cause degradation of pectic material (Selvendran and O'Neill, 1987; Ryden and Selvendran, 1990). Maybe this was due to interference of contaminating suspended material from the residue after centrifugation. Another possibility is that the galactose in the buffer fraction is a small neutral fraction corresponding with the neutral fraction found by Ryden and Selvendran (1990). In both cases this fraction accounted for about 1.5% of the total cell wall material.

The ratio of neutral sugars to uronic acid increased with more severe extraction conditions, comparing the compositions of both Na_2CO_3 fractions and the KOH fraction with each other (Table 2). This increase in ratio is correlated with the presence of more PG-resistant material (Figure 1). These observations implied the presence of more branched rhamnogalacturonan regions in the consecutive Na_2CO_3 and KOH fractions. The pectic polysaccharides in these fractions are slightly more methylated and acetylated than in the initial CWM, because relatively higher amounts of uronic acid than methyl esters and acetyl groups were extracted with buffer and CDTA from the CWM (Table 1). Furthermore, it was observed that incubation with RGase led to the formation of material with an increasing retention time (due to the presence of shorter homogalacturonan regions between branched rhamnogalacturonan regions) and release of more RGase oligomers for the consecutive fractions, which is in agreement with the above results. Similar observations were made for fractions of apple cell wall material (Schols et al., 1995). During incubation with RGase only small amounts of oligomers were formed. Incubation of CDTA-insoluble apple cell wall material (Renard et al., 1993) and fractions of cell wall material from apple (Schols et al., 1995) with RG also released low amounts of oligomers compared with RGase incubation of (modified) hairy regions from apple (Schols et al., 1990, 1995). The presence of relatively high amounts of the mentioned hexamer and octamer after treatment with RGase was also reported by Schols et al. (1995) for the hairy regions isolated from the alkali fraction and residue of apple cell walls.

The following differences between the cultivars Irene and Nicola were found with respect to the pectic polysaccharides extracted by buffer, CDTA, and cold Na_2CO_3. When the two cultivars were compared, the ratio of ARA+GAL to uronic acid was higher for cv. Irene while the ratio of uronic acid to rhamnose was higher for cv. Nicola (Table 2). Furthermore, the CDTA fraction had a higher DA for cv. Irene compared with cv. Nicola (Table 1). These results indicate that the buffer, CDTA, and cold Na_2CO_3-soluble pectic polysaccharides of cv. Irene contain more and/or longer side chains than those of cv. Nicola. Such a difference in structure was also observed comparing the HPSEC elution patterns of the saponified CDTA fractions for both cultivars. The undigested, saponified CDTA fraction of cv. Nicola contained more material with retention times between 20 and 26 min than this fraction of cv. Irene. This material was relatively more degraded after incubation with PG for cv. Nicola than for cv. Irene (Figure 1). Since the CDTA fractions had a low DM (ca. 12%), saponification and subsequent incubation with PG gave similar results. These observations demonstrate the presence of more and/or longer homogalacturonan regions for cv. Nicola.

Comparison of the sugar composition of the Na_2CO_3 (20 °C), KOH fractions, and residues of both cultivars revealed differences between pectic polysaccharides and the xyloglucan−cellulose network in the primary cell walls of both cultivars.

The branched pectic polysaccharides extracted by Na_2CO_3 at 20 °C form a relative similar weight fraction of the cell walls of both cultivars. However, a higher ratio of ARA+GAL to uronic acid for cv. Nicola (Table 2) and a higher ratio of uronic acid to rhamnose for cv. Irene (Table 1) are an indication for the presence of more and/or longer side chains in the pectic polysaccharides for cv. Nicola than for cv. Irene.

The yield of the KOH fraction was 7.7% higher for cv. Nicola than for cv. Irene due to the extraction of relative more pectic polysaccharides and glucans (Table 1). An indication for a difference in structure between the pectic polysaccharides extracted by KOH for both cultivars is the presence of more soluble material in the KOH fraction of cv. Nicola than in this fraction of cv. Irene (Figure 1).

For cv. Irene 50% of the CWM remained in the residue, which is about 24% more than for cv. Nicola (Table 1). This difference is mainly due to the presence of more uronic acid, arabinose, and galactose in the residue of cv. Irene compared to the residue of cv. Nicola. This may be an indication that for cv. Irene the neutral sugar side chains are more strongly and/or to a larger extent bound to or entangled in the xyloglucan or cellulose material than for cv. Nicola.

The differences found between the pectic polysaccharides of both cultivars may influence the solubilization of pectic polysaccharides during cooking of potatoes. In our previous paper (van Marle et al., 1997) observations made by transmission electron microscopy (TEM) showed that at least part of this cell wall material solubilized during cooking originated from the middle lamellae. The solubilized material was relatively more branched and higher methylated and acetylated for cv. Irene than for cv. Nicola (van Marle et al., 1997). These results agree with the data obtained by the fractionation study. The pectic polysaccharides extracted by buffer, CDTA, and cold Na_2CO_3 contained more branched rhamnogalacturonan regions for cv. Irene than cv. Nicola.

Since non-cooked cells of cv. Irene had significantly more CWM per unit cell surface area than the cells of cv. Nicola (van Marle et al., 1997) and since comparable amounts of pectic polysaccharides were extracted from the CWM with buffer, CDTA, and cold Na_2CO_3 for both cultivars, the cell walls remaining after these extractions still contained more CWM per unit of cell surface area for cv. Irene than for cv. Nicola. Therefore the

remaining cell walls of cv. Irene will be thicker and/or more compact compared with the cell walls of cv. Nicola. For cv. Nicola, from the remaining cell walls more branched pectic polysaccharides (higher ratio of ARA+GAL to uronic acid) were extracted with Na_2CO_3 (20 °C) (Table 2) and relative higher amounts of pectic material were extracted with KOH (Table 1). This is an additional indication that the primary cell walls of cv. Nicola have a less compact structure than the ones of cv. Irene. In the model proposed by Carpita and Gibeaut (1993), the cellulose−xyloglucan network is embedded in a pectin matrix, which defines, among other functions, the porosity of the cell wall. It can be proposed that for cv. Irene in the cell walls remaining after extraction with buffer, CDTA, and cold Na_2CO_3, the cellulose−xyloglucan construction is embedded in a less porous pectin matrix than for cv. Nicola. A similar postulation can be made comparing the structure of the cell walls remaining after cooking for both cultivars. These results agreed with the observations made by cryoscanning electron microscopy showing cooked cells with more intact cell walls for cv. Irene than for cv. Nicola (van Marle et al., 1992).

In conclusion, during cooking more branched pectic polysaccharides were solubilized for cv. Irene than for cv. Nicola, which reflects the observed differences in composition and structure of the pectic polysaccharides extracted by buffer, CDTA, and cold Na_2CO_3 between both cultivars (Tables 1 and 2). These differences were quite small and probably give a small contribution to the explanation of the large differences in texture development. However, the difference in total amount of CWM and the difference in structure of the cell walls remaining after extraction with buffer, CDTA, and cold Na_2CO_3 between both cultivars were more pronounced and may play a role in texture development. However, physical measurements of the intercellular forces and the strength of cell walls are necessary to confirm the above postulations.

Although, relative small differences between the structures of pectic polysaccharides in the cell walls and middle lamellae were found for both cultivars, when using the ratio of ARA+GAL to uronic acid as an indication for the abundance and/or length of neutral sugar side chains, these differences were confirmed by the enzymic degradation studies.

ABBREVIATIONS USED

CWM, cell wall material; UA, uronic acid; ARA, arabinose; GAL, galactose; GLU, glucose; MAN, mannose; RHA, rhamnose; XYL, xylose; PG, polygalacturonase; RGase, rhamnogalacturonase; CDTA, 1,2-cyclohexylenedinitrilotetraacetic acid; HPSEC, high-performance size-exclusion chromatography; HPAEC, high-performance anion-exchange chromatography; NSP, non-starch polysaccharides; DP, degree of polymerization; DM, degree of methylation; RI, refractive index; DA, degree of acetylation.

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