Carbohydrate Research, 172 (1988) 217–227
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ISOLATION AND CHARACTERISATION OF THE CELL-WALL FIBRES OF CARROT*

PATRICE MASSIOT, XAVIER ROUAU, AND JEAN-FRANCOIS THIBAULT
Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, Rue de la Géraudière, F-44072 Nantes (France)

(Received April 29th, 1987; accepted for publication, July 8th, 1987)

ABSTRACT

Cell-wall fractions have been prepared from an alcohol-insoluble residue of carrot root by treatment with (*a*) Pronase to remove the cytoplasmic proteins, (*b*) hot dilute acid and cold dilute alkali to give pectin-free residues, and (*c*) concentrated alkali to leave the α-cellulose and lignin. The purified cell-wall material still contained ~1% of protein and was composed mainly of cellulose, lignin, methyl-esterified galacturonic acid, and smaller amounts of galactose and arabinose. Methylation analysis of the insoluble residues indicated the presence, in order of decreasing concentration, of rhamnogalacturonan with the rhamnosyl residues carrying side chains at position 4, cellulose, (1→4)-linked galactan, (1→5)-linked arabinan, (1→4)-linked xylan, (1→4)-linked mannan, and xyloglucan.

INTRODUCTION

Carrots are potential sources of dietary fibres and pectic substances[1–4]. Dietary fibres, which consist of the part of the plant which cannot be hydrolysed by the human digestive enzymes[5] and are made up mainly of cellulose, hemicelluloses, pectins, and lignin, probably play a role in preventing many organic diseases and in infant nutrition [6,7]. Pectins, mainly used as gelling agents, are usually extracted from citrus peels or apple marks, but alternative sources such as carrot[2] or beet[8,9] may be found.

The cell wall is responsible *in vivo* for the firmness and cohesiveness of fruit and vegetable tissues and is subject to chemical and enzymic degradations during ripening, storage, and processing, and therefore influences the texture of processed products[10,11]. We now report a study of the structural features of carrot cell-wall polymers; the pectins and hemicelluloses are characterised in the following paper[12]. Hot water, hot ammonium oxalate, alkali[13–14], and enzymic degradations[15] have been used to fractionate the cell-wall material of carrot root. Since carrot cell walls appear to be a potential source of pectins, the effect of hot acid treatment[16] on

* Structural Study of the Cell Wall of Carrot (*Daucus carota* L.), Part I.

0008-6215/88/$03.50

cell-wall material has been investigated.

EXPERIMENTAL

Plant material. — Carrot roots (*Daucus carota* L.) of the "Nantaise" variety were harvested in January 1986, 28 weeks after sowing.

Preparation of the alcohol-insoluble residue (AIR). — In order to obtain cell walls with the minimum of enzymic degradation, fresh carrots (100 g) were rapidly cut into small pieces and blended for 10 min in boiling aqueous 96% ethanol (500 mL). After filtration, the insoluble residue was washed with acetone, and then mixed using a Polytron mixer (Kinematic) in aqueous 70% ethanol at 45°. The residue was washed with ethanol until the filtrate was colourless, then with acetone and ether, and air-dried. The average particle size was < 1 mm.

Isolation of cell-wall material (CWM). — The AIR (20 g) was suspended in 0.2M acetate buffer (2 L, pH 5.0 or 7.5) at 25° or 45°, Pronase (Boehringer-Mannheim, 400 mg) was added, and the suspension was stirred for 24 h and then filtered through a G4 sintered-glass filter (pore size: 10–15 μm). This operation was carried out twice. The residue was washed extensively with water. Half of the CWM was washed with aqueous 96% ethanol, acetone, and ether, and then air-dried. The other half was used for sequential extraction.

Sequential extraction of CWM. — Undried CWM was dispersed in 0.05M hydrochloric acid and heated for 30 min at 85°. The suspension was filtered through a G4 sintered-glass filter. This extraction was then repeated twice. Finally, the acid-insoluble residue (HR) was washed extensively with water. A part of HR was dried by solvent exchange and then air-dried.

Undried HR was stirred with 0.05M sodium hydroxide for 30 min at 4° under argon (3 times). The suspension was centrifuged (6,000*g*, 10 min) and the pellet was washed extensively with water. A part of the dilute-alkali-insoluble residue (OHR) was dried by solvent exchange, and then air-dried. Undried OHR was stirred with 4M sodium hydroxide containing 0.01M sodium borohydride for 2 h at room temperature under argon. The mixture was filtered as above and the extraction was repeated twice. The concentrated-alkali-insoluble residue (COHR) was dried as above.

All the soluble fractions were collected, neutralised, concentrated, dialysed, and freeze-dried[12].

Analytical methods. — All values were calculated on a moisture free-basis.

Polysaccharides were treated for 30 min with aqueous 72% H_2SO_4 at room temperature and then hydrolysed with M H_2SO_4 (2 h, 100°). Sugars were converted[18] into alditol acetates and analysed by g.l.c. on a column (180 × 0.2 cm i.d.) of SP 2340 at 225°, using nitrogen as carrier gas and *myo*-inositol as the internal standard. Cellulosic glucose was determined by the difference between the contents of glucose obtained before and after the pre-hydrolysis step.

Uronic acids were determined colorimetrically[19] with the 3-hydroxybiphenyl reagent after hydrolysis of the insoluble material as described above, and using galacturonic acid as standard. Galacturonic acid and neutral sugars (expressed as galactose) in the soluble fractions were determined by the automated 3-hydroxybiphenyl[20] and orcinol methods[21], respectively, the latter being corrected for interfering galacturonic acid.

Uronic acids in insoluble fractions were identified, after pre-hydrolysis with 13M trifluoracetic acid for 1 h at 20° followed by hydrolysis with 2M acid for 2 h at 120°[22], by t.l.c. on Silica Gel F-1500 (Schleicher and Schüll), using 1-propanol–ethyl acetate–ethanol–pyridine–acetic acid–water (7:3:3:2:2:5) and detection with thymol (0.5%) in ethanol–sulfuric acid (95:5).

Methanol and acetic acid contents were determined, after treatment of the polysaccharides with 0.4M NaOH in water–2-propanol (1:1) for 2.5 h, by h.p.l.c.[23] using a Waters Model 590 instrument equipped with a column (30 × 0.78 cm i.d.) of Aminex HPX 87H (9 μm, Bio-Rad) and elution with 0.01M H_2SO_4 at 0.6 mL/min. Refractive index detection was used.

Ester-linked phenolic acids were saponified with 2M NaOH for 2 h at 35° under argon. After acidification (pH 2.0) with 12M HCl, phenolic acids were extracted by ether and separated by h.p.l.c., using a column (25 × 0.46 cm i.d.) of Rsil C18 HL (5 μm, RSL) and elution with water–methanol–acetic acid (73:26:1) at 0.9 mL/min. The effluent was monitored by absorption at 280 nm.

Nitrogen was determined by the Kjeldahl method and protein contents were estimated as N × 6.25. Amino acid analyses were carried out, after sample hydrolysis with 7.5M HCl in a sealed tube for 24 h at 110°, using a Kontron Liquimat III amino acid analyser and detection at 480 and 870 nm after reaction with ninhydrin.

Determination of lignin, starch, and ashes. — Lignin contents were determined by the method of Klason[24], starch by the D-glucose oxidase–peroxidase system after heat-gelatinisation and hydrolysis by amyloglucosidase[25], and ash after incineration overnight at 550° and for 1 h at 800°.

Methylation analysis. — Cell-wall polysaccharides were methylated by the Hakomori technique[26] as adapted by Lomax *et al.*[27] and Harris *et al.*[28] for secondary cell-walls. Dry samples (10 mg) were ground (3 min) in liquid nitrogen using a Spex 6700 freeze-mill. After oven-drying overnight at 40° *in vacuo*, anhydrous methyl sulphoxide (1 mL) was added under argon and the mixture was ultrasonicated for 30 min. Freshly prepared sodium methylsulphinylmethanide was then added under argon, and the mixture was ultrasonicated for 30 min and shaken overnight at room temperature. Methyl iodide (1 mL) was added in portions of 0.2 mL to the ice-cooled sample in order to allow slow dissolution. After stirring for 1 h at room temperature, chloroform–methanol (2:1) was added, followed by water (3 mL). The mixture was centrifuged (1,000 *g*, 2 min), the upper aqueous phase was removed, the washing was repeated 4 times, and then 2,2-dimethoxypropane (2 mL) and 18M acetic acid (20 μL) were added. The suspension was concentrated to dryness at 40°. The methylated polysaccharides were hydrolysed by 2M trifluoroacetic acid

after the addition of quebranchitol (0.25 mg; 2-*O*-methyl-*chiro*-inositol) as internal standard. The resulting sugars were converted into alditol acetates[28] and analysed by g.l.c., using a Delsi 30c gas chromatograph with on-column injection facilities, flame-ionisation detector, and capillary columns (50 m × 0.32 mm i.d.) of OV1 (40→190° at 15°/min) and CP SIL 88 and SP 1000 (40°→210° at 15°/min). The carrier gas was helium. Partially methylated alditol acetates were identified by comparison (*a*) of their retention coefficients calculated[29] using 2,3,4,6-tetra-*O*-methyl-D-glucitol diacetate and quebrachitol penta-acetate as standards with values given by Lomax *et al.*[27] and (*b*) with chromatograms of partially methylated alditol acetates obtained from polysaccharides of known structure.

RESULTS AND DISCUSSION

Preparation of the carrot cell-wall material (CWM). — The yield of alcohol-insoluble residue (AIR) was 24.3 g/kg of fresh carrot, corresponding to 26.1 g/100 g of dry matter. The AIR of carrot contained structural polysaccharides and lignin together with starch, proteins, and pigments such as carotenoids. In order to achieve maximum solubilisation of cytoplasmic material with minimum loss of cell-wall polysaccharides, the conditions of proteolysis (Table I) were studied in detail; at the optimum pH and temperature (7.5 and 45°, respectively), the proteolysis efficiency of Pronase was 92% after treatment for 24 h, but 56.5% of the uronic acids were solubilised. This solubilisation, also found with cabbage[30], may be ascribed to β-elimination reactions of methylated pectins. In order to minimise this degradation, proteolysis was carried out after decreasing the temperature and pH to 25° and 5.0, respectively. After 24 h, 20.6% of uronic acids had been solubilised and the proteolysis efficiency was 82%. There was no further solubilisation of uronic acids after 48 h and there was 1.2% of residual protein. The use[31] of sodium dodecyl sulfate (1%) to facilitate the removal of cytoplasmic protein did not improve the proteolytic treatment. Pronase in acetate buffer extracted more pectic polysaccharides than did the buffer alone, indicating their possible association in complexes with proteins[32].

TABLE I

SOLUBILISATION OF PROTEINS AND POLYSACCHARIDES FROM AIR UNDER DIFFERENT CONDITIONS OF PRONASE TREATMENT

Temperature (degrees)	25	25	25	25	25	45
pH	5	5	5	5	5	7.5
Time (h)	24	24	24	24	48	24
SDS (% w/v)	0	1	0	1	0	0
Pronase (mg/g of AIR)	0	0	0.02	0.02	0.02	0.02
Protein solubilisation (%)	48	57	82	82	89	92
Uronic acids solubilised[a]	15.2	13.6	20.6	23.8	20.6	56.5
Neutral sugars solubilised[b]	6.9	5.7	8.6	7.9	8.9	11.6

[a] As a % of the total uronic acids of AIR. [b] As a % of the total neutral sugars of AIR.

TABLE II

CHEMICAL COMPOSITION (% DRY MATTER) OF THE ALCOHOL-INSOLUBLE RESIDUE (AIR) OF CARROT, CELL-WALL MATERIAL (CWM) AND INSOLUBLE FRACTIONS OBTAINED BY SEQUENTIAL EXTRACTION

Fraction	*AIR*	*CWM*	*HR*[a]	*OHR*[b]	*COHR*[c]
Recovery	100	75.4	57.0	32.8	24.9
Uronic acids[d]	31.5	34.3	31.5	6.4	5.8
Total neutral sugars	40.6	47.4	54.1	82.2	83.3
Rhamnose[d]	1.6 (1.6)	1.8 (2.0)	1.6 (1.9)	0.8 (0.8)	0.5 (0.5)
Fucose[d]		0.2 (0.1)		0.1	
Ribose[d]	0.1 (0.1)	0.1			
Arabinose[d]	4.1 (4.1)	4.6 (5.3)	2.3 (2.3)	1.8 (1.8)	1.2 (1.0)
Xylose[d]	1.6 (1.1)	2.0 (1.8)	2.5 (1.7)	4.1 (3.5)	0.8 (0.5)
Mannose[d]	1.8 (0.8)	2.0 (0.6)	2.5 (0.5)	4.2 (0.6)	0.3 (-)
Galactose[d]	6.2 (6.0)	7.2 (7.0)	6.9 (6.9)	3.5 (3.5)	3.0 (3.0)
Glucose[d]	25.2 (2.8)	29.5 (1.8)	38.3 (1.9)	67.7 (4.0)	78.0 (5.5)
Protein (N × 6.25)	8.0	1.2	1.1	0.9	0.3
Lignin	6.4	6.4	8.9	8.0	11.8
Ash	8.4	6.1	0.7	1.7	2.1
Methanol	4.7	4.5	3.2	<0.1	<0.1
Acetic acid	2.8	3.7	3.1	0.5	<0.1
Phenolic acids	1.1	0.1	0.3	0.3	0.1

[a] Acid-insoluble residue. [b] Alkali-insoluble residue. [c] Concentrated-alkali-insoluble residue. [d] Digestion with aqueous 72% sulfuric acid followed by dilution and hydrolysis; values in parentheses are after hydrolysis with M H_2SO_4.

Because of the low starch content (0.3%) in the AIR, no amylolytic treatment was carried out. The yield in CWM was 75.4 g/100 g of AIR with recoveries of 82% and 88% in uronic acids and neutral sugars, respectively (Table II).

Composition of AIR and CWM. — The compositions of AIR and CWM are shown in Table II. The high contents (31.5–34.3%) of galacturonic acid, which are similar to those obtained from onion[33] and are higher than those from beet or apple[34], suggested the presence of an important amount of pectic substances. Galactose and arabinose were the main non-cellulosic sugars (Table II) and are generally associated with pectins. The content of glucose was high (AIR, 25.2%, CWM, 29.5%); the bulk originated from cellulose, but a fraction (11% and 6%, respectively) was not of cellulosic origin because it was removed by mild acid hydrolysis. The sugar contents determined with or without a pre-hydrolysis step were different for xylose and mannose, but similar for rhamnose, arabinose, and galactose. This result suggested the presence of xylans and mannans tightly bound to the cellulosic fibres. The calculations showed that rhamnose, arabinose, and galactose were preponderantly solubilised by the proteolysis and indicated them to be part of water-soluble pectins.

The protein content decreased to 1.2% in CWM, but the sum of the amino acids was much lower (Table III) although cysteine, methionine, and tryptophan were not determined. A fraction of the nitrogen probably originated from nucleic

TABLE III

AMINO ACID COMPOSITION[a] (MG/100 G) OF AIR, CWM, AND INSOLUBLE FRACTIONS OBTAINED BY SEQUENTIAL EXTRACTION

Fraction	*AIR*	*CWM*	*HR*[b]	*OHR*[b]	*COHR*[b]
Asp	560	65	35	40	15
Thr	265	35	25	25	10
Ser	295	45	40	40	15
Glu	640	65	60	45	20
Pro	200	40	30	20	
Gly	280	50	30	40	15
Ala	345	45	50	35	20
Val	345	45	50	35	20
Ileu	250	30	30	25	10
Leu	375	50	40	15	20
Tyr	140	25	20	20	10
Phe	190	25	20	20	15
Lys	345	45	30	20	15
His	110	30	20	20	10
Arg	375	35	30	20	10
Hyp	350	115	80	70	35
Total	5065	745	590	490	240

[a] Cysteine, methionine, and trytophan were not determined. [b] See footnotes to Table II.

acid. Aspartic acid (11.0% of the total amino acids), glutamic acid (12.6%), leucine (7.4%), and arginine (7.4%) were the main amino acids in AIR. The contents of aspartic and glutamic acid decreased slightly in CWM, but that of hydroxyproline increased markedly (AIR, 6.9%; CWM, 15.4%). These amino acids, already found in purified CWM of carrot[13], may be implicated in the association with polysaccharides, *e.g.*, as a part of cell-wall proteoglycan[35,36].

The contents of lignin of AIR and CWR were high, but were probably overestimated because of an incomplete removal of other polymers, such as highly crystalline cellulose which is resistant to hydrolysis.

The contents of methanol and acetic acid were high. Assuming that methanol is ester-linked to the galacturonic acid residues, then 90% of the galacturonic acid in AIR and 80% in CWM were esterified. All of the acetic acid was recovered from CWM, suggesting that the solubilised pectins were not highly acetylated.

The contents of phenolic acids decreased markedly after treatment with Pronase, indicating that they could be associated with the protein fraction and/or the solubilised polysaccharides. In the AIR, the preponderant phenolic acids were *p*-hydroxybenzoic acid (46% of the phenol content), previously detected in carrot[37], *p*-hydroxyphenylacetic acid (20%), and ferulic acid (10%).

The acid-insoluble residue (HR). — Mild acid treatment (0.05M HCl) is known to remove large amounts of pectic material, probably by cleavage of the cross-links of the matrix polymers, especially those involving arabinose and rhamnose

residues[16], and other linkages such as ionic or hydrogen bonds[35]. However, for the carrot, only small amounts of polysaccharides were solubilised, since 70% of the galacturonic acid and 85% of the neutral sugars initially present in the CWM were recovered in HR (Table II).

Recoveries of rhamnose and galactose (Table II) were similar to that of galacturonic acid. Recovery of arabinose was lower (38%), but those of xylose, mannose, and glucose were much higher (95–98%). Thus, treatment with acid extracted mainly rhamnogalacturonan together with associated neutral polysaccharides (arabinan, galactan, or arabinogalactan) to leave a residue where the cellulosic matrix (95% of glucose was from cellulosic origin) was still present with hemicelluloses and an important fraction of pectins. The molar ratio methanol/galacturonic acid decreased to 0.6, suggesting that highly methyl-esterified pectic substances were preferentially solubilised. Almost all of the ash was removed by this acid treatment. Part of the protein rich in hydroxyproline and aspartic acid was removed (Table III) and was probably linked by ionic bonds to polysaccharides, as previously reported for hydroxyproline-rich cell-wall glycoprotein from carrot[38].

The dilute-alkali-insoluble residue (OHR). — The treatment with 0.05M NaOH cleaves such alkali-labile linkages as esters. A low temperature (4°) was chosen in order to minimise β-elimination of pectins and extraction was carried out under an inert gas in order to avoid oxidation. Only 8% of the galacturonic acids from CWM were recovered in OHR. The contents of rhamnose and galactose decreased in similar proportion, whereas the arabinose-containing polymers were solubilised to a lesser extent since 45% of the arabinose was recovered. These results showed that the bulk of the pectic substances had been solubilised. The contents of xylose, mannose, and glucose remained constant. Therefore, OHR mainly consisted of cellulose, other glucans (6% of glucose content), and mannose-, xylose-, and galactose-containing polysaccharides.

Of the lignin present in CWM, 60% was recovered, so that part was alkali-soluble as reported for Graminae[39]. Only slight differences were observed in the contents of amino acids of HR and OHR, indicating that structural proteoglycans were still present in these cell-wall fractions.

The concentrated-alkali-insoluble residue (COHR). — In order to remove the "hemicellulosic" fraction, OHR was treated with 4M sodium hydroxide in the presence of sodium borohydride under argon to avoid oxidative degradation. COHR was composed mainly of cellulose (72.5%) and lignin (11.8%); almost all of the arabinose and galactose residues present in OHR were recovered, indicating that they were strongly bound to the remaining polymers. The treatment solubilised mannose (95%), xylose (85%), and glucose (15%) present in OHR, and t.l.c. of a hydrolysate of this fraction revealed galacturonic acid as the sole uronic acid. A pectic fraction was therefore strongly bound to remaining fibres and resisted extraction, as observed also for cabbage[30] and suspension-cultured Rosa cell-walls[40]. A small protein fraction was detected in COHR, indicating that significant proportions of proteoglycans were strongly cross-linked to the cell-wall polysaccharides[13],

TABLE IV

PATIALLY METHYLATED ALDITOL ACETATES DERIVED FROM METHYLATED POLYSACCHARIDE-FRACTIONS OF CARROT AND IDENTIFIED BY G.L.C.

Derivative[a]	*AIR*	*CWM*	*HR*	*OHR*	*COHR*
2,3,4,6-Me_4-Glc[b]	1.0[c]	2.0	0.7	0.6	1.3
2,3,6-Me_3-Glc	49.8	46.9	65.6	75.8	85.7
2,3-Me_2-Glc	1.8	2.8	1.6	1.3	1.6
2,6-Me_2-Glc	0.5	0.6			
3,6-Me_2-Glc	0.9	0.8	1.2	1.2	0.7
Glc	1.8				1.7
Total[d]	54.8 (62.3)	53.1 (63.8)	69.1 (71.2)	78.9 (82.3)	91.0 (93.0)
2,3,4,6-Me_4-Gal	0.7	1.2	2.2	0.3	1.6
2,3,4-Me_3-Gal	1.2	1.0	0.6	0.6	0.5
2,3,6-Me_3-Gal	9.8	9.3	6.1	2.3	1.8
2,4,6-Me_3-Gal	1.2	0.3			
2,3-Me_2-Gal	1.1	0.9	0.8	1.0	
2,6-Me_2-Gal	0.6	0.6			
Gal	1.3				
Total	15.9 (15.3)	13.3 (14.0)	9.7 (12.8)	4.2 (4.3)	3.9 (3.6)
2,3,5-Me_3-Ara	4.1	3.8	1.6	0.5	0.9
2,3-Me_2-Ara	6.9	8.5	4.4	4.6	0.6
2,5-Me_2-Ara	1.7	1.9			
2-Me-Ara	0.7	0.6			
3-Me-Ara	0.5				
Ara	0.7	0.3			
Total	14.6 (10.3)	15.1 (9.5)	6.0 (4.3)	5.1 (2.2)	1.5 (1.5)
2,3,4-Me_3-Xyl	0.6	0.7	1.1	0.4	2.0
2,3-Me_2-Xyl	2.4	3.7	4.4	4.1	0.7
2-Me-Xyl	1.2	1.0	0.8	0.3	
3-Me-Xyl	0.6	0.4	0.1		
Total	4.8 (3.9)	5.8 (4.4)	6.4 (4.6)	4.8 (5.0)	2.7 (1.0)
2,3,4,6-Me_4-Man	0.2	0.8	0.5	tr[e]	
2,3,6-Me_3-Man	4.8	5.4	5.4	5.0	0.3
2,3-Me_2-Man	1.0	2.4	1.2	1.3	0.6
2,6-Me_2-Man	0.2	0.7	0.9		
Total	6.2 (4.5)	9.3 (4.5)	8.0 (4.6)	6.3 (5.1)	0.9 (0.4)
3,4-Me_2-Rha	0.5	1.6	tr		
2-Me-Rha	0.2				
3-Me-Rha	0.9	1.0	1.3		
4-Me-Rha	0.2	0.6	tr		
Rha	0.5				
Total	2.3 (3.9)	3.2 (3.9)	1.3 (2.9)	-(1.0)	-(0.6)

[a] Some minor components, the identities of which were not confirmed, are omitted. [b] 2,3,4,6-Me_4-Glc denotes 1-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, *etc.* [c] % of total area of identified peaks. [d] Values in parentheses are based on analysis of alditol acetates. [e] Trace.

e.g., by covalent bonds[41].

Methylation analysis of AIR, CWM, and the cell-wall fractions. — There was good agreement between the results of the analyses of alditol acetates and partially methylated alditol acetates (Table IV) for AIR, CWM, and residues obtained after sequential extraction. However, especially for AIR and CWM, the contents of glucose were apparently underestimated by methylation analysis. Similar observations were made on CWM of cabbage[30] in contrast to results obtained by other workers[27,42]. The formation of 2,6-di-*O*-methyl-, 3,6-di-*O*-methyl- and unmethylated-glucitol derivatives was probably due to incomplete methylation of cellulose and could explain why the sum of the "branch points" was sometimes higher than the sum of the terminal residues (mainly in AIR). Despite the dramatic decrease in particle size obtained by cryo-milling, the accessibility of the cellulose molecules to the methylation agents may be hindered by the pectic matrix. Furthermore, the cellulose of secondary cell walls has a high degree of crystallinity[43] which could prevent a complete methylation.

(1→4)-Linked glucopyranose accounted for ~90% of the total glucose in AIR and CWM, and ~95% in the other fractions, indicating a cellulose content of ~25% in the cell wall of carrot. The small but significant amounts of 2,3-di-*O*-methylglucitol and 2,3,4-tri-*O*-methylxylitol derivatives formed from all the residues are consistent with the presence of xyloglucans, which are generally the main hemicelluloses of dicotyledonous primary cell walls[44] and are known to be linked by hydrogen bonds to cellulose.

The AIR, CWM, HR, and OHR fractions contained small proportions of (1→4)-linked mannan with a d.p. of 50–100, 25% of which carried 6-linked side-chains. (1→4)-Linked glucosyl residues may be interspersed in the mannan backbone, and terminal galactosyl groups found in significant amounts in these fractions could be attached[45] at position 6. According to Stevens and Selvendran[13], this mannose-containing polysaccharide was more readibly solubilised by 4M alkali, which is typical of cell-wall hemicelluloses.

The formation of 2,3-di-*O*-methylxylitol derivatives indicated the presence of a linear xylan backbone. The total amount of (1→4)-linked xylopyranosyl residues did not decrease during the graded extraction, which implies the enrichment of HR and OHR fractions in xylans. Nevertheless, 50% of these sugar residues were extracted during the last treatment. Hence, some of the (1→4)-linked xylan chain can be linked to cellulose by hydrogen bonds, and this explains the strong association observed between xylose-containing polysaccharides and cellulose[43]. Another usual typical substituent, 4-*O*-methylglucuronic acid, was not identified by t.l.c.

The main part of the 2,3-di-*O*-methylarabinitol derivatives could reflect an arabinan backbone which was slightly branched as indicated by the formation of 2-*O*-methyl-, 3-*O*-methyl, and unmethylated-arabinitol derivatives from AIR and CWM. Since extraction with dilute acid and dilute alkali removed most of the arabinose-containing polysaccharides, they were probably present as side chains of rhamnogalacturonan, as reported by Aspinall *et al.*[14] for carrot pectins.

The presence of a (1→4)-linked galactan was detected in the pectin-rich fractions. The 2,3-di-*O*-methyl- and 2,6-di-*O*-methyl-galactitol derivatives, previously found in carrot[13] and field-bean hulls[46], are characteristic of a type I arabinogalactan. Minor amounts of 2,3,4-tri-*O*-methyl- and 2,4,6-tri-*O*-methyl-galactitol and 2,5-di-*O*-methylarabinitol derivatives, formed from AIR and CWM, have been derived from a type II arabinogalactan either in association with pectins, as observed in dicotyledonous cell wall[47], or as part of an arabinogalactan–protein complex[32] as suggested by its solubilisation from cell walls during the proteolysis step.

As expected in pectin-rich fractions, a 3,4-di-*O*-methylrhamnitol derivative was found, corresponding to a kink in the galacturonan backbone. Associated neutral polysaccharides of variously linked arabinosyl and galactosyl residues were attached mainly at position 4 of the rhamnosyl residues, as noticed in carrot pectins[13]. Minor amounts of 2-*O*-methyl-, 4-*O*-methyl-, and 2,3,4-tri-*O*-methyl-rhamnitol derivatives were formed from AIR, but not from the other residues. Similar derivatives of rhamnose were previously found by Darvill *et al.*[48] in rhamnogalacturonan II of suspension-cultured sycamore cell walls. The fact that rhamnosyl residues were present in minute amounts in OHR and COHR suggested that galacturonic acid, detected by t.l.c. in these fractions, could have been derived from the homogalacturonan fraction, as already observed in Rosa cell walls[40].

Mature-carrot cell walls contain all the polysaccharides usually found in dicotyledonous cell walls, namely, cellulose (~25%), hemicelluloses (10–15%) as xyloglucans, xylans, and mannans, and pectic material with associated arabinose- and galactose-containing polysaccharides (45–50%) and probably complexed with proteins and polyphenolic material. The pectins of carrot appeared to be highly methyl-esterified and rich in galacturonic acids. The characterisation of the soluble fractions of graded extraction, especially of pectin fractions, is dealt with in the following paper[12].

ACKNOWLEDGMENTS

The authors thank Dr. J. F. Drilleau and Dr. A. Baron (Station de Recherches Cidricoles et Biotransformation des Fruits et Légumes, INRA, Le Rheu) for the preparation of the AIR, and Dr. A. H. Gordon (The Rowett Research Institute, Aberdeen, Scotland) for a gift of quebrachitol.

REFERENCES

1 E. Leclercq, *Process Biochem.*, 20 (1985) 75–78.
2 N. A. Kirtschev and C. G. Kratchanov, *Z. Lebensm.-Unters. Forsch.*, 170 (1980) 31–33.
3 J. A. Robertson, W. G. Brydon, K. Tadesse, P. Wenham, A. Walls, and M. A. Eastwood, *Am. J. Clin. Nutr.*, 32 (1979) 1889–1892.
4 J. A. Robertson, M. A. Eastwood, and M. M. Yeoman, *J. Sci. Food Agric.*, 30 (1979) 388–394.
5 H. Trowell, D. A. T. Southgate, T. M. S. Wolever, A. R. Leeds, M. A. Gussull, and D. J. A. Jenkins, *Lancet*, 1 (1976) 967.

6 L. DOBSZAY AND K. SARKANY, *Medicina*, (1975) 190–242.
7 J. H. CUMMINGS, D. A. T. SOUTHGATE, W. BRANCH, H. HOUSTON, D. J. A. JENKINS, AND W. P. T. JAMES, *Lancet*, 1 (1978) 5–9.
8 F. M. ROMBOUTS AND J.-F. THIBAULT, *Carbohydr. Res.*, 154 (1986) 177–187.
9 F. M. ROMBOUTS AND J.-F. THIBAULT, *Carbohydr. Res.*, 154 (1986) 189–203.
10 F. M. ROMBOUTS AND W. PILNIK, *Process Biochem.*, 19 (1978) 9–13.
11 A. G. J. VORAGEN AND W. PILNIK, *Fluess. Obst.*, 48 (1981) 261–264.
12 P. MASSIOT, X. ROUAU, AND J.-F. THIBAULT, *Carbohydr. Res.*, 172 (1988) 229–242.
13 B. J. H. STEVENS AND R. R. SELVENDRAN, *Carbohydr. Res.*, 128 (1984) 321–333.
14 G. O. ASPINALL, H. K. FANOUS, AND A. K. SEN, *ACS Symp. Ser.*, 214 (1983) 33–48.
15 H. KONNO AND Y. YAMASAKI, *Plant Physiol.*, 69 (1982) 864–868.
16 J. N. BEMILLER, *Adv. Carbohydr. Chem.*, 22 (1967) 25–108.
17 J. F. SAEMAN, W. E. MOORE, R. L. MITCHELL, AND M. A. MILLET, *Tappi*, 37 (1954) 336–343.
18 A. B. BLAKENEY, P. J. HARRIS, R. J. HENRY, AND B. A. STONE, *Carbohydr. Res.*, 113 (1983) 291–299.
19 N. BLUMENKRANTZ AND G. ASBOE-HANSEN, *Anal. Biochem.*, 54 (1983) 484–489.
20 J. F. THIBAULT, *Lebensm. Wiss. Technol.*, 12 (1979) 247–251.
21 M. T. TOLLIER AND J. P. ROBIN, *Ann. Technol. Agric.*, 28 (1979) 1–15.
22 D. FENGEL AND G. WEGENER, *Adv. Chem. Ser.*, 181 (1979) 145–158.
23 A. G. J. VORAGEN, H. A. SCHOLS, AND W. PILNIK, *Food Hydrocolloids*, 1 (1986) 65–70.
24 P. KLASON, IN DUNOD (Ed.), *La Chimie du Bois*, Paris, 1931, pp. 174–185.
25 P. COLONNA, A. BULEON, AND C. MERCIER, *J. Food Sci.*, 46 (1981) 88–93.
26 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
27 J. A. LOMAX, A. H. GORDON, AND A. CHESSON, *Carbohydr. Res.*, 122 (1983) 11–22.
28 P. J. HARRIS, R. J. HENRY, A. B. BLAKENEY, AND B. A. STONE, *Carbohydr. Res.*, 127 (1984) 59–73.
29 J. A. LOMAX AND J. CONCHIE, *J. Chromatogr.*, 236 (1982) 385–394.
30 B. J. H. STEVENS AND R. R. SELVENDRAN, *J. Sci. Food Agric.*, 31 (1980) 1257–1267.
31 J. M. BRILLOUET, X. ROUAU, B. CARRE, E. LORTA, C. HOEBLER, AND J. L. BARRY, *J. Agric. Food Chem.*, (submitted).
32 R. R. SELVENDRAN, *J. Cell Sci. Suppl.*, 2 (1985) 51–88.
33 A. T. MANKARIOS, M. A. HALL, M. C. JARVIS, D. R. THREFALL, AND K. FRIEND, *Phytochemistry*, 19 (1980) 1731–1733.
34 A. G. J. VORAGEN, J. P. J. TIMMERS, J. P. H. LINSEN, H. A. SCHOLS, AND W. PILNIK, *Z. Lebensm.-Unters. Forsch.*, 177 (1983) 251–256.
35 S. C. FRY, *Annu. Rev. Plant Physiol.*, 37 (1986) 165–186.
36 D. A. T. LAMPORT, IN R. D. PRESTON (Ed.), *Adv. Bot. Res.*, 2 (1965) 151–218.
37 R. D. HARTLEY AND P. J. HARRIS, *Biochem. Syst. Ecol.*, 9 (1981) 189–203.
38 M. M. BRYSK AND M. J. CHRISPEELS, *Biochim. Biophys. Acta*, 257 (1972) 421–432.
39 E. BECKMAN, O. LIESCHE, AND F. LEHMANN, *Biochem. Z.*, 139 (1923) 491–498.
40 G. CHAMBAT AND J.-P. JOSELEAU, *Carbohydr. Res.*, 85 (1980) c10–c12.
41 S. C. FRY, *Biochem. J.*, 204 (1982) 449–455.
42 A. CHESSON, A. H. GORDON, AND J. A. LOMAX, *Carbohydr. Res.*, 141 (1985) 137–147.
43 A. DARVILL, M. MCNEIL, P. ALBERSHEIM, AND D. P. DELMER, IN N. E. TOLBERT (Ed.), *The Biochemistry of Plants*, Vol. 1, Academic Press, New York, 1980, pp. 91–162.
44 G. O. ASPINALL, IN J. PREISS (Ed.), *The Biochemistry of Plants*, Vol. 3, Academic Press, New York, 1980, pp. 473–500.
45 M. MCNEIL, A. G. DARVILL, S. C. FRY, AND P. ALBERSHEIM, *Annu. Rev. Biochem.*, 53 (1984) 625–663.
46 P. V. SALIMATH AND R. N. THARANATHAN, *Carbohydr. Res.*, 104 (1982) 341–347.
47 K. KEEGSTRA, K. W. TALMADGE, W. D. BAUER, AND P. ALBERSHEIM, *Plant Physiol.*, 51 (1973) 188–196.
48 A. G. DARVILL, P. ALBERSHEIM, M. MCNEIL, J. M. LAU, W. S. YORK, T. T. STEVENSON, J. THOMAS, S. DOARES, D. J. COLLINS, P. CHELF, AND K. DAVIS, *J. Cell Sci. Suppl.*, 2 (1985) 203–217.