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Characterisation and enzymatic hydrolysis of cell-wall polysaccharides from different tissue zones of apple

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Cell-wall polysaccharides from different tissue zones, namely epidermis, the outer parenchyma, the parenchyma of the carpels zone, the carpels and the core line, were isolated as alcohol-insoluble solids (AIS), extracted with cyclohexane-diaminotetraacetic acid, then hot dilute hydrochloric acid, and analysed, then purified by ion-exchange chromatography. In both zones of parenchyma, the cell-wall material represented about 80% of the total cell-wall material from the whole fruit. The pectins from the outer parenchyma accounted for 70% of the total, whereas the carpels zone contained a large proportion of cellulose and hemicelluloses. However, there was no change in galacturonic acid concentration. The enzymatic solubilisation of tissues or AIS was higher in the parenchyma zones than in the others. Nevertheless, the depolymerisation of the soluble pectins from parenchyma zones with an endopolygalacturonase required the action of pectin methylesterase. The depolymerisation of pectins from the other zones, however, did not.

INTRODUCTION

In apple processing, enzymatic treatment of the crushed fruit leads to a lower degree of degradation of the peel and the core than the rest of the fruit (Reising, 1990). Some processes deal only with the edible pulp of the fruit, e.g. for the production of stewed fruit or fruit salad. These distinct parts, peel, pulp and core of the apple fruit, correspond to tissues with different botanical definitions. In the apple, the five ovaries of the flower, together with closely associated parts, are embedded in tissue which, along with the carpel tissue, becomes fleshy and edible. Figure 1 shows the separate tissue zones in diagrammatic form. Their anatomic origins are different: the epidermis and outer parenchyma zones are tissues derived from the fusion of the calyx, corolla and stamens of the flower; the inner zones correspond to tissue derived from ovaries and carpels (Hulme & Rhodes, 1971).

In freshly harvested apples, concentrations of major components are different for each of these zones. It has been found that the amounts of total sugars, soluble in the tonoplasm (Yamaki, 1984), increase from the core to the outside and also increase from the stem to the calyx end (Smock & Neubert, 1950). Moreover, the pulp is poorer in total organic acids than the outer zone (Ulrich, 1970); ascorbic acid is largely confined to the peel of the fruit (Hulme & Rhodes, 1971). The amounts of phenolic compounds in the skin are higher than those in the flesh (Burda *et al.*, 1990). No investigation has assessed whether these chemical differences extended to the cell-wall polysaccharides. Much of the research done on apple cell-wall polysaccharides has dealt with their composition, structure or changes during ripening (Knee, 1973*a,b*, 1978*a,b*; Knee *et al.*, 1975; de Vries *et al.*, 1981, 1982, 1983*a,b*, 1984; Aspinall & Fanous, 1984; Stevens & Selvendran, 1984; Renard *et al.*, 1990, 1991*a,b,c,d*, 1993). The characterisation of the cell-wall material from the different zones of the fruit may provide additional information on the possibility of finding uses for the discarded fractions.

We now report on the isolation, fractionation and enzymatic hydrolysis of the cell-wall polysaccharides from different tissue zones of apple fruit.

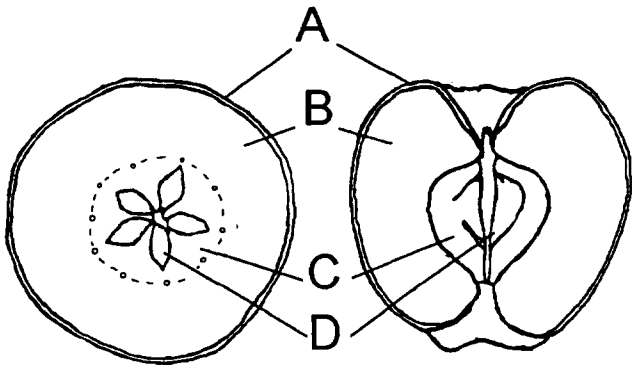

Fig. 1. Sections of mature apple: A, epidermis zone; B, outer parenchyma; C, parenchyma of the carpels zone; D, carpels and core line.

EXPERIMENTAL

Preparation of alcohol-insoluble solids (AIS)

Ripe apples of the Judeline variety were obtained from orchards in the Department of Ille et Vilaine (France) and were stored at 4°C. Out of a calibrated batch (55–60 mm), 3 kg of starch-free apples (negative iodine test) were randomly chosen and manually cut into four parts (Fig. 1): the epidermis zone (A), the outer parenchyma (B), the parenchyma of the carpels zone (C) and the carpels and the core line (D). Stems and pips were discarded. The fractions were immersed in aqueous 96% ethanol (1 : 4, w/v), ground in a Waring blender for 20 s and heated to boiling for 10 min. The mixture was filtered through a G3 sintered-glass filter and washed with 70% ethanol until the filtrate became colourless and gave a negative reaction in the phenol–sulphuric acid test (Dubois *et al.*, 1956). The AIS were dried by solvent exchange (96% ethanol, acetone) and air-dried at 35°C.

Enzymes

The endopolygalacturonase isolated from *Aspergillus niger* was provided by Megazyme (North Rocks, Australia). The endoglucanase was purified from *Trichoderma reesei* (Massiot, 1992). UM10, a polygalacturonase-rich preparation (from *A. niger*) and SP249, a preparation for liquefaction (from *A. aculeatus*), were provided by Novo Industri (Bagsvaerd, Denmark). CPE, a pectin methylesterase-rich preparation (from *A. niger*) was provided by Gist-brocades (Seclin, France) and showed no polygalacturonase activity under the conditions of our experiment.

Chemical extraction

For each AIS, the sequential extraction was carried out three times. AIS (1 g) was suspended in a solution (120 ml) of cyclohexanediaminotetraacetic acid (CDTA), 20 mM (pH 5·0), at 25°C, and stirred mechanically for 30 min. After filtration through a G3 sintered-glass filter, the residue was resuspended in a fresh CDTA solution (three extractions). The final residue was washed with distilled water. All the extracts (CDTA soluble pectin; CSP) were pooled, concentrated under vacuum (5×10^3 Pa), extensively dialysed in a 12 000 mol.wt cut-off dialysis tube (Spectrum Medical Industries, Los Angeles, USA) against deionised water at 4°C and then freeze-dried. The CDTA insoluble residue was resuspended in 50 mM hydrochloric acid (120 ml) and heated for 30 min at 85°C. The suspension was filtered through a G3 sintered-glass filter. The extraction was then repeated twice, and the final residue was washed with distilled water. All the extracts (HCl soluble pectin; HSP) were pooled and brought to pH 4·5 with M sodium hydrogen carbonate, then this fraction was treated as the CSP fraction. The lyophilised fractions (CSP and HSP) were dissolved in a 50 mM sodium acetate buffer (pH 5·0), analysed and purified by anion-exchange chromatography.

Enzymatic treatments

The apple zones A to D (10 g) were immersed in 50 ml of a mixture of 1% (w/v) ascorbic acid, 1% (w/v) citric acid and 0·1% (w/v) sodium chloride, then homogenised in a Waring blender for 5 s. After filtration through a G3 sintered-glass filter, the pulp was suspended in 50 ml of a 0·05 M sodium acetate buffer at pH 4·5. Enzyme preparations with 1000 nkat of polygalacturonase activity (UM10 or SP249) or 300 nkat of pectin methylesterase activity (CPE) were added and the suspension was stirred for 2 h at 40°C. After filtration through a G3 sintered-glass filter, the residue was washed with distilled water, then dried by solvent exchange (96% ethanol and acetone) and weighed. Each hydrolysis was duplicated. The yields of liquefaction (with SP249) or maceration (with UM10) were expressed as dry residue weight/AIS weight ratio of the corresponding tissue zone.

The AIS from the apple zones A to D (10 mg) were suspended with 5 ml of a 50 mM sodium acetate buffer (pH 4·5) in glass tubes fitted with Teflon-lined screw caps. Enzyme preparations with 15 nkat of polygalacturonase activity (UM10, SP249 or endopolygalacturonase), 5 nkat of pectin methylesterase activity or 15 nkat of endoglucanase activity were added and the suspensions were stirred at 40°C. After 0·25 to 5 h reaction, the suspensions were heated for 5 min at 95°C, centrifuged at 450 *g* for 5 min, then filtered on 3 μm Millipore filters. Filtrates were analysed for galacturonic acid. Each experiment was duplicated and controls were carried out without enzymes.

In glass tubes fitted with Teflon-lined screw caps, 2 ml of a solution of pectic fractions (containing 5 μmol/ml of

galacturonic acid in a 50 mM sodium acetate buffer, pH 4·5) were stirred with endopolygalacturonase (2·5 nkat) with or without pectin methylesterase (1 nkat). After 30 min of reaction at 40°C, the tubes were treated as above, and the filtrates were analysed by high performance size-exclusion chromatography.

Chromatography

High-performance ion-exchange chromatography (HPIEC)

An anion-exchange column TSK DEAE 5PW (75 × 7·5 mm, Tosohaas, Stuttgart, Germany) was connected to a high-performance liquid chromatography (HPLC) system (Kontron Instruments, Zurich, Switzerland). The eluent was 0·05 M sodium acetate (pH 6·0) for 10 min, then a linear gradient of 0·05–0·5 M sodium acetate (pH 6·0) for 47 min with a flow rate of 0·6 ml/min. Samples of 200 µl containing 3 µmol of galacturonic acid were injected. The eluate was continuously monitored for galacturonic acid at 520 nm, using the automated *m*-hydroxydiphenyl method (Thibault, 1979) and for total sugars, at 430 nm, using the automated orcinol method (Tollier & Robin, 1979).

High-performance size-exclusion chromatography (HPSEC)

The molecular weight distribution of polysaccharides was determined using a HPLC system involving a laboratory data control programmable pump equipped with four Bio-Gel TSK columns (300 mm × 7·8 mm each) in series (50, 40, 30 and 25 PWXL; Tosohaas, Stuttgart, Germany), in combination with a TSK XL guard column (40 mm × 6 mm) at 40°C. The eluent was 0·4 M acetic acid/sodium acetate (pH 3·6) with a flow rate of 0·7 ml/min. Samples of 100 µl containing 3 µmol of galacturonic acid were injected. The eluate was continuously monitored for galacturonic acid at 520 nm using the automated *m*-hydroxydiphenyl method (Thibault, 1979).

Preparative anion-exchange chromatography

Solutions (50 ml) of CSP or HSP (1 mg/ml) each were loaded on to a column (1·2 cm × 12 cm) of DEAE–Trisacryl (Industries Biologiques Françaises, Villeneuve-la-Garonne, France) equilibrated and eluted with a 50 mM sodium acetate buffer (pH 5·0) at 25 ml/h. The fractions (10 ml) were assayed for galacturonic acid and neutral sugars. Pectic material bound on the gel was then eluted with a sodium acetate buffer (pH 5·0) with an appropriate ionic strength calculated from HPIEC analysis. The purified pectin was extensively dialysed against deionised water and freeze-dried.

Analytical methods

The AIS were ground (3 min) in a MM2 mixer mill (Retsch, Haan, Germany). The individual neutral sugars were analysed by gas chromatography (capillary column of 30 m × 0·25 mm i.d. coated with DB225, 0·15 µm film thickness; J & W Scientific, Folsom, USA) at 215°C, using hydrogen as carrier gas, after sulphuric acid hydrolysis (Seaman *et al.*, 1954) and derivatisation to alditol acetates (Hoebler *et al.*, 1989). *Myo*-inositol was used as internal standard. The galacturonide content was estimated colorimetrically at 520 nm with *m*-hydroxydiphenyl (Blumenkrantz & Asboe-Hansen, 1973) after sulphuric acid hydrolysis (Ahmed & Labavitch, 1977). In the soluble fractions, the galacturonic acid and neutral sugars (expressed as arabinose) were determined by the automated *m*-hydroxydiphenyl (Thibault, 1979) and orcinol (Tollier & Robin, 1979) methods, respectively, the latter being corrected for interfering galacturonic acid.

The degree of methoxylation was estimated by determining the methanol content released after alkaline hydrolysis of pectins. The methanol was measured by gas chromatography (Carbowax 20M capillary column of 50 m × 0·32 mm i.d., 0·2 µm film thickness; AML Chromato, Peyrelevade, France) at 70°C using hydrogen as carrier gas, after extraction by steam distillation from a pectic solution (2 mg/ml) in 0·2 M NaOH, for 1 h, at room temperature. Propanol was used as internal standard.

Nitrogen was determined according to Moll *et al.* (1975) and protein content was estimated as N × 6·25. The lipids were estimated by weighing the dry matter extracted from the AIS with boiling chloroform/methanol (2 : 1) in a Soxhlet-type apparatus (Matzke & Riederer, 1991) for 4 h (twice).

RESULTS AND DISCUSSION

Distribution and composition of cell-wall material

Table 1 summarises the distribution of weight and AIS in the different zones obtained after dissection of the fruit. The outer parenchyma (B) is the major tissue zone of the fruit; it corresponded to more than 80% of the dry matter. The edible zones (B and C) represented 90%, whereas the skin and carpels zones corresponded to 9% of the fruit. De Vries *et al.* (1981) found a pulp weight of 70–72% of fresh matter, which is less than the weight from zone B, but their scheme of dissection was not indicated. The cell-wall material, expressed as the AIS, represented, in the whole fruit, 12·6% of dry matter, which corresponds to the results of previous studies on apple cell-wall (Knee, 1973*a*; de Vries *et al.*, 1981; Voragen *et al.*, 1983; Renard *et al.*, 1990). The AIS from zones B and C represented 79% of the total AIS, whereas those from the epidermis and carpels zones represented 11 and 9·5%, respectively.

The four tissue zones of the apple differed from one another in their proportion of cell walls: zones B and C

Table 1. Distribution of weight and alcohol-insoluble solid (AIS) in the different zones of Judeline apple

Zone	Weight		AIS
	g/100 g fresh matter	g/100 g dry matter	g/100 g dry matter
Epidermis zone (A)	4·0 (1·3)[a]	5·1 (1·7)	1·4 (0·2)
Outer parenchyma (B)	84·0 (2·1)	81·9 (2·0)	8·8 (0·5)
Parenchyma of the carpels region (C)	8·1 (0·6)	7·5 (0·6)	1·2 (0·1)
Carpels and core line (D)	3·4 (0·7)	3·9 (0·8)	1·2 (0·1)
Seeds	0·4 (0·1)	1·4 (0·4)	nd
Stem	0·1 (<0·1)	0·2 (<0·1)	nd
Total	100	100	12·60

[a]Standard deviations in parentheses, determined on three batches.
nd: not determined.

Table 2. Composition of AIS in different tissue zones of apple (see Fig. 1)

Tissue zone	Yield[a]	Sugar composition[b]								Proteins[b]	Lipids[b]
		Rha	Ara	Xyl	Man	Gal	Glc	Gal. A	Total		
A	274	15	47	34	19	54	154	265	588	57	290
B	107	13	83	74	24	98	296	287	875	27	55
C	160	11	48	88	23	48	301	283	802	44	62
D	308	13	32	135	28	27	344	253	832	55	38

[a]mg/g of dry matter of tissue.
[b]mg/g of AIS.

contained less cell-wall material than zones A and D (Table 2). The high level of AIS from the epidermis zone was the consequence of its content in lipids (29%), corresponding to the cutin substances in the skin. The carbohydrate contents were 9·4, 12·6, 16·1 and 25·6% of dry matter in the zones B, C, A and D, respectively, suggesting that the concentration of cell-wall polysaccharides was the greatest in the carpels zone and the least in the zone of the outer parenchyma. The monosaccharides composition of each tissue zone indicated that the carbohydrate polymers were mostly pectic polysaccharides and glucans. The four AIS contained high and equivalent amounts of galacturonic acid (25·3–28·7%), indicating that the distribution of pectic substances was similar to the distribution of the AIS in the fruit: 71·3% of the galacturonic acid was present in zone B, 10·5% in zone A, 9·6% in zone C and 8·6% in zone D. Moreover, arabinose and galactose, the main non-cellulosic sugars were concentrated in the zone of the outer parenchyma, 82 and 84%, respectively. Hemicellulosic polymers were also present, especially in zone D, with a high level of xylose (13·5%). The glucose content indicated a gradient in the glucan concentration: the AIS from zone A contained only 15% of glucose, whereas those from zones B and C contained 30%; in zone D, one-third of AIS was glucose, probably from cellulose and xyloglucans (Renard *et al.*, 1991*c*). The zone of carpels contained a higher content of cell-wall polysaccharides, especially cellulose, than the other zones. The AIS contained a relatively small proportion of proteins (2·7–5·7%), especially in the zone of the outer parenchyma, compared with the results (7·3–7·6%) obtained in previous studies (Voragen *et al.*, 1983; Renard *et al.*, 1990).

Isolation and characterisation of pectic polysaccharides

The soluble fractions CSP and HSP were analysed by an ion-exchange chromatography (Fig. 2). For the CSP fractions, the elution patterns of galacturonic acid containing polysaccharides were similar, with a main population eluted with the sodium acetate gradient (0·13–0·15 M). Nevertheless, the CSP fraction from zone B showed a peculiar elution pattern of the total sugars: a peak of neutral sugars was eluted with 0·05 M sodium acetate, whereas no peak was eluted with 0·5 M sodium acetate; moreover, the main peak contained less galacturonic acid than the corresponding peaks from the other zones. The HSP fractions exhibited identical peaks of neutral sugars, but the main peak was eluted with the sodium acetate, 0·19, 0·22, 0·24 and 0·3 M for the D, C, A and B fractions, respectively. The elution pattern of the CSP fraction from zone B and the HSP fractions suggested the presence of neutral polysaccharides associated with rhamnogalacturonans, as arabinans, galactans or arabinogalactans (de Vries *et al.*, 1983*a*). The main populations (bars on Fig. 2) of the CSP and HSP fractions were isolated by preparative chromatography.

The recovery of the purified pectins from the four zones (Table 3) indicated that the CSP amounts were larger than the HSP ones. In this experiment, CDTA, a chelating agent, often used for the extraction of pectins (Selvendran & O'Neil, 1987), solubilised the major

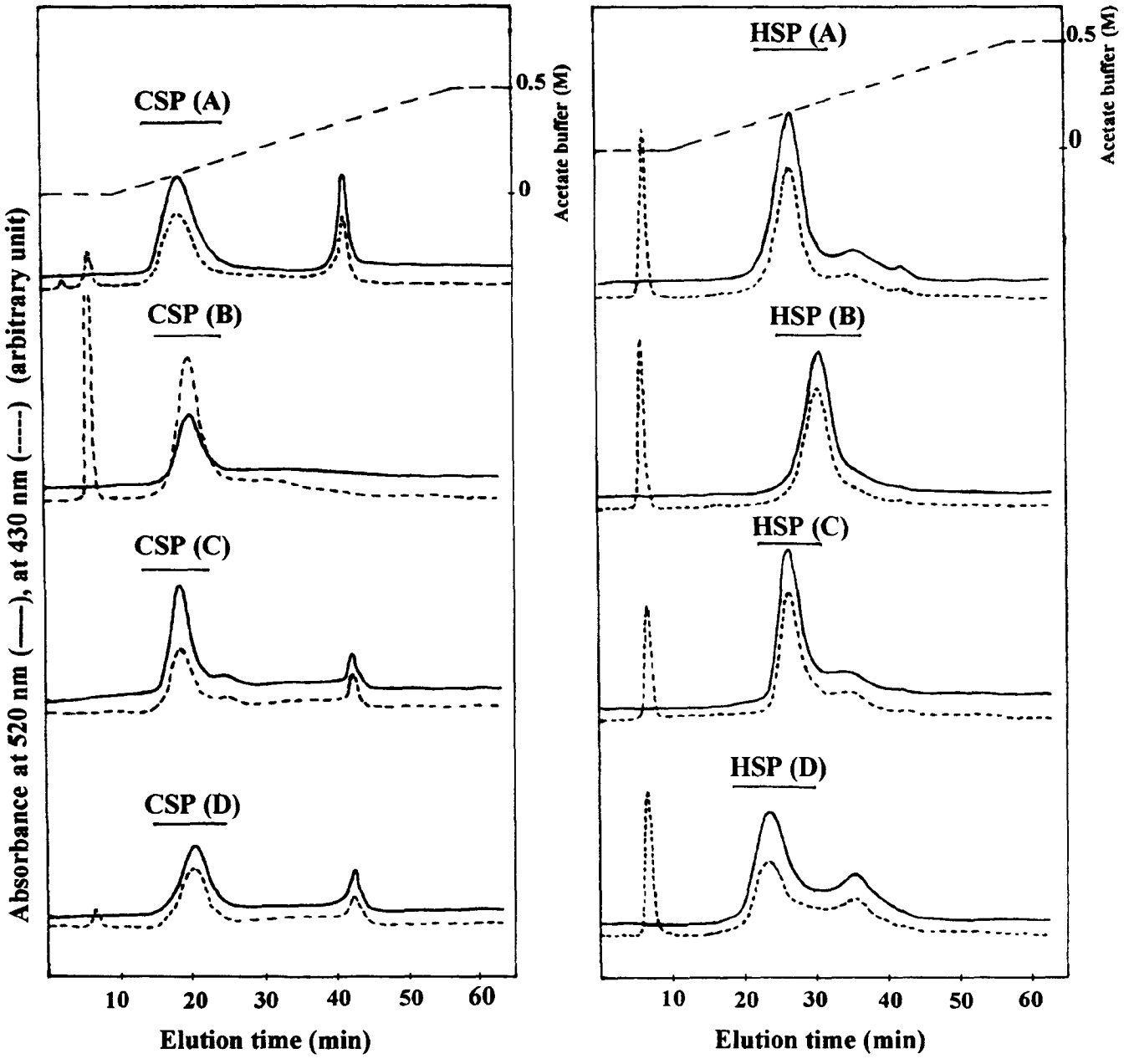

Fig. 2. HPIEC of cyclohexanediaminotetraacetic acid-soluble pectin (CSP) and HCl-soluble pectin (HSP) from different tissue zones of apple (see Fig. 1). (——) absorbance at 520 nm (galacturonic acid); (------) absorbance at 430 nm (total sugars).

proportion of the pectic substances. The hot dilute acid did not extract a large part of pectins, in agreement with a study on carrots (Massiot *et al.*, 1988), but in contrast to other studies on beets (Rombouts & Thibault, 1986) or grapes (Saulnier & Thibault, 1987). Nevertheless, the CSP quantities were similar to those obtained by Stevens & Selvendran (1984) with water and oxalate extraction, and the HSP quantities were comparable with those of Renard *et al.* (1990). The CSP fractions from zones A, C and D contained a large amount of galacturonic acid (80–86%) compared with the zone of the outer parenchyma (62·8%). However, galactose and especially arabinose contents in zone B were larger than in the other zones. This result denoted that the side chains of this pectic fraction could be made of arabinans, galactans and arabinogalactans. The compositions of the HSP fractions were similar, containing mainly galacturonic acid (64·3–71·8%), arabinose (8·4–10·7%) and galactose (7·3–13·2%). In contrast to the CSP fractions, the HSP fractions showed a low galacturonic acid/rhamnose molar ratio (10–15), suggesting the presence of branched rhamnogalacturonan with rhamnosyl residues as branching points of the side chains, corresponding to the hairy region of pectin (de Vries *et al.*, 1982). The degrees of methoxylation of the CSP fractions corresponded to the literature (de Vries

Table 3. Monosaccharide composition of solubilised cell-wall polysaccharides purified by ion-exchange chromatography in different tissue zones of apple (see Fig. 1)

Fraction	Tissue zone	Yield (g% AIS)	Sugar composition (mol%)								Degree of methoxylation
			Rha	Ara	Xyl	Man	Gal	Glc	Gal. A	Total (mg/g)	
CSP[a]	A	19·2	1·5	9·6	0·8	0·5	6·3	1·3	80·0	582	70
	B	16·8	1·8	22·7	1·0	0·2	11·1	0·4	62·8	722	71
	C	16·9	1·9	5·9	0·6	tr	5·4	0·2	86·0	516	62
	D	13·0	2·4	7·4	1·3	1·0	5·8	1·3	80·8	428	65
HSP[b]	A	8·6	4·9	9·7	2·9	0·7	8·2	1·8	71·8	650	52
	B	12·5	5·7	9·0	4·5	0·4	13·2	2·9	64·3	795	41
	C	11·1	6·2	10·7	4·6	0·4	8·4	2·3	67·4	759	55
	D	7·9	7·1	8·4	4·2	0·8	7·3	1·9	70·3	692	49

[a]CSP: Cyclohexanediaminotetraacetic acid soluble pectin.
[b]HSP: HCl soluble pectin.

et al., 1981; Aspinall & Fanous, 1984; Voragen *et al.*, 1983; Stevens & Selvendran, 1984) and were slightly higher in zones A and B (70 and 71%, respectively). The degrees of methoxylation of HSP fractions were low, especially in the zone of the outer parenchyma, suggesting that these fractions were pectins from primary cell-wall material (Selvendran, 1985) in which free carboxyl groups were implicated in linkages (ionic, ester) with other polymers, e.g. hemicelluloses or proteins.

The composition of the pectic fractions confirmed the presence of highly esterified slightly branched rhamnogalacturonan in the CSP fractions, corresponding to the pectins of the middle lamella and the presence of highly branched rhamnogalacturonan in the HSP fraction corresponding to the pectins of the primary cell-wall (Selvendran, 1985). However, the CSP pectic fraction from zone B attracted attention by its high content of neutral sugar chains, as shown by the HPIEC pattern.

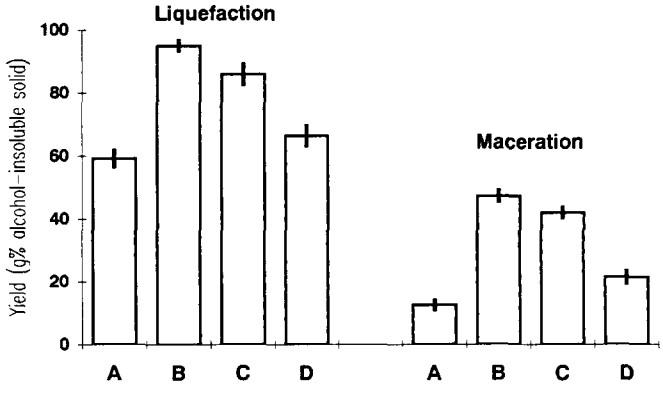

Fig. 3. Solubilisation of cell-wall material (as alcohol-insoluble solid) when apple tissues were treated with liquefying (SP249) or macerating (UM10) preparations (see experimental): A, epidermis zone; B, outer parenchyma tissue; C, parenchyma of the carpels zone; D, carpels and core line; bars represent two standard deviations.

Enzyme hydrolysis of cell-wall polysaccharides

When the apple tissues were treated with enzyme preparation for liquefaction (Fig. 3), the cell-wall materials were solubilised with different yields: 95, 86, 66 and 59% for zones B, C, D and A, respectively. The low proportion of carbohydrates (59%) and the high lipid content (29%) in the AIS from zone A partly explained this yield of enzyme hydrolysis. The sequence was the same with the maceration treatment, but the yields were lower: 47, 42, 22 and 13%, respectively. The pectins of zones B and C were partly solubilised by the action of polygalacturonases only.

The solubilisation of pectins, expressed as galacturonic acid, from the AIS with a mixture of enzymes is presented in Fig. 4. With polygalacturonases (a), the kinetics were close to each other, with limits around 40–50% after 2 h of reaction. A combined action of polygalacturonases and pectin methylesterases (b) increased these values, especially for the AIS from zone B. The sequence of the zones was D, A, C and B, in rising order of solubilisation. A mixture of cell-wall polysaccharide-degrading enzymes (c) increased the limits of solubilisation, but the order of the zones was the same as above.

With purified enzymes, less cell-wall material was solubilized (Fig. 5). The endopolygalacturonase alone (a) released only 15 to 20% of galacturonic acid containing pectic material. The addition of pectin methylesterase to the endopolygalacturonase (b) substantially increased the solubilisation of galacturonic acid containing pectic material from the AIS (B) and (C), giving more than 20% after 5 h of hydrolysis. The action of endoglucanase alone (c) released pectins (5–15%) as previously observed (Ella Missang *et al.*, 1993), but the combined action of the pectinases (d) did not modify the kinetics of solubilisation with regard to the series (b). These data suggested that the action of this pure cellulase did not facilitate the release of pectins, even in the cellulose-rich carpels zone (D). Nevertheless, a liquefying enzyme preparation containing a mixture of cellulases and pectic enzymes

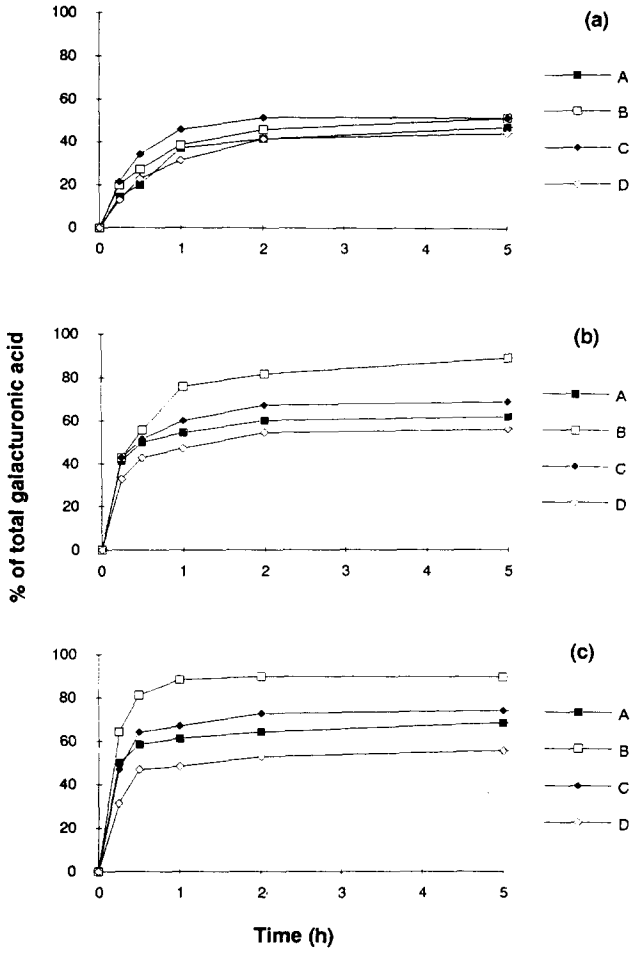

Fig. 4. Content of galacturonic acid in the soluble fraction after hydrolysis of AIS from different tissue zones (see Fig. 1) of apple with (a) polygalacturonases, (b) polygalacturonases and pectin methylesterase, and (c) pectinases and cellulases (SP249). Assay values were corrected by the control values.

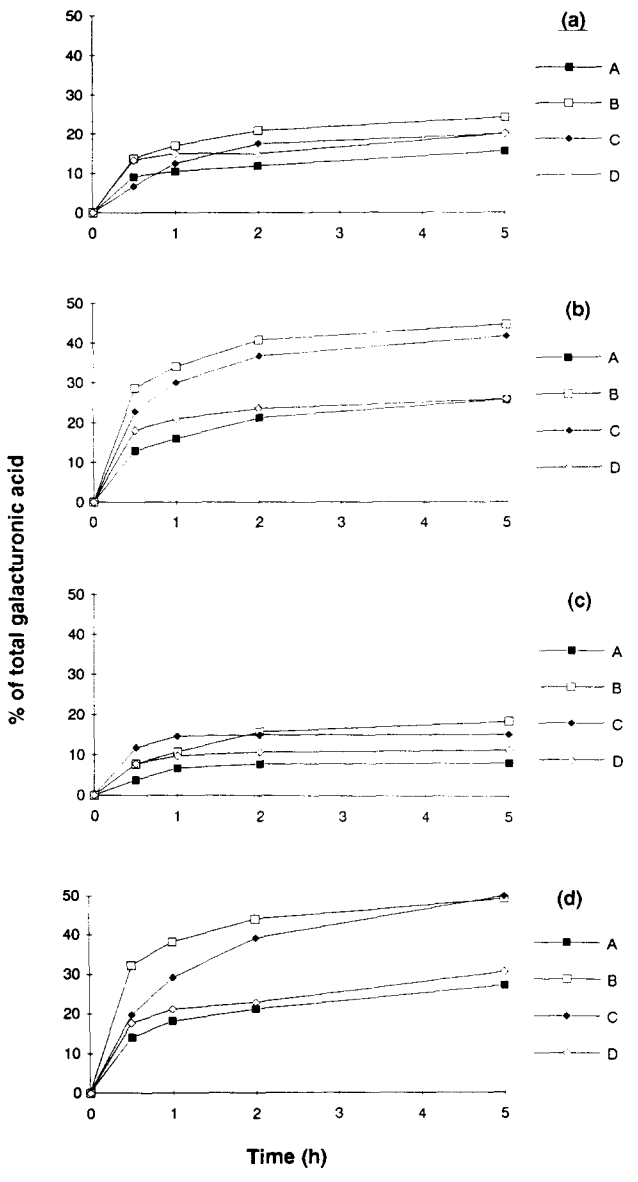

Fig. 5. Content of galacturonic acid in the soluble fraction after hydrolysis of AIS from different tissue zones (see Fig. 1) of apple with (a) endopolygalacturonase (endoPG), (b) endo PG and pectin methylesterase (PME), (c) endoglucanase (EG), and (d) endoPG and PME and EG. Assay values were corrected by the control values.

enhanced the level of pectin solubilisation of the other zones. The solubilisation of pectins in zone D was limited to 50–55%, in spite of the action of different types of cellulases which acted in synergy with pectinases (Renard *et al.*, 1991*a*). The linkages of pectic polysaccharides with the hemicelluloses, e.g. xyloglucans in this cell-wall material could explain this low solubilisation.

Figure 6 shows the evolution of the molecular weight distribution of the CSP and HSP fractions after hydrolysis by the endopolygalacturonase with or without pectin methylesterase activity. The CSP pectic fractions were polydisperse, and showed one main galacturonic acid peak with similar apparent molecular weights ($0{\cdot}25 < K_{av} < 0{\cdot}3$). After 30 min of reaction with the endopolygalacturonase, the CSP fractions from zones A and D were partially hydrolysed, whereas the fractions from zones B and C were not: peaks appeared at K_{av}, corresponding to oligogalacturonic acids, but the K_{av} of the main peak were not modified, indicating that the apparent molecular weights were unaltered, and, therefore, the enzymes acted at the ends of the polysaccharide. On the other hand, in the four patterns, the combined action of endopolygalacturonase and pectin methylesterase led to the disappearance of the polymers along with an increase in the population of oligomers. The HSP fractions from zones A and B exhibited lower molecular weights and were more polydisperse than the CSP fractions, but the fractions HSP from zones C and D were eluted with K_{av} similar to those of the corresponding CSP fractions. In contrast to the CSP fractions, the four HSP fractions were degraded with the

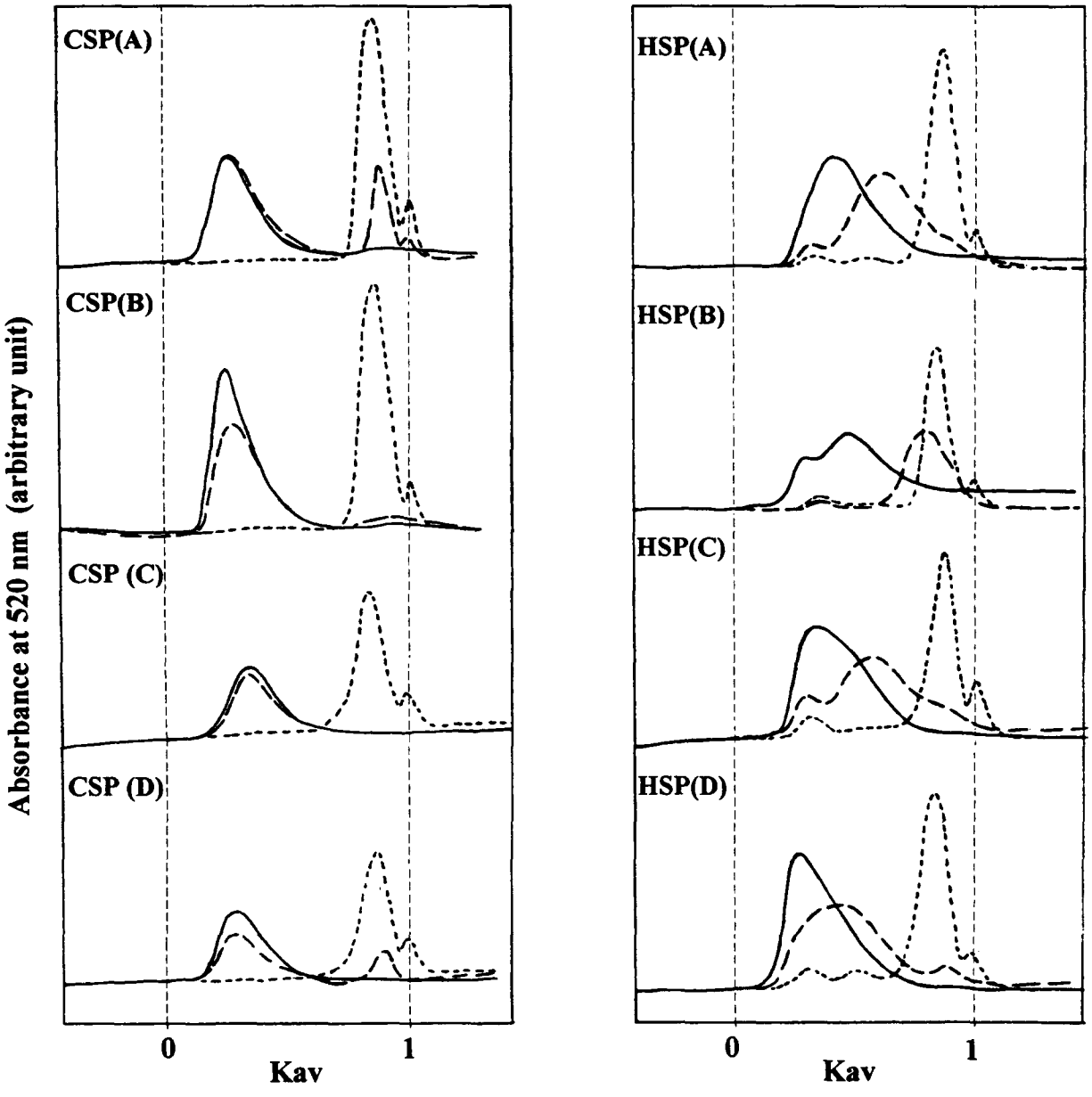

Fig. 6. HPSEC of cyclohexanediaminotetraacetic acid-soluble pectin (CSP) and HCl-soluble pectin (HSP) from different tissue zones (see Fig. 1) of apple (——) or after hydrolysis with endopolygalacturonase (— — —) or with endopolygalacturonase and pectin methylesterase (------).

endopolygalacturonase, especially the CSP fraction from the zone of the outer parenchyma (B). Endopolygalacturonase and pectin methylesterase activities led to the production of oligogalacturonides in the four cases.

The enzymatic solubilisations of pectic substances from the AIS were high in zones B and C. In these fractions, the addition of pectin methylesterase substantially increased the action of polygalacturonases. The fact that the CSP pectic fractions from zones B and C were not modified by the action of the endopolygalacturonase alone suggested that these fractions were structurally different. In particular, the high degree of methoxylation of both fractions and the significant proportion of neutral sugars in the CSP fraction (zone B) could limit the action of the endopolygalacturonase. If the CSP fractions corresponded to the pectins of the middle lamella (Selvendran, 1985), finding the most maceration of the tissue by the polygalacturonases in zone B was paradoxical because the action of endopolygalacturonase on the CSP pectin (B) was the most limited. Nevertheless, the cell-wall material as AIS from zones B and C was the most highly solubilised with the pectinases. These results indicated that the main factor in the degradation of the pectic cement was the solubilisation of the pectins and not their depolymerisation. This phenomenon seemed to be similar to the softening of tissues during the maturation of fruit, in

which polygalacturonase activity alone was not directly correlated with pectin solubilisation (Brady, 1992).

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