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Enzymic Degradation of Isolated Pectic Substances and Cell Wall from Pulp of Grape Berries

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SUMMARY

Pectic substances sequentially extracted with water (WSP), oxalate (OXP), acid (HP) and alkali (OHP) from an alcohol-insoluble residue (AIR) from pulp of grape berries, and further purified, were degraded with two pectic enzymes, namely endopolygalacturonase (endopg) and endo-pectinylase (endopel). Degradation products were fractionated by gel permeation chromatography (Bio-gel P2, Sephadex G100, Sepharose CL 6B) or by ion-exchange chromatography (DEAE-Sephacel). The results suggest that pectic substances from grape berries are constituted of 'smooth' homogalacturonan areas, interspersed with very densely branched 'hairy' regions where neutral sugar side-chains are located. WSP on one hand and HP, OXP, OHP on the other hand were differentiated by their side-chain length. Treatment of AIR previously extracted with water and oxalate by endopel are reported and degradation products are compared with HP 'hairy' fragments.

INTRODUCTION

Pectic substances are composed of a rhamnogalacturonan backbone in which rhamnosyl residues are interspersed in chains of $\alpha(1\text{-}4)$-linked galacturonic acids partly esterified by methanol (Aspinall, 1980; Dey & Brinson, 1984). Neutral sugars, mainly arabinose and galactose, are found as side chains of varying length (Aspinall, 1980). Rhamnosyl residues are the main branching point of side chains (Albersheim, 1978),

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but some sugars, such as xylose, may be bound to galacturonic acid residues. These structural features have been established from permethylation analysis of pectin fragments released enzymatically from cell walls and of pectins degraded by mild acid hydrolysis. Other studies, using enzymes as analytical tools on isolated pectins, have led some workers (De Vries *et al.*, 1982; Thibault, 1983*a*, Rouau & Thibault, 1984; Rombouts & Thibault, 1986) to distinguish 'hairy' and 'smooth' regions in the pectin molecule, because side-chains were found to be concentrated on small areas of the rhamnogalacturonan backbone. In this paper, pectic substances sequentially extracted from pulp of grape berries by water (WSP), oxalate (OXP), hot dilute HCl (HP), and cold dilute NaOH (OHP) and further purified (Saulnier & Thibault, 1987) were submitted to endopg and endopel action, which effected hydrolysis of the $\alpha(1-4)$ galacturonic acid bonds and a β-elimination reaction, respectively. These isolated pectins contained galacturonic acid (63–54% dry weight) and some neutral sugars (25–17% dry weight) composed mainly of arabinose and galactose but also of rhamnose, xylose and minor amounts of glucose and mannose, and they were characterized by a high degree of esterification with the exception of OHP. Results were compared to those obtained by the action of endopel on grape berry cell walls.

MATERIALS AND METHODS

Pectic substances

An alcohol-insoluble residue (AIR) was prepared from the pulp of grape berries and the pectic substances were sequentially extracted with water (WSP), oxalate (OXP), hot HCl (HP) and cold NaOH (OHP), and further purified as previously described (Saulnier & Thibault, 1987). Samples were dissolved in distilled water prior to enzymic degradation or de-esterified as follows. Pectins (2 mg ml^{-1}) were dissolved in 0·05 M NaOH under magnetic stirring (2 h at 2°C), then pH was adjusted to 5–6 with 0·1 M HCl and solutions were extensively dialyzed against distilled water and concentrated under vacuum (at < 40°C) to obtain appropriate concentration.

Enzymes and degradation conditions

Endopolygalacturonase (endopg, E.C. 3.2.1.15) (Thibault & Mercier, 1977, 1978) and endopectinylase (endopel, type 2, E.C. 4.2.2.10) (Van Houdenhoven, 1975) were purified from *Aspergillus niger* preparations.

Endopg hydrolysis (2 nkat ml^{-1}) was performed, on pectins previously de-esterified, in NaOAc buffer (0·05 M, pH 4.2) at 30°C for 24 h (Thibault, 1983a). The extent of hydrolysis was determined by measuring reducing sugars. Degradation of pectins by endopel (8·3 nkat ml^{-1}) was performed in citrate buffer (0·01 M, pH 5.2) at 30°C for 24 h (Rombouts & Thibault, 1986) and the reaction was monitored by measuring absorbance at 235 nm of aliquots diluted tenfold in distilled water and the percentage degradation was calculated using a molar extinction coefficient of 5500 (Edstrom & Phaff, 1964).

The degradation of the cell wall of grape berries by endopel was performed as follows: AIR (4 g) was first sequentially extracted, as described by Saulnier & Thibault (1987), with water, oxalate and citrate buffer, in order to remove soluble pectic substances (WSP, OXP and citrate-soluble), then the residue was suspended in 200 ml of citrate buffer (0·01 M, pH 5.2) under magnetic stirring at 30°C and endopel was added (4.2 nkat). The degradation was monitored by measuring galacturonic acid content and absorbance at 235 nm of aliquots periodically removed. After 48 h the mixture was centrifuged (30 min, 3000 g), the supernatant was concentrated under vacuum (at <40°C) filtered through a 3·0-μm Millipore sieve and frozen for subsequent analysis.

Analytical methods

Galacturonic acid and neutral sugars were determined by automated *m*-phenylphenol (Blumenkrantz & Asboe-Hansen, 1973; Thibault, 1979) and orcinol (Tollier & Robin, 1979) methods, respectively (responses were corrected for mutual interferences). Reducing sugars were determined by the method of Nelson (1944) using galacturonic acid as a standard. Neutral sugars were released by TFA hydrolysis (2 M, 1·25 h at 120°C) of pectin fragments, and analyzed by gas-liquid chromatography (GLC) as their alditol acetate derivatives (Selvendran *et al.*, 1979).

Gel permeation chromatography

Chromatography using Bio-gel P2 was performed as described previously (Thibault, 1980), the product of an enzymic digestion of a pectin (2 mg ml^{-1}) being injected (0·5 ml) onto the column (203 cm × 2 cm) and eluted by an NaOAc buffer (0·1 M, pH 3·6) at 50 ml h^{-1}. The eluate was continuously analyzed for galacturonic acid and neutral sugar content by the *m*-phenylphenol and orcinol methods, respectively.

The proceeds of enzymic digestion of pectins (1–2 mg ml^{-1}) were also injected (1–2 ml) onto columns containing Sephadex G100 (V_0: 30 ml, V_t: 84 ml), Sepharose CL 6B (V_0: 43 ml, V_t: 108 ml) and Sepharose CL

2B (V_0: 74 ml, V_t: 190 ml) and were eluted by an NaOAc buffer (0·1 M, pH 4·0) at 15–20 ml h^{-1}. Fractions (3–4 ml) were collected and analyzed for their galacturonic acid and neutral sugar content by the *m*-phenylphenol and orcinol methods, respectively.

The neutral sugar composition of fractions isolated by gel permeation chromatography was determined by aggregating aliquots of concerned fractions. Such aggregates were extensively dialyzed against distilled water, evaporated to dryness and analyzed by GLC.

Ion-exchange chromatography

Fractions derived from endopg WSP and HP digestions and isolated at the void volume of Sephadex G100 were chromatographed on DEAE-Sephacel. The fractions eluted below K_{av} 0·2 on Sephadex G100 were pooled, extensively dialyzed against distilled water and evaporated to dryness below 40°C, an accurate amount of water was then added and the solution (1 ml) was loaded onto the DEAE column (7·5 cm × 1·6 cm) and washed with 50 ml of NaOAc buffer (0·05 M, pH 4·8). The bound material was eluted using 90 ml of a linear NaOAc buffer gradient (0·05 M–0·8 M, pH 4·8). Fractions (3 ml) were collected and analyzed for their galacturonic acid and neutral sugar content as described earlier. The neutral sugar compositions of the isolated fractions were determined as for gel permeation products.

RESULTS AND DISCUSSION

Enzymic degradation of isolated pectins

Enzymic action
Prior to degradation by endopg, WSP, OXP and HP were de-esterified in order to maximize hydrolysis, as endopg is unable to cleave linkages between methyl galacturonate residues (Rexova-Benkova & Markovic, 1976). The de-esterification process was assumed to have no effect on sugar composition (Rouau & Thibault, 1984; Rombouts & Thibault, 1986). However, slight losses of arabinose and galactose were observed for WSP (81% and 88% recovered, respectively). Although the temperature was kept low (2°C) a decrease in M_w was evident as attested by higher K_{av} values on Sepharose 2B CL for de-esterified WSP and HP compared to native pectins and probably arose from a β-elimination reaction during the de-esterification process (Albersheim *et al.*, 1960). The extent of degradation was high (45%, 40%, 39% and 38% for WSP,

OXP, HP and OHP, respectively), as compared to sugar-beet pectins (Rombouts & Thibault, 1986) (range 29·3% to 35·7%) but lower than with a commercial polygalacturonic acid (Thibault, 1983b) (57·5%). The action of endopg is hampered by rhamnosyl residues and neutral sugar side chains, and the high degree of hydrolysis of the four samples shows that substantial homogalacturonan regions are present. WSP, which had the lowest rhamnose content and the highest neutral sugar content (Saulnier & Thibault, 1987) was more extensively degraded than other samples.

Endopel, which catalyzed the β-elimination reaction between methyl-esterified galacturonic residues, was not assayed with OHP since it was de-esterified during the extraction step. WSP, OXP and HP were highly esterified (Saulnier & Thibault, 1987) (77% to 68%), and the percentages of galacturonosyl bonds broken were similar (16%, 14% and 18% for WSP, OXP and HP, respectively) and higher than for sugar-beet pectins (Rombouts & Thibault, 1986). Endopel is blocked by steric factors and required a certain sequence of methyl-esterified galacturonosyl residues (Rexova-Benkova & Markovic, 1976; Pilnik & Rombouts, 1981), which explained the lower extent of degradation than with endopg.

Characterization of degradation products

Products of enzymic degradation have been submitted to gel permeation chromatography on various supports.

The proceeds of the four digestions of pectin by endopg had similar elution patterns on Biogel P2 (Fig. 1); four peaks were separated. The first one, at the void volume of the column, contained some galacturonic acid residues and all the neutral sugars, which corresponded to side chains and rhamnosyl residues. The others were oligogalacturonides identified as tri-, di- and galacturonic acids. The action of endopg was almost complete and trigalacturonic acid was found to be the most abundant final product (Table 1) as previously reported (Thibault, 1983b). These neutral sugars excluded from the column, were associated with only 7% of the galacturonic acids for WSP and about 22% for the other fractions (Table 1). The results obtained were in good agreement with the extents of hydrolysis determined above, and confirmed that side chains were not distributed randomly, but on small areas of the rhamno-galacturonan backbone.

There was a general trend towards separation of the products into two large peaks on Sephadex G100 for digestion by both endopg and endopel (Fig. 2). Large fragments of pectin molecule were isolated at the excluded volume ($< K_{av}$ 0·2), these populations had equivalent amounts
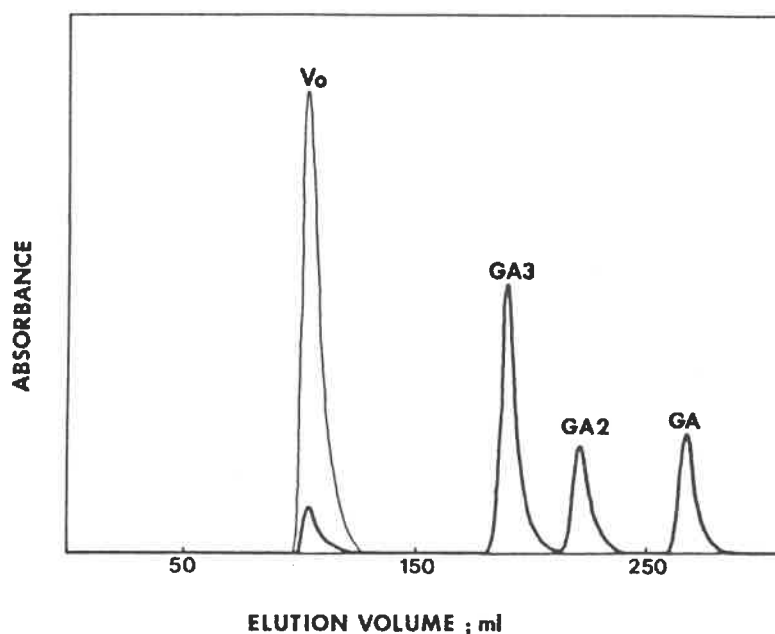
Fig. 1. Elution profiles after chromatography on Bio-gel P2 of WSP, OXP, HP and OHP endopg digestion. V_0, Void volume; GA3, GA2 and GA peaks corresponding to tri-, di- and galacturonic acid, respectively. (————), Orcinol response for neural sugars (corrected from galacturonic acid response); (————), m-phenylphenol response for galacturonic acid.

TABLE 1

Percentage of Galacturonic Acid Detected in Peaks Fractionated on Bio-gel P2 from WSP, OXP, HP and OHP Endopg Digestion[a]

	V_0	GA3	GA2	GA
WSP	7·0	52·8	20·2	19·8
OXP	22·3	42·6	16·9	18·2
HP	23·7	43·2	17·0	16·2
OHP	21·6	47·0	17·0	14·0

[a] Results are expressed as % of total galacturonic acid found in peaks V_0, GA3, GA2 and GA.

of galacturonic acid and neutral sugars for OXP, HP and OHP, whereas they were richer in neutral sugars than in galacturonic acid for WSP. The sugar content and composition (Table 2) were similar for digestion by endopg and endopel, and these peaks contained 80% of the total neutral sugars for all the samples and from 3·7% (WSP) to 15%, (HP, OHP) of the total galacturonic acid, with an intermediate value of 11% for OXP. Slight differences in M_w distribution were found for $K_{av} > 0.2$, between
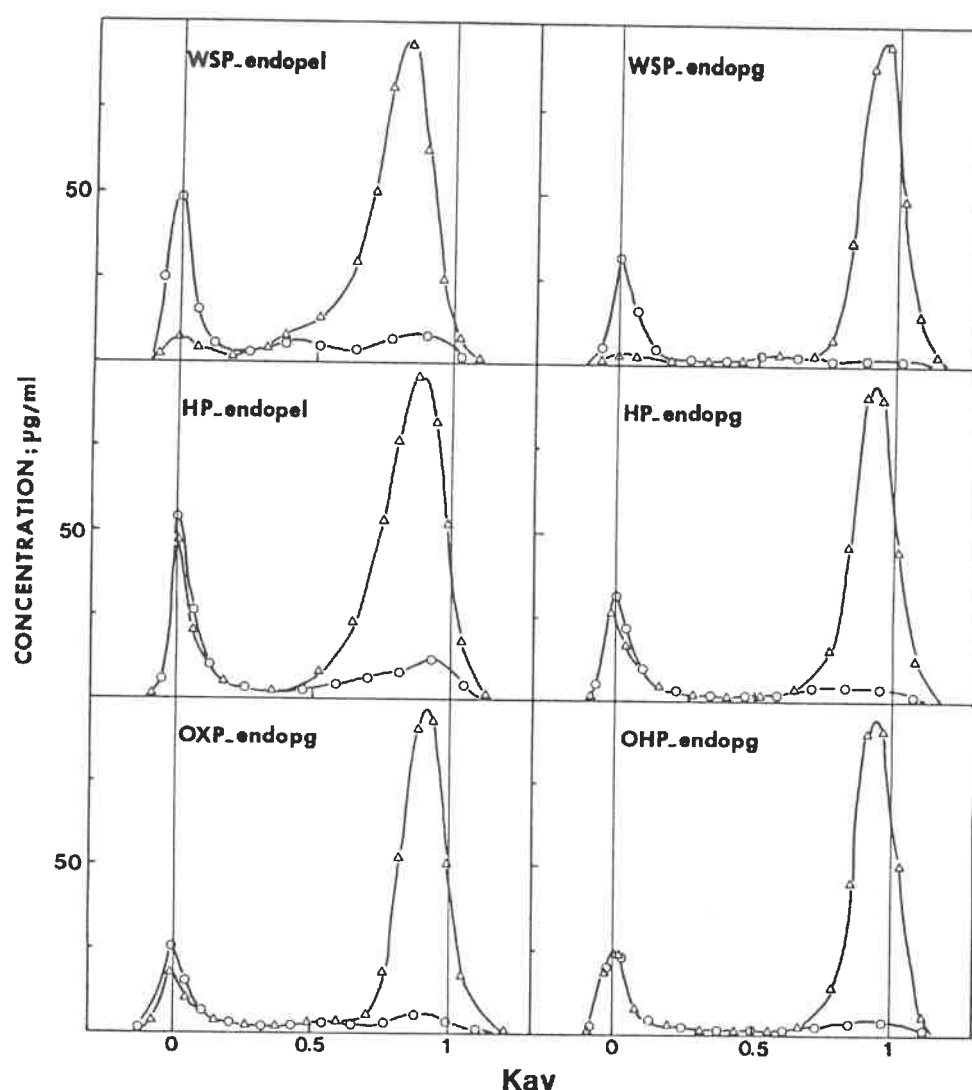
Fig. 2. Elution profiles after chromatography on Sephadex G100 of the degradation products from WSP, OXP, HP and OHP endopg and endopel digestions. —△—, anhydrogalacturonic acid; —○—, neutral sugars determined by *m*-phenylphenol and orcinol method, respectively.

the products of digestion by endopg and endopel reflecting the different action pattern of the two enzymes. The populations eluted in this fractionation range were composed of small fragments of pectin molecules in which were found the remaining neutral sugars, or of oligo-galacturonides which were found close to the total volume.

Sepharose CL 6B separates a population of higher M_w than Sephadex G100. Endopel and endopg digestion gave similar elution patterns (Fig. 3) as for those with Sephadex G100. WSP exhibited a peak rich in neutral sugars and poor in galacturonic acid at K_{av} 0·45. The elution pattern for OXP, HP and OHP were closely similar; below K_{av} 0·7, the

TABLE 2
The Sugar Composition and Content of the Products of Digestion with Endopel and Endopg of WSP, OXP, HP and OHP Samples Fractionated on Sephadex G100 and Sepharose CL6B[a]

	WSP		HP		OXP endopg	OHP endopg
	endopel	*endopg*	*endopel*	*endopg*		
Fractions recovered below K_{av} 0·2 on Sephadex G100						
GA[b]	3·7	4·2	15·0	15·2	11·0	15·7
NS[b]	82·0	82·0	83·0	80·0	82·0	67·0
Rha	7·4	6·8	28·6	24·8	28·1	32·1
Ara	42·1	42·6	36·4	38·4	33·6	21·2
Xyl	1·5	1·2	5·0	6·1	3·4	8·3
Man	0·7	1·9	0·6	1·6	1·2	0·0
Gal	42·3	44·0	20·7	21·3	20·5	23·7
Glc	5·9	3·0	7·5	3·0	13·7	14·3
Fractions recovered below K_{av} 0·6 on Sepharose CL 6B						
GA[b]	5·2	3·0	—	16·0	10·7	20·0
NS[b]	82·0	60·0	—	71·0	80·0	81·0
Rha	7·2	7·3	—	28·9	25·2	28·0
Ara	32·3	40·2	—	24·8	35·7	21·2
Xyl	4·2	6·0	—	5·2	4·6	10·0
Man	3·4	2·5	—	1·0	1·0	1·0
Gal	36·8	44·7	—	28·8	23·7	33·5
Glc	15·7	3·5	—	11·3	9·5	6·1

[a] Results are expressed as % in mole.
[b] % in weight of eluted material. GA: galacturonic acid; NS: neutral sugars; determined by the *m*-phenylphenol and the orcinol method, respectively.

ratio of neutral sugars to galacturonic acid was constant (= 1). A peak mainly composed of galacturonic acid was eluted close to the total volume. The composition of neutral sugars of the products eluted below K_{av} 0·7 was roughly similar to that found at the void volume of Sephadex G100 (Table 2).

A consistent excess of glucose was observed in all the samples analyzed, which has also been previously reported (Rombouts & Thibault, 1986); a 'bleeding' of Sephadex gel has been proposed to explain this artifact, but this is not an adequate explanation since we have observed this artifact with Sepharose CL 6B gel which is free from glucose.

The results obtained show that fractions rich in neutral sugar side chains, which have been called 'hairy' regions in previous studies (De
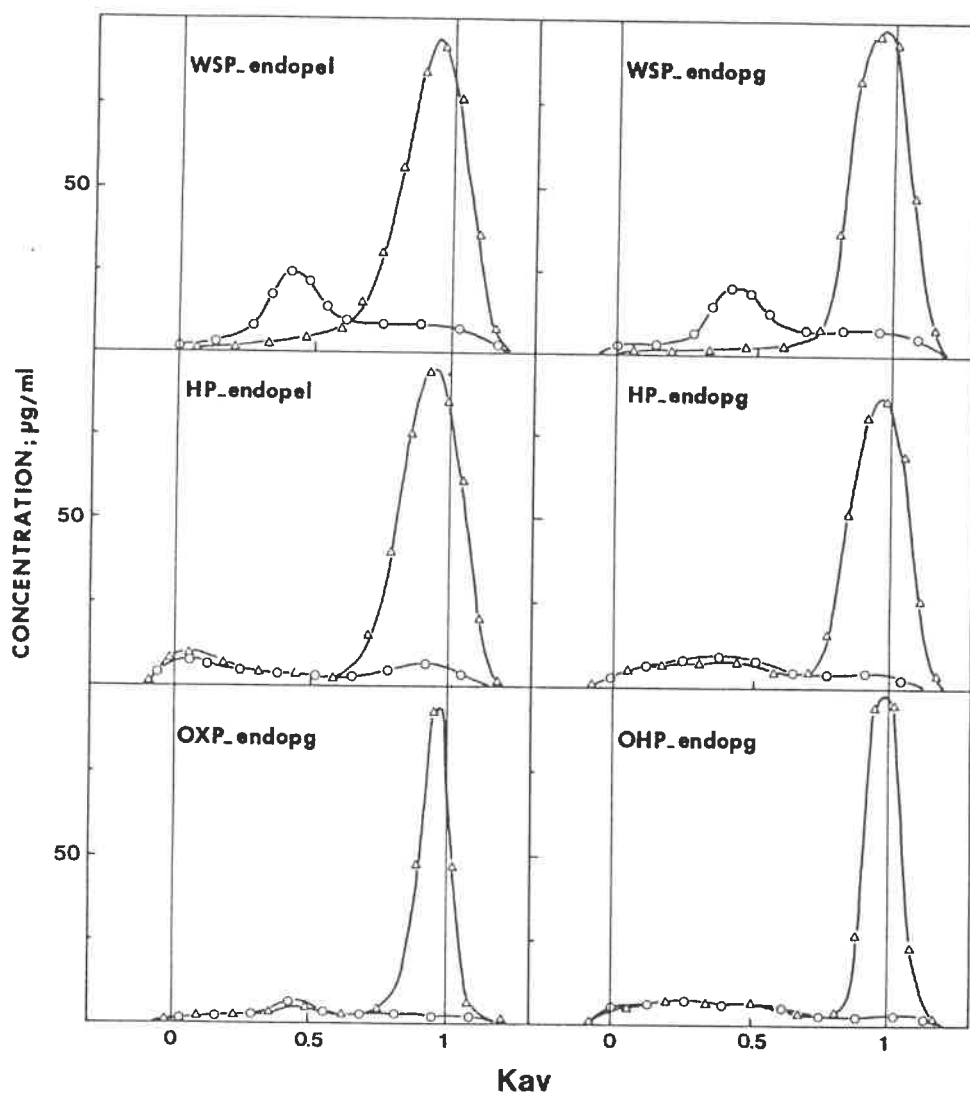
Fig. 3. Elution profiles after chromatography on Sepharose CL 6B of the degradation products from WSP, OXP, HP and OHP endopg and endopel digestions. —△—, anhydrogalacturonic acid; —○—, neutral sugars determined by *m*-phenylphenol and orcinol method, respectively.

Vries *et al.*, 1982; Thibault, 1983*a*; Rombouts & Thibault, 1986), are isolated. Endopg and endopel gave the same degradation products with minor differences in galacturonic acid content as reported for sugar-beet pectins (Rombouts & Thibault, 1986). These 'hairy' fragments seemed to be large molecules, and two main kinds were isolated: on one hand WSP fragments, which were rich in neutral sugars and poor in galacturonic acid and well separated on Sepharose CL 6B; on the other hand OXP, HP and OHP fragments which had equivalent amounts of neutral sugar and galacturonic acid. In subsequent investigations we have studied in greater detail WSP and HP 'hairy' fragments.

TABLE 3

Sugar Composition of 'Hairy' Fragments Isolated at V_0 using Sephadex G100 after Endopg Digestion of WSP and HP, and Subsequent Chromatography by DEAE-Sephacel[a]

| | WSP 'hairy' fragments | | | | HP 'hairy' fragments | | | |
| | Initial[b] | DEAE-chromatography | | | Initial[b] | DEAE-chromatography | | |
		(0–50 ml)	(70–100 ml)	(> 100 ml)		(0–50 ml)	(70–100 ml)	(> 100 ml)
GA[c]	100	3·6	11·0	85·4	100	0·5	5·5	94·0
NS[c]	—	40·0	38·5	21·5	—	7·1	12·3	80·6
Rha	65·1	1·4	4·9	16·2	83·8	3·4	16·4	36·7
Ara	372·2	27·7	36·4	34·9	106·6	20·1	16·2	32·4
Xyl	13·7	16·0	3·1	9·4	17·6	13·2	12·4	5·7
Man	6·2	0·4	1·1	0·9	2·0	2·3	4·8	1·4
Gal	373·4	39·5	40·9	15·8	60·6	21·5	24·5	16·1
Glc	-52·3	29·4	14·6	22·8	22·0	39·5	25·7	7·7

[a] Results are expressed as % in mole.
[b] Expressed as mole of sugars per 100 moles of galacturonic acid.
[c] % in weight of eluted material; GA: galacturonic acid; NS: neutral sugars; determined by the *m*-phenylphenol method and as alditol acetate, respectively.

WSP and HP 'hairy' fragments

The sugar content and composition of WSP and HP fragments eluted at the void volume of Sephadex G100 after digestion by endopg are reported in Table 3. The neutral sugar:galacturonic acid ratio was about 9 for WSP, and the recovery study showed that 55% of the total rhamnose, 22% of the total xylose and almost all of the arabinose and galactose of the initial WSP was recovered in these fragments. If we assumed that side chains were mainly arabinogalactans and that all the arabinose and galactose moieties were bound to rhamnosyl residues, a mean degree of polymerization (DP) of 11 is calculated for side chains. HP 'hairy' fragments had a neutral sugar:galacturonic acid ratio of 3 and a mean DP, calculated as above, of 3; no significant enrichment or loss of neutral sugars was found compared to the initial pectins, except for glucose. These results suggested that WSP 'hairy' fragments are composed of small parts of the rhamnogalacturonan backbone with long side chains of arabinogalactan, while HP fragments are larger and composed of a rhamnogalacturonan backbone rich in rhamnose with short side chains.

Both the WSP and HP 'hairy' fragments isolated in the way previously described were chromatographed on DEAE-Sephacel (Fig. 4) and the sugar composition of the eluted peaks was determined (Table 3). Some material, mainly composed of neutral sugars with very low amounts of associated galacturonic acid, was not bound on the column for the two samples, but this material represented 40% of the total neutral sugars and 3·6% of the total galacturonic acid for WSP and minor amounts for HP (7% of the neutral sugars and 0·5% of galacturonic acid). Two peaks were eluted with the acetate buffer gradient for WSP: the first one at low ionic strength was rich in neutral sugars and poor in galacturonic acid; the second one at high ionic strength (0·6 M) was characterized by a neutral sugar:galacturonic acid ratio close to 1 and contained the major part of the overall galacturonic acid content (85% of total galacturonic acid). These two peaks were also characterized by an increasing rhamnose content compared to the unbound population (Table 3). The HP fragments were eluted in a mean peak at high ionic strength (0·6 M) of neutral sugar:galacturonic acid ratio close to 1 which contained almost all the galacturonic acid (94% of total galacturonic acid) and the major part of the neutral sugar (80·6%). A minor peak was eluted at low ionic strength. Owing to their different galacturonic acid contents and to their hypothetical structure, WSP and HP 'hairy' fragments would have a different elution pattern on ion-exchange chromatography, the first one being, roughly, not bound on the exchanger and the second one eluted
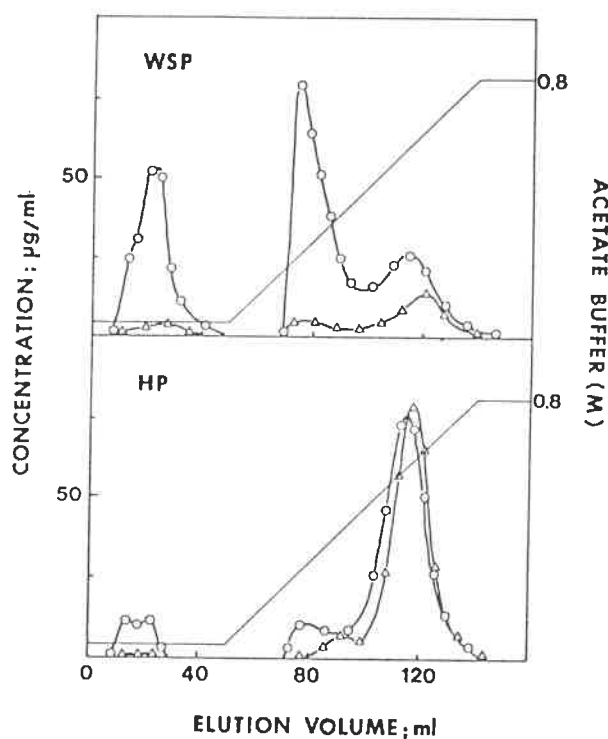
Fig. 4. Elution profiles after chromatography on DEAE-Sephacel of WSP and HP 'hairy' fragments isolated on V_0 of Sephadex G100 after endopg digestion. —△—, anhydrogalacturonic acid; —○—, neutral sugars determined by *m*-phenylphenol and orcinol method, respectively.

with an ionic-strength gradient, which was partly observed. Moreover, these results show that the elution on the ion exchanger is affected by the galacturonic acid and the rhamnose content of the fragments which are correlated with the side-chain length.

Although purified pectins were used for enzymic degradation, these materials were highly heterogeneous (Saulnier & Thibault, 1987), which was reflected in the analyses of the degradation products obtained. WSP 'hairy' fragments which had a relatively homogeneous M_w distribution (Sepharose CL 6B), were highly heterogeneous on ion-exchange chromatography, while HP 'hairy' fragments displayed the opposite behaviour. Nevertheless, a scheme (based on studies of rhamnogalacturonan by Albersheim, 1978) which gives the main structural features of the 'hairy' fragments isolated can be proposed, namely: WSP is composed of a very small portion of rhamnogalacturonan backbone on which long side chains are bound, while HP is composed of large fragments of the rhamnogalacturonan backbone, rich in rhamnose, on which short side chains are bound. It is likely that WSP 'hairy' fragments are a more complex mixture of probably coexisting regions with side

chains of variable length and composition as reflected by ion-exchange chromatography.

Enzymic degradation of grape-berry cell wall

Pectins are found in two forms in the AIR. On one hand, some pectic material is soluble in water or in the presence of calcium chelating agents and are extracted apparently without degradation. On the other hand, the main portion of pectic material is insoluble and must be extracted with hot acids (Pilnik & Voragen, 1970); this extraction involves the degradation of acid-sensitive side chains (arabinans) while the rhamno-galacturonan is only little affected (Be Miller, 1967).

Endopectinylase treatment

Endopel has been used to release pectic materials from an AIR of apple (Voragen *et al.*, 1980). We have used this enzyme as an alternative to acid treatment after a sequential extraction with water, oxalate and then citrate which was the buffer chosen for the enzyme action. The time-course of the endopel reaction was followed and a plateau was reached after 24 h in the conditions used (Fig. 5). The extract was highly esteri-fied and endopel released large amounts of pectic substances, but less than that by an acid treatment (67% by weight of the total galacturonic acid released with acid) but more neutral sugars (130% by weight of the total neutral sugars released by the use of acid) from the AIR. The neutral sugar composition (Table 4) shows that the extract was very rich in arabinose and had similar amounts of rhamnose, xylose and galactose.
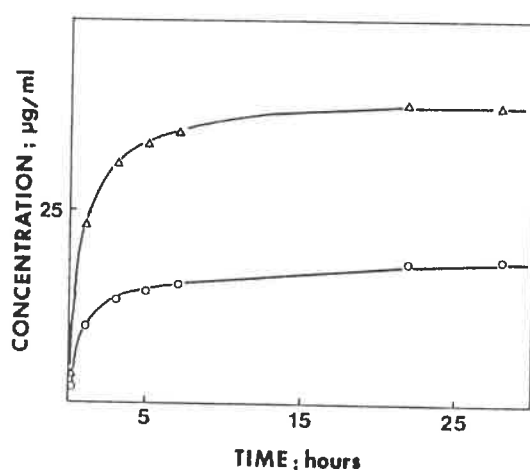

Fig. 5. Time-course of degradation of AIR from pulp of grape berry by endopel. — △ —, anhydrogalacturonic acid; —o—, neutral sugars determined by *m*-phenylphenol and orcinol method, respectively.

TABLE 4

Sugar Composition of Endopel AIR Digestion and of Fractions Recovered after Chromatography on Sephadex G100[a]

	Crude extract	Fractions recovered on Sephadex G100		
		$K_{av} < 0 \cdot 2$	$0 \cdot 2 < K_{av} < 0 \cdot 7$	$K_{av} > 0 \cdot 7$
GA[b]	—	20·0	17·0	63·0
NS[b]	—	70·0	18·0	12·0
Rha	11·5	20·4	22·1	9·0
Ara	58·0	53·7	29·9	21·3
Xyl	11·6	2·7	14·5	20·3
Man	1·7	1·3	3·5	6·2
Gal	10·4	11·8	16·0	13·5
Clc	6·8	10·1	14·0	29·7

[a] Results are expressed in % of mole.
[b] % in weight of eluted material; GA: galacturonic acid, NS: neutral sugars; determined by the *m*-phenylphenol and as alditol acetate, respectively.
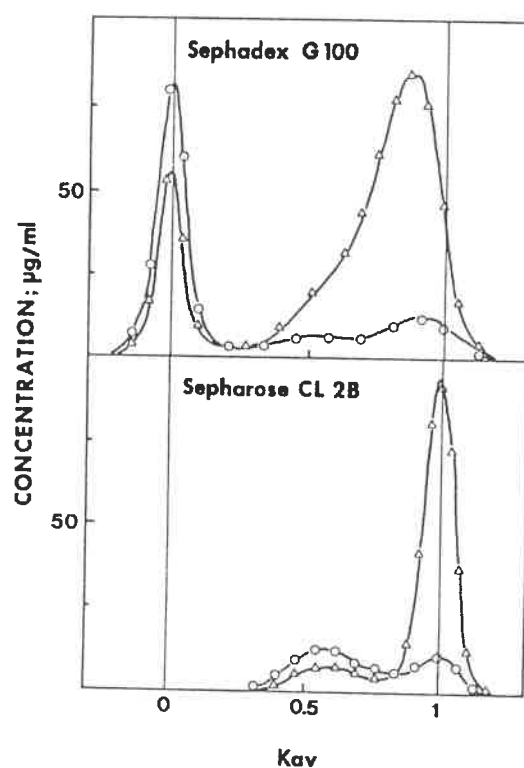

Fig. 6. Elution profiles after chromatography on Sephadex G100 and Sepharose CL 2B of the degradation products from endopel AIR digestion. —△—, anhydrogalacturonic acid; —○—, neutral sugars determined by *m*-phenylphenol and orcinol method, respectively.

Analysis of degradation products

The pectic substances released by endopel were analyzed on Sephadex G100 and Sepharose CL 2B columns (Fig. 6). The large degraded pectin molecules were eluted at the void volume of Sephadex; they were rich in neutral sugars, especially in rhamnose and arabinose (Table 4), and probably corresponded to rich side-chain areas of the rhamnogalacturonan backbone. This fraction had a high M_w, and was eluted between K_{av} 0·3 and 0·8 on Sepharose CL 2B, where 65% of the neutral sugars and 16% of the galacturonic acid of the initial material (w/w) was found. Most of the galacturonic acid was eluted close to the total volume on Sephadex and on Sepharose.

The fractions from the endopel cell-wall digestion isolated on Sephadex and Sepharose gels were related to the HP 'hairy' fragments, their neutral sugar compositions were similar, except for the arabinose content which was higher for the endopel than for the HP fragments (Tables 3 and 4). A DP of 3 can be calculated for the endopel fragments isolated at the void volume of Sephadex, which was higher than that for the HP 'hairy' fragments but much lower than that of WSP. Hence it can be presumed that endopel releases HP-type 'hairy' fragments with undegraded arabinans side-chains.

CONCLUSION

The aim of this paper was to investigate the fine structure of purified pectins with enzymes. The results have shown that pectin molecules are composed of 'smooth' and 'hairy' areas. The WSP and HP pectins are differentiated by their sugar composition and the structure of these regions. Common trends were found between the HP 'hairy' fragments and the pectic substances isolated from the endopel grape cell-wall digestion. It appears from these results that pectin insolubility could be related to the proportion of the 'hairy' regions on the rhamnogalacturonan backbone. We shall investigate further the linkages occurring in side chains by methylation analysis.

ACKNOWLEDGMENTS

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