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Chemical Characterization of Water-Soluble Pectin in Papaya Fruit

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ABSTRACT

Water-soluble pectin was extracted from ripe papaya fruit pulp and fractionated by ion-exchange chromatography and gel filtration. Sugar analysis of fractions obtained revealed that the major component was a rhamnogalacturonan with low content of arabinose and galactose residues (2–3%). About 50% of the galactopyranosyluronic acid residues were methyl-esterified and ^1H *and* ^{13}C *NMR-analysis established that these ester groups were randomly distributed. COSY-NMR was used to distinguish between resonances for protons in free or esterified uronic acid residues.*

INTRODUCTION

Pectins are common components in the cell walls of fruits and have important nutritional and technological properties, mainly because of their ability to form gels (Pilnik & Voragen, 1970). Characteristic structural features for this group of heteropolysaccharides are a main chain of (1→4)-linked α-D-galactopyranosyluronic acid residues interspersed with α-L-rhamnopyranosyl residues (Aspinall, 1980; Dey & Brinson, 1984). Some of the rhamnose residues are branched with side

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chains comprising different amounts of neutral sugar residues, mainly arabinose and galactose. In addition, methyl and other ester groups are often present (Stephen, 1983).

Few studies have dealt with the chemical characterization of papaya polysaccharides. The structure of acidic polysaccharides, present as contaminants in papain, has been investigated by methylation analysis (BeMiller & Dikko, 1986). The sugar composition and degree of esterification (DE) has been determined (Voragen *et al.*, 1983) for an aqueous ethanol-insoluble fraction of papaya fruit. Biswas *et al.* (1969, Biswas & Rato, 1969*a*) reported that $(1\rightarrow3)$ and $(1\rightarrow4)$-linked galacturonans as well as a galactan (Biswas & Rao, 1969*b*) were present in a water-soluble fraction isolated from unripe papaya fruit. The aim of the present investigation was to fractionate and elucidate the chemical structure of water-soluble pectin in the edible part of papaya fruit.

MATERIALS AND METHODS

General methods

All weights reported are on a dry weight basis. Concentrations were performed under diminished pressure below 40°C or by freeze-drying. Dialysis was performed using Spectrapor membranes with molecular weight cut-off at 12 000 Da. Optical rotations were measured at room temperature in water ($c = 1{\cdot}0$) on a Perkin Elmer 141 polarimeter. ^{13}C (101 MHz) and ^{1}H (400 MHz) NMR spectra were recorded without measurement of pD at 85°C in D_2O on a Varian 400 VXR instrument using sodium 3-(trimethylsilyl)-propionate-d_4 as internal reference. The pulse width (^{13}C NMR) was set at 45° (8 μs) with a repetition time of 1·5 s and a waltz modulator was used for broadband decoupling. Two-dimensional NMR was performed with COSY (36 μs-pulse; 90-t1-90-t2) and delayed COSY (0·3 s) was used to observe the weak coupling between H-4 and H-5 in galactosyl uronic acid residues. Gas–liquid chromatography (GLC) of neutral polysaccharide constituents was performed as alditol acetates (Theander & Westerlund, 1986) on a Cp-Sil 88 column (9·5 m × 0·25 mm i.d., helium flow approx. 1 m/s), using programming at 4°C/min from 170°C (3 min initial temperature) to 220°C (1 min final temperature).

Samples

Papaya was purchased at a market in Dhaka, Bangladesh. The pulp of the fruit was sliced and extracted by sequential reflux with 80% aqueous (v/v) ethanol and chloroform (Rahman *et al.*, 1991). The extractive-free residue isolated accounted for 18% of the dry original pulp. Fully methyl-esterified citrus pectin (DE 95) was obtained from a previous investigation (Westerlund *et al.*, 1989).

Extraction of water-soluble papaya pectin

The extracted residue (11·2 g) was suspended in acetate buffer (240 ml, 0·1 M, pH 5·0) at 96°C for 30 min with thermostable α-amylase Termamyl 120L (0·200 ml, 28·8 kN-units, Novo A/S, Copenhagen Denmark). After cooling and incubation with amyloglucosidase (0·200 ml, crystal suspension, 28 U, Boehringer Mannheim, FRG) overnight, the suspension was centrifuged (200 × *g* for 15 min). The residue was further washed with water (2 × 100 ml), recovered by centrifugation, and the supernatants combined. After concentration, dialysis (against running tap water for 24 h and then distilled water for 24 h), cation-exchange with Dowex 50W-X8 (H^+) and freeze-drying, the crude water-soluble pectin isolated accounted for 3·8% of the original dry papaya pulp.

Fractionation of water-soluble papaya pectin

The crude water-soluble pectin (2·04 g) was dissolved in water and fractionated on a QAE-Sephadex A-50 anion exchange column (22 × 7 cm) using stepwise elution with water and piperazine buffers (pH 6·0; 0·1 M, 0·3 M and 0·5 M, respectively) according to Sun *et al.* (1987). The total contents of carbohydrates (Dubois *et al.*, 1956) and uronic acid residues (Blumenkrantz & Asboe-Hansen, 1973) were monitored in fractions (18 ml) of the effluent by colorimetric methods. Six main fractions (F1–F6) were isolated, as shown in Fig. 1, by pooling, cation-exchange, dialysis against distilled water and freeze-drying. A small amount of carbohydrate material was eluted with 0·5 M piperazine buffer but this was not further investigated.

Gel filtration of fractions

Samples (4·0 mg) of F3–F6 were separately dissolved in 0·25 M aqueous sodium chloride (2 ml), kept at 4°C overnight and then run on Superose 12 using the same solvent as eluent (Fig. 2). The column (30 × 1·5 cm) was eluted at a rate of 0·5 ml/min and was calibrated with glucose and dextran standards T10 and T150 (Pharmacia, Uppsala, Sweden).

Preparative gel filtration of the major water-soluble pectic component (2 × 100 mg), present in F5, was performed on a column (95 × 2·5 cm) of

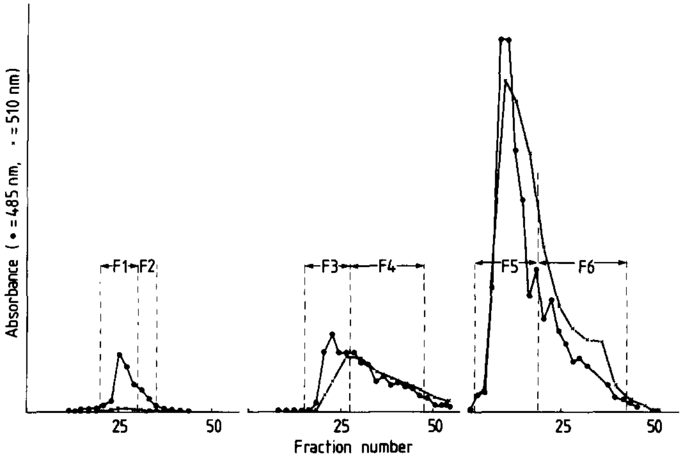

Fig. 1. Fractionation of crude water-soluble papaya pectin on QAE-Sephadex A-50. Fractions were eluted with water (F1–F2), 0·1 M (F3–F4) and 0·3 M piperazine buffers (F5–F6). Colorimetric estimation of uronic acids (×——×) and neutral sugars (•——•).

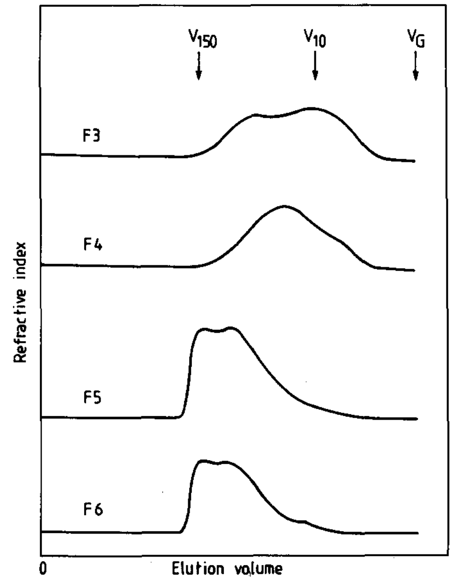

Fig. 2. Analytical gel filtration on Superose 12 of water-soluble pectin fractions (F3–F6) obtained by QAE fractionation as shown in Fig. 1. Arrows show the mean elution volume for the calibration standards (V_G = glucose, V_{10} = dextran T10 and V_{150} = dextran T150).

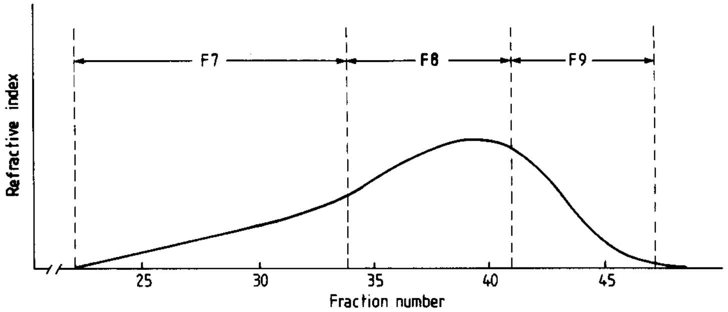

Fig. 3. Preparative gel filtration (10-ml fractions) on Sephacryl S-400 of the major water-soluble pectin fraction (F5), obtained by QAE fractionation as shown in Fig. 1.

Sephacryl S-400, calibrated with glucose and dextran standard T2000, using 0·25 M sodium chloride as eluent. The broad peak obtained (Fig. 3), was divided into three subfractions (F7–F9) which were isolated by dialysis against distilled water and freeze-drying.

RESULTS AND DISCUSSION

The chemical composition of the edible part of fresh papaya fruit established that uronic acids, determined colorimetrically (Blumenkrantz & Asboe-Hansen, 1973), and glucose, determined as the alditol acetate after acid hydrolysis (Theander & Westerlund, 1986), were the main polysaccharide residues present (Table 1). This finding indicates that pectins and cellulose are major components of the polysaccharides in the fresh fruit. The predominant carbohydrates are, however, free sugars (Rahman *et al.*, 1991), as indicated by the high amount of hydrophilic extractives. In the present study, the procedure used for isolation of crude water-soluble pectin from papaya was similar to that developed for analysis of water-soluble dietary fibre (Theander & Westerlund, 1986). The resulting preparation was further fractionated on an anion exchange column, using stepwise elution with water and piperazine buffers as previously described for the purification of a tobacco rhamnogalacturonan (Sun *et al.*, 1987). The colorimetric elution profiles for the six main fractions collected (Fig. 1) showed that only small amounts of mainly neutral polysaccharides were eluted with water (F1–F2), whereas the bulk of pectic material was released by elution with 0·1 M (F3–F4) and 0·3 M (F5–F6) piperazine buffers. The recovery of polysaccharides loaded onto the column was 90%. Uronic acid residues (85%) predominated in the crude water-soluble pectin fraction with smaller

TABLE 1
Chemical Composition of the Edible Part of Fresh Papaya Fruit[a]

Components	*Content (g/kg fresh weight)*
Water	898·00
Hydrophilic extractives[b]	89·00
Lipophilic extractives[c]	2·00
Starch	trace
Dietary fibre components	12·00
Klason lignin	0·20
Uronic acids	5·05
Neutral polysaccharide residues	
Rhamnose	0·20
Fucose	0·10
Arabinose	0·20
Xylose	0·62
Mannose	0·45
Galactose	0·48
Glucose	4·69

[a] Calculated in part from Rahman *et al.* (1991).
[b] Soluble in hot 80% aqueous ethanol.
[c] Soluble in hot chloroform.

amounts of galactose (6%), rhamnose (4%) and arabinose (3%), and traces of other sugar residues also present (Table 2). Arabinose and galactose residues were predominant components in the two neutral fractions (F1 and F2) isolated in low yield. In the other fractions a successive decrease occurred, essentially in order of elution, for arabinose, galactose and xylose residues, whereas uronic acid residues increased. This indicated that the amount of rhamnogalacturonans in the fractions increased in order of elution. It is not clear if the high value for galactose residues in F1–F2 was due to the presence of galactans, such as that previously isolated from the pulp of unripe papaya (Biswas & Rao, 1969*b*), and/or arabinogalactans.

Analytical gel filtration of F3–F6 on Superose 12 showed (Fig. 2) that the average molecular weight of these polydisperse pectin fractions increased in the order F3 to F6. The major pectin fraction (F5) was subjected to preparative gel filtration on Sephacryl S-400. The broad peak obtained, ranging from the void to the included volume of the column, was divided into three subfractions, F7–F9 (Fig. 2). The polysaccharide content in these subfractions was close to 100%, with uronic acid residues predominating and only small amounts (5–8%, Table 3) of

TABLE 2

Carbohydrate Yields (g/kg Dry Fruit) and Polysaccharide Composition (rel %) for Water-soluble Fractions of Pectins

	Crude water-soluble fraction	*QAE-Fractions*[a]					
		F1	*F2*	*F3*	*F4*	*F5*	*F6*
Carbohydrate yield	23·0	0·3	0·1	1·5	1·6	12·4	4·9
Polysaccharide composition							
Rhamnose	4·0	0·6	2·0	2·0	3·0	2·0	3·0
Arabinose	3·0	33·0	38·0	9·0	3·0	1·0	0·5
Xylose	1·0	2·0	12·0	2·0	0·4	0·3	0·3
Mannose	<0·3	5·0	4·0	0·1	0·6	<0·1	<0·1
Galactose	6·0	54·0	39·0	19·0	5·0	2·0	2·0
Glucose	1·0	5·0	6·0	7·0	0·5	0·3	0·6
Uronic acids	85·0	<0·1	<0·1	64·0	88·0	94·0	94·0

[a] Obtained as described in Fig. 1.

TABLE 3

Analysis Data for Pectin Fractions Isolated by Preparative Gel Filtration of the Major Pectic QAE Fraction

	Fractions[a]		
	F7	*F8*	*F9*
Yield (g carbohydrate/kg dry fruit)	2·9	5·9	2·3
$[\alpha]_{578}$	+255	+212	+201
Degree of esterification[b]	50	49	44
Polysaccharide composition (rel %)			
Rhamnose	2	2	3
Arabinose	0·7	2	0·7
Xylose	0·6	0·2	0·2
Mannose	0·3	0·1	0·1
Galactose	2	3	1
Glucose	1	0·3	0·4
Uronic acids	94	94	94

[a] Obtained as described in Fig. 3.

[b] Calculated from the intensity of the two signals for C-1 (esterified and unesterified galactopyranosyluronic acid residues) in ^{13}C NMR spectra.

neutral sugar residues present. Main differences observed were a slightly higher content of arabinose and galactose residues in F8 compared to the other fractions. The lowest molecular-weight fraction (F9) had a higher content of rhamnose residues and lower DE values and optical rotation than the other subfractions.

Grasdalen *et al.* (1988) have shown by one-dimensional ^{1}H NMR-analysis that methyl ester groups were randomly distributed in apple pectins of varying DE, when the samples were prepared by alkaline de-esterification of fully esterified apple pectin. It has been shown that the pD of the solution affects the chemical shift, particularly for H-5 in free uronic acid residues by a significant deshielding below pD 5 (Jaques *et al.*, 1979). In the present study ^{1}H NMR spectra of the fractions isolated by preparative gel filtration (that of F8 is shown in Fig. 4) exhibited a pattern in the low-field region (peaks 1–10) in good agreement with that

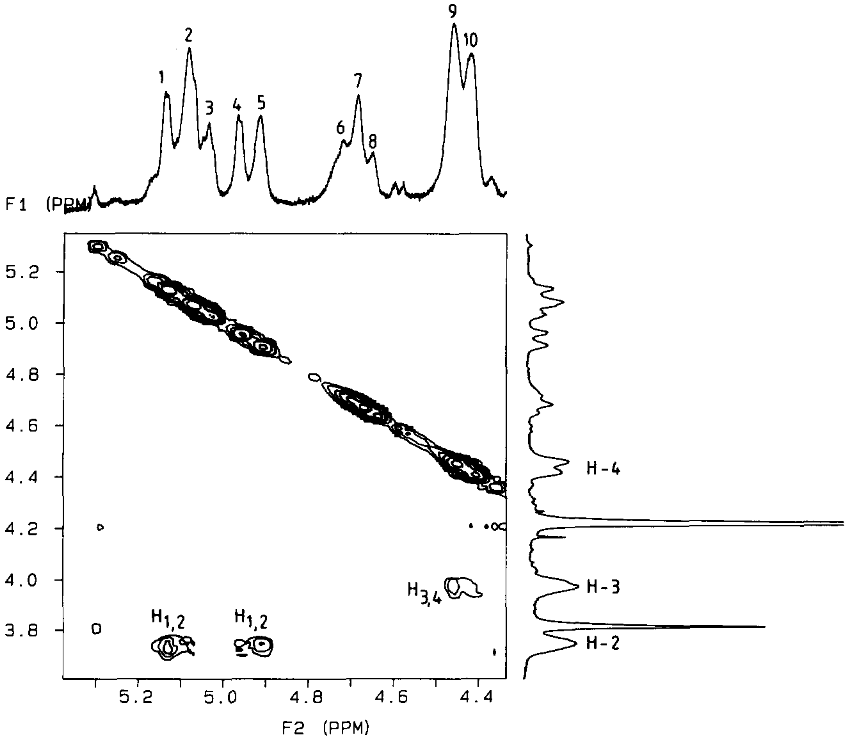

Fig. 4. ^{1}H COSY-NMR spectrum of the major fraction (F8) of papaya pectin obtained on gel filtration as shown in Fig. 3. The peaks are numbered according to Grasdalen *et al.* (1988) and are assigned to the ring protons in esterified and unesterified galactopyranosyluronic acid residues as follows: peaks 1–3 (overlap H-1 and H-5), peaks 4–5 (H-1), peaks 6–8 (H-5) and peaks 9–10 (H-4).

previously published (Grasdalen *et al.*, 1988) for a sample of apple pectin with DE 56, analysed at pD 5. This finding strongly suggests that the present spectra were recorded under similar conditions (pD 5) and that the papaya pectins in F7–F9 therefore should also have random distribution of methyl ester groups and similar DE value. The peaks observed in the low-field region arise from H-1, H-4 and H-5 in methyl-esterified as well as unesterified galactopyranosyluronic acid residues (Grasdalen *et al.*, 1988).

Evaluation of COSY experiments (Kessler *et al.*, 1988) performed on isolated papaya pectin, F8 (Fig. 4) and on a reference sample of fully esterified citrus pectin (Fig. 5) made it possible to differentiate main signals of H-1 from those of H-5. Thus, the COSY spectrum of fully esterified citrus pectin showed that the resonance around 4·9 ppm was due to H-1 in esterified sugar residues, since it was correlated to H-2. In a delayed COSY of this sample a weak correlation for the signal centred at 5·1 ppm (H-5) to that at 4·4 ppm (H-4) was also observed, showing

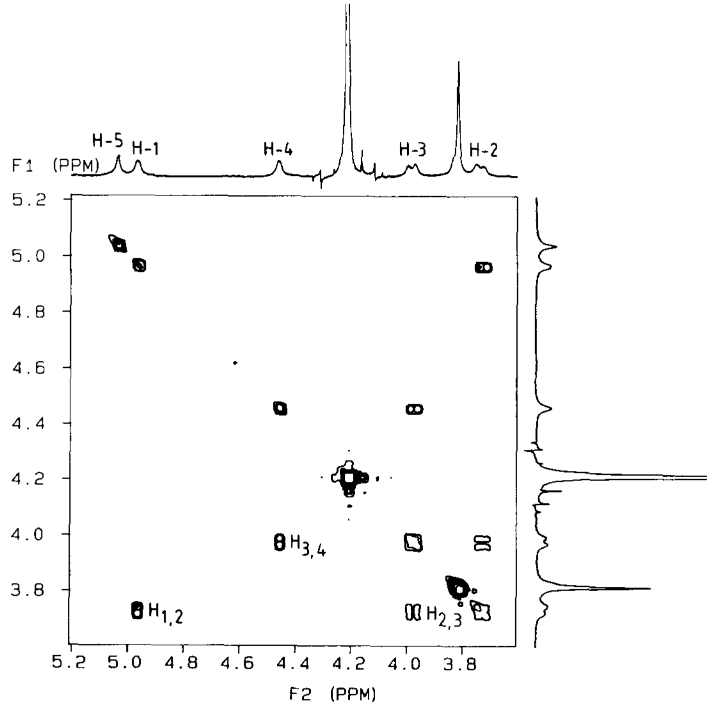

Fig. 5. ^{1}H COSY-NMR spectrum of fully methyl-esterified citrus pectin. The coupling between H-4 and H-5 is weak in galactopyranosyl residues and is not observed under the experimental conditions used.

that H-5 resonated down-field of H-1. The occurrence of major correlations for H-1 around 4·9 and 5·1 ppm in the papaya pectin (Fig. 4) showed, by comparison with the reference sample, that H-1 was moved downfield when present in unesterified sugar residues. H-5 in such residues, on the other hand, was moved upfield as shown by the presence of significant multiple peaks around 4·65 ppm which, in the delayed COSY correlated to the peaks at 4·4 ppm (H-4). Based on these findings, the positions originally assigned for H-1 and H-5 in esterified galactopyranosyluronic acid residues (Grasdalen *et al.*, 1988) are to be reversed.

The high-field ^{13}C NMR spectra of F7–F9 (F8 is shown in Fig. 6) were very similar and all showed predominant resonances from carbons in free and methyl-esterified galactopyranosyl uronic acid residues in accordance with those previously observed in pectins (Sun *et al.*, 1987; Westerlund *et al.*, 1991). Signals for C-2 to C-5 appeared in the region 70–82 ppm, and those for C-1 at 102·9 (esterified residues) and 102·2 (unesterified residues) ppm. The signal at 55·7 ppm originated from the methyl group in the esterified glycosyluronic acid residues. The multiple appearance of signals from the ester carbonyls around 173·5 ppm is due to influences from neighbouring sugar residues (free or esterified galactosyluronic acid) and the broad peak observed around 177 ppm arises from the carboxyl carbons (Westerlund *et al.*, 1991). Integration of

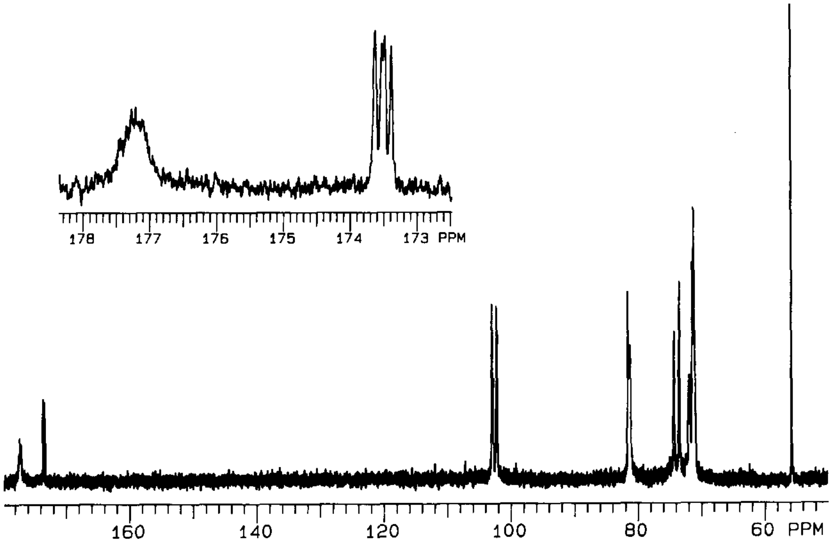

Fig. 6. ^{13}C NMR spectrum of the major fraction (F8) of papaya pectin obtained on gel filtration as shown in Fig. 3.

the signals for C-1 in esterified and unesterified sugar residues as well as of carboxyl and ester carbonyl carbons revealed that DE varied from 44 to 50 in the three fractions (Table 3). The fact that carbonyl carbons in ester groups appeared as four signals of approximately equal intensity (see the expanded low-field region) further confirmed that the methyl ester groups were randomly distributed in the polymer (Westerlund *et al.*, 1991). Only traces of signals from neutral sugar residues were observed in the spectra, further confirming the low content of these residues in the main fraction of papaya pectin. Trace resonances at 18 ppm were assigned to C-6 in rhamnopyranosyl residues and those at 22 ppm to the presence of acetyl groups in the pectin fractions.

No evidence was obtained for the presence of (1→3)-linked galacturonans in water-soluble papaya pectin, which is in contrast to previous findings (Biswas *et al.*, 1969; Biswas & Rao, 1969*a*). The DE value of the papaya pectin was lower in the present study than previously observed (Westerlund *et al.*, 1991) for papaya (DE 70), but this divergency may be due to differences in, for example, source, botanical origin, ripeness or the isolation procedure used.

In conclusion, the edible part of ripe papaya contained significant amounts of water-soluble pectins. This pectin had a wide molecular weight distribution and contained low amounts of neutral sugar residues. The distribution of methyl ester groups in the polymer was random.

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REFERENCES

Aspinall, G. O. (1980). Chemistry of cell-wall polysaccharides. In *The Biochemistry of Plants*, ed. J. Preiss, Vol. 3. Academic Press, New York, pp. 473–500.

BeMiller, J. N. & Dikko, S. B. (1986). Structural analysis of papaya polysaccharide II. *Carbohydr. Res.*, **158**, 173–81.

Biswas, A. B. & Rao, C. V. N. (1969*a*). Enzymic hydrolysis and Barry degradation studies on pectic acid from the pulp of unripe papaya (*Carica papaya*) fruit. *Indian J. Chem.*, **7**, 592–3.

Biswas, A. B. & Rao, C. V. N. (1969*b*). Structural investigation of the galactan component of the pectic substance from *Carica papaya*. *Aust. J. Chem.*, **22**, 2001–4.

Biswas, A. B., Mukherjee, A. K. & Rao, C. V. N. (1969). Pectic acid from the pulp of unripe papaya (*Carica papaya*) fruit. *Indian J. Chem.*, **7**, 588–91.

Blumenkrantz, N. & Asboe-Hansen, D. (1973). New method for quantitative determination of uronic acids. *Anal. Biochem.*, **54**, 484–9.

Dey, P. M. & Brinson, K. (1984). Plant cell-walls. *Advances in Carbohydrate Chemistry and Biochemistry*, **42**, 265–382.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–6.

Grasdalen, H., Bakøy, O. E. & Larsen, B. (1988). Determination of the degree of esterification and the distribution of methylated and free carboxyl groups in pectins by ^{1}H-NMR spectroscopy. *Carbohydr. Res.*, **184**, 183–91.

Jaques, L. W., Macaskill, J. B. & Weltner, W. (1979). Electric field theory applied to hydrogen-1 and carbon-13 nuclear magnetic resonance spectra of carbohydrates. *J. Phys. Chem.*, **83**, 1412–21.

Kessler, H., Gehrke, M. & Griesinger, C. (1988). Two-dimensional NMR spectroscopy: Background and overview of the experiments. *Angew. Chem. Int. Ed. Engl.*, **27**, 490–536.

Pilnik, W. & Voragen, A. G. J. (1970). Pectic substances and other uronides. In *The Biochemistry of Fruits and their Products*, Vol. 1, ed. A. C. Hulme. Academic Press, New York, pp. 53–87.

Rahman, S. M. M., Mosihuzzaman, M. & Westerlund, E. (1991). Free sugars and dietary fibre in some fruits of Bangladesh. *Food Chem.* (in press).

Stephen, A. M. (1983). Other plant polysaccharides. In *The Polysaccharides*, Vol. 2, ed. G. O. Aspinall. Academic Press, New York, pp. 97–193.

Sun, H. H., Wooten, J. B., Ryan, W. S., Bokelman, G. H. & Åman, P. (1987). Structural characterization of a tobacco rhamnogalacturonan. *Carbohydr. Polym.*, **7**, 143–58.

Theander, O. & Westerlund, E. (1986). Studies on dietary fiber. 3: Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.*, **34**, 330–6.

Voragen, F. G., Timmers, J. P. J., Linssen, J. P. H., Schols, H. A. & Pilnik, W. (1983). Methods of analysis for cell-wall polysaccharides of fruit and vegetables. *Z. Lebensmittelunters. u. Forsch.*, **177**, 251–6.

Westerlund, E., Åman, P., Andersson, R. E. & Andersson, R. (1991). Investigation on the distribution of methyl ester groups in pectin by high-field ^{13}C NMR. *Carbohydr. Polym.*, **14**, 179–87.