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Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure☆,☆☆

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Abstract

Pectin was acid extracted from orange albedo by microwave heating under pressure. Extraction times ranged from 2.5 to 8 min. Solubilized pectin was characterized for molar mass (M), rms radius of gyration (R_g) and intrinsic viscosity [η] by HPSEC with online light scattering and viscosity detection. M, R_g and [η] all decreased with increasing extraction time. Nevertheless, at heating times of 2.5 and 3.0 min, M, R_g and [η] were significantly higher than a commercial citrus pectin when the albedo:solvent ratio was 1:25 (w/v). At the heating time of 2.5 min M_w was 3.6×10^5, R_{gz} was 38 nm and $[\eta]_w$ was 10.8 dL/g. Chromatography revealed that solubilized pectin distributions were bimodal in nature and that the low-molar-mass fraction increased at the expense of the high-molar-mass fraction with increasing extraction time. Scaling law exponents revealed that the high-molar-mass fraction was extremely compact in shape, whereas the low-molar-mass fraction was more asymmetric in shape. Possibly these results indicated that at short extraction times, pectin was solubilized as compact aggregated network structures that were broken down to their more asymmetric components with increased heating times. Published by Elsevier Science Ltd.

Keywords: Pectin; Microwave extraction; Orange albedo; HPSEC; Molar mass; Radius of gyration; Intrinsic viscosity; Light scattering

1. Introduction

Over the years there has been a significant amount of research on pectin because it is a gelling and texturizing agent in foods, a dietary fiber, an important component in the cell walls of higher plants, and a substance with a growing number of recognized pharmacological activities. The structure and function of pectin has been reviewed thoroughly and a few selected reviews are given here [1–4].

It is now well accepted that pectin is a group of complex anionic polysaccharides whose backbone consists primarily of blocks of (1→4)-linked homogalacturonan interrupted by single (1→2)-linked rhamnose units [5]. More recently it has been determined that a much smaller percentage of the pectin backbone contain regions of rhamnogalacturonans with rhamnose side chains and xylogalacturo-

☆ The albedo or mesocarp is the white portion of the peel that consists of loose tissue with large intercellular spaces containing large amounts of pectin and hesperidin. See W.B. Sinclair, *The Biochemistry and Physiology of the Lemon and Other Citrus Fruits*, University of California, Division of Agriculture and Natural Resources, Publ. No. 3306, 1984, pp. 45 and 79.

☆☆ Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture above others of a similar nature not mentioned.

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nans in which xylose is the side chain [6]. The homogalacturonan blocks are copolymers of galacturonic acid and its methyl ester. In many naturally occurring pectins, 50% or more of the carboxyl groups are methyl esterified. Pectin also contains the neutral sugars galactose and arabinose. These often occur as side chains linked to the rhamnogalacturonan portion of the pectin backbone.

The solution properties of pectin have been studied frequently, often in an effort to obtain a better understanding of its functional properties [7]. Several authors have recognized the existence of pectin aggregates in solution as early as the 1970s [8–10]. These and subsequent studies are important in that pectin aggregates in solution under non-gelling conditions could be considered as precursors to gelation. As such, the nature of these aggregates could provide a basis for understanding whether or not a particular pectin would be a good gelling agent. Some of the most convincing evidence for the aggregation of pectin in solution comes from visualizing pectin aggregates by electron microscopy [11–14]. In addition to electron microscopy [13,14], we have provided evidence for the aggregation of pectin by membrane osmometry and end group analysis [15], by high-performance chromatography with [16–18] and without [19–21] online viscometry detection, and by IR spectroscopy [22].

The high value of food-grade pectin has prompted research on seeking methods of pectin extraction that increase the yield and quality of solubilized pectin. Conventionally, pectin is extracted in heated water (ca. 85 °C) from citrus peels at an acid pH range of 1–3 for periods in excess of 30 min. Manabe et al. [23] used microwave energy to extract pectin from mandarin orange pulp. They found that by using microwave energy, they could extract about 5% more pectin in 15 min than could be extracted by conventional methods in 60 min. Furthermore after 10 min, when 95% of the pectin has been extracted, microwave-extracted pectin had higher relative viscosity, anhydrogalacturonic content and degree of esterification than pectin extracted by conventional heating. These results appear to indicate that rapid heating with microwave energy has the potential to increase yield and quality of extracted pectin.

In this report we extract pectin from orange albedo by microwave heating in pressure-resistant, microwave-transparent cells under conditions of controlled pressure and temperature. Previously, it has been shown that similar types of cells could be used to rapidly solubilize high-molar-mass starches from their granules [24]. The molecular size, mass, intrinsic viscosity, anhydrogalacturonic acid content, neutral sugar content and degree of esterification of solubilized pectins were determined to measure the effect of microwave heating under pressure on pectin structure.

2. Experimental

Pectin.—Early Valencia oranges (EVO) were picked on 3/21/96 and received on 3/25/96. We thank Dr Karel Grohmann and Fred Osick of CSPL, ARS, USDA in Winter Haven, FL for supplying the oranges. Upon arrival, the flavedo was stripped from the skin with a potato peeler, followed by removal of the albedo with a paring knife. After cutting the albedo into small pieces, it was stored at −20 °C in sealed polyethylene bags until extraction.

Extraction.—A flow chart for the extraction and analysis of pectin from the albedo is given in Fig. 1. Microwave heating was performed in a CEM, model MDS-2000 microwave sample preparation system. Samples were irradiated with 630 W of microwave power at a frequency of 2450 MHz. The oven contained a circular, 360° rotating carousel that had the capacity to hold up to 12 microwave pressure digestion vessels. One vessel was equipped with temperature- and pressure-sensing devices that measured and controlled the temperature and pressure within the cell. The maximum pressure level was set at 50 ± 2 psi and the maximum temperature was set at 195 °C. For each experiment, six equally spaced cells were placed in the carousel. Experiments were run with HCl at pH values of 1.0, 1.5 and 2.0. Cells were loaded with either 1 or 5 g of albedo dispersed in 25 mL of acid solution. In one set of experiments the same

residue was extracted three times to determine the effect of repeated extractions on pectin structure and yield.

Carbohydrate analysis.—Selected fractions were assayed for total neutral sugars by the phenol–H_2SO_4 (PS) method [25] and for galacturonic acid by the Blumenkrantz–Asboe-Hansen (BAH) method [26]. The degree of methyl esterification for pectin was determined by hydrolyzing pectin with base, oxidizing the methanol released with alcohol oxidase (EC 1.1.3.13), and then quantitating the hydrogen peroxide released in the methanol oxidation by allowing hydrogen peroxide to react with the chromagen, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in the presence of peroxidase (EC 1.11.1.17) [27]. The enzymatic method for the determination of degree of esterification was tested on a high methoxyl commercial citrus pectin and gave results that agreed with the manufacturer's specifications within experimental error.

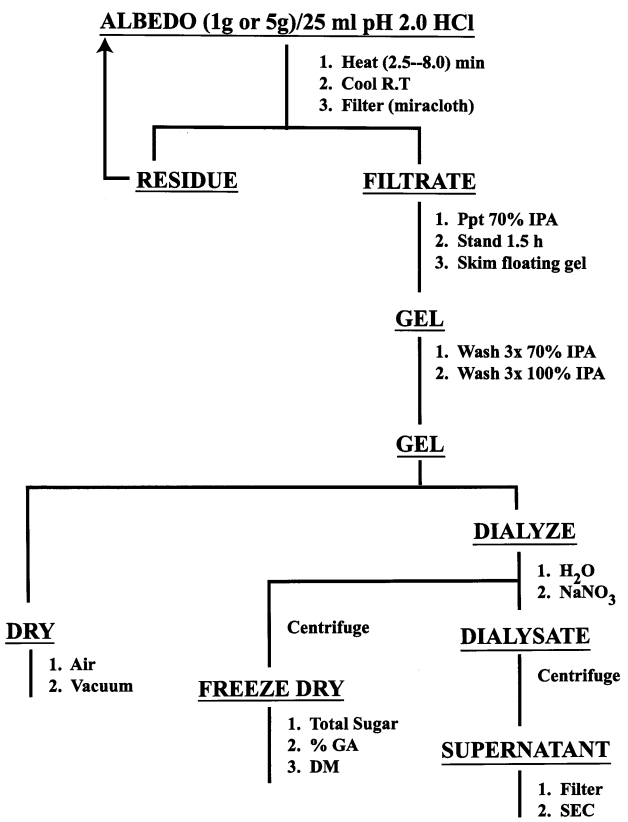

Fig. 1. Flow chart for pectin extraction, isolation and analysis.

Preparation of pectin for chromatography.—For commercial citrus pectin samples (Sigma), 20 mg of sample was dissolved in 10 mL of 0.05 M $NaNO_3$, placed in a covered beaker, stirred for 0.5 h and refrigerated overnight. For alcohol-precipitated EVO pectin, 0.5–1 g of the alcohol gel was dissolved in 10–20 mL of water, placed in a covered beaker and stirred at room temperature for 2 h, dialyzed for 1 day against water and 2 days against 0.05 M $NaNO_3$ (two changes of outside solvent/day). The dialysis tubing was Spectra/Por 6 with a molecular-weight cutoff of 10,000 Da. The dialyzed solutions were centrifuged at 50,000*g* for 10 min, and the supernatant was collected and refrigerated overnight. Immediately prior to analysis by chromatography, each sample was stirred for 15 min, and filtered with a 0.2-μm, sterile Millex 6v filter.

Chromatography.—Sample injection volume was 100 μL. The mobile phase was 0.05 M $NaNO_3$ filtered with a 0.4-μm Nucleopore (Costar Corp.) membrane filter before degassing. The nominal flow rate was 0.7 mL/min. Columns were thermoregulated at 45 °C by immersing them in a water bath.

The chromatography system consisted of a model KT-35 Shodex degasser (JM Science Inc.) connected in series to a model 1050 autosampler and pump (Hewlett–Packard Corp.), inline 0.2 μm vv Durapore membrane filter housed in a high pressure holder (Millipore Corp.), 15′ stainless steel warming coil, i.d. 0.04″ (1.02 mm), two (10 × 3.2 mm i.d.) Synchropak cartridge guard columns, one pre- and one post-column set (SynChrom, Inc.), three chromatography columns, model Dawn F multiangle laser light-scattering detector (MALLS) fitted with a helium–neon laser ($\lambda = 632.8$ nm) and a K-5 flow cell (Wyatt Tech.), a model 100 differential pressure viscometer detector (DP, Viscotek Corp.), and an ERC-7510 model differential refractive index monitor (DRI) (ERMA Optical Works, Ltd). The serially placed chromatography columns were two PL-Aquagel OH-60 and one OH-40 (Polymer Labs). The exclusion limits for these columns as specified by the manufacturer for polyethylene glycol are 2×10^7 and 1×10^5 g/mol, respectively. Each column was 7.5 mm i.d. × 300 mm length.

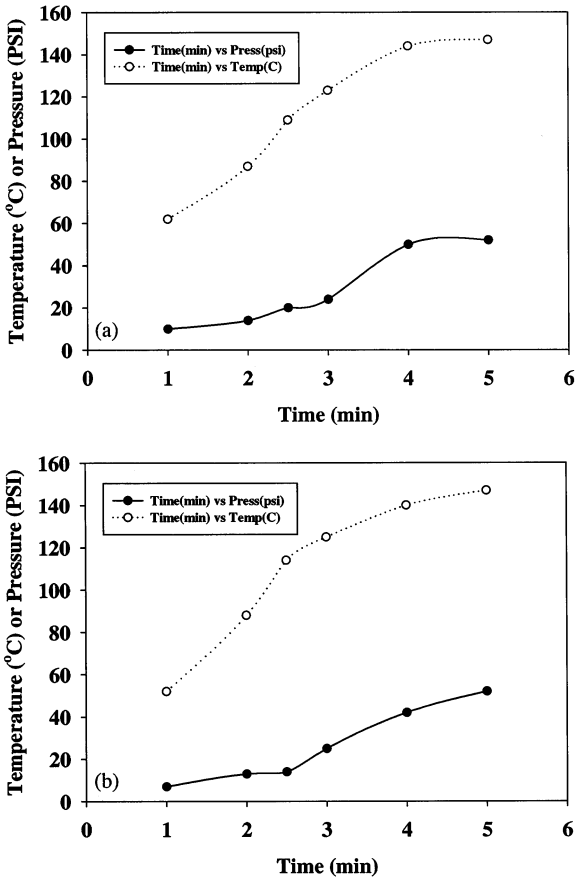

Fig. 2. Temperature and pressure as a function of heating time during pectin extraction. Solvent HCl, pH 2. (a) Sample load: 1 g/25 mL of solvent. (b) Sample load: 5 g/25 mL of solvent.

The electronic outputs from the MALLS at 90° scattering angle, DRI and DP detectors were sent to a Viscotek DM 400 model data manager, which in turn was interfaced to an Intel 486 computer containing Viscotek TriSec 3.0 GPC software. Simultaneously, the electronic outputs from the MALLS at 15 light-scattering angles and the DRI were sent to an A/D board housed in the MALLS, which in turn was interfaced to a second Intel 486 PC loaded with ASTRA™ (v. 2.11) and ASTRA™ (v. 4.2) software (Wyatt Tech.). The data was collected with ASTRA™ (v. 2.11) software and processed with ASTRA™ (v. 4.2) software. The DRI response factor was measured by injecting a series of known NaCl concentrations directly into the detector cell with a syringe. This response factor was obtained from the slope of the linear plot between NaCl concentration and RI response. The factor to correct the Rayleigh ratio at 90° (R_{90}) for instrument geometry was obtained by measuring the scattering intensity of toluene at 90° and tested with pullulan standards [24]. The responses to scattered light intensity of the photodiodes arrayed around the scattering cell at angles other than 90° were normalized to the diode at 90° with a P-50 pullulan standard. The scattering angles in degrees available for intensity measurements were 22.26, 29.11, 36.46, 44.72, 54.19, 65.02, 77.11, 90.00, 102.89, 114.98, 125.81, 135.28, 143.54, 150.89, and 157.74. As suggested by Jeng and Balke [28], molar masses and radii were extracted from data fit to Debye equations. Data were found to be best fitted by linear-least-squares to a first-order Debye equation. There was no ad hoc deletion of scattering angles in fitting the Debye equations, but closeness of line fit to the reduced excess light scattering at a particular angle was weighted. The weighting factor was based on the standard deviation of the scattering at each angle as compared with the average standard deviation of all detectors fitted. The standard deviation of the scattering intensity at the four lowest scattering angles was greatest. Generally, for these angles the standard deviation decreased with increasing scattering angle. The practical effect of this method was that in some cases the scattering intensity of light from the lower scattering angles fell far from the fitted line. The viscometer was checked with pullulan standards to insure that intrinsic viscosities were measured accurately. The concentration of EVO pectin was obtained from the area of its DRI chromatogram. This concentration was calculated using ASTRA™ software by inputing the concentration dependence of the refractive index (dn/dc). A dn/dc value of 0.146 mL/g at 670 nm was determined using acid-extracted lime pectin as the source. The method for measuring dn/dc has been described previously [29].

3. Results and discussion

In Fig. 2(a,b) are plots of temperature and pressure against time of microwave irradiation for sample loads of 1 and 5 g per 25 mL of acid solution at pH 2. The 1-g sample reached

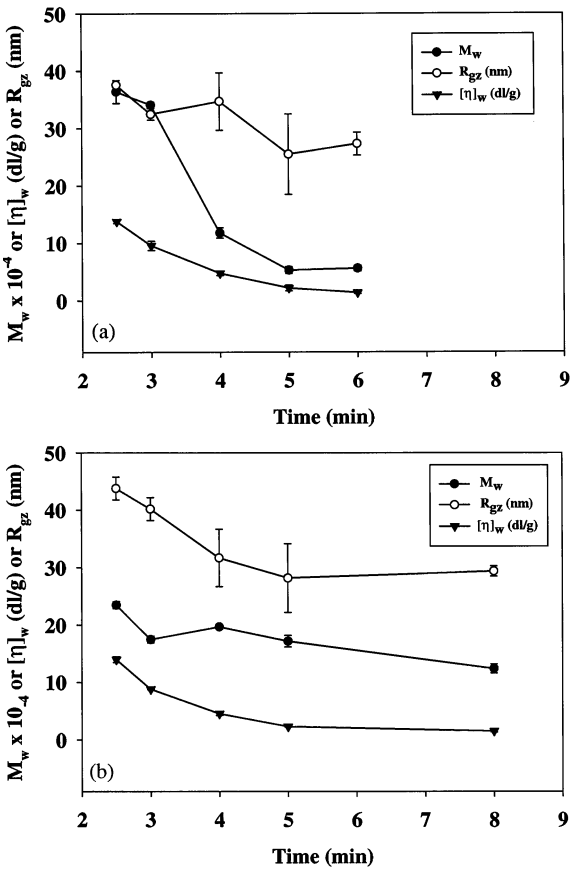

Fig. 3. Effect of heating time on pectin properties. (a) Sample load: 1 g/25 mL of solvent. (b) Sample load: 5 g/25 mL of solvent.

the maximum pressure limit more quickly than the 5-g sample. The maximum recorded temperature for both sample loads was 147 °C, but the 1-g sample again reached this temperature more quickly than the 5-g sample load. This latter result follows from the proportionality between time of heating (t) and weight of sample, i.e., solvent plus solid sample (m), as given by Eq. (1) [30].

$$t = C_p \Delta T m / P \quad (1)$$

where C_p is the heat capacity of the sample, ΔT is the change in temperature of the sample during irradiation and P is the average power absorbed by the sample. Thus, the smaller sample load will reach its temperature plateau more rapidly than the larger sample load. Since change in pressure is proportional to change in temperature when both are allowed to change freely, the smaller sample load also reached its pressure plateau more rapidly than the larger sample.

In Fig. 3(a,b), the effect of microwave heating time on weight-average molar mass (M_w), weight-average intrinsic viscosity $[\eta]_w$ and z-average rms radius of gyration (R_{gz}) of the isolated pectins was measured as determined by HPSEC with online multiangle light-scattering and viscometry detection. The properties of pectins with sample loads in the microwave of 1 g/25 mL (Fig. 3(a)) and 5 g/25 mL (Fig. 3(b)) are shown. For both sample loads, all three properties have a downward trend with increasing time of heating. Manabe et al. [23] found a similar trend for relative viscosity when they used microwave energy in an open vessel to extract pectin from the pulp of mandarin oranges. In that study they irra-

Table 1
Molecular properties of selected pectins

Sample	1 g/25 mL [a]			5 g/25 mL [a]		
	$M_w \times 10^{-5}$	R_{gz} (nm)	$[\eta]_w$ (dL/g)	$M_w \times 10^{-5}$	R_{gz} (nm)	$[\eta]_w$ (dL/g)
2.5 [b]	3.6(0.2) [c]	38(1)	10.8(0.1)	2.4(0.1)	44(2)	13.8(0.2)
2.5 [b,d]	3.4(0.1)	40(2)	10.3(0.1)			
3.0 [b]	3.4(0.1)	33(1)	7.7(0.5)	1.8(0.1)	40(2)	8.8(0.2)
CCP [e]	2.8(0.1)	38(1)	5.5(0.2)			
4.0 [b]	1.2(0.1)	35(5)	4.8(0.1)	2.0(0.1)	37(3)	4.5(0.3)

[a] Albedo/solvent extraction ratio.
[b] Time of microwave heating.
[c] Standard deviation of triplicate analysis.
[d] Dialyzed against 0.1% EDTA prior to dialysis against 0.05 M $NaNO_3$.
[e] Commercial citrus pectin control.

Table 2
Percentage of sugars in orange albedo pectin

Ext. time (min)	1 g/25 mL [a]				5 g/25 mL [a]			
	AGA [b]	NS [c]	AGAP [d]	DE [e]	AGA [b]	NS [c]	AGAP [d]	DE [e]
0 (dry albedo)	49(1)	7.3(0.4) [f]	88		49(1)	7.3(0.4)	88	
2.5	103(3)	4.2(0.2)	96	91(1)	84(2)	16(1)	84	91(1)
3.0	98(6)	7.2(0.1)	93	93(2)	96(1)	10(2)	91	69(1)
4.0	84(3)	11(1)	88	73(2)	84(1)	7.4(0.5)	92	80(2)

[a] Albedo/solvent extraction ratio.
[b] Anhydrogalacturonic acid.
[c] Neutral sugars.
[d] Anhydrogalacturonic acid in pectin.
[e] Degree of esterification.
[f] Standard deviation of triplicate analysis.

diated the pulp for three 5 min periods or a total of 15 min.

In this study, the highest values of properties were obtained after 2.5 min of heating for both sample loads. For heating times less than 2.5 min, insufficient quantities of pectin were obtained for analysis. Nevertheless, as shown by the data in Table 1, samples heated for 2.5 and 3 min gave M_w and $[\eta]_w$ values that were higher than those of the commercial citrus pectin (CCP) when the sample load was 1 g/25 mL. For the 5 g/25 mL sample load, $[\eta]_w$ was higher but M_w was lower. No difference in $[\eta]_w$ and M_w was found at 2.5 min heating time regardless of whether or not the pectin was dialyzed against 0.1% EDTA after dialyzing against water and prior to dialyzing against 0.05 M $NaNO_3$, thus indicating that the pectin was not aggregated due to Ca^{2+} crosslinks. At heating times of 4 min or higher (see Table 1 and Fig. 3(a,b)), $[\eta]_w$ and M_w values were lower than found for CCP.

In Table 2 are percentages of sugar and degrees of esterification (DE) for various samples measured here. The percentage of anhydrogalacturonic acid in pectin, AGAP, ranged from about 84% to about 96%. There was no discernible trend with heating time, although the AGAP at sample loads of 1 g/25 mL may have been slightly higher than the values found at sample loads of 5 g/25 mL. DE values ranged from 69 to 96% with no discernable trend with heating time, but slightly lower values of DE at the higher sample load, i.e., 5 g/mL. AGAP and DE were appreciably higher for the flash-extracted pectin in our fresh frozen orange albedo than for pectin extracted by conventional heating methods in fresh orange peels as reported in the literature [31]. In the conventionally heated pectin, AGAP and DE were about 71%.

In Table 3 are the percentage recoveries of pectin in dried albedo and of the total pectin in the albedo. Loss of moisture on drying the albedo was 70.8%. Total pectin in the albedo was obtained by combining percentages of anhydrogalacturonic acid and neutral sugars assayed colorimetrically in the albedo. Percentage pectin extracted was obtained by weighing and drying the material precipitated by alcohol from the acid extract. When the albedo:solvent ratio was 1:25 (w/v), 11.3% of the dried albedo or 20.2% of the pectin in the

Table 3
Percentage recovery of pectin in orange albedo

Ext. time (min)	1 g/25 mL [a]		5 g/25 mL [a]	
	Albedo [b]	Pectin [c]	Albedo [b]	Pectin [c]
2.5	4.3	7.7	2.9	5.2
3.0	11.3	20.2	3.3	5.9
4.0	8.3	14.8	8.0	14.3
5.0	11.3	20.2	11.0	19.6
6.0	11.3	20.2		
8.0			8.6	15.3

[a] Albedo/solvent extraction ratio.
[b] Percentage of dried albedo.
[c] Percentage of pectin in dried albedo (55.9% pectin).

Table 4
Molecular properties of sequentially extracted pectin [a]

Ext. no. [b]	$M_w \times 10^5$	R_{gz} (nm)	$[\eta]_w$ (dL/g)
1	2.6(0.2) [c]	37(4)	9.9(0.1)
2	2.4(0.1)	37(1)	8.3(0.2)
3	2.7(0.2)	34(4)	6.7(0.4)

[a] Albedo/solvent extraction ratio 1:25 (w/v).
[b] Extraction sequence.
[c] Standard deviation of triplicate analysis.

Table 5
Percentage recovery of pectin from sequential extraction [a] (2.5 min)

No.	Albedo [b]	Pectin [c]
1	7.2	12.9
2	2.8	5.0
3	5.3	9.5
Total	15.3	27.4

[a] Albedo/solvent ratio 1 g/25 mL.
[b] Percentage of dried albedo.
[c] Percentage of pectin in dried albedo (55.9% pectin).

albedo was recovered after 3 min of heating. At an albedo:solvent ratio of 5:25 (w/v), 11.0% of the dried albedo or 19.6% of the pectin in the albedo was recovered after 6 min of heating. Based on results from recovery data and on the molecular properties data (see Fig. 3), the 1:25 albedo:solvent ratio and 3 min of heating are the conditions of choice. The yield of pectin from the albedo (fresh weight basis) at 3 min of microwave heating was 3.30% as compared with a literature value of 3.36% for fresh orange peels heated by conventional methods [31].

In an effort to assess the effect of repeated extractions on pectin properties and yield, at an albedo:solvent ratio of 1:25, the same residue was extracted three times for a duration of 2.5 min per extraction by following the scheme outlined in Fig. 1. The data in Table 4 reveal that M_w and R_{gz} remained relatively constant for the three extractions, whereas $[\eta]_w$ decreased with an increasing number of extractions. Even in the case of the third extraction, $[\eta]_w$ was appreciably higher than the values obtained at 4 min or longer times of heating (see Fig. 3(a) and Table 2). From these results, we may conclude that some pectin already extracted and in solution is being de-

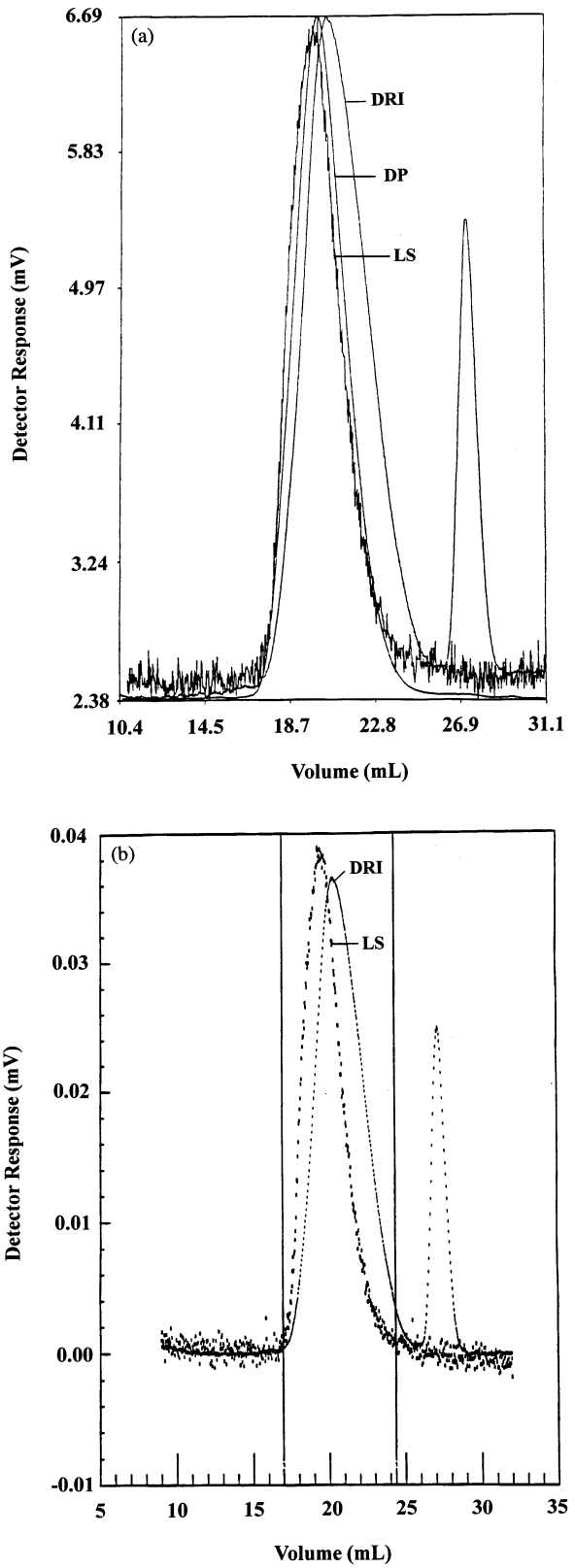

Fig. 4. Chromatograms for the pectin extracted after 2.5 min of microwave heating. (a) Generated by Trisec software, scattering intensity at 90°, LS, the DP and the DRI. (b) Generated by ASTRA™ software, scattering intensity at 90°, LS, and the DRI. Vertical lines indicate limits of integration.

Table 6
Comparison of pectin molecular properties [a]

Ext. time (min)	MALLS [b]		LS–viscometry [c]			
	$M_w \times 10^{-5}$	R_{gz} (nm)	$M_w \times 10^{-5}$	R_{gz} (nm)	$[\eta]_w$ (dL/g)	a [d]
2.5	3.6(0.2) [e]	38(1)	4.2(0.1)	62(1)	10.8(0.1)	0.71
3.0	3.4(0.1)	33(1)	4.2(0.1)	58(2)	7.7(0.5)	0.63
4.0	1.2(0.1)	35(5)	1.9(0.1)	39(3)	4.8(0.1)	0.75
5.0	0.53(0.06)	26(7)	0.53(0.01)	19(1)	1.8(0.2)	0.98
6.0	0.56(0.05)	27(2)	0.31(0.02)	15(1)	1.4(0.1)	0.99

[a] Albedo/solvent extraction ratio 1:25 (w/v).
[b] Determined by multiangle laser light scattering.
[c] Determined by combination of light scattering at 90° and viscometry.
[d] Mark–Houwink constant.
[e] Standard deviation of triplicate analysis.

graded as other pectin is being extracted. Pectin recoveries for the multiple extraction are given in Table 5. As can be seen from the data in Tables 4 and 5, with short multiple microwave extractions pectins can be obtained with better properties and higher yields than pectins obtained with conventional heat extraction.

In Fig. 4(a) are typical superimposed chromatograms of the scattering intensity at 90° (left), the DP (middle) and the DRI (right) for the pectin extracted after 2.5 min of microwave heating. In Fig. 4(b), are superimposed chromatograms of the scattering intensity at 90° (left), and the DRI (right) for the same data that was used to obtain the comparable chromatograms in Fig. 4(a). The chromatograms in Fig. 4(a) were generated by TriSec software, whereas the chromatograms in Fig. 4(b) were generated by ASTRA™ software. The basic concept of the LS (light scattering)–viscometry method is to calculate R_g from the Pititsyn–Eisner equation:

$$R_g = (1/6)^{1/2}([\eta]M/F)^{1/3} \quad (2)$$

where F is obtained from

$$F = 2.86 \times 10^{21}(1 - 2.63e + 2.86e^2) \quad (3)$$

and

$$e = (2a - 1)/3 \quad (4)$$

Here a is the exponent of the Mark–Houwink equation:

$$[\eta] = K'M^a$$

The viscosity $[\eta]$ and the Mark–Houwink exponent, a, of the fractionated polymer are measured with an online differential-pressure viscometer and the molar mass, M, of the fractionated polymer by an online light-scattering detector at the 90° scattering angle.

Using the LS–viscometry method and employing TriSec software, it is possible to calculate molar masses, intrinsic viscosities and radii of gyration from the data that generated the chromatograms in Fig. 4(a) [32]. Employing ASTRA™ software and using scattering intensities at all 15 angles at which the instrument is capable of making measurement, it is possible to calculate molar masses and radii of gyration from the data that generated the chromatograms in Fig. 4(b) [33].

Table 6 contains a comparison of data obtained from MALLS against data obtained from LS–viscometry. Results from both methods showed a decrease in M_w and R_{gz} with increasing time of microwave irradiation. Discrepancies in M_w between the two methods ranged from negligible to 50–60%, whereas discrepancies in R_{gz} ranged from about 11 to 60%. Measurements of M_w on a series of polybutadiene standards by LS–viscometry were found to be systematically high with discrepancies between 3 and 41% [32]. Generally speaking, one would expect the LS–viscometry method to give more accurate values at low molar mass and R_{gz}, whereas MALLS would be more accurate at high molar mass and R_{gz}. This expectation is based on the concept

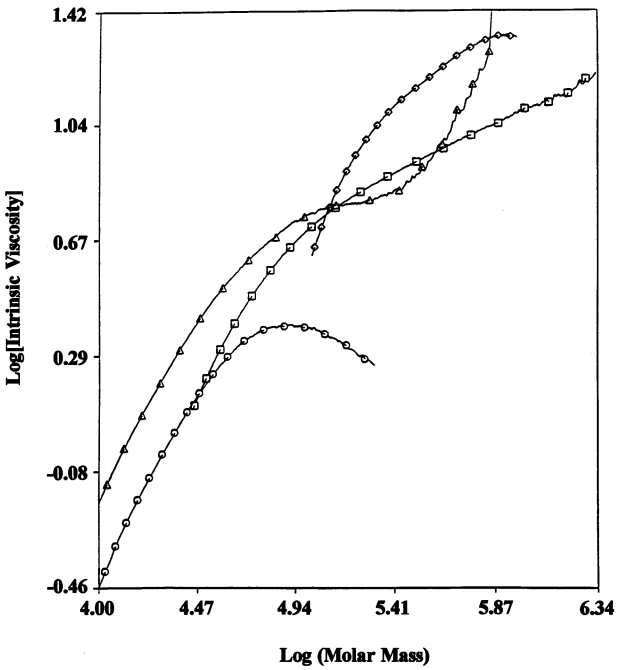

Fig. 5. Overlaid Mark–Houwink curves for pectins that were extracted after 2.5 (◇), 4.0 (□), 5.0 (△) and 6.0 (○) min, respectively.

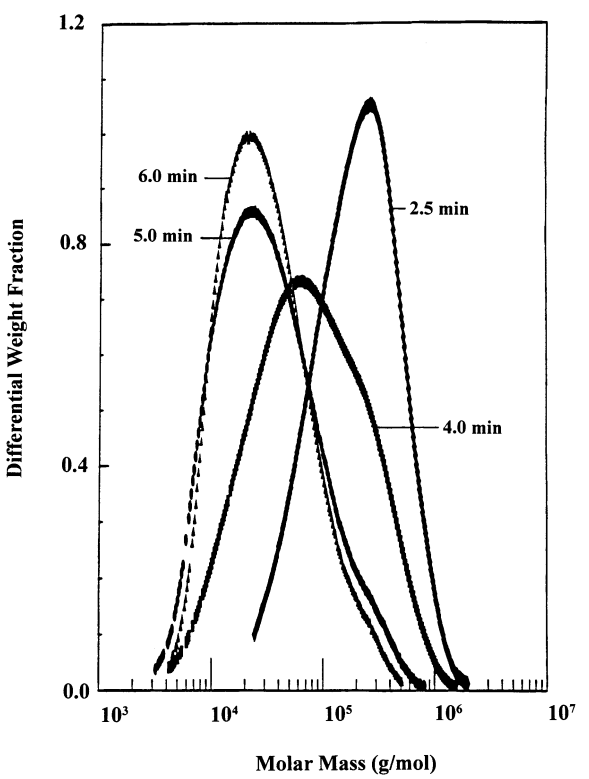

Fig. 6. Overlaid differential weight fraction against molar mass curves for pectins extracted after 2.5, 4.0, 5.0 and 6.0 min, respectively.

that viscometry is more sensitive than light scattering at low molar mass and size, whereas at high molar mass and size, angles lower than 90° are necessary for accurate determinations. Based on the data in Table 6, it would appear that for most cases both methods give reasonable values for molar mass, but molar mass values by the LS–viscometry method are systematically higher than by the MALLS method. Previously, for orange peel pectin, using HPSEC with universal calibration and online viscometry, we found about 25 nm for R_{gz} [21], about 2.1 dL/g for $[\eta]_w$ and about 1.2×10^5 for M_w [17]. The values of M_w and $[\eta]_w$ for this sample extracted for 1 h with ammonium oxalate at 90 °C fall somewhere between the values of our albedo samples that were extracted for 4 and 5 min, respectively. The value of R_{gz} falls between the 4- and 5-min values obtained by the LS–viscometry method, but not the MALLS method. Thus, in the case of R_{gz}, the two lowest values of R_{gz} by the MALLS method may be too high. Comparison of R_{gz} values at 2.5 and 3 min of microwaving reveals that the LS–viscometry method gives significantly higher values than the MALLS method. Possibly, in this case, the LS–viscometry method gives values of R_{gz} that are too high because of the reliance on estimated values of molar mass from the light-scattering intensity at 90°.

In the last column of Table 6 are Mark–Houwink (MH) constants obtained from the slopes of plots of log $[\eta]$ against log M. These values were obtained from plots such as those in Fig. 5 by employing Trisec software to determine the best linear least squares line through each data set. The MH values increased with increased heating time. This indicates that pectin becomes more rod-like in character with decreasing molar mass.

In Fig. 6 are overlaid differential weight fraction against molar mass curves for pectins that were extracted after 2.5, 4.0, 5.0 and 6.0 min, respectively. The differential molar mass distributions demonstrate that with increased heating, the distributions become bimodal. The bimodal nature of the sample microwaved for 6 min is also shown in Fig. 7. This Figure contains the chromatogram generated by the light scattered at 90° overlaid on the chromatogram generated by DRI detector. Previously, with images from electron microscopy

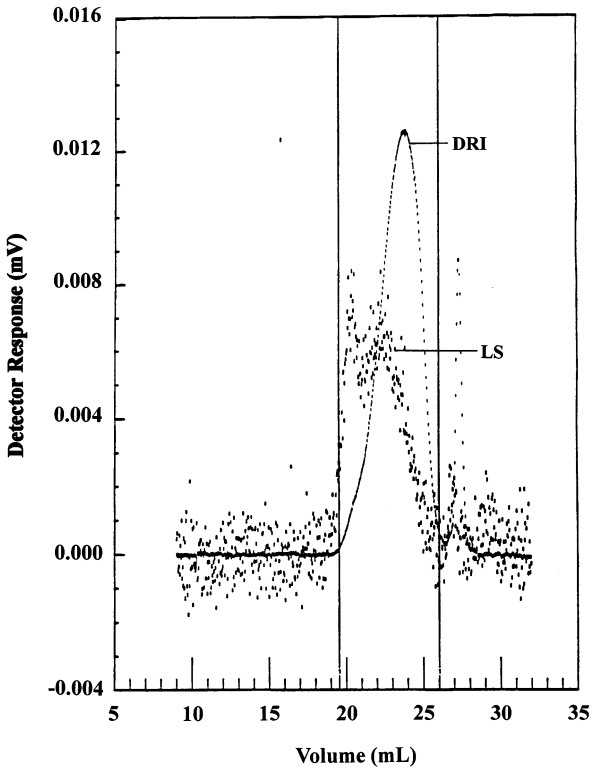

Fig. 7. Chromatograms for the pectin extracted after 6.0 min of microwave heating. Generated by ASTRA™ software, scattering intensity at 90°, LS, and the DRI. Vertical lines indicate limits of integration.

[13,14], we have demonstrated that dilute solutions of pectin in salt may exist as rods, segmented rods and kinked rods. These are components of microgels with an internal network structure that we have observed when pectin is dissolved under conditions of low ionic strength. The microgels were of the order of 1000 nm in diameter, whereas the dissociated components ranged from about 20–300 nm in contour length. A more detailed plausible explanation for increases in the MH constant with increases in heating time found in Table 6 is as follows. Flash extraction solubilizes pectin, which is a mixture of partially dissociated network structure (loose microgels) and network components fully dissociated from the network in the form of rods, segmented rods, and kinked rods. In this study, it appears that pectin extracted at a heating time of 2.5 min is almost entirely in the form of microgels. Thus, in the case of the pectin extracted for 2.5 min, the proportion of microgels to dissociated components is so large that the chromatogram generated by the light scattered at 90° is dominated by scattering from microgels (see Fig. 4). As heating time increases, the proportion of fully dissociated components increases at the expense of microgels. At a heating time of 6 min, the scattering from the dissociated components approaches that of the microgels, and the 90° light-scattering chromatogram is visibly bimodal (see Fig. 7). As seen by the DRI trace in Fig. 7, only a small portion of the sample is comprised of microgels. Nevertheless, because the microgels are significantly larger than the dissociated components, they scatter almost as much light at 90°. The general trend with heating is that high molar mass, relatively compact networks are being converted to less compact dissociated components. Hence, the MH constant increases with increasing heating time.

The MH values in Table 6 are average values for the entire distribution. In view of the evidence already cited, the curvilinear nature of

Table 7
Molecular properties of bimodal fractions

Fraction	Ext. time (min)	wt.% [a]	$M_w \times 10^{-5}$	R_{gz} (nm)	$[\eta]_w$ (dL/g)	a [b]
1 [c]	4.0	26(1) [d]	3.9(0.2)	36(3)	9.7(0.1)	0.45(0.01)
1	5.0	11(1)	3.3(0.1)	32(11)	4.4(0.6)	0.14(0.04)
1	6.0	8(1)	2.6(0.4)	26(5)	1.2(0.1)	
2	4.0	74(1)	0.78(0.03)	24(1)	3.3(0.1)	0.92(0.01)
2	5.0	89(5)	0.31(0.01)	13(1)	1.4(0.2)	1.2(0.2)
2	6.0	92(1)	0.25(0.01)	12(1)	1.2(0.1)	1.2(0.1)

[a] Weight percent of fraction.
[b] Mark–Houwink constant.
[c] M_w and R_{gz} in Fraction 1 distributions were calculated using ASTRA™ software, whereas M_w and R_{gz} in Fraction 2 distributions were calculated using Trisec software.
[d] Standard deviation of triplicate analysis.

the MH plots could be further evidence of the bimodal or possibly multimodal nature [17,20, 21] of the pectin distributions under study. The question arises as to whether approximating the entire distribution with a single MH exponent will lead correctly to the conclusion that the distribution is becoming more asymmetric with increased heating time. One method of checking the validity of this conclusion is to approximate the distribution with two MH exponents and determine if the results are consistent with the conclusion based on a single MH exponent for the entire distribution. In the case of pectins heated from 4 to 6 min, the bimodal nature of the distribution is clearly delineated by the light-scattering chromatogram. Therefore, we integrated the light-scattering chromatograms by parts using elution volumes coincident with the minimum in the 90° curve to separate the distributions. In this way we were able to calculate more accurately the average molecular parameters for the molecules in each distribution of the bimodal. The results of these calculations are given in Table 7. The M_w and R_{gz} from Fraction 1 were calculated using the MALLS method, whereas M_w and R_{gz} from Fraction 2 were calculated using the LS–viscometry method. The weight percent (wt.%) of pectin in Fraction 1 decreases from about 26 to 8% with heating time, whereas it increases from 74 to 92% in Fraction 2, indicating that Fraction 2 is increasing at the expense of Fraction 1. M_w, $[\eta]_w$, and R_{gz} decrease in both fractions with heating time. The MH exponents (a values) were 0.45 and 0.14 in Fraction 1, whereas the values were 0.92, 1.2 and 1.2, respectively,

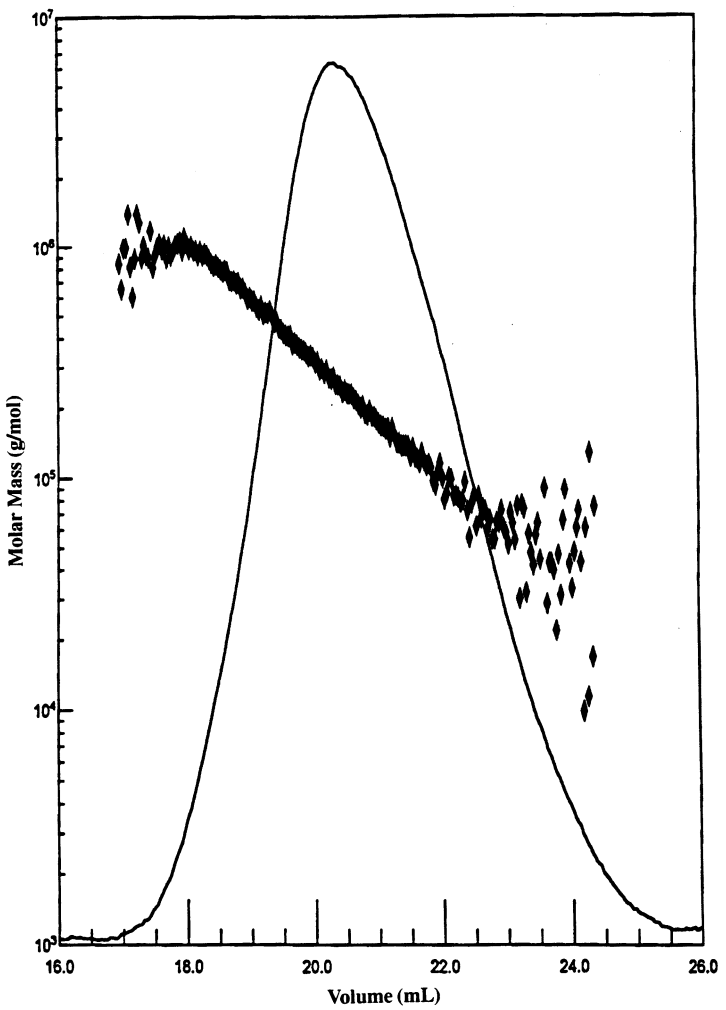

Fig. 8. Log molar mass plotted against elution volume (line) superimposed on DRI chromatogram (curve) for pectin heated for 2.5 min.

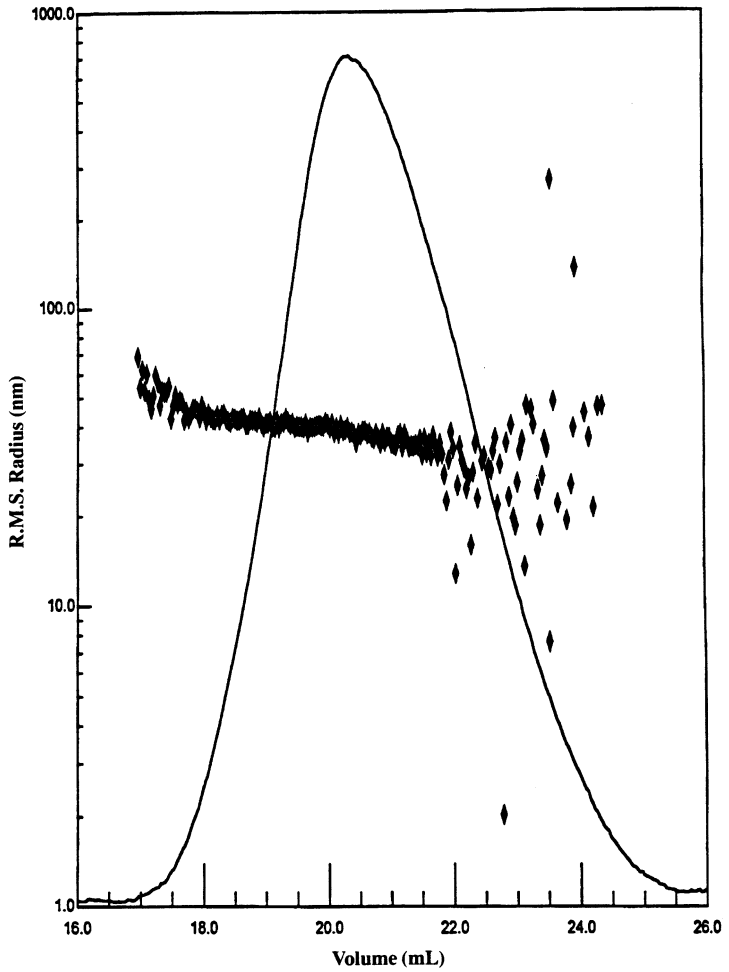

Fig. 9. Log rms radius of gyration plotted against elution volume (line) superimposed on DRI chromatogram (curve) for pectin heated for 2.5 min.

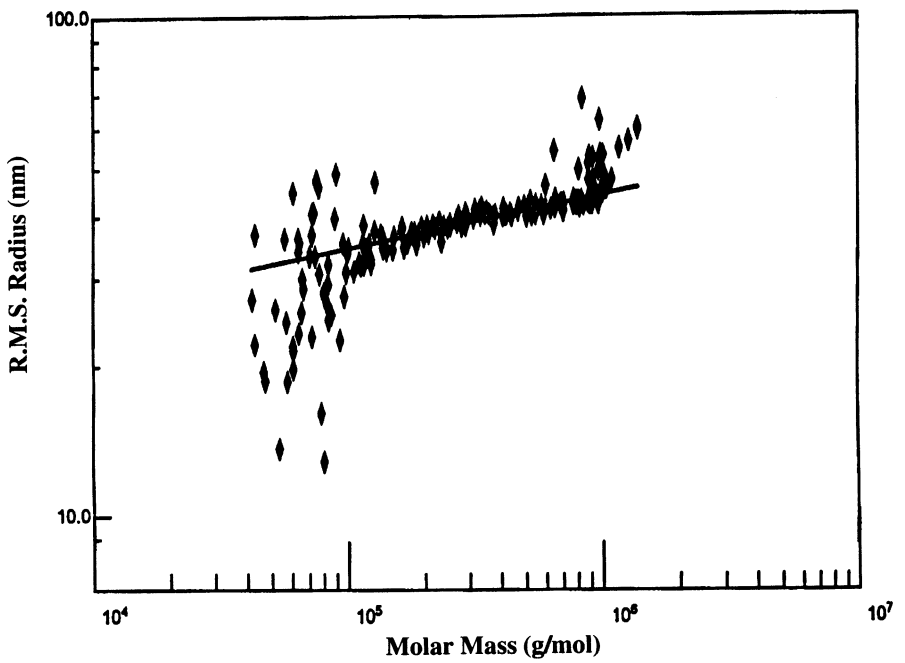

Fig. 10. Log rms radius of gyration plotted against log molar mass for pectin heated for 2.5 min.

in Fraction 2. The MH values that were below 0.5 for Fraction 1 and near or above 1 for Fraction 2 pectins are consistent with our interpretation of microgels in Fraction 1 and dissociated components in Fraction 2. The increase of Fraction 2 at the expense of Fraction 1 and the concomitant increase in the MH constant are consistent with the interpretation that relatively more compact microgels are being dissociated into their less compact components with increased time of heating.

In Figs. 8 and 9, log molar mass and log rms radius of gyration are plotted against elution volume for pectin heated for 2.5 min, whereas log rms radius of gyration is plotted against log molar mass in Fig. 10. Over the molar mass range 10^5–10^6, the rms radius changes by about 10 nm. The slope of the line is about 0.11, which is consistent with the formation of microgels [33]. This value of 0.11 for the slope is also consistent with the low values of *a* found for Fraction 1 MH plots in Table 6.

4. Conclusions

We have demonstrated that pectin, flash (rapidly) extracted from orange albedo by microwave heating under pressure has increased molar mass, size and intrinsic viscosity when compared with pectin extracted by conventional heating techniques. Based on evidence from size-exclusion chromatography with online molar mass and viscosity detection, we attribute the above increases in molecular properties to the solubilization of large, relatively compact, aggregated network structures when pectin is rapidly released from its plant matrix. Prolonged heating tends to dissociate these aggregates into smaller but more asymmetric components of the network.

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