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Component and Global Average Radii of Gyration of Pectins from Various Sources*

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ABSTRACT

Pectins solubilized from beet pulp, the peels of grapefruit, oranges, mandarin oranges, pomegranates and artichokes, the skin of garlic and peas, carrot and colocasia wastes, and garlic foliage were analysed by high-performance size-exclusion chromatography. Fractions were detected continuously by differential refractive index. The partially resolved components in each chromatogram were determined by computer-aided curve fitting. In all cases, the experimental chromatograms were fitted by a linear combination of five macromolecular species. The radius of gyration of each component and the number-, weight-, and Z-average radii of gyration were determined for pectins. Pectins were grouped according to the radius of gyration ratios of the fitted components. The most common set of implied size ratios was 1:2:4:8:15 which was found for carrot, beet, orange, artichoke, colocasia, mandarin orange and mango pectin. Several additional sets of implied size ratios such as 1:2:3:6:12, 1:2:3:6:13 and 1:2:5:10:17 were found. Based on these and earlier studies it was inferred that pectin may possess quaternary structure organized on the basis of size components and that quaternary structure may vary with pectin source.

*Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

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INTRODUCTION

Recently, in a study of pectins extracted from the cell walls of tomatoes and analysed by high-performance size-exclusion chromatography (HPSEC), it was found that the experimental chromatograms of all pectin fractions could be reconstructed as a linear combination of five macromolecular-sized species (Fishman *et al.*, 1989*a*). Universal calibration of the chromatographic columns in radius of gyration with a series of pullulan and dextran standards (Fishman *et al.*, 1987) revealed that the relative sizes of these pectins were 1:2:4:8:16. Here, we report the findings from this same method applied to pectins extracted from a variety of fruits and vegetables.

MATERIALS AND METHODS

Extraction of pectins

All pectins were extracted from fruit and vegetable by-products obtained from Egyptian food factories. Pectin sources extracted were beet pulp, the peels of mangoes, oranges, mandarin oranges, grapefruits, pomegranates and artichokes, the skin of garlic and peas, carrot and colocasia wastes, and garlic foliage. Typically, the pectin source was extracted for 1 h at 90°C with 0·5% (w/v) ammonium oxalate solution, precipitated with acidified alcohol, and dried (El-Atawy, 1984). Then 1 g dried pectin was dissolved in 60 ml of 0·01 M sodium phosphate buffer containing 0·01 M EDTA (pH = 6·05), stirred overnight at 4°C, dialysed against four changes of water over 48 h, centrifuged at 32 000 g for 1 h at 5°C to remove trace insolubles and lyophilized.

Gas chromatography

Pectins were methylated by 2 M HCl in methanol which was prepared by the dropwise addition of 6 ml acetyl chloride to 30 ml stirred methanol surrounded by an ice bath. These intermediate methyl glycosides were converted to the trimethyl silylate derivatives by the procedure which follows and analysed by GC for their constituent sugars. A 10-mg sample was weighed out in a 5-ml vial. A 0·5-ml aliquot of erythritol solution (4 mg/ml) was added and the mixture blown dry with a stream of air while being heated on a water bath. The methanolic HCl (0·5 ml) was added to the dry residue, the vial was sealed with a screw-top cap, and the sample maintained at 108°C in a heating block for 16 h.

Methanol (1 ml) was added to the cooled solution, which was then neutralized with silver carbonate. After centrifugation on a desk-top centrifuge, the clear supernatant was decanted into a 5-ml vial, dried and silylated with 0·5 ml Tri-sil 'Z' (Pierce Chemicals), the vial resealed and it was heated at 75°C for 0·5 h. A 1-μl sample was injected onto a 30-m J&W DB-1 bonded-phase fused silca capillary column. Gas chromatography was with a Perkin-Elmer, model Sigma 3B, and a flame ionization detector. Helium was the carrier gas and the derivatized sugars were detected by flame ionization. The detector was interfaced with an IBM 9000 lab computer which was driven by IBM software. The sugar composition of the various pectins is in Table 1. Neutral sugar reproducibility was $\pm 10\%$, by weight. Total recoveries ranged from 40% to 78% of the weighed sample.

HPSEC

The calibration curve for the column set is shown in Fig. 1. The column set consisted of micro-bondagel E-High, E-1000 (Waters Assoc., Milford, Mass.) and a Synchropak GPC-100 (Synchrom Inc., Linden, Ind.) in series. Column dimensions were $300 \times 3{\cdot}9$ mm for the micro-bondagel columns and $250 \times 4{\cdot}6$ mm for the Synchropak column. As

TABLE 1
Sugar Content[a] of Pectins

Sample	*GalA*[b]	*Ara*	*Gal*	*Rha*	*Glu*	*Xyl*	*Man*	*Tot*[c]	*Tot*[d]
Pomegranate	92·0	3·0	1·5	1·2	2·4	0·0	0·0	77·9	95·4 ± 0·3
Carrot	68·5	9·4	13·5	4·8	0·8	0·0	3·3	75·6	94·7 ± 0·8
Beet	83·6	3·0	6·8	6·0	0·7	0·0	0·0	70·3	96·9 ± 2·6
Orange	83·8	6·8	5·5	4·0	0·0	0·0	0·0	64·9	98·8 ± 0·5
Artichoke	91·7	3·4	1·8	1·4	1·6	0·0	0·0	73·3	92·7 ± 1·4
Colocasia	85·9	3·5	2·0	2·0	6·7	0·0	0·0	40·5	93·4 ± 1·2
Mandarin orange	87·3	4·1	6·0	2·6	0·8	0·0	0·2	66·6	91·8 ± 1·5
Mango	87·8	3·8	2·8	1·8	1·6	1·4	0·8	49·8	98·4 ± 0·5
Grapefruit	92·7	3·1	1·7	2·5	0·0	0·0	0·0	71·6	103·2 ± 0·1
Pea skin	81·1	4·4	4·0	2·0	6·8	0·4	1·3	54·5	70·3 ± 1·4
Garlic skin	93·3	0·0	2·0	2·8	0·0	0·0	0·0	59·7	97·4 ± 0·8
Garlic foliage	94·5	0·0	1·6	3·6	0·4	0·0	1·4	50·7	97·4 ± 0·8

[a]Percentage of total sugars.
[b]Gal A, galacturonic acid; Ara, arabinose; Gal, galactose; Rha, rhamnose; Glu, glucose, Xyl, xylose; Man, mannose.
[c]Percentage recovery from GC analysis.
[d]Percentage recovery from HPSEC analysis.

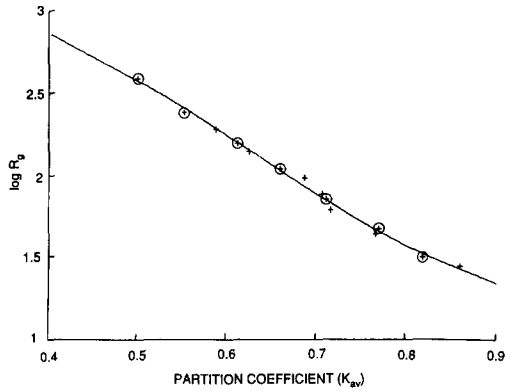


Fig. 1. Calibration curve. Standards in order of elution. In legend: P, pullulan; T, dextran. P800, 3·52 mg/ml; P400, 5·52 mg/ml; T500 16·9 mg/ml; P200, 9·24 mg/ml; T250, 16·7 mg/ml; P100, 13·9 mg/ml; T110 17·4 mg/ml; T70 20·4 mg/ml; P50, 20·8 mg/ml; T40 23·4 mg/ml; T20 23·1 mg/ml; P20 20·9 mg/ml; P10 20·0 mg/ml; T10 24·8 mg/ml. Mobile phase, 0·05 M NaCl; nominal flow rate, 0·05 ml/min; injection volume, 100 μl; detection, differential refractive index. On graph: ⊕, pullulan; +, dextran.

determined from the quarter-band width at half-height for sucrose, the column set was determined to have $5{\cdot}8 \times 10^4$ theoretical plates (Balke, 1984). Detection was by differential refractometry (Erma Optical Co., Tokyo). Columns were thermostatted at $35 \pm 0{\cdot}003$°C by immersing in a water bath. The refractometer had its own thermostatted cell, also set at 35°C. Injections were with a 100-μl sample loop.

To determine sample recoveries, areas of chromatograms such as those in Fig. 2 were calibrated by chromatographing known quantities of sodium polygalacturonate (NaPG) and high-methoxy sodium pectinate (NaHMP) derived from lime. For NaPG over the concentration range 1·03–5·03 mg/ml, the linear least-squares relationship for area in millivolt–millilitres (A, mv-ml) against concentration (c, mg/ml), was found to be:

$$A = 119{\cdot}8c - 37{\cdot}6 \qquad (1)$$

The standard error of A calculated from linear regression was 4·7 and the regression coefficient was 0·9995. Each concentration was run twice. A similar experiment in 0·05 M $NaNO_3$ was performed with NaHMP. In this case, the slope was 119·7 and the intercept was −11.3. Correlation coefficient and standard error in A were 0·9992 and 4·9, respectively. Thus, the negative intercept was treated as a systematic error of unknown origin and the regression line was forced through zero at its origin by setting the intercept to zero.

By assuming that the various pectins gave a differential refractive index (DRI) response identical to that of NaPG (Fishman *et al.*, 1989*a*), percentage recoveries of these pectins were calculated from the area of their chromatograms — eqn (1) with zero intercept — and the weight of injected sample. Recoveries were nearly quantitative with the exception of pea-skin pectin.

Curve fitting

Determination of overlapped components from chromatograms in which they were partially resolved was accomplished with the aid of ABACUS, version D.2 (Damert, 1984). ABACUS is a user-interactive command-driven program which fits chromatograms with a series of Gaussian-shaped peaks. Other peak shapes can be chosen, but we have found that narrow pullulan standards gave peak shapes which approached Gaussian. The position established by elution pattern and area of each peak within the experimental chromatogram is determined by minimizing the sum of the squares of the residuals between heights at each point of the calculated curve as compared to the experimental curve. Table 2 contains radii of gyration of the pectin components obtained by computer-aided curve fitting of chromatograms such as those in Fig. 2. Chromatograms (run in triplicate) for each pectin source were fitted to a linear combination of five macromolecular components. The radius of gyration for each component was found from the partition coefficient (K_{av}) of its peak maximum, by assuming that pectin components coelute on the column set with pullulans and dextrans of identical radii. Averaged values of R_g for the individual components agreed within experimental error with those found for tomatoes at three stages of maturity except for the component from peak 4 (Fishman *et al.*, 1989*a*). The R_g values of tomato pectin are included in Table 2 for purposes of comparison. The experimental chromatogram, the component peaks and the calculated curve at each point where it differs from the experimental curve is displayed on computer terminal and can be printed. Least-square minimization is by a non-linear approach (Draper & Smith, 1966). The number of peaks is estimated prior to the iteration. Three parameters can be iterated for each peak, namely the peak mean, height at maximum and width. Although ABACUS in its most general form does not yield a unique solution, it was found through iteration that all chromatograms could be fitted with five peaks and the same value for the component peak width at half-height. Furthermore, peak positions for each of the five component peaks clustered around a relatively narrow range of retention times as manifested by a variation of 10% or

TABLE 2
Radii of Gyration of Resolved Components[a] Peak

Sample	*1*	*2*	*3*	*4*	*5*
Pomegranate	458 ± 9	261 ± 9	134 ± 9	62·1 ± 5·1	26·8 ± 2·3
Carrot	400 ± 11	213 ± 8	104 ± 4	53·3 ± 3·0	26·3 ± 1·0
Beet	400 ± 13	211 ± 10	113 ± 7	57·9 ± 3·9	26·6 ± 2·7
Orange	391 ± 2	198 ± 4	103 ± 3	52·3 ± 2·9	24·3 ± 0·6
Artichoke	386 ± 3	210 ± 1	110 ± 1	59·1 ± 0·6	27·5 ± 2·3
Colocasia	381 ± 13	206 ± 7	107 ± 3	57·2 ± 1·3	28·9 ± 1·8
Mandarin orange	374 ± 13	194 ± 8	99 ± 1	49·5 ± 2·9	25·4 ± 1·2
Mango	366 ± 8	195 ± 6	104 ± 4	54·8 ± 3·2	27·1 ± 1·0
Grapefruit	347 ± 7	184 ± 6	102 ± 2	55·8 ± 1·5	26·9 ± 1·7
Pea skin	336 ± 9	175 ± 5	94 ± 1	53·8 ± 1·0	28·3 ± 0·7
Garlic skin	336 ± 6	174 ± 4	95 ± 1	55·2 ± 2·6	29·3 ± 1·9
Garlic foliage	293 ± 5	149 ± 12	77 ± 1	44·8 ± 3·6	23·5 ± 1·6
Tomato[b]	405 ± 32	206 ± 14	103 ± 7	46·6 ± 2·3	25·6 ± 0·7
Average R_g[c]	372 ± 39	198 ± 27	104 ± 13	54·6 ± 4·8	26·7 ± 2·1
Ratio	13·9	7·02	3·65	1·93	1
Implied ratio	14	7	4	2	1

[a]Angstroms.
[b]Data from Fishman *et al.* (1989*a*).
[c]Excludes data for tomatoes.

less for the deviation in radii of gyration for each of the components (Table 2).

Calculation of radius of gyration (R_g) and weight fraction (w_i)

Number-, weight- and *Z*-average radii of gyration for each of the pectins has been calculated according to eqns (2)–(4), respectively. These averages are defined in a manner analogous to the number-, weight- and

Fig. 2. Chromatograms of pectins. Mobile phase, 0·05 M NaCl; nominal flow rate, 0·5 ml/min; injection volume, 100 μl; detection, differential refractive index; thick line, experimental; thin line, calculated; peak components referred to in the text and in Fig. 3 by number are 1–5, left to right. (a) Pomegranate: 2·64 mg/ml; (b) carrot: 2·60 mg/ml; (c) beet pulp: 2·58 mg/ml; (d) orange: 2·57 mg/ml; (e) artichoke: 2·64 mg/ml; (f) colocasia: 2·69 mg/ml; (g) mandarin orange: 2·60 mg/ml; (h) mango: 2·51 mg/ml; (i) grapefruit: 2·70 mg/ml; (j) pea skin: 2·50 mg/ml; (k) garlic skin: 2·58 mg/ml; (l) garlic foliage: 2·67 mg/ml.

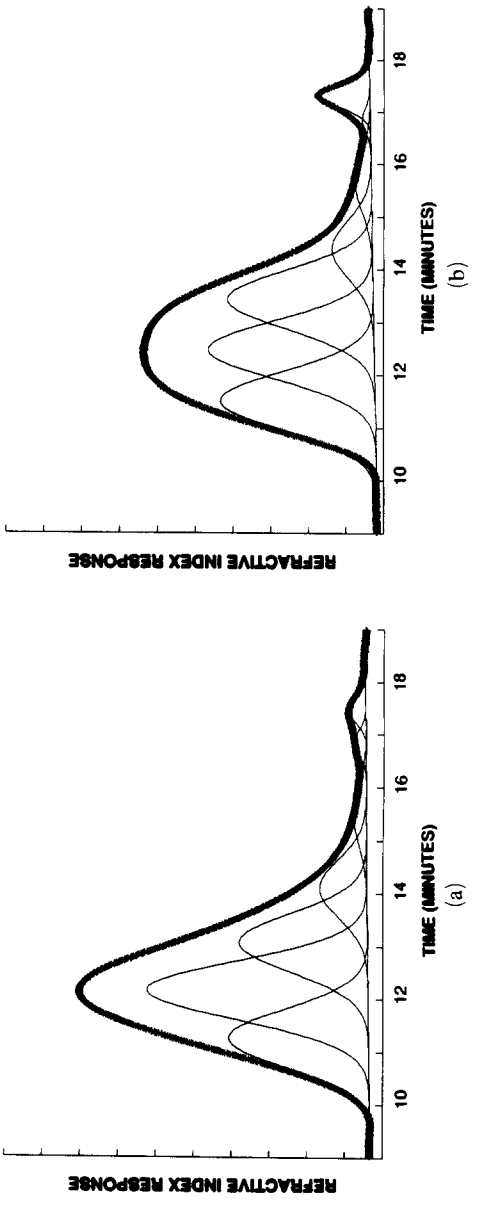
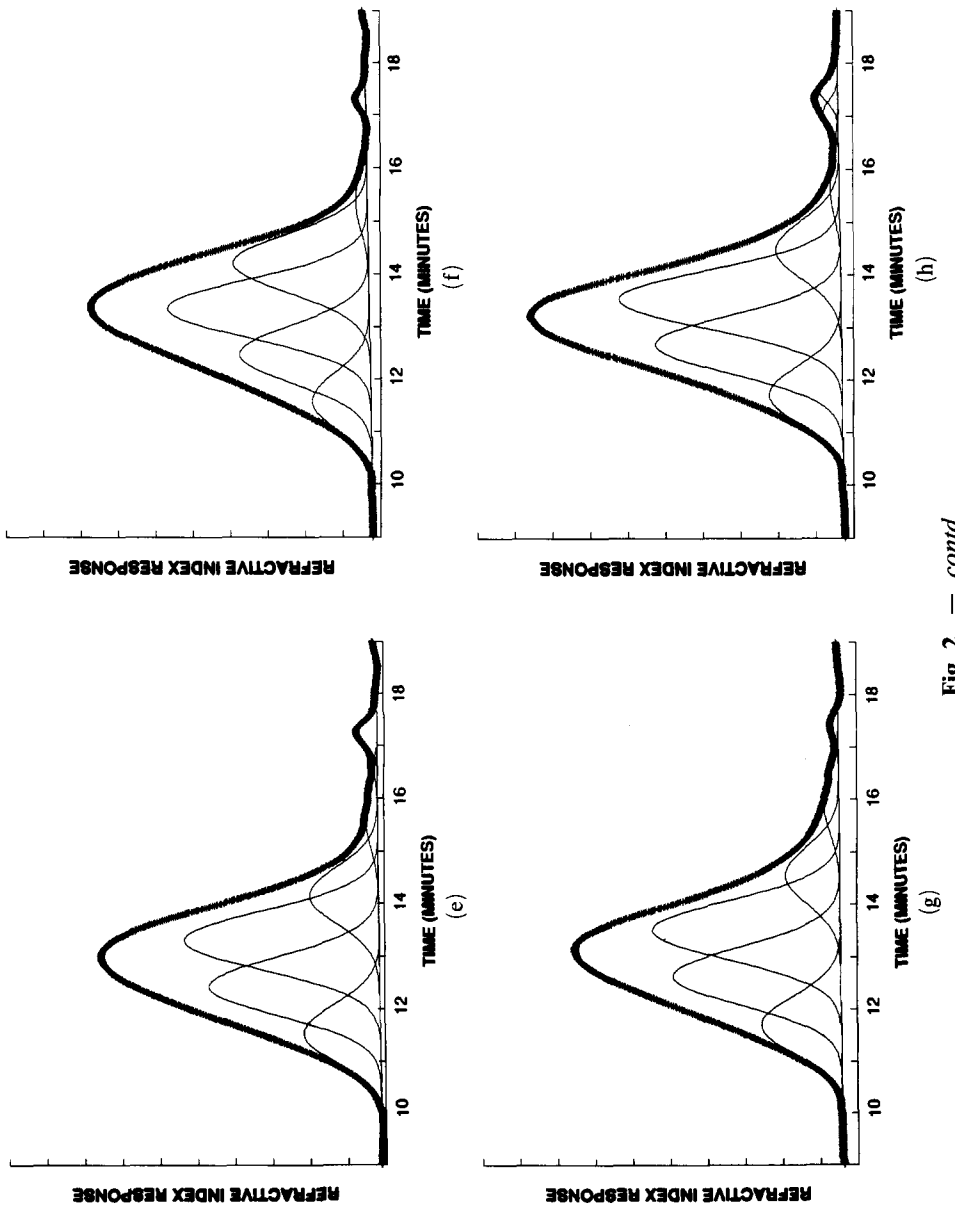


Fig. 2. — *contd.*



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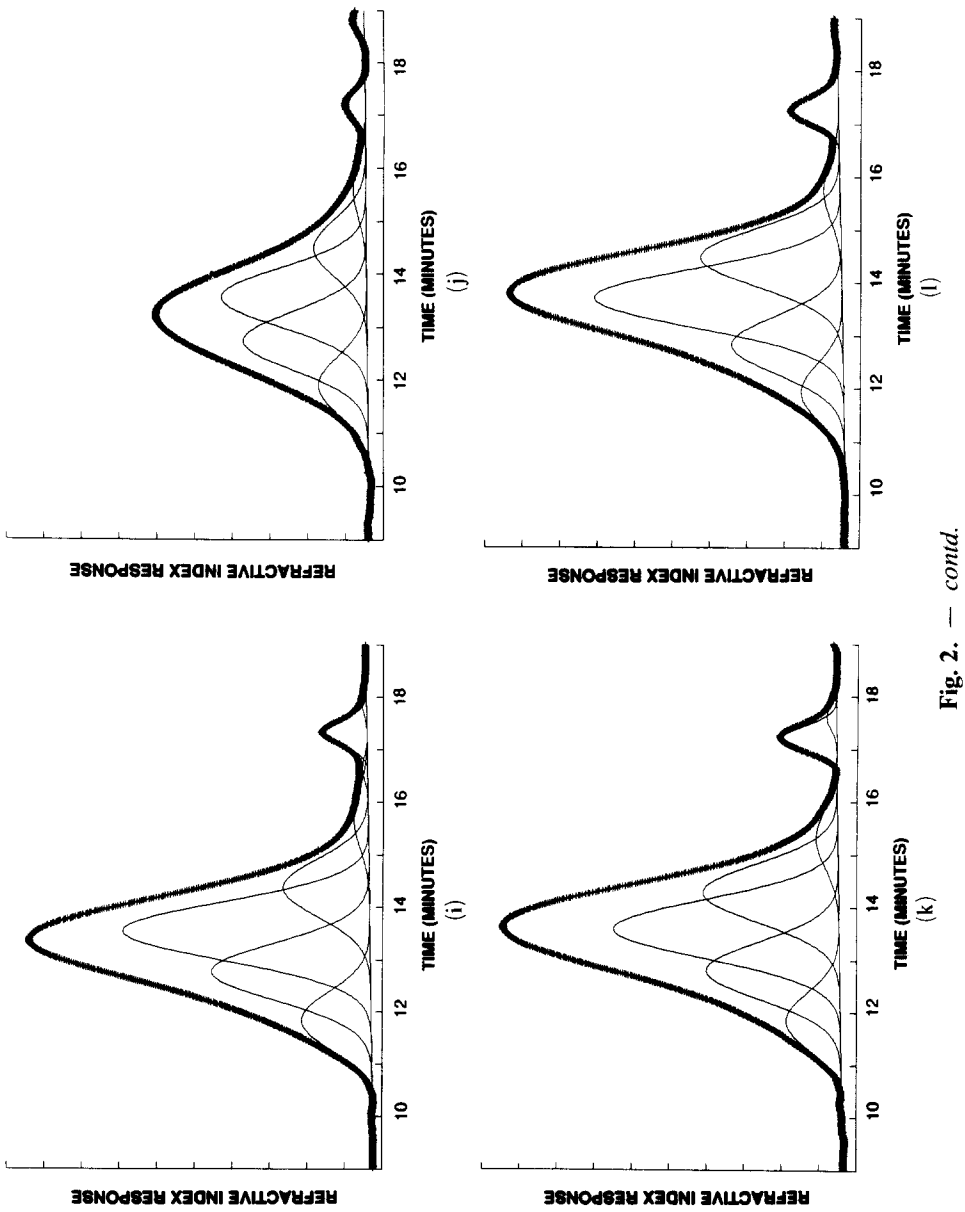

Fig. 2. — *contd.*

Z-average molecular weights with the radius of gyration replacing the molecular weight (Flory, 1953).

$$R_{gn} = 1/\Sigma(R_{gi}/w_i) \tag{2}$$

$$R_{gw} = \Sigma w_i R_{gi} \tag{3}$$

$$R_{gz} = \Sigma w_i R_{gi}^2/\Sigma w_i R_{gi} \tag{4}$$

These global averages were obtained by summing over the five macromolecular-sized species in each distribution and therefore are governed by the weight fraction, w_i, and the size of the five subunits.

Weight fractions of pectin components were calculated from the refractive index height at peak maximum (h_i) of the resolved components in the HPSEC chromatogram (Fig. 2), according to eqn (5).

$$w_i = h_i/\Sigma h_i \tag{5}$$

RESULTS AND DISCUSSION

Figure 2(a)–(l) contains HPSEC chromatograms of the various pectins, detected by DRI. Percentage recoveries calculated from DRI areas according to eqn (1) are contained in the last column of Table 1. Table 1 also contains the sugar content of the pectins as a percentage of total sugar recovered from the gas chromatography analysis. The percentage of recovered pectin based on amounts derivatized for gas chromatography is appreciably lower than values from refractive index detection of the HPSEC chromatograms. Possibly, derivatization was incomplete or these pectins contained significant percentages of moieties other than sugars. Nevertheless, except for carrot pectin, galacturonic acid content ranged from about 81% to 93% of sugars recovered from GC analysis. These percentages are typical for pectins. Also typical is the presence of rhamnose, galactose and arabinose as the most abundant neutral sugars (Jarvis, 1984).

Comparison of tomato pectin components with those studied here revealed that the standard deviation for the five components ranged from about 8% to 3% in the case of tomatoes and from about 14% to 8% for the pectins in this study (see Table 2). The rounded or implied ratio of the smallest-sized component (component 5 or the last eluted from the column set) to the others was 1:2:4:8:16 or 2^n for tomatoes, whereas it was 1:2:4:7:14 in this study. In an effort to group pectins whose mean component R_g had a standard deviation comparable to that of tomatoes, pectins from different sources were grouped by application

of Bonferroni T statistics (Miller, 1980) to the R_g values for each of the components in Table 2. It was found that the pectins could be subdivided into four groups according to significant differences in the R_g value of component 1. Group 1 included only pomegranate pectin. Group 2 encompassed carrot, beet, orange, artichoke, colocasia, mandarin orange, and mango pectins. Group 3 included grapefruit, pea-skin, and garlic-skin pectin. Group 4 included only garlic-foliage pectin. In the case of garlic foliage, the R_g of all components differed significantly from those of the other pectins, whereas for pomegranate pectin the R_g of components 1–4 differed from all other pectins. The R_g of component 5 from pomegranate pectin only differed from that of garlic foliage pectin. Grapefruit pectin could have been grouped with either group 2 or 3, but was included with 3 because the R_g of component 1 was closer to that of group 3 pectins than of group 2 pectins. Table 3 contains peak ratios and averaged radii according to group. The standard deviation of R_g for the means of the various grouped components is 9% or less. Four sets of implied ratios were obtained — namely, 1:2:5:10:17, 1:2:4:8:15, 1:2:3:6:12 and 1:2:3:6:13. Members of the largest group (2) have group values of R_g which do not differ appreciably from those found for tomatoes, but the largest component has an implied ratio of 15 rather than 16. The foregoing indicates that pectins from a variety of sources consist of five components. In this study 7 of the 11 have size components which do not differ statistically from each other as a group at the 95% confidence level. In 10 of 11, component 5 does not differ statistically in size. Nevertheless, the several implied ratios are evidence that the size polydispersity of pectin may vary with source.

Even though the size distribution of pectin is composed of a limited number of components, further diversity can occur through variation of the amount of each component. This diversity can be quantified by determining the weight fraction of each macromolecular component, w_i. The validity of eqn (5) rests upon the assumptions that each component has the same peak shape and that the quarter bandwidth (sigma) is constant throughout the length of the column. As in the case of the tomato fractions studied previously, the macromolecular portion of all the chromatograms could be fitted with a linear combination of five Gaussian peaks which had one sigma value — namely, 0·274 ml. This value was relatively close to the value of $0{\cdot}254 \pm 0{\cdot}007$ ml which was the averaged sigma value of the narrow pullulan standards, P800, P400, P200, P100 and P50. Most of the chromatograms had a small peak which eluted at or near the included volume. The origin of this small peak has not been determined. Possibly, it is a small pectic fragment(s) which was released from the pectin as it passed through the column.

TABLE 3
Pectins Grouped by Implied Ratios of R_g

Peak	*1*	*2*	*3*	*4*	*5*
Sample			*Ratios group 1*		
Pomegranate	17·1	9·8	5·0	2·3	1
Average R_g	458 ± 9	262 ± 9	134 ± 9	62·1 ± 5·1	26·8 ± 2·3
Average ratio	—	—	—	—	—
Implied ratio	17	10	5	2	1
			Ratios group 2		
Carrot	15·2	8·1	4·0	2·0	1
Beet	15·0	8·1	4·2	2·2	1
Orange	16·1	8·2	4·3	2·2	1
Artichoke	14·0	7·7	4·0	2·1	1
Colocasia	13·2	7·1	3·7	2·0	1
Mandarin orange	14·7	7·7	3·9	1·9	1
Mango	13·5	7·2	3·8	2·0	1
Average R_g^3	386 ± 15	204 ± 10	106 ± 5	54·9 ± 4·0	26·6 ± 2·0
Average ratio	14·5 ± 1	7·7 ± 0·4	4·0 ± 0·2	2·1 ± 0·1	1
Implied ratio	15	8	4	2	1
			Ratios group 3		
Grapefruit	12·9	6·8	3·8	2·1	1
Pea skin	11·8	6·2	3·3	1·9	1
Garlic skin	11·4	5·9	3·2	1·9	1
Average R_g^3	340 ± 9	178 ± 7	96·8 ± 4·1	55·0 ± 1·9	28·2 ± 1·8
Average ratio	12·0 ± 0·8	6·3 ± 0·8	3·4 ± 0·3	2·0 ± 0·1	1
Implied ratio	12	6	3	2	1
			Ratios group 4		
Garlic foliage	12·5	6·3	3·3	1·9	23·5
Average R_g^3	293 ± 6	149 ± 12	77·1 ± 5·3	44·8 ± 3·6	23·5 ± 1·6
Average ratio	—	—	—	—	—
Implied ratio	13	6	3	2	1

These were fitted with one or two Gaussian peaks which had the same sigma values as glucose: 0·116 ± 0·008 ml.

Figures 3(a)–(d) contain the weight fractions of pectins grouped in the same fashion as found in Table 3. Each distribution may be envisioned as a chromatogram with baseline separation of components. Thus the shape of the chromatograms in Fig. 2 can be explained by referring to the distribution of component weight fractions because of the propor-

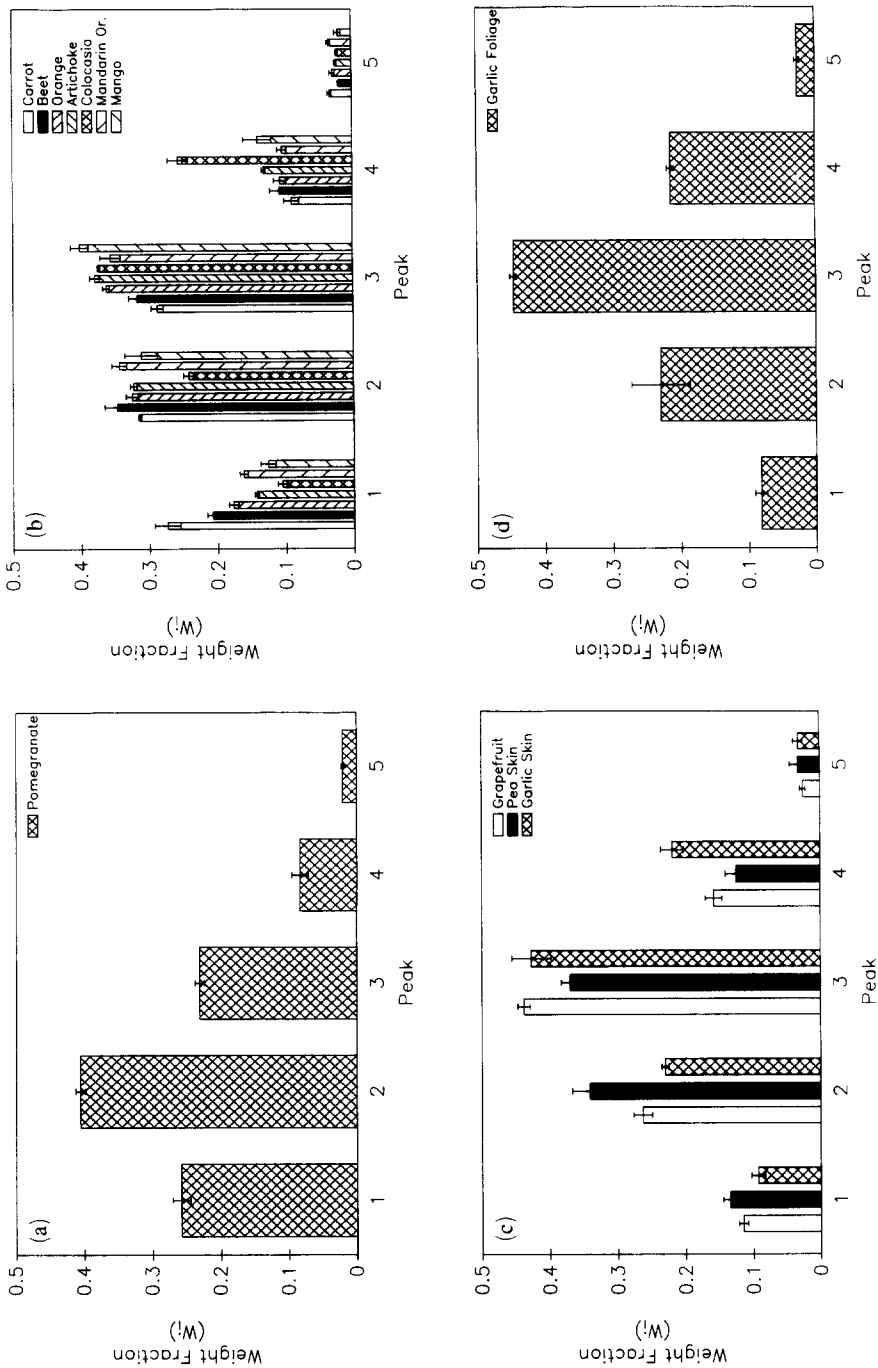

Fig. 3. Size distribution of pectins grouped by implied size ratio. (a) 1:2:5:10:17; (b) 1:2:4:8:15; (c) 1:2:3:6:12; (d) 1:2:3:6:13.

tionality between w_i and refractive index response. The chromatogram of pomegranate pectin — Fig. 2(a) — is skewed to the left because component 2 has the largest value of w_i, and the w_i of components 3–5 decrease in order — Fig. 3(a). In the case of carrot pectin whose distribution is contained in Fig. 3(b), the weight fractions of the first three components are about equal, followed by components 4 and 5 whose w_i decreases in order. Thus the w_i distribution and the chromatogram — Fig. 2(b) — tend to be initially flat at the top, but overall skew to the left. The chromatogram of garlic-skin pectin — Fig. 2(k) skews to the right because the w_i values of components 2 and 4 are symmetrical about 3, and component 1 has a larger w_i value than component 5 — Fig. 3(c). Table 4 contains the global averages calculated according to eqns (2)–(4). For the most part, R_{gn} is close to the radius of component 3, because the weight fraction is often greatest for this component. Usually, R_{gw} falls between the radius values of components 2 and 3, whereas R_{gz} is close to or greater than the radius of component 2.

CONCLUSION

We have shown that HPSEC chromatograms of pectins from a wide variety of sources could be reconstructed with a linear combination of

TABLE 4
Global Average Radii of Gyration[a]

Sample	R_{gn}[b]	R_{gw}[c]	R_{gz}[d]
Pomegranate	168 ± 6	261 ± 6	331 ± 6
Carrot	127 ± 2	213 ± 2	290 ± 4
Beet	132 ± 3	199 ± 4	267 ± 6
Orange	114 ± 2	178 ± 1	248 ± 1
Artichoke	119 ± 1	174 ± 1	235 ± 2
Colocasia	98 ± 1	145 ± 1	211 ± 4
Mandarin orange	108 ± 2	167 ± 1	230 ± 1
Mango	110 ± 3	157 ± 3	214 ± 2
Grapefruit	102 ± 1	142 ± 1	195 ± 1
Pea skin	90 ± 2	125 ± 3	176 ± 2
Garlic skin	90 ± 2	125 ± 2	176 ± 2
Garlic foliage	74 ± 1	103 ± 1	148 ± 1

[a]Angstroms.
[b]Number average.
[c]Weight average.
[d]*Z*-average.

just five macromolecular-sized species. This type of analysis should provide more accurate size distributions of pectins for correlations with its properties as a structural and metabolic component in plants, and as a functional and nutritional component in foods. Furthermore, these studies and earlier ones on tomato pectin may imply that pectin possesses a quaternary structure organized on the basis of these five components. In the case of tomatoes, these components could be dissociated by dialysis against salt which indicated that components comprised of pectic polymers held together at least partially by non-covalent forces. This last conclusion is consistent with the well-established propensity for pectin to exist as aggregates (Jordan & Brandt, 1978; Davis *et al.*, 1980; Fishman *et al.*, 1986, 1989*a*, *b*). Although the most prevalent set of implied size ratios appears to be 1:2:4:8:15, other ratios such as 2^n, 1:2:3:6:12, to name two of the more likely may also exist. Thus pectin quaternary structure may vary with plant source.

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