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Relationships between textural changes and the changes in linkages of pectic substances of sweet pepper during cooking processes, and the applicability of the models of interactions between pectin molecules

C. Y. Chang,[a]* L. R. Lai[b] & W. H. Chang[b]

[a]Department of Food Engineering, Da-Yeh Institute of Technology, Dah-Tseun, Chang-Hwa, Taiwan

[b]Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan

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Pectins in the alcohol-insoluble solids of sweet pepper (*Capsicum annuum* L.) were fractionated by sequential extraction with various solvents, and the changes in molecular size of the pectins in each fraction before and after further treatment with either heat or chelating agents were also investigated. Gel permeation chromatography was used to elucidate the changes in linkages of pectins and the textural change of sweet pepper tissue during cooking processes, and to search for the real existence of some sub-types in an elaborate model proposed for the interactions between A-, B-, and C-types of pectins. Based on the observed correlations between the contents of pectin fractions and the firmness of sweet pepper tissue, it can be deduced that the major forces for maintaining the texture of sweet pepper during cooking processes come from the pectin molecules linked by heat-labile bonds and covalent bonds. Moreover, from the molecular-size distribution of each pectin fraction before and after further treatment with heat or chelating agents, it can be concluded that the models proposed for the interactions between pectin molecules and other cell-wall constituents in vegetable tissues are reasonable and applicable.

INTRODUCTION

Most vegetables precooked at a moderate temperature for a suitable period of time and then cooked in boiling water showed higher firmness than those directly cooked without precooking (Chang *et al.*, 1986; Wu & Chang, 1990). This firming effect of precooking accords with the action of pectinesterase on the cell-wall materials, particularly pectic substances, and results in de-esterification of pectin molecules and subsequent formation of calcium bridges between free carboxyl groups of adjacent pectin molecules (Hoogzand & Doesburg, 1961; Hsu *et al.*, 1965; Bartolome & Hoff, 1972; Lee *et al.*, 1979; Van Buren, 1979). In order to understand the details of chemical changes in pectic substances which cause textural changes of vegetable tissues during cooking processes, we first proposed a simple, basic model (Fig. 1) consisting of five types of pectin interactions in the vegetable tissues; namely, S-type pectin which is extractable with cold water; A-type pectin extractable with cold solutions of chelating agents; B-type pectin extractable with hot water; C-type pectin which is co-stabilised by heat-labile bonds and Ca-bridges, and is extractable with hot solutions of chelating agents; and P-type pectin extractable with hot, dilute acid or alkali solutions (Chang *et al.*, 1993). Then, in order to better describe the detailed interactions between pectin molecules as well as other cell-wall constituents of different vegetables, we further proposed an elaborate model (Fig. 2) containing 12 sub-types, (a) to (l), of more complicated pectin interactions based on the simple, basic model, and it was found that in the tissue of snap bean pods, the sub-types (g) and (l) are the main, but not exclusive, structures contributing to its texture. Evidence for this came partly from the

* To whom correspondence should be addressed.

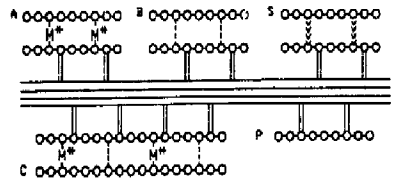

Fig. 1. A simple, basic model proposed for the bondings and interactions between the long chains of pectin molecules in vegetable tissues. o-o-o, pectin polymer; ≡, other cell wall materials; <<<<<, Van der Waals forces → CWP; -M⁺⁺-, metal-ion bridge → CHP; - - - -, heat-labile bond → HWP; =, covalent bond → HAP.

difference in calcium distribution among different pectin fractions obtained from the alcohol-insoluble solids (AIS) of the tissue before and after different cooking treatments, partly from the positive or negative values obtained by calculation for the C-type pectin according to the simple, basic model, and more importantly from the changes in molecular size of the pectins in each

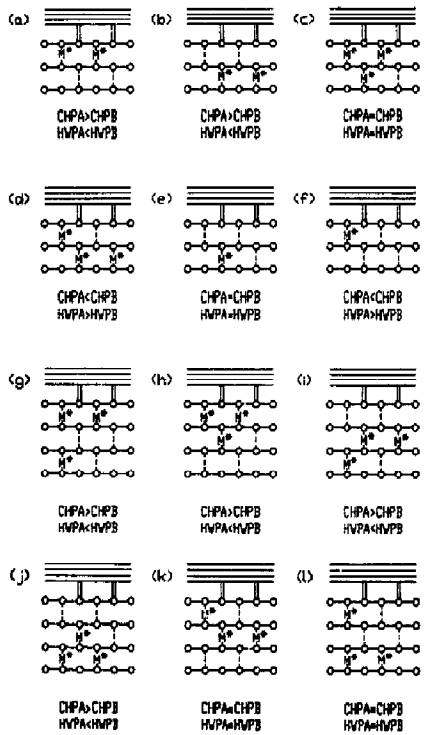

Fig. 2. Elaborate models proposed for the interactions between A-, B- and C-types of pectins, as shown in Fig. 1, in vegetable tissues, together with the comparison of the magnitude of CHP and HWP fractions between the two pectin extraction sequences, A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB → HAPB). (* Denotes incapable of comparison.)

pectin fraction before and after further treatment of the pectin fraction with either heat or chelating agent. In this study, pectins in the AIS of sweet peppers, after different cooking treatments, were subjected to the same types of experiments in order to examine the applicability of the methodology and the proposed models to other vegetables.

MATERIALS AND METHODS

Materials

Fresh sweet peppers (*Capsicum annuum* L.) were obtained from a local wholesaler. Raw materials of normal size and maturity, and free from decay or mechanical damage were selected for use. Both ends of the peppers were cut off, and only the middle part was used in the experiment. The trimmed sweet peppers were further sliced into five to eight pieces of about 6–7 cm length and about 2 cm width.

Methods

Texture measurement

A rheometer (Model NRM-3002D, Fudoh Kogyo KK, Japan), mounted with a cylinder-like plunger (adapter No. 6) of 3 mm diameter and 8 cm length, was used to measure the firmness of sweet peppers. The flat base, on which the sample was horizontally placed, moves upward to the plunger at a speed of 30 cm/min to measure the maximum puncture-through force as an index of firmness of the sample. For each sample, measurements were taken for each of five pieces of the sample, and the average of 15 measured values was expressed as relative firmness by taking the firmness of the fresh sample as 100.

Preparation of AIS

Sweet pepper tissue (100–200 g) was homogenized with five volumes of 95% ethanol, and the mixture was kept at 60°C for 40 min to facilitate protein coagulation and subsequent filtration. After cooling to room temperature, the suspension was filtered through filter paper, and the residue was sequentially washed twice with 80% ethanol and once with diethyl ether, and then dried in an oven at 40°C to obtain the AIS.

Pectin fractionation and analysis

Four pectin fractions were obtained by sequential extraction of the AIS by continuous shaking with different solvents by two sequences, A and B, as shown in Fig. 3. These procedures were modified from that of Manabe (1980), and the conditions were worked out after a series of experiments with several different vegetables (Chen, 1987; Tseng, 1987; Wang, 1987; Tu, 1989; Lai, 1991; Chang *et al.*, 1993). After each step of extraction and before proceeding to the next step of extraction, the residue was filtered, thoroughly washed with cold water, and the washings were combined with

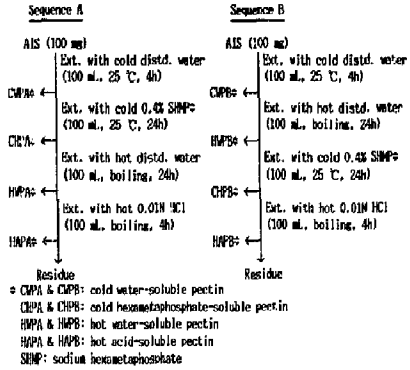

Fig. 3. Flow diagram for sequential extraction of pectin fractions by two different sequences, A and B, from the AIS of sweet peppers.

the respective extract. Triplicate samples of each pectin fraction extracted were then analyzed for their pectin content by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and the results were averaged and expressed as anhydrogalacturonic acid (AGA).

Gel permeation chromatography of pectins

The pectins in each fraction were subjected to gel permeation chromatography by using a Fractogel TSK HW-65(F) column (1·6 cm i.d. × 60 cm) and eluting with distilled water at a flow rate of 15 ml/h, and 3 ml fractions were collected.

Statistical analysis

The pectin fractionation was carried out in triplicate. Data were analysed by an ANOVA and Duncan's multiple-range test (mean comparison) by using the Statistical Analysis System program (SAS, 1985).

RESULTS AND DISCUSSION

The relationship between the changes in contents of pectin fractions extracted from the AIS by different sequences and the textural changes of sweet pepper

The content of each pectin fraction extracted by the sequence A (CWPA → CHPA → HWPA → HAPA) from the AIS of sweet peppers before and after different cooking treatments is shown together with the relative firmness of the tissues in Fig. 4, and the correlation coefficients between the contents of pectin fractions and the relative firmness of the tissues are shown in Table 1. In Fig. 4, the relative firmness values of the fresh and the precooked samples were not much different from each other. However, the relative firmness of the precooked and subsequently cooked sample was much higher than that of the directly cooked sample. This

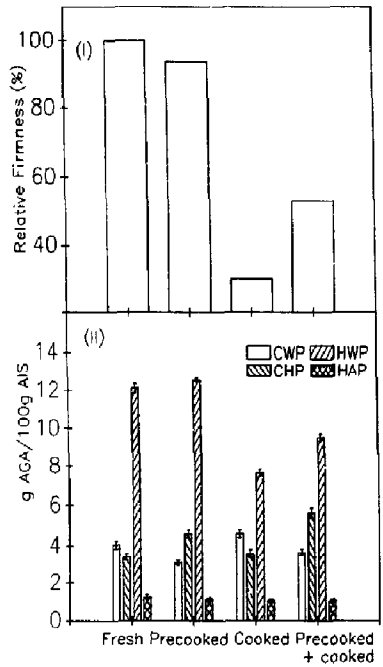

Fig. 4. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence A (CWP → CHP → HWP → HAP) from the AIS of sweet pepper before and after 10-min cooking in boiling distilled water, with or without 30-min precooking in distilled water at 70°C.

revealed that sweet peppers have the same firming effect of precooking treatment as some other vegetables such as mung bean sprouts (Chang *et al.*, 1993), radish (Chen, 1987), and snap bean pods (Chang *et al.*, 1993). In Table 1, the content of CWPA was negatively correlated to the firmness of the tissues ($P<0.05$), and the contents of both HWPA and HAPA were positively correlated to the firmness of the tissues ($P<0.01$ and $P<0.05$, respectively), while the content of CHP, which is linked by Ca-bridges and extracted with cold solutions of chelating agents, was not significantly correlated to the firmness ($P>0.05$). These results apparently contradict the theory of the firming effect of precooking treatment of vegetables, which was also observed in the case of mung bean sprouts (Chang *et al.*, 1993).

In order to investigate the reason for the contradictory results described above, the sequence B of pectin extraction (CWPB → HWPB → CHPB → HAPB) was tried again, as we did in the case of mung bean sprouts. The content of each pectin fraction extracted from the AIS of sweet peppers by the sequence B is shown together with the relative firmness of the tissues in Fig. 5, and the correlation coefficients between the contents of pectin fractions and the relative firmness of the tissues are shown in Table 1. The values of CWPB in Fig. 5

are practically identical to those of CWPA in Fig. 4, while the values of HAPB in Fig. 5 are almost the same as those of HAPA in Fig. 4, and therefore it appears in Table 1 that the CWPB values are negatively correlated to the tissue firmness ($P<0.05$), while the HAPB values are positively correlated to the tissue firmness ($P<0.01$). However, the values of CHPB in Fig. 5 are all much higher than those of CHPA in Fig. 4, while the relationships between the HWPB and HWPA values are reversed. The CHP in the sequence B, similar to that in the sequence A, was still found no significant correlation to the tissue firmness, while the HWP in the sequence B was the same as that in the sequence A with positive correlation to the tissue firmness. These results obtained from sweet pepper were similar to those obtained for mung bean sprout and snap bean pod (Chang *et al.*, 1993), and are worthy of investigation.

Table 1. The correlation coefficients between the firmness and the contents of various pectin fractions extracted by different sequences, A and B, from the AIS of sweet pepper before and after different cooking treatments

Pectin fraction	Correlation coefficient to firmness
Extraction sequence A[a]	
CWPA	-0·6002*
CHPA	-0·1329
HWPA	0·9833**
HAPA	0·6202*
Extraction sequence B[a]	
CWPB	-0·6002*
HWPB	0·9712**
CHPB	-0·2455
HAPB	0·8669**
Calculated value for C-type pectin	
CHPB-CHPA	-0·1299
HWPA-HWPB	0·4789

[a]A: CWPA → CHPA → HWPA → HAPA.
[b]B: CWPB → HWPB → CHPB → HAPB.
*$P<0.05$.
**$P<0.01$.

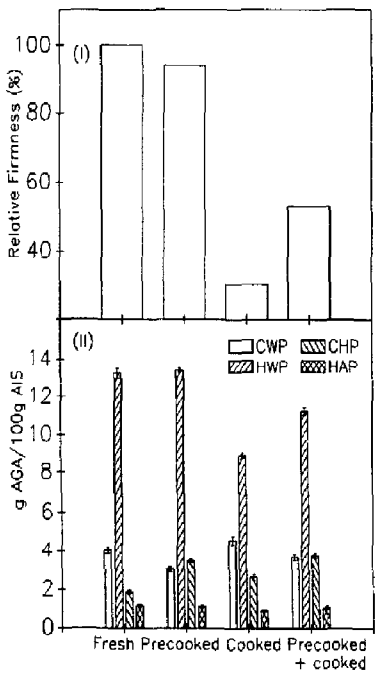

Fig. 5. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence B (CWP → HWP → CHP → HAP) from the AIS of sweet pepper before and after 10-min cooking in boiling distilled water, with or without 30-min precooking in distilled water at 70°C.

The amount of C-type pectin and the relationships between the amount of C-type pectin and the tissue firmness of sweet pepper

According to the simple, basic model of pectin interactions (Fig. 1), two kinds of bond (heat-labile bond and divalent-metal-ion bridge) co-exist in the C-type pectin. Therefore, the pectin fractionation would be affected by the sequence of pectin extraction. If a cold solution of chelating agents is used before hot water for pectin extraction (the sequence A), the C-type pectin will fall into the HWPA fraction. On the other hand, if hot water is used before a cold solution of chelating agents (the sequence B), the C-type pectin will fall into the CHPB fraction. Thus, the amount of C-type pectin can be estimated by calculation of the difference, either (CHPB–CHPA) or (HWPA–HWPB) (as shown in Table 2) (Chang *et al.*, 1993). The contents of various pectin fractions and the calculated amounts of C-type pectin in the AIS of sweet peppers before and after different cooking treatments are listed in Table 2. As shown in Table 2, the amounts of C-type pectin estimated for each cooking treatment using the above two methods of calculation were not significantly different ($P>0.05$). But the calculated amounts were all negative, which was also observed in the AIS of mung bean sprouts and snap bean pods after direct cooking (Chang *et al.*, 1993). This strongly implies the presence of more complicated bondings and/or interactions between pectin molecules and other cell wall constituents, as described in the elaborate model (Fig. 2), in the AIS of sweet pepper. But which of the 12 sub-types in this model really exists in substantial amounts in the tissue of sweet pepper is a question which needs further research and will be discussed in the following sections.

The correlation coefficients between the amounts of C-type pectin and the firmness of sweet pepper tissues before and after different cooking treatments are shown in Table 1. No significant correlations exist between the amounts of C-type pectin and the firmness of the sweet pepper tissues. However, the contents of the HWP and HAP fractions in both pectin extraction sequences, A and B, had significantly positive correlations to the firmness of the tissue. These results revealed that the major forces to maintain the texture of sweet pepper

Table 2. Calculation of various pectin fractions in the AIS of sweet peppers before and after different cooking treatments in distilled water

Pectin fraction[a]	Pectin type[b]	Content (wt%) in the AIS of sweet peppers[c]			
		Fresh	Precooked[d]	Cooked[d]	Precooked + cooked
CHPA	A	3·38	4·59	3·57	5·63
CHPB	A + C	1·88	3·47	2·64	3·76
HWPA	B + C	12·13	12·54	7·63	9·53
HWPB	B	13·23	13·40	8·87	11·26
CHPB-CHPA	C	-1·50a	-1·13b	-0·93c	-1·87d
HWPA-HWPB	C	-1·11a	-0·86b	-1·24c	-1·73d

[a]The pectin fractions were obtained by extraction according to the sequences of:
A: CWPA → CHPA → HWPA → HAPA
B: CWPB → HWPB → CHPB → HAPB.
[b]See Fig. 1 for the pectin types.
[c]Means with the same following letter in each column are not significantly different ($P > 0.05$).
[d]Precooked: 70°C, 30 min; cooked: boiling, 10 min.

are from the pectin molecules linked by heat-labile bonds (HWP) and covalent bonds (HAP), and very different from those in snap bean pods, which are from the C-type pectins co-stabilised by heat-labile bonds and divalent metal ion bridges.

Gel permeation chromatography of the cold chelating agent-soluble pectin and the hot water-soluble pectin extracted by different sequences from the AIS of sweet peppers

The pectin fractions extracted from sweet peppers before and after different cooking treatments were analyzed by gel permeation chromatography on Fractogel TSK HW-65(F) in order to investigate the chemical changes within pectin molecules in the sweet pepper tissues during cooking processes so that a judgement could be made of the most probable sub-types in the elaborate model of pectin interactions. Since the CHP and HWP fractions extracted from the AIS of sweet peppers were not only different from each other, but also different depending on the extraction sequences, that is, CHPA is different from CHPB, and HWPA is different from HWPB, the molecular sizes of these pectin fractions were analysed first, and the results are shown in Figs 6 and 7, respectively. In Fig. 6, it is observed that all the four CHPA fractions extracted from the AIS of sweet peppers by the sequence A contained mainly high-molecular-weight pectin molecules together with very small amounts of medium- and low-molecular-

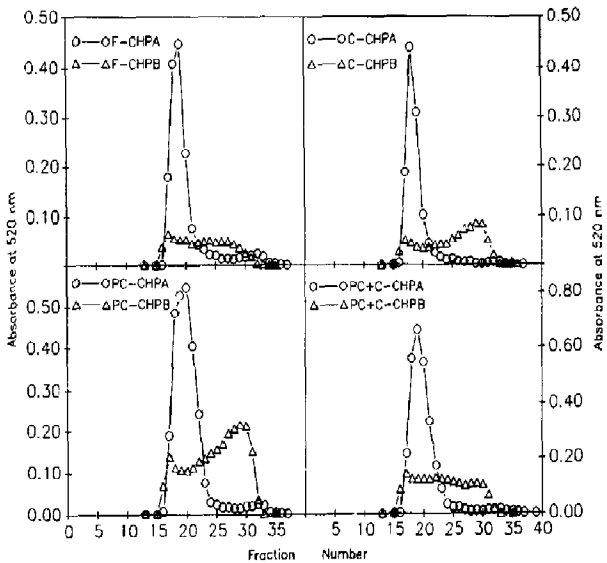

Fig. 6. Gel permeation chromatograms on Fractogel TSK HW-65 (F) of cold Na hexametaphosphate-soluble pectins (CHP) extracted by the sequences A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB →HAPB) from the AIS of sweet pepper before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 30 min; C: cooked by boiling for 10 min; both in distilled water.

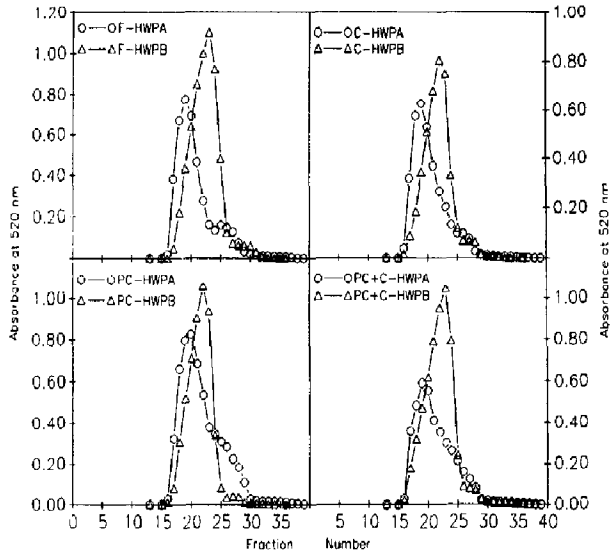

Fig. 7. Gel permeation chromatograms on Fractogel TSK HW-65 (F) of hot water-soluble pectins (HWP) extracted by the sequences A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB → HAPB) from the AIS of sweet pepper before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 30 min; C: cooked by boiling for 10 min; both in distilled water.

weight pectin molecules, whereas the CHPB fractions extracted by the sequence B contained much smaller amounts of high-molecular-weight and much larger amounts of medium- and low-molecular-weight pectin molecules. These results clearly indicate that most of the pectins solubilised by direct treatment of the AIS with chelating agent are large molecules consisting of many medium or small pectin molecules, which were linked to each other by heat-labile bonds; therefore if the AIS was subjected to heat-treatment before extraction with chelating agent (sequence B), the pectin molecules solubilised were much smaller in size.

With respect to the HWP fractions (Fig. 7), the molecular-size distributions of the four HWPA fractions extracted from the AIS of sweet peppers by the sequence A were almost the same, and all contained appreciable amounts of medium- and low-molecular-weight pectin molecules. In the four HWPB fractions, the molecular-size distributions were slightly shifted from a high-molecular-weight fraction to medium- and low-molecular-weight fractions by comparing with the HWPA fractions, and were obviously not different between cooking treatments.

Most probable sub-types of and evidence for the interactions between A-, B-, and C-types of pectins existing in sweet pepper

Since the simple, basic model for the interactions between pectin molecules could not explain the negative values calculated for the amount of C-type pectin in the AIS of sweet peppers, and the molecular-size distributions in the CHP and HWP fractions extracted by the sequences A and B from the AIS of sweet peppers were different from each other, we now try to use the elaborate model here to explain these uncommon results and examine the applicability of this model to other vegetables. During the process of pectin extraction by the sequence B (CWPB → HWPB → CHPB → HAPB), HWPB was extracted before CHPB, and thus the heat-labile bonds in the C-type pectin would have been broken during HWPB extraction and the remaining Ca bridges would be broken during the following CHPB extraction, and thus the C-type pectin would consequently fall into the CHPB fraction. The medium- and low-molecular-weight pectin molecules in the CHPB fractions in Fig. 6 can therefore be temporarily considered as C-type pectin, which would be extracted with hot water after chelating-agent extraction by the sequence A. However, the HWPA fractions in Fig. 7 contained much smaller amounts of medium- and low-MW pectin molecules than those in the CHPB fractions (Fig. 6). Thus, it can be deduced that the C-type pectins in the AIS of sweet pepper are not medium or small molecules but are large molecules like those in the AIS of snap bean pods, and that the appearance of medium- and low-molecular-weight pectin molecules in the CHPB fractions is due to the interactions between A-, B-, and C-types of pectins as shown in Fig. 2.

In view of the appearance of medium- and low-MW pectin molecules in the CHPB fractions, the sub-types possibly existing in the sweet pepper tissues are (a), (c), (d), (f), (g), (h), (k), and (l) as shown in Fig. 2. In addition, the negative calculated values of CHPB-CHPA or

HWPA–HWPB for the amount of C-type pectin reveal that the sub-types (a), (b), (g), (h), (i), (j), (k), and (l) also possibly exist in the sweet pepper tissues. From these two observations, it can be concluded that the

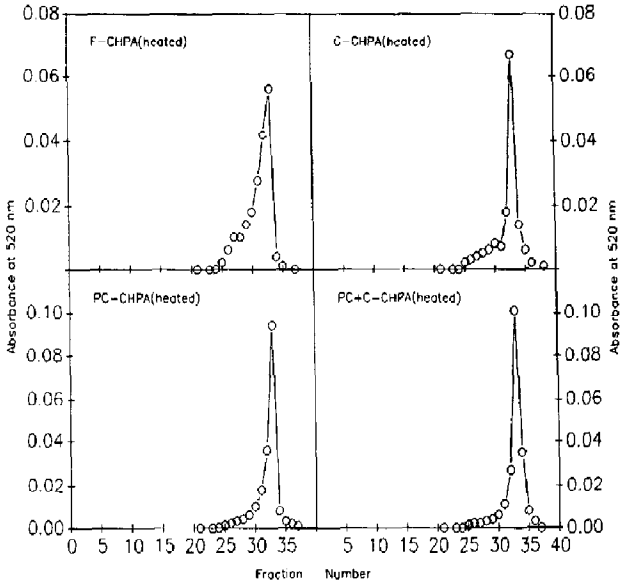

Fig. 8. Gel permeation chromatograms on Fractogel TSK HW-65 (F) of the products of 24 h boiling-water heating of the cold Na hexametaphosphate-soluble pectins (CHP in Fig. 6) which were extracted by the sequence A (CWPA → CHPA → HWPA → HAPA) from the AIS of sweet pepper before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 30 min; C: cooked by boiling for 10 min; both in distilled water.

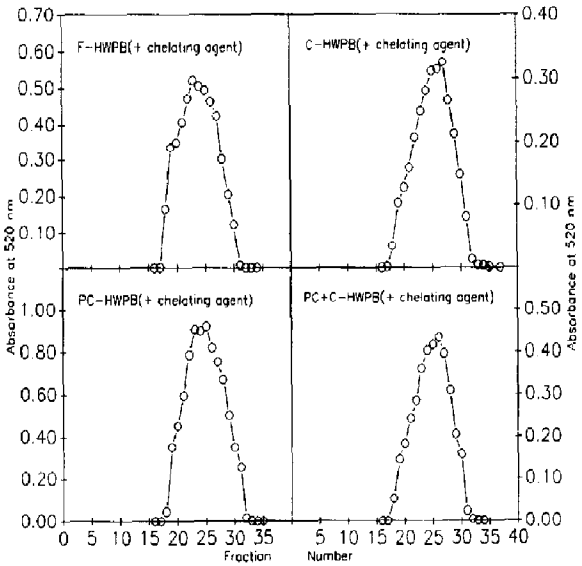

Fig. 9. Gel permeation chromatograms on Fractogel TSK HW-65 (F) of the products of 0·4% Na hexametaphosphate treatment of the hot water-soluble pectins (HWP in Fig. 7), which were extracted by the sequence B (CWPB → HWPB → CHPB → HAPB) from the AIS of sweet pepper before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 30 min; C: cooked by boiling for 10 min; both in distilled water.

most probable sub-types of pectin interactions existing in large amounts in the sweet pepper tissues are sub-types (a), (g), (h), (k), and (l) in Fig. 2.

If the sub-types (a), (g), (h), (k), and (l) really exist in substantial amounts in the sweet pepper tissues, then, if the CHPA and the HWPB fractions are subjected to further treatments with heat in boiling water and with chelating agents, respectively, appreciable amounts of medium- and low-molecular-weight pectin molecules should appear on gel permeation chromatography. In Figs 8 and 9, it is observed, as expected, that the molecular-size distribution of pectins in the CHPA fractions shifted from a high-molecular-weight fraction (Fig. 6) to medium- and low-MW fractions (Fig. 8) after boiling for 24 h in distilled water, and the molecular-size distribution of pectins in the HWPB fractions also shifted from a high-molecular-weight fraction (Fig. 7) to medium- and low-molecular-weight fractions (Fig. 9) after treatment with chelating agent. These results are in accordance with those mentioned above, and can be taken as further evidence for the existence of sub-types (a), (g), (h), (k), and (l) of pectin interactions in the sweet pepper tissues.

CONCLUSION

In this study, the estimated amounts of C-type pectin by calculation of either (CHPB-CHPA) or (HWPA–HWPB) in the AIS of sweet pepper before and after different cooking treatments were not significantly different from each other ($P > 0.05$). This result strongly confirms again that the simple, basic model for the bondings and/or interactions between pectin molecules and other cell wall constituents, particularly for the presence of C-type pectin, is reasonable and reliable. Moreover, from the comparison of the molecular size of pectins extracted from the AIS of sweet peppers before and after further treatment with either heat or chelating agent, it has been proven that among the 12 sub-types of pectin interactions in the elaborate model we proposed, (a), (g), (h), (k), and (l) sub-types really exist in substantial amounts in the tissue of sweet peppers. This also means that the elaborate model for the interactions between A-, B-, and C-types of pectins is applicable and reliable.

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