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Models for the interactions between pectin molecules and other cell-wall constituents in vegetable tissues

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In experiments to study the relationships between textural change and chemical changes in pectins of vegetables after different cooking treatments, pectins in the alcohol-insoluble solids (AIS) of the vegetables were fractionated into four groups according to their solubility in different extractants, and their yields were correlated with the firmness of the tissues. Based on the correlations observed for various vegetables, a simple model consisting of five types of basic pectin interactions in the tissues was proposed first. It includes S-type pectin extractable with cold water, A-type pectin extractable with cold chelating-agent solutions, B-type pectin extractable with hot water, C-type pectin extractable with hot chelating-agent solutions, and P-type pectin extractable with hot dilute acid or alkali solutions, among which C-type pectin was obtained by calculation rather than direct determination. It was found later that, in some cases, the amount of C-type pectin thus obtained came out as a negative value, which is apparently unreasonable. We therefore proposed, by speculation, an elaborate model consisting of twelve sub-types of complicated pectin interactions, which are based on the simple model, in order to explain the unreasonable situation resulting from the calculated value of C-type pectin content. Using snap-bean pods as sample, we now present several experiments to support the real existence of some of the complicated pectin interactions previously proposed. The supporting data came from the analysis and comparison of the amounts of bound calcium in the pectin fractions extracted and from the measurement and comparison of the size of pectin molecules extracted before and after further treatment by either heating or calcium removal.

INTRODUCTION

Many researches in our laboratory have revealed that most vegetables precooked at a moderate temperature for a suitable period of time and then cooked in boiling water showed greater firmness than those directly cooked without precooking (Chang *et al.*, 1986; Wu & Chang, 1990). This firming effect of precooking was in accordance with the result of the action of pectinesterase on the cell-wall materials, particularly pectic substances, which resulted in de-esterification of pectin molecules and the 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). However, the details of the chemical changes in pectic substances that caused textural changes in vegetable tissues during heating processes are not fully understood. In this study, pectins in the alcohol-insoluble solids of the vegetables after different cooking treatments were fractionated by sequential extraction with various extractants, and the correlations between their yields and the firmness of the tissues were investigated. We attempted to establish a model for the basic linkages and/or interactions between the long chains of pectin molecules, and also between pectin molecules and other cell-wall components, so that the model could be used to describe and/or to predict the chemical basis of textural changes in vegetable tissues during cooking processes.

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MATERIALS AND METHODS

Materials

Fresh radish (*Raphanus sativus* L.) roots and snap-bean (*Phaseolus vulgaris* L.) pods were obtained from a local wholesaler, while mung-bean (*Vigna radiata*) sprouts

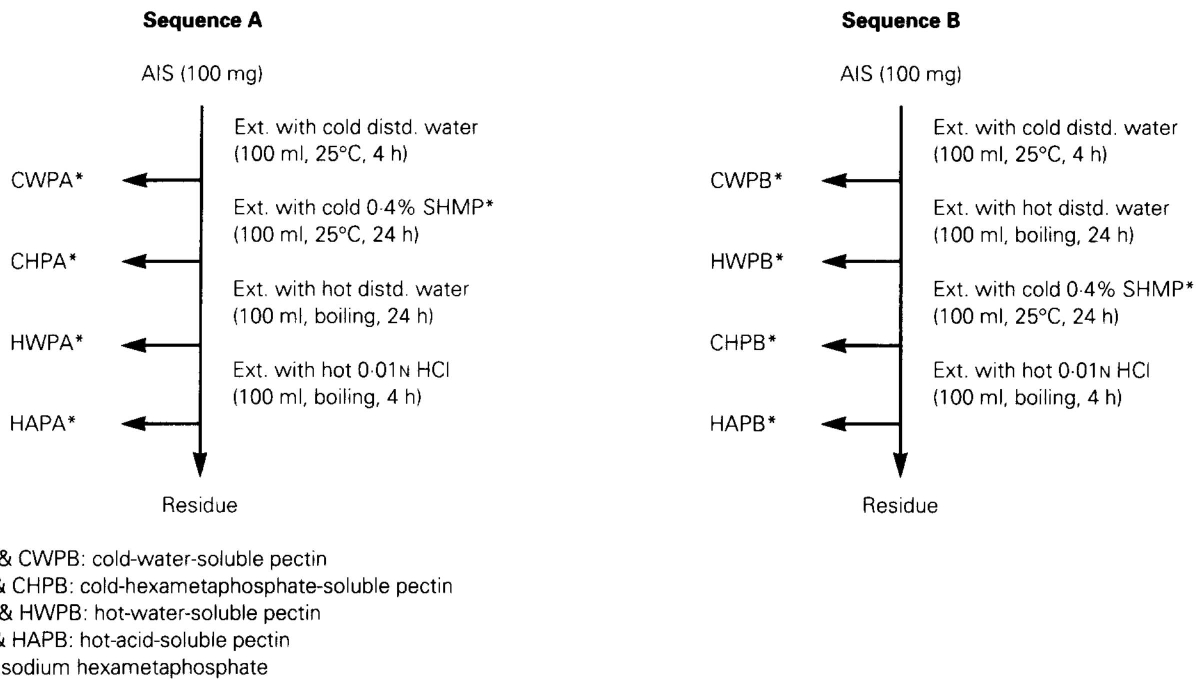

Fig. 1. Flow diagrams for sequential extraction of pectin fractions by two different sequences from the alcohol-insoluble solids (AIS) of vegetables.

were grown in a germination chamber. Materials of normal size and maturity, and free from decay or mechanical damage, were selected for use. Radish roots were peeled; both ends of the vegetables were cut off, and only the middle parts of them were used in the experiment. The trimmed radish was further sliced into a thickness of about 1·0 cm.

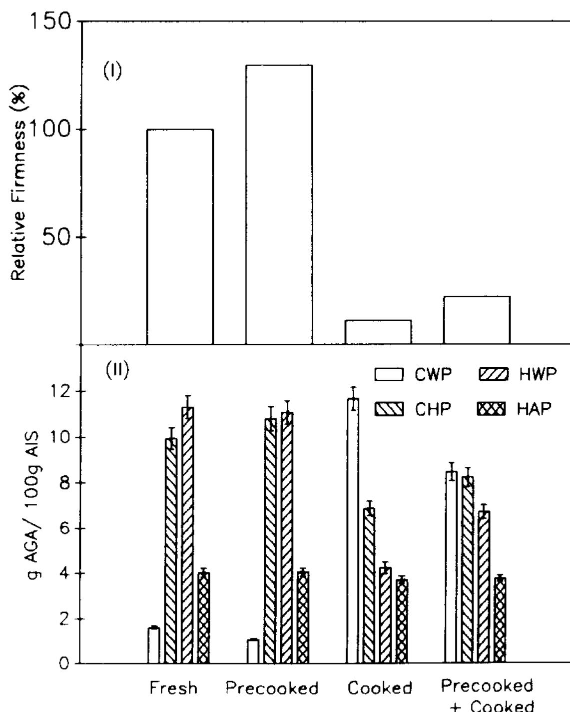

Fig. 2. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence A (CWP → CHP → HWP → HAP) from the AIS of radish cubes before and after 30-min cooking in boiling distilled water, with or without 30-min precooking in distilled water at 60°C.

Methods

Texture measurement

A Rheometer (Model NRM-3002D, Fudoh Kogyo KK, Japan), mounted with a plunger (adapter No. 6) for radish and snap bean, and a cutter (adapter No. 14) for mung-bean sprouts, was used to measure the firmness of the samples. The flat base, on which the sample was placed, moves upward to the plunger or cutter at a speed of 30 cm/min to measure the maximum puncture force or cutting resistance as an index of firmness of the sample. For every sample, from two to four measurements were taken for each of between four and ten pieces of the sample, and the average of sixteen or more measured values was expressed as relative firmness by taking the firmness of the fresh sample as 100.

Preparation of alcohol-insoluble solids (AIS)

Vegetable 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, 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. 1. These procedures were modified from that of Manabe (1980), and the conditions were worked out after a series of experiments with several vegetables

(Chen, 1987; Tseng, 1987; Wang, 1987; Tu, 1989; Chang, 1990; Lai, 1991). After each step of extraction and before proceeding to the next step of extraction, the residue was filtered and thoroughly washed with cold water, and the washings were combined with 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).

Measurement of calcium and magnesium ions

The pectin fractions were wet-digested (Osborne & Voogt, 1978) for the determination of divalent metal ions (Ca and Mg) by atomic-absorption spectrophotometry (aa/ae Spectrophotometer, IL151, Instrumentation Laboratory, USA).

Gel-permeation chromatography of pectins

The pectins in each fraction were further 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 and metal-ions measurement were carried out in triplicate. Data were analyzed by an ANOVA and Duncan's multiple-range test (mean comparison) by using the General Linear Model procedure of the Statistical Analysis System program from SAS Institute, Inc., Raleigh, NC, USA.

RESULTS AND DISCUSSION

The relationships between the changes in the contents of pectin fractions extracted from the AIS by the sequence A and the textural changes of vegetables

Pectin fractions were extracted by the sequence A (CWP → CHP → HWP → HAP) from the AIS of radish after different cooking treatments, and their contents and the firmness of the tissues are shown together in Fig. 2 for comparison. Figure 2 shows that the contents of CHP are positively correlated with the firmness of the tissues, whereas the contents of CWP are negatively correlated with the firmness. These phenomena can be explained by the firming effect of the precooking treatment as described by many researchers (Hoogzand & Doesburg, 1961; Hsu *et al.*, 1965; Bartolome & Hoff, 1972; Lee *et al.*, 1979; Van Buren, 1979; Manabe, 1980; Chang *et al.*, 1986; Wu & Chang, 1990). In a similar experiment with mung-bean sprouts (Fig. 3), however, it was the contents of HWP instead of CHP that were found to show positive correlation with the firmness of the tissues, although the contents of CWP were still found to have negative correlation with the firmness. These results cannot be explained by the firming effect of the precooking treatment as described above. The results of another experiment with snap-bean pods (Fig. 4) showed less similarity to radish and more similarity to mung-bean sprouts, and hence they also cannot be explained by the firming effect of the precooking treatment.

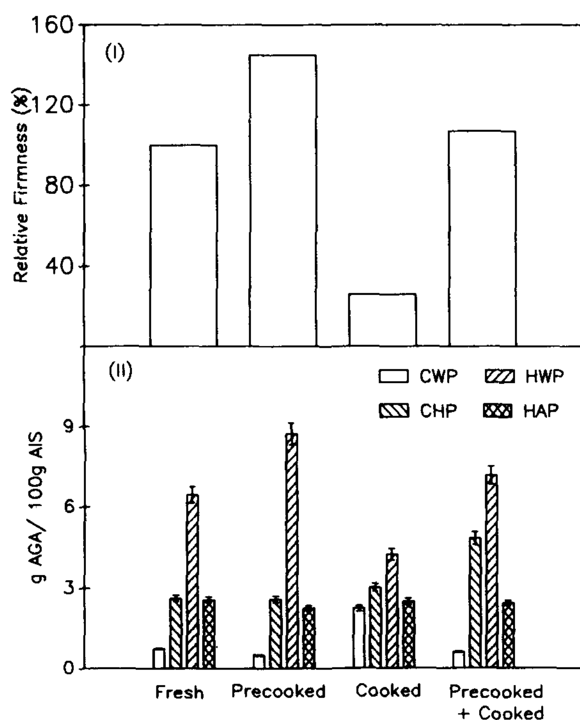

Fig. 3. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence A (CWP → CHP → HWP → HAP) from the AIS of mung-bean sprouts before and after 30-min cooking in boiling distilled water, with or without 20-min precooking in distilled water at 60°C.

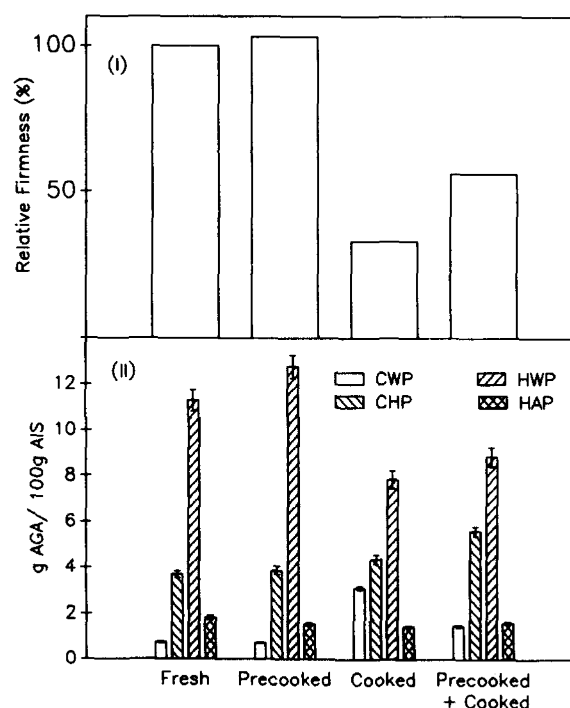

Fig. 4. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence A (CWP → CHP → HWP → HAP) from the AIS of snap-bean pods before and after 15-min cooking in boiling distilled water, with or without 20-min precooking in distilled water at 70°C.

The relationships between the changes in the contents of pectin fractions extracted from the AIS by the sequence B and the textural changes of vegetables

In order to investigate the reason for the contradictory results described above, the sequence B of pectin extraction (CWP → HWP → CHP → HAP) from the AIS of mung-bean sprouts and snap-bean pods after different cooking treatments was tried. The changes in contents of the pectin fractions extracted by the sequence B from the AIS and the firmness of the tissues of mung-bean sprouts and snap-bean pods are shown in Fig. 5 and Fig. 6, respectively. By comparing the two figures with Fig. 3 and Fig. 4, respectively, the following trends were observed.

(1) The contents of CWP in Fig. 5 and Fig. 6 are practically identical to those in Fig. 3 and Fig. 4, respectively, and are all negatively correlated to the tissue firmness of mung-bean sprouts and snap-bean pods, respectively (also see Table 1 for snap-bean pods), before and after various cooking treatments.

(2) The contents of HAP in Fig. 5 for mung-bean sprouts were not only independent of the cooking treatments, but also not much different from each other. The same trend is also observed in Fig. 3, but the HAP contents in Fig. 5 are apparently higher than those in Fig. 3, indicating that the pectin in the AIS of mung-bean sprouts was more easily and better extracted with cold hexametaphosphate solution and hot water by the sequence A (CHP → HWP) than by the sequence B (HWP → CHP), whereas the opposite phenomena are observed for the pectin of snap-bean pods in Fig. 4 and Fig. 6.

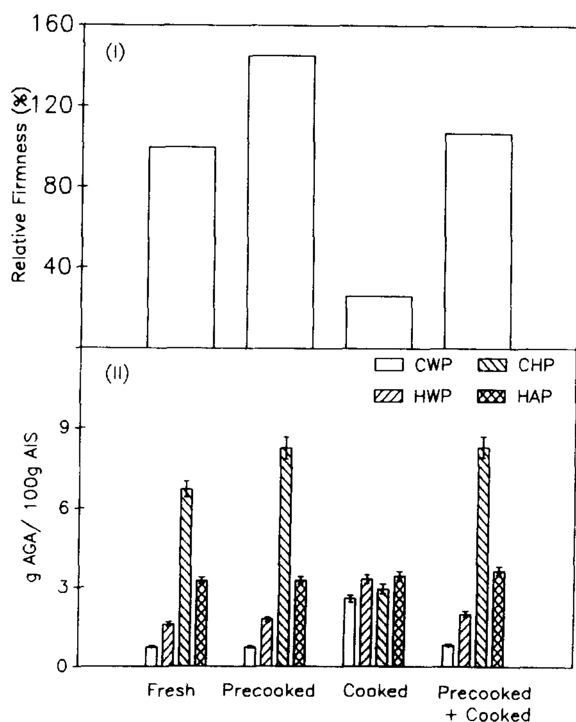

Fig. 5. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence B (CWP → HWP → CHP → HAP) from the AIS of mung-bean sprouts before and after 30-min cooking in boiling distilled water, with or without 20-min precooking in distilled water at 60°C.

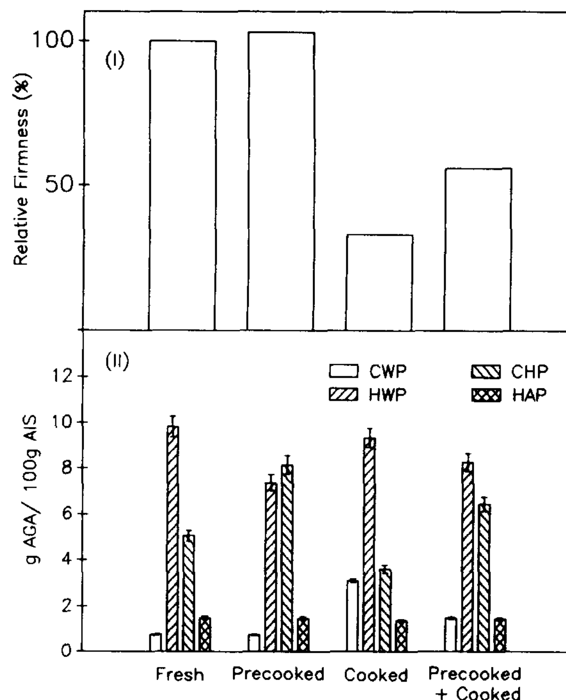

Fig. 6. Changes in firmness (I) and the contents of various pectin fractions (II) extracted by the sequence B (CWP → HWP → CHP → HAP) from the AIS of snap-bean pods before and after 15-min cooking in boiling distilled water, with or without 20-min precooking in distilled water at 70°C.

(3) The contents of CHP and HWP had different correlations with the firmness of the tissues depending on the extraction sequence, as shown in Table 1, and the results were compared and summarized as follows.

(a) The contents of CHP extracted by the sequences A and B from the AIS of mung-bean sprouts (Fig. 3 and Fig. 5, respectively) or snap-bean pods (Fig. 4 and Fig. 6) after different cooking treatments were different from each other, and so were the contents of HWP.

(b) In the AIS of both mung-bean sprouts and snap-bean pods before and after precooking, the amounts of CHP extracted by the sequence B were much larger than those extracted by the sequence A, whereas the situation was entirely reversed for the HWP fractions, and the increment was further increased by the precooking treatment for both the CHP and the HWP fractions.

(c) In the AIS of mung-bean sprouts after direct cooking, the amount of HWP extracted by the sequence B (Fig. 5) was smaller than that extracted by the sequence A (Fig. 3), but the amounts of CHP extracted by the two sequences were not very different from each other. In the AIS of snap-bean pods after direct cooking, however, the amount of HWP extracted by the sequence B (Fig. 6) was larger than that extracted by the sequence A

Table 1. The correlation coefficients between firmness and the contents of various pectin fractions extracted by different sequences from the AIS of snap-bean pods after various cooking treatments

Pectin fraction	Correlation coefficient to firmness
Extraction sequence A[a]	
CWPA	−0·835 2**
CHPA	0·468 6
HWPA	0·952 7**
HAPA	0·573 6
Extraction sequence B[a]	
CWPB	−0·835 2**
HWPB	−0·192 5
CHPB	0·470 4
HAPB	0·615 8
Calculated value	
CHPB − CHPA	0·743 7**
HWPA − HWPB	0·798 0**

[a] Extraction sequences
A: CWPA → CHPA → HWPA → HAPA
B: CWPB → HWPB → CHPB → HAPB
** $p < 0{\cdot}01$.

(Fig. 4), whereas the amount of CHP extracted by the sequence B was smaller than that extracted by the sequence A.

(d) In the AIS of mung-bean sprouts after precooking and subsequent cooking, the amount of CHP extracted by the sequence B (Fig. 5) was much larger than that extracted by the sequence A (Fig. 3), but the amount of HWP extracted by the sequence B was much smaller than that extracted by the sequence A. In the AIS of snap-bean pods after precooking and subsequent cooking, however, the amounts of both CHP and HWP were not much different between the two extraction sequences.

(e) From the results of statistical analysis shown in Table 1, it is clear that only the contents of CWP were negatively correlated ($p < 0{\cdot}01$) with the firmness of snap-bean pods, whereas the contents of HWPA, but not HWPB, were positively correlated with the firmness ($p < 0{\cdot}01$). These results strongly indicate that the correlation between the contents of various pectin fractions and the firmness cannot be simply discussed and judged on the basis of information obtained by any one of the two pectin-fractionation procedures.

A hypothetical model for the bondings and/or interactions between the long chains of pectin molecules and other cell-wall constituents of vegetables tissues

A hypothetical model, as shown in Fig. 7, was proposed to describe the bondings and/or interactions between the long chains of pectin molecules and other cell-wall constituents and also to explain the mechanism of the firming effect of the precooking treatment on the texture of vegetable tissues. In Fig. 7, there are five types of molecular interactions proposed for the pectin molecules in plant tissues, namely: (i) *P-type pectin*, linked by covalent bonds to other cell-wall constituents, such as cellulose, hemicellulose, lignin, or proteins, and therefore extractable only with hot dilute acid or alkali solutions; (ii) *S-type pectin*, linked by weak bonds or Van der Waals forces to other pectin molecules or other cell-wall constituents, and therefore extractable with cold water; (iii) *A-type pectin*, linked by ionic bridges of divalent-metal ions, such as Ca^{2+} or Mg^{2+}, to other pectin molecules, and therefore extractable with cold solutions of chelating agents; (iv) *B-type pectin*, linked by heat-labile bonds, such as intensive H-bondings, to other pectin molecules or other cell-wall constituents, and therefore extractable with hot water; (v) *C-type pectin*, linked and costabilized by heat-labile bonds and divalent-metal-ion bridges to other pectin molecules, and therefore extractable only by simultaneous treatment with heat and chelating agents.

Because of the coexistence of two kinds of bond, i.e. heat-labile bonds and divalent-metal-ion bridges, in the C-type pectin, the result of 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 from the AIS (the sequence A), the metal-ion bridges present in the C-type pectin molecules can be disrupted by the former, whereas the heat-labile bonds will survive. The C-type pectin will therefore remain in the AIS during CHP extraction in sequence A and will then be solubilized by hot water during the following HWP

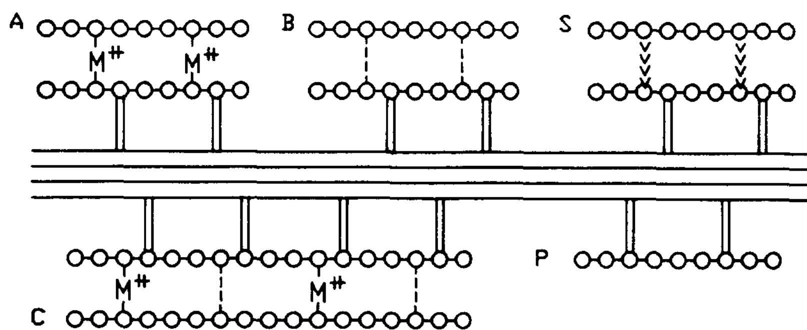

Fig. 7. A hypothetical model proposed for the bondings and interactions between the long chains of pectin molecules in vegetable tissues. o–o–o pectin polymer; ≡ other cell-wall materials; <<<<< Van der Waals forces—CWP; $-M^{++}$ metal-ion bridge—CHP; ----- heat-labile bond—HWP; = covalent bond—HAP.

extraction and become a part of HWPA. On the other hand, if hot water is used before a cold solution of chelating agents (the sequence B), the C-type pectin will remain in the AIS during HWP extraction and will then be solubilized by chelating agents and fall into the CHPB fraction.

On the basis of the above discussion, it can be deduced that the amount of CHPA, which does not include the C-type pectin, must be smaller than that of CHPB, which does include the C-type pectin, whereas the amount of HWPA, which includes the C-type pectin, must be larger than that of HWPB, which does not include the C-type pectin; thus the amount of the C-type pectin can be estimated by calculation of the difference, either (CHPB − CHPA) or (HWPA − HWPB) as shown in Table 2.

Evidence for the existence of C-type pectin and the relationships between the content of C-type pectin and the tissue firmness of snap-bean pods

The contents of various pectin fractions and the calculated amounts of C-type pectin in the AIS of snap-bean pods before and after different cooking treatments are listed in Table 2. As shown in Table 2, the amounts of C-type pectin estimated by calculation of the difference of either (CHPB − CHPA) or (HWPA − HWPB) for each cooking treatment were not significantly different from each other ($p > 0.05$), and the amounts of (CHPA + HWPA) and (CHPB + HWPB), both of which theoretically include A-, B-, and C- types of pectin all together according to Fig. 7, were not significantly different from each other ($p > 0.05$) or between cooking treatments. These results strongly imply that the model proposed in Fig. 7 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 overall. However, the calculated amounts of C-type pectin in the AIS of snap-bean pods after direct cooking were negative, which is apparently unreasonable and may be due to the presence of more complicated bondings and/or interactions between pectin molecules and other cell-wall constituents in the AIS. Thus, it may be necessary to make some modifications of this model, and also to find new evidence to support the modifications by further research.

The amounts of C-type pectin estimated by calculation of the differences (CHPB − CHPA) and (HWPA − HWPB) were both positively correlated with the firmness of snap-bean pods before and after different cooking treatments, with correlation coefficients of 0·7437 and 0·7980 ($p < 0.01$), respectively (Table 1). Another pectin fraction, the content of which is positively correlated ($p < 0.01$) with the firmness of snap-bean pods, was HWPA, indicating that hot-water-soluble pectin (B-type), when it coexists with C-type pectin, also contributes markedly to the firmness of the tissue. It can therefore be concluded that the content of C-type pectin is most intimately correlated with the changes in firmness of snap-bean pods before and after different cooking treatments.

An elaborate model for the interactions between pectin molecules and other cell-wall constituents in vegetable tissues

Because the hypothetical model we had proposed for the basic interactions between pectin molecules and other cell-wall constituents in vegetable tissues (as shown in Fig. 7) could not explain the negative values calculated for the content of C-type pectin in the AIS of snap-bean pods after a direct cooking treatment, we now propose an elaborate model for the pectin interactions, which includes twelve sub-types of complicated interactions between A-, B-, and C-type pectins as shown in Fig. 8. Actually, the twelve sub-types of complicated interactions are the combinational arrangements of A-, B-, and C-type pectin interactions linked outside the P-type pectin interaction, which is the most stable and directly attached to the insoluble cell-wall constituents.

Table 2. Calculation of various pectin fractions in the AIS of snap-bean pods after different cooking treatments in distilled water

Pectin fraction[a]	Pectin type[b]	Content (wt%) in the AIS of snap-bean pods[c]			
		Fresh	Precooked[d]	Cooked[d]	Precooked + cooked
CHPA	A	3·78	4·21	4·60	5·74
CHPB	A + C	5·54	8·81	3·77	5·92
HWPA	B + C	10·95	12·80	7·90	9·23
HWPB	B	9·48	7·35	9·42	8·33
CHPB − CHPA	C	1·76a	4·60b	−0·83c	0·18d
HWPA − HWPB	C	1·47a	5·45b	−1·52c	0·90d
CHPA + HWPA	A + B + C	14·73e	17·01f	12·50g	14·98h
CHPB + HWPB	A + B + C	15·02e	16·15f	13·18g	14·25h

[a] The pectin fractions were extracted by the sequences of:
A: CWPA → CHPA → HWPA → HAPA
B: CWPB → HWPB → CHPB → HAPB
[b] See Fig. 7 for the pectin type.
[c] Means with the same letter in each column are not significantly different at $p < 0.05$.
[d] Precooked: 70°C, 20 min; cooked: boiling, 15 min.

Different pectin interactions in each sub-type can be broken by different treatments, in which different solvents are used at different temperatures, so that pectin fractionation by sequential and differential extraction can be achieved. Thus the amounts of various pectin fractions can be determined by two different sequences of extraction with these solvents, namely, sequence A (CWPA → CHPA → HWPA → HAPA) and sequence B (CWPB → HWPB → CHPB → HAPB). The amounts of S-, A-, B-, and P-type pectin can be obtained directly from the results of such fractionation experiments, namely, from the amounts of (i) CWPA or CWPB, (ii) CHPA, (iii) HWPB, and (iv) HAPA or HAPB fractions, respectively, whereas the amount of C-type pectin can only be evaluated by calculation from the amounts of either CHPA and CHPB (CHPB − CHPA) or HWPA and HWPB (HWPA − HWPB). In Fig. 8, the magnitudes of CHPA and HWPA are also compared, respectively, with those of CHPB and HWPB, as shown below the drawing of each sub-type of pectin interaction. Thus, it can be seen from Fig. 8 that the values calculated for the amounts of C-type pectin in sub-types (*a*), (*b*), (*g*), (*h*), (*i*), and (*j*), and possibly (*k*) and (*l*) as well, will be negative, because CHPA is larger than CHPB, whereas HWPA is smaller than HWPB. Thus the elaborate model of pectin interactions can be used to explain the reason why the calculated value for the amount of C-type pectin in the AIS of vegetables could be negative.

Although part of the pectin interactions in the elaborate model can be used to explain the negative calculated value for the amount of C-type pectin, there should be some direct evidence to support the existence of such pectin interactions. In this study, therefore, we also present some supporting data for the real existence of some of the complicated pectin interactions we propose. The evidence came from the analysis and comparison of the amounts of bound calcium in some of the pectin fractions extracted, and also from the analysis and comparison of molecular size, by gel-permeation chromatography before and after further treatment with heat or Ca-removal by chelating agents, of the pectin molecules contained in some of the pectin fractions extracted.

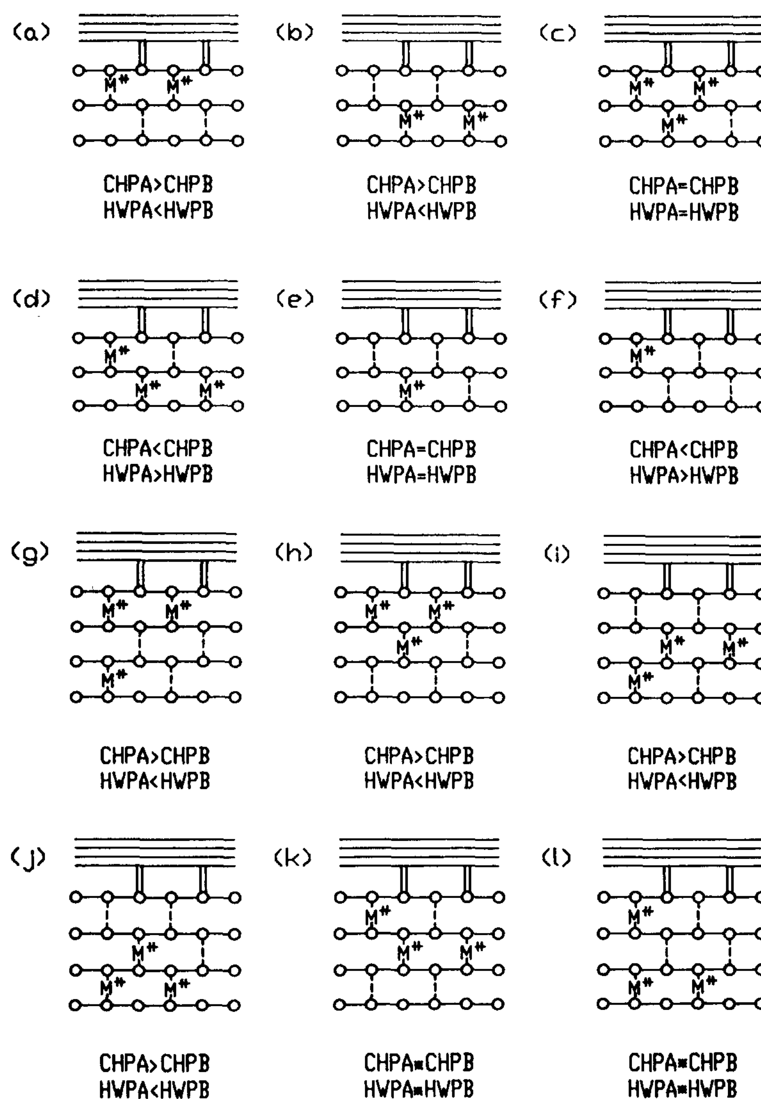

Fig. 8. Models proposed for the interactions between A-, B-, and C-types of pectins, as shown in Fig. 7, in vegetable tissues. Comparison of the magnitude of CHP and HWP fractions between two pectin-extraction sequences, A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB → HAPB). (* Denotes incapable of comparison.)

The contents of calcium and magnesium ions in the pectin fractions extracted from the AIS of snap-bean pods before and after different cooking treatments

The pectin fractions extracted from the AIS of snap-bean pods were wet-digested and analyzed for the contents of Ca and Mg ions by atomic-absorption spectrophotometry. Since the contents of Mg ions were too low to be detected by atomic absorption, only the contents of Ca ions are shown in Table 3, with the following deductions.

(1) The presence of Ca ions only in the CWPA and CHPA fractions extracted by sequence A, and in the CWPB, HWPB, and CHPB fractions extracted by sequence B, but not in the HWPA, HAPA, and HAPB fractions, indicates that all the extractable Ca ions were extracted before and during the step of chelating-agent extraction. Furthermore, the fact that the Ca contents found in the residues of all AIS after pectin extraction by the sequence B were slightly lower than those extracted by the sequence A, and that there were fewer Ca ions found in the residues of AIS after precooking and/or cooking treatments, indicates that the removal of AIS-bound Ca ions by treatment with chelating agent is easier and more complete if the AIS is extensively treated with hot or boiling water prior to chelating-agent extraction.

(2) Since the AIS was prepared by treatment and thorough washing with 80% ethanol, the Ca ions present in the CWP fraction must be bound rather than free. It is noteworthy that there may be 12 more kinds of interactions between A-, S-, and C-types of pectins present in the AIS of vegetable tissues, analogous to those shown in Fig. 8, except that all or some of the heat-labile bonds are replaced by cold-water-destructible interactions, such as Van der Waals forces. However, the presence of such weak interactions in the AIS will not affect the results of pectin fractionation by the analysis we have proposed.

(3) Although hot water cannot break the Ca bridges, the HWPB fractions extracted by sequence B from the AIS of fresh and cooked samples contained large amounts of Ca ions, whereas the CHPB fractions from the same samples contained far fewer Ca ions. These results indicate that the probability of the existence of (*b*), (*e*), (*g*), (*i*), (*j*) and (*l*) sub-types of pectin interactions, as shown in Fig. 8, in the AIS of snap-bean pods is great.

(4) When the pectin in the AIS was fractionated by sequence A, the Ca-ion contents of the CHPA fractions were found to be increased by the precooking treatment, which is apparent by comparing the Ca-ion contents of CHPA fractions from the precooked and the precooked-and-cooked samples with those from the fresh and the cooked samples, respectively, whereas the Ca-ion contents of the CWPA fractions were decreased by the precooking treatment. If we combine these facts, together with another observation that the precooking treatment resulted in a decrease of CWP contents and increases of CHP and HWP contents in the AIS of snap-bean pods, it seems reasonable to attribute the firming effect of precooking to the following chemical changes in the tissues. The pectins initially belonging to B- or S-type in Fig. 7 were converted to C- or A-type pectins by the action of pectinesterase and the formation of Ca bridges, which resulted in the firming of the tissue. In other words, the water-destructible interactions (S-type) and the heat-labile bonds (B-type) in the pectins of sub-types (*a*), (*b*), (*e*), (*f*),

Table 3. The Ca-ion contents of various pectin fractions extracted from the AIS of snap-bean pods after different cooking treatments

Pectin fraction[a]	Ca-ion content (m mol/100 g AIS)[b]			
	Fresh	Precooked[c]	Cooked[c]	Precooked + cooked
CHPA	5·20 ± 0·22	3·98 ± 0·17	4·56 ± 0·27	3·58 ± 0·20
CHPB	5·20 ± 0·22	7·25 ± 0·31	6·44 ± 0·20	7·49 ± 0·34
HWPA	#	#	#	#
HWPB	#	#	#	#
Residue A	1·84 ± 0·13	1·08 ± 0·10	0·67 ± 0·09	1·08 ± 0·11
CWPB	5·01 ± 0·21	3·57 ± 0·18	4·34 ± 0·20	3·53 ± 0·17
HWPB	5·84 ± 0·23	4·73 ± 0·16	6·37 ± 0·21	5·46± 0·20
CHPB	1·25 ± 0·10	3·42 ± 0·15	0·73 ± 0·08	3·38 ± 0·13
HAPB	#	#	#	#
Residue B	0·96 ± 0·08	0·69 ± 0·08	#	#
Total[d]	13·24 ± 0·43	12·47 ± 0·34	11·78 ± 0·35	12·77 ± 0·53

[a] The pectin fractions were extracted by the sequences of:
A: CWPA → CHPA → HWPA → HAPA → Residue A
B: CWPB → HWPB → CHPB → HAPB → Residue B
[b] Values are mean ± SD of three independent experiments.
[c] Cooking treatments: precooked: 70°C, 20 min; cooked: boiling, 15 min; both in distilled water.
[d] The total Ca-ion contents were determined directly for the AIS of snap-bean pods before and after different cooking treatments.
#The Ca-ion content was too low to be determined by atomic-absorption spectrophotometry.

and (*g*) through (*l*) were changed to A-type and C-type pectin interactions, respectively, by the formation of new Ca bridges. This prediction was supported by the observation that the Ca-ion contents of the CHPB fractions were increased more significantly by the precooking treatment, whereas those of both the CWPB and HWPB fractions were decreased.

However, it is noteworthy that the formation of such Ca bridges is not necessarily dependent on free Ca ions, which are soluble in 80% ethanol, because it was also observed that the total Ca-ion contents of the four AIS samples before and after different cooking treatments were not much different from each other (Table 3). It therefore also seems reasonable to predict that there must be a change during the precooking treatment from some kinds of bound Ca ions, which are not linking different pectin molecules together and are thus not contributing much to the firmness of the tissue, to Ca bridges between different pectin molecules that are contributing much to the firmness of the tissue.

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

The pectin fractions extracted from snap-bean pods 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 tissue of snap-bean pods during cooking processes. Because the CHP and the HWP fractions extracted from the AIS of snap-bean pods had different correlation coefficients with the firmness of the tissue, depending on the extraction sequences, the molecular sizes of these pectin fractions were analyzed first, and the results are shown in Fig. 9 and Fig. 10, respectively.

In Fig. 9, it may be observed that all the four CHPA fractions extracted from the AIS of snap-bean pods before and after different cooking treatments contained mainly large-MW pectin molecules together with very small amounts of medium- and small-MW pectin molecules, whereas the CHPB fractions contained much larger amounts of medium- and small-MW pectin molecules. The weight percentages of large-MW (fractions 16–21 in Fig. 9), medium-MW (fractions 22–27), and small-MW (fractions 28–32) pectin molecules in the AIS of each sample were calculated and are listed in Table 4. The results indicate that, in the CHPA fractions, the percentage of large-MW pectin molecules ranged from 90 to 96%, with the highest value observed for the AIS of precooked sample, whereas, in the CHPB fractions, it ranged from 52 to 66%, with the lowest value observed for the AIS of the directly cooked sample. It is particularly noteworthy that the CHPB fraction extracted from the AIS of the precooked-and-cooked sample contained a higher percentage of large-MW pectin molecules (61·4%) than that from the directly cooked sample (52·3%) and that the latter contained much higher percentages of

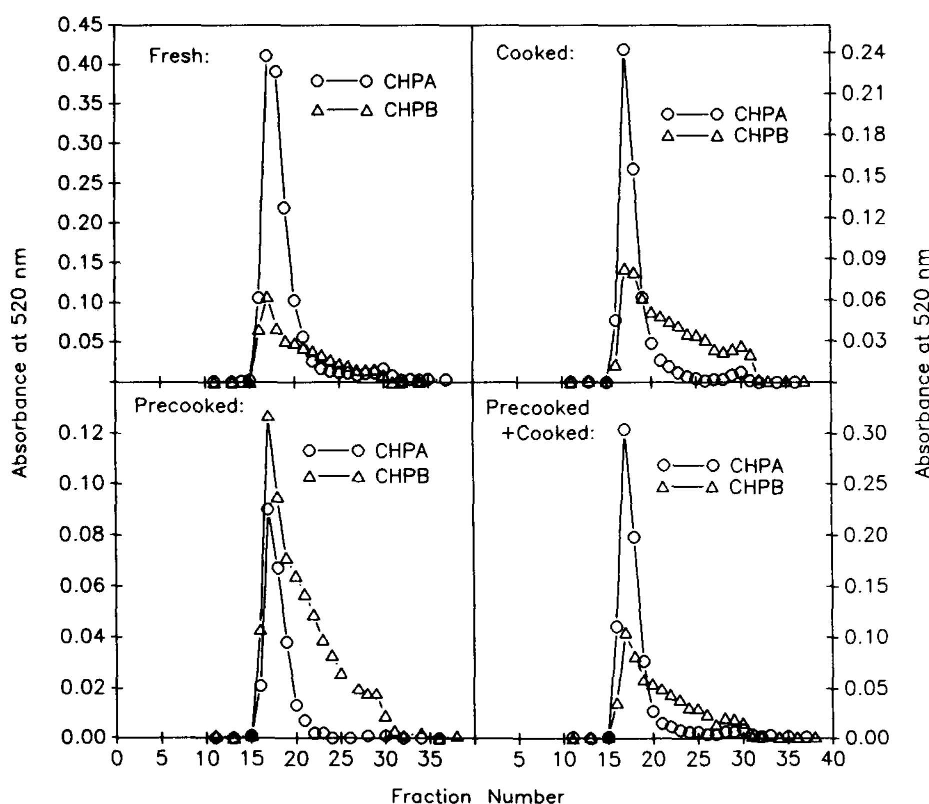

Fig. 9. Gel-permeation chromatograms on Fractogel TSK HW-65 (F) of cold Na-hexametaphosphate-soluble pectins (CHP) extracted by the sequences A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB → HAPB) from the AIS of snap-bean pods before and after different cooking treatments. Precooked: 70°C, 20 min; cooked: boiling, 15 min; both in distilled water.

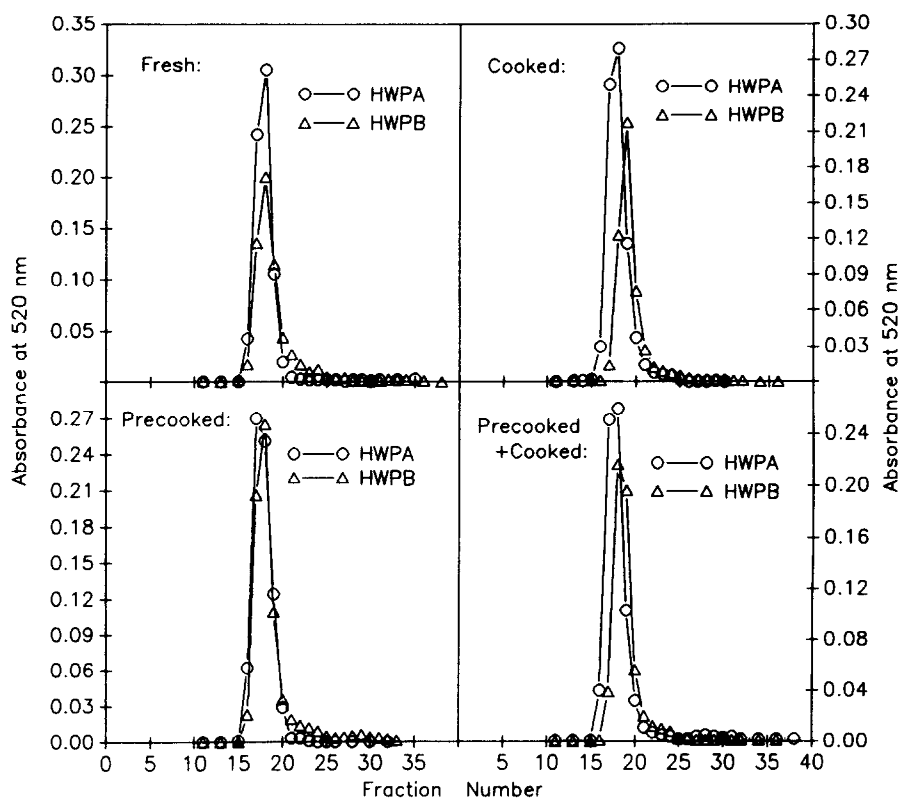

Fig. 10. Gel-permeation chromatograms on Fractogel TSK HW-65 (F) of hot-water-soluble pectins (HWP) extracted by the sequences A (CWPA → CHPA → HWPA → HAPA) and B (CWPB → HWPB → CHPB → HAPB) from the AIS of snap-bean pods before and after different cooking treatments. Precooked: 70°C, 20 min; cooked: boiling, 15 min; both in distilled water.

medium-MW (32·6%) and small-MW (15·1%) pectin molecules. These results strongly suggest that the bean pods after precooking have a firmer texture not only because of the increase in Ca bridges between pectin molecules, but also because of large-MW pectin molecules arising through the formation of new bonds or linkages between pectin molecules that cannot be broken even by cooking.

With respect to the HWP fractions, the HWPB fractions contained slightly lower percentages of large-MW pectin molecules and higher percentages of medium- and small-MW pectin molecules than the HWPA fractions (Fig. 10 and Table 5). By considering the characteristics of the C-type pectin, which is co-stabilized by Ca bridge and heat-labile bonds, the high percentages of large-MW pectin molecules in the HWPA fractions (94–99·5% in Table 5) in comparison with the much lower percentages of large-MW pectin molecules in the CHPB fractions (52–66% in Table 4), clearly indicate that Ca bridges are contributing much more than heat-labile bonds to the inter-molecular linking of pectin molecules.

Table 4. The molecular-size distribution, as analyzed by gel-permeation chromatography on Fractolgel TSK HW-65 (F), of pectins in the cold Na-hexametaphosphate-soluble fractions (CHP) extracted from the AIS of snap-bean pods before and after different cooking treatments

Sample	Pectin fraction	Percentage (wt%) in CHP		
		Large MW 16–21[a]	Medium MW 22–27[a]	Small MW 28–32[a]
Fresh	CHPA[c]	90·1	6·3	3·6
	CHPB[c]	65·9	27·2	6·9
Precooked[b]	CHPA	95·9	2·9	1·2
	CHPB	65·4	27·9	7·4
Cooked[b]	CHPA	92·7	4·7	2·5
	CHPB	52·3	32·6	15·1
Precooked	CHPA	91·4	5·0	3·6
+ cooked	CHPB	61·4	28·9	9·7

[a] Fractions numbers in gel-permeation chromatography in Fig. 9.
[b] Cooking treatments: precooked: 70°C, 20 min; cooked: boiling, 15 min; both in distilled water.
[c] The pectins were extracted from the AIS of snap-bean pods by the sequences of:
A: CWPA → CHPA → HWPA → HAPA
B: CWPB → HWPB → CHPB → HAPB.

Table 5. The molecular-size distribution, as analyzed by gel-permeation chromatography on Fractolgel TSK HW-65 (F), of pectins in the hot-water-soluble fractions (HWP) extracted from the AIS of snap-bean pods before and after different cooking treatments

Sample	Pectin fraction	Percentage (wt%) in CHP		
		Large MW 16–21[a]	Medium MW 22–27[a]	Small MW 28–32[a]
Fresh	HWPA[c]	97·6	1·9	0·5
	HWPB[c]	90·0	8·7	1·3
Precooked[b]	HWPA	99·5	0·5	0
	HWPB	90·6	7·2	2·2
Cooked[b]	HWPA	97·1	2·8	0·1
	HWPB	90·9	7·9	1·2
Precooked	HWPA	94·2	3·9	1·9
+ cooked	HWPB	92·8	6·3	0·9

[a] Fractions numbers in gel-permeation chromatography in Fig. 10.
[b] Cooking treatments: precooked: 70°C, 20 min; cooked: boiling, 15 min; both in distilled water.
[c] The pectins were extracted from the AIS of snap-bean pods by the sequences of:
A: CWPA → CHPA → HWPA → HAPA
B: CWPB → HWPB → CHPB → HAPB.

Most probable sub-types of and evidence for the interactions between A-, B-, and C-types of pectins existing in snap-bean pods

During the process of pectin extraction by the sequence B, 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 will consequently fall into the CHPB fraction. The medium- and small-MW pectin molecules in the CHPB fractions in Fig. 9 and Table 4 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. 10 and Table 5 contained very small amounts of medium- and small-MW pectin molecules. Thus it can be deduced that the C-type pectins in the AIS of snap-bean pods are not medium or small molecules but are large molecules and that the appearance of medium- and small-MW pectin molecules in the CHPB fractions is due to the interactions between A-, B-, and C-types of pectin as shown in Fig. 8.

In view of the appearance of medium- and small-MW pectin molecules in the CHPB fractions, the sub-types possibly existing in the tissue of snap-bean pods are (*a*), (*c*), (*d*), (*f*), (*g*), (*h*), (*k*), and (*l*) as shown in Fig. 8. In addition, there are other observations in this series of experiments that can be used to judge or predict the possibly existing sub-types of pectin interactions, such as the calculated value of CHPB − CHPA or HWPA − HWPB for the content of C-type pectin and the contents of Ca ions in the HWPB fractions as shown in Table 3. All these items are listed together in Table 6, which results in an overall conclusion that the most probable sub-types of pectin interactions existing in large amounts in the tissue of snap-bean pods are sub-types (*g*) and (*l*) in Fig. 8.

If the sub-types (*g*) and (*l*) are really existing in substantial amounts in the tissue of snap-bean pods, then, if the CHPA and the HWPB fractions are subjected to further treatments with heat in boiling water and with chelating agents, respectively, there must appear appreciable amounts of medium- and small-MW pectin molecules on gel-permeation chromatography. In Fig. 11, it is observed as expected that the molecular-size

Table 6. Most probable sub-types of interactions between A-, B- and C-types of pectins existing in large amounts in the tissue of snap-bean pods before and after different cooking treatments

Bases for judgement	Probable sub-types[a]			
	Fresh	PC[b]	C[b]	PC + C
1. According to the calculated value of CHPB − CHPA or HWPA − HWPB (see Table 2)	(*d*), (*f*), (*k*), (*l*)	(*d*), (*f*), (*k*), (*l*)	(*a*), (*b*), (*g*), (*h*) (*i*), (*j*), (*k*), (*l*)	(*d*), (*f*), (*k*), (*l*)
2. Ca-ion content in HWPB (see Table 3)	(*b*), (*e*), (*g*), (*i*), (*j*), (*l*)	(*b*), (*e*), (*g*) (*i*), (*j*), (*l*)	(*b*), (*e*), (*g*), (*i*), (*j*), (*l*)	(*b*), (*e*), (*g*), (*i*), (*j*), (*l*)
3. Medium and small pectin molecules in CHPB (see Fig. 9)	(*a*), (*c*), (*d*), (*f*), (*g*), (*h*), (*k*), (*l*)	(*a*), (*c*), (*d*), (*f*), (*g*), (*h*), (*k*), (*l*)	(*a*), (*c*), (*d*), (*f*), (*g*), (*h*), (*k*), (*l*)	(*a*), (*c*), (*d*), (*f*), (*g*), (*h*), (*k*), (*l*)
Most probable sub-types	(*l*)	(*l*)	(*g*), (*l*)	(*l*)

[a] See Fig. 8 for the sub-types of interactions between A-, B-, and C-types of pectins.
[b] PC: precooked (70°C, 20 min); C: cooked (boiling, 15 min);both in distilled water.

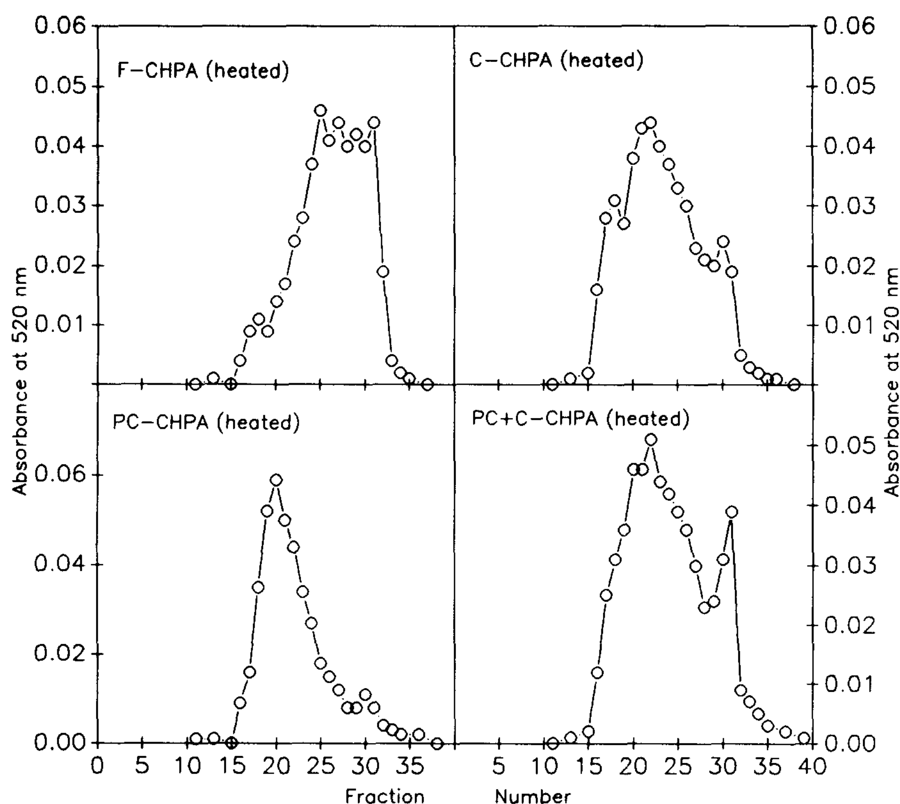

Fig. 11. Gel-permeation chromatograms on Fractogel TSK HW-65 (F) of the products of 24-h boiling water heating of the cold Na-hexametaphosphate-soluble pectins (CHP in Fig. 9), which were extracted by the sequence A (CWPA → CHPA → HWPA → HAPA) from the AIS of snap-bean pods before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 20 min; C: cooked by boiling for 15 min; both in distilled water.

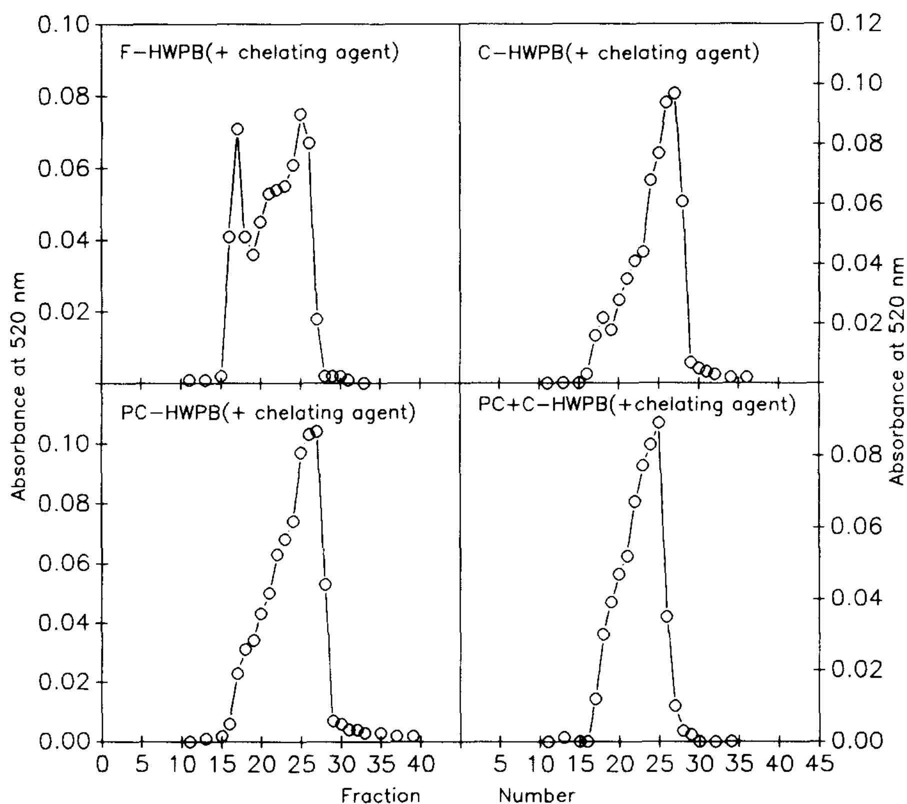

Fig. 12. Gel-permeation chromatograms on Fractogel TSK HW-65 (F) of the products of 0·4% Na-hexametaphosphate treatment of the hot-water-soluble pectins (HWP in Fig. 10), which were extracted by the sequence B (CWPB → HWPB → CHPB → HAPB) from the AIS of snap-bean pods before and after different cooking treatments. F: fresh; PC: precooked at 70°C for 20 min; C: cooked by boiling for 15 min; both in distilled water.

distribution of pectins in the CHPA fractions shifts from a large-MW fraction (Fig. 9) to medium- and small-MW fractions after boiling for 24 h in distilled water, whereas in Fig. 12 it is observed that the molecular-size distribution of pectins in the HWPB fractions also shifts from a large-MW fraction (Fig. 10) to medium- and small-MW fractions after treatment with chelating agents. These results accord with those mentioned above, and can be taken as further evidence for the existence of sub-types (*g*) and (*l*) of pectin interactions in the tissue of snap-bean pods.

CONCLUSION

The simple model for the basic interactions between pectin molecules and other cell-wall constituents is sufficient for depicting the mode of pectin interactions, and to explain the correlation between the chemical changes and textural changes of vegetable tissues during different cooking treatments, only when there is no or little involvement of more complicated pectin interactions. However, if there is much involvement of such interactions, particularly when a large amount of C-type pectin is involved, the calculated content of C-type pectin may turn out to be negative, and it is then necessary to use the elaborate model to explain the complicated pectin interactions. In such cases, moreover, the judgement between the twelve sub-types of interaction will require additional information such as the calcium content and molecular size of the extracted pectin fractions before and after further treatments by either heating or calcium removal.

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REFERENCES

Bartolome, L. G. & Hoff, J. E. (1972). Firming of potatoes: biochemical effects of precooking. *J. Agric. Food Chem.*, **20**, 266–70.

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

Chang, C. Y. (1990). Studies on the relationships between textural changes and chemical changes in pectins of snap bean pods during cooking processes, and on the models of interactions between pectin molecules. Doctoral dissertation, Graduate Institute of Food Science & Technology, National Taiwan University, Taipei, Taiwan.

Chang, W. H., Liau, L. L. & Shiau, S. Y. (1986). Effect of cooking on the texture and chemical composition of cucumber and radish. In *Role of Chemistry in the Quality of Processed Food*, ed. O. R. Fennema, W. H. Chang & C. Y. Lii. Food & Nutrition Press, Inc., Westport, CT, USA, pp. 303–13.

Chen, J. Y. (1987). Studies on the correlation between texture changes and chemical compositions during drying and cooking processes of radish. Master's thesis, Graduate Institute of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan.

Hoogzand, C. & Doesburg, J. J. (1961). Effect of blanching on texture and pectin of canned cauliflower. *Food Technol.*, **15**, 160–3.

Hsu, C. P., Deshpande, S. N. & Desrosier, N. W. (1965). Role of pectin methylesterase in firmness of canned tomatoes. *J. Food Sci.*, **30**, 583–5.

Lai, L. R. (1991). Studies on the cooking characteristics and the factors related to the firming effect of precooking of pea pods—pectin and pectinesterase. Master's thesis, Graduate Institute of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan.

Lee, C. Y., Bourne, M. C. & Van Buren, J. P. (1979). Effect of blanching treatments on the firmness of carrots. *J. Food Sci.*, **44**, 615–16.

Manabe, T. (1980). Studies on the firming mechanism of Japanese radish root by pre-heating treatment. *Nippon Shokuhin Kogyo Gakkaishi*, **27**, 234–9.

Osborne, D. R. & Voogt, P. (1978). The Analysis of Nutrients in Food, Academic Press, New York, NY, USA.

Tseng, C. Y. (1987). Studies on the relationships between texture changes and chemical compositions during drying and cooking processes of calabash and Kanpyo (dry calabash strips). Master's thesis, Graduate Institute of Food Science & Technology, National Taiwan University, Taipei, Taiwan.

Tu, Y. Y. (1989). Studies on the relationship between texture changes and chemical composition during cooking and soaking processes of carrot. Master's thesis, Graduate Institute of Food Science & Technology, National Taiwan University, Taipei, Taiwan.

Van Buren, J. P. (1979). The chemistry of texture in fruits and vegetables. *J. Texture Studies*, **10**, 1–23.

Wang, R. L. (1987). Studies on the relationship between texture changes and chemical compositions during heating and pickling processes of mustard stem slices. Master's thesis. Graduate Institute of Home Economics, Chinese Culture University, Taipei, Taiwan.

Wu, A. & Chang, W. H. (1990). Influence of precooking on the firmness and pectic substances of three stem vegetables. *Internat. J. Food Sci. & Tech.*, **25**, 558–65.