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Characterization of the pectin extracted from citrus peel in the presence of citric acid

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ABSTRACT

Pectins were extracted from the peel of *Citrus unshiu* Marcovitch × *C. nobilis* Loureiro (Kara mandarin) in the presence of citric acid at different pH and temperature. Treatment with citric acid, particularly, at neutral pH gave a significant increase in viscosity. The citric acid treatment at high temperature led to decreased viscosity although the viscosity remained higher than that of untreated pectin. Pectin methylesterase activity was inhibited at high citric acid concentrations. The increase in viscosity of treated pectin was proportional to the amounts of citric acid bound to pectin. The pectin with citric acid treatment had a low degree of methylation (8.4%) and a high distribution (50 to 2000 kDa) of molecular mass than untreated pectin. Analysis of chemical composition showed that the pectin with citric acid treatment contained lower protein and galacturonic acid, and higher neutral sugars, particularly, galactose than the untreated pectin. The citric acid treatment at neutral pH prior to extraction is an attractive procedure for low methoxy pectin without lowering its molecular weight.

1. Introduction

Pectin is an acidic hydrocolloid that is widely used as a food ingredient for its gelling property. The primary structural feature of pectin is a linear chain of poly-α-(1 → 4)-D-galacturonic acid with varying degrees of methyl esterification (DE) Pectin gelation depends on its DE and molecular size (Thakur, Singh, & Handa, 1997). The pectin classes based on the DE are high methoxy (HM) pectins and low methoxy (LM) pectins. HM pectins can be deesterified with acid, alkali, ammonia, or pectin methyl esterase to form LM pectins (May, 1990; Ralet et al., 2001).Generally, commercial pectin is extracted by treating the raw material with hot dilute mineral acid at acidic pH about 2. The extracted pectin is altered with enzymatical and chemical means in response to industrial demand, for example, deesterification of HM pectin to LM pectin.

Chemical deesterification of pectin coincides with decreased molecular weight due to depolymerization of the pectin backbone by β-elimination (Renard & Thibault, 1996). On the other hand, enzymatic deesterification performed with pectin methyl esterase is an attractive alternative without lowering molecular weight (Ralet et al., 2001; Hotchkiss et al., 2002). At present there is no adequate preparation of chemically deesterified pectin while preserving its molecular weight.

In the present work we have examined the effects of citric acid which is present in high concentrations in the juice from citrus fruits on viscosity of pectin. The objective of this work was to provide a procedure for chemical modification of pectin with preserving its molecular weight. The citric acid treatment prior to heat extraction at acidic pH was efficient in altering the physicochemical properties of pectin; the pectin with citric acid treatment in our work contained lower galacturonic acid concentrations compared to transacylated pectin catalyzed by pectinesterase with high molecular mass (Lee, Wu, Lee, Jiang, & Chang, 2003).

2. Materials and methods

2.1. Materials

The fruits of *Citrus unshiu* Marcovitch × *C. nobilis* Loureiro (Kara mandarin) were collected in January 2006 from Kumano, Mie prefecture, Japan. After the juice extraction process, the peel sample was air-dried in an oven at 40 °C for 48 h. The dry sample was ground with a fiber mixer. All chemicals used were of the purest grade from Wako Pure Chemicals (Osaka, Japan).

2.2. Extraction and purification of pectin

Ten grams of dried peel powder were suspended in distilled water and adjusted to pHs from 5.8 to 8.0 and concentrations of citric acid that varied from 0.05 to 1 M. The suspensions were heated to three different temperatures, 50, 65, and 80 °C. Samples were held at each temperature for 2 h and then cooled to room temperature. The suspensions were adjusted to pH 2.2 with conc. HCl and heated for 15 min in a boiling water bath. The crude ex-

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tracts were cooled to room temperature and centrifuged at 10,000 rpm for 15 min. The supernatant was adjusted to pH 7.0 with 4 M NaOH. Ethanol was added to the solution at final concentrations of 60% (v/v) and the precipitate was collected by centrifugation, washed twice with 80% ethanol, and air-dried at 50 °C for 14 h. The dry precipitate was dissolved in distilled water and dialyzed against water. The dialyzed solution was freeze-dried. The control sample without citric acid treatment was directly extracted at pH 2.2 and followed the same procedure described above.

2.3. Viscosity measurement

Viscosity measurement was performed by using a sine-wave viscometer (SV-10, A&D, Tokyo, Japan) with a water-jacket assembly (AX-SV-37, A&D). The vibration frequency was a constant 30 Hz with negligible degradation of pectin structure. Pectin was dissolved in distilled water at room temperature at the concentrations of 1.0% (w/v). The viscosities of pectins were measured at 25 °C.

2.4. Pectin methylesterase activity assay

Pectin methylesterase (PME) was extracted from dried peel powder according to the same procedure for pectin extraction described earlier. Samples (4 g) were suspended in distilled water and adjusted to the interest pH from 5.8 to 8.0 and concentrations of citric acid varied from 0 to 1 M. The suspensions were heated to three different temperatures, 50, 65, and 80 °C, respectively. Samples at each temperature were constant for 2 h and then cooled to room temperature. The suspensions were centrifuged at 12,000g for 20 min at 4 °C. The supernatants were collected and then concentrated with Biomax-10 (Ultrafree-15, Millipore Corporation, Billerica, USA). The concentrated solution was used as a sample for PME assay. PME was assayed by the enzyme producing methanol. Two milliliters of citrus pectin (2 mg/mL) in 50 mM phosphate buffer (pH 7.5) was mixed well and incubated with 0.5 mL of enzyme sample for 10 min at 37 °C. The reaction mixture in a screwed cap tube was immediately heated at 100 °C in boiling water for 5 min to inactivate PME. The sample was then cooled in ice and 0.5 mL of the solution was reacted with 0.5 mL of alcohol oxidase (1.0 U/mL) at 25 °C for 20 min. Two milliliters of coloring reagent (0.02 M acetylacetone/2.0 M ammonium acetate/0.05 M acetic acid) was added to the reaction mixture and incubated at 60 °C for 15 min. The color of the reaction mixture which developed at absorbance 412 nm was recorded, while methanol solutions (0.5–10 μg/mL) were used to construct the standard curve for the determination of the PME assay. One unit of activity was defined as the amount of enzyme forming 1 μmol of methanol per minute. Protein concentration of enzyme sample was measured using a Bio-Rad protein assay kit (Nippon Bio-Rad, Tokyo, Japan).

2.5. Determination of ash, protein and moisture content

Ash content was determined by incinerating 1 g of sample in a furnace at 600 °C for 4 h. The subsequent ash was cooled and stored in a desiccator with P_2O_5 until weighing. Nitrogen (N) was determined by the Kjeldahl procedure and protein content was estimated as N × 6.25. The moisture content was calculated as the weight loss after drying at 130 °C for 3 h.

2.6. Determination of anhydrouronic acid, total sugars, and citric acid

Total anhydrouronic acid (AUA) content of polysaccharide was determined by the xylenol method (Walter, Fleming, & MacFeeters, 1993). Sample (500 μL) in 1% (w/v) NaCl was mixed with 4.0 mL of sulfuric acid in an ice bath, and heated in a boiling water bath for 10 min. The solution was then mixed with 200 μL of glacial acetic acid containing 0.1% (w/v) xylenol, and rested for 10 min at room temperature. The reaction mixture was then measured at absorbance 450–415 nm, while the galacturonic acid solutions (10–100 μg/mL) were used to construct the standard curve for the determination of AUA content.

The content of total sugars of polysaccharide was determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using D-galactose as a standard.

Citric acid was measured by HPLC (Japan Spectroscopic Co., Ltd 880-Type, Japan) on a Shodex Ionpak C-811 (Showa Denko, Tokyo, Japan). Five mg of polysaccharide was dissolved in 6 N HCl (0.5 mL) and heated at 100 °C for 16 h, and then dried with a rotary evaporator at 50 °C. The dried sample was resuspended in distilled water (1 mL) for HPLC analysis. The column was eluted with 3 mM $HClO_4$ at a flow rate of 1.0 mL/min. Detection was done by monitoring the absorbance at 445 nm to elute organic acids with 0.2 mM bromothymol blue and 15 mM Na_2HPO_4.

2.7. Sugar composition analysis

One mg of sample was hydrolyzed in 400 μL of 4 M trifluoroacetic acid for 16 h at 100 °C. The hydrolysate was dried with a rotary evaporator at 40 °C and sugars were converted to UV derivatives with an ABEE labeling kit as described by Yasuno, Kokubo, and Kamei (1999). Aliquot (40 μL) of the ABEE reagent solution was added to sample, and the mixture was then incubated at 80 °C for 1 h. After cooling to room temperature, each 200 μL of chloroform and distilled water were added to the mixture. The reaction mixture was vigorously shaken and then centrifuged to separate it into two layers. The upper aqueous layer was used for HPLC analysis with a Waters 2697 Alliance and 996-photodiode array detector (Waters, Milford, MA) using a Cosmosil 5C18-MS column (2 mm × 150 mm, Nakalai Tesque, Kyoto, Japan). The flow rate was kept constant at 0.25 mL/min and the elution was under isocratic condition of 3% (v/v) of acetonitrile containing 0.01% trifluoroacetic acid at 40 °C. The ABEE-labeled sugars were monitored by measuring the absorbance at 308 nm.

2.8. Determination of degree of methylation

Degree of methylation (DM) in polysaccharide was determined by the modified alcohol oxidase method (Klavons & Bennett, 1986). Two mL of citrus pectin (0.2 mg/mL) in 50 mM phosphate buffer (pH 7.5) was mixed well and incubated with 1 mL of pectinesterase 10 U/mL (E.C.3.1.1.11, Sigma – Aldrich, Japan) for 60 min at 37 °C. The reaction mixture in a screwed cap tube was immediately heated at 100 °C in boiling water for 5 min to inactivate pectinesterase. The sample was then cooled in ice and 0.5 mL of the solution was reacted with 0.5 mL of alcohol oxidase (1.0 U/mL) at 25 °C for 20 min. Two mL of coloring reagent (0.02 M acetylacetone/2.0 M ammonium acetate/0.05 M acetic acid) was added to the reaction mixture and incubated at 60 °C for 15 min. The color of the reaction mixture which developed at absorbance 412 nm was recorded, while methanol solutions (0.5–10 μg/mL) were used to construct the standard curve for the determination of the methyl ester linkages in polysaccharide. DM was calculated as molar ratio (%) of methanol to uronic acid.

2.9. FT-IR spectroscopy

FT-IR spectrum of polysaccharide was obtained at a resolution of 1 cm^{-1}. Sample was incorporated with KBr (spectroscopic grade) and pressed into a 3 mm pellet. The 256 scans were entered before Fourier transformation. Spectra were recorded in the transparent mode from 4000 to 400 cm^{-1}, using a Spectrum 2000 (PerkinElmer Ink., MA, USA).

2.10. Size-exclusion chromatography

Sepharose CL-4B (GE Healthcare, Piscataway, NJ, USA) was used for size-exclusion chromatography. The column (11 mm × 47.5 cm) was eluted by 50 mM phosphate buffer (pH 6.8) containing 20 mM NaCl at the flow rate of 0.15 mL/min at 23 °C. The column was calibrated with Dextran 2000, 500, 70, and 40 (GE Healthcare, Piscataway, NJ, USA). Pectin that was dissolved in the same buffer (up to 1.0 mg/mL) was injected into the column and then 1.0 mL fractions were collected. The fractions were assayed for total sugars and uronic acid as described earlier.

3. Results and discussion

3.1. Effect of pH, temperature, and citric acid concentrations

The citric acid treatment led to increased viscosity of pectins in the pH region from 5.8 to 8.0 compared to untreated pectin (Fig. 1a). The viscosity of pectin treated at pH 7.0 was ten times than that of untreated pectin. The viscosity gradually increased from pH5.8 to pH 7.0 but not after pH 7.6. At alkaline pH-values pectin is rapidly deesterified and degradation occurs even at room temperature (Sriamornsak, 2003). However, the effect of citric acid treatment on increased viscosity was greater than rapid loss of viscosity due to alkaline pH.

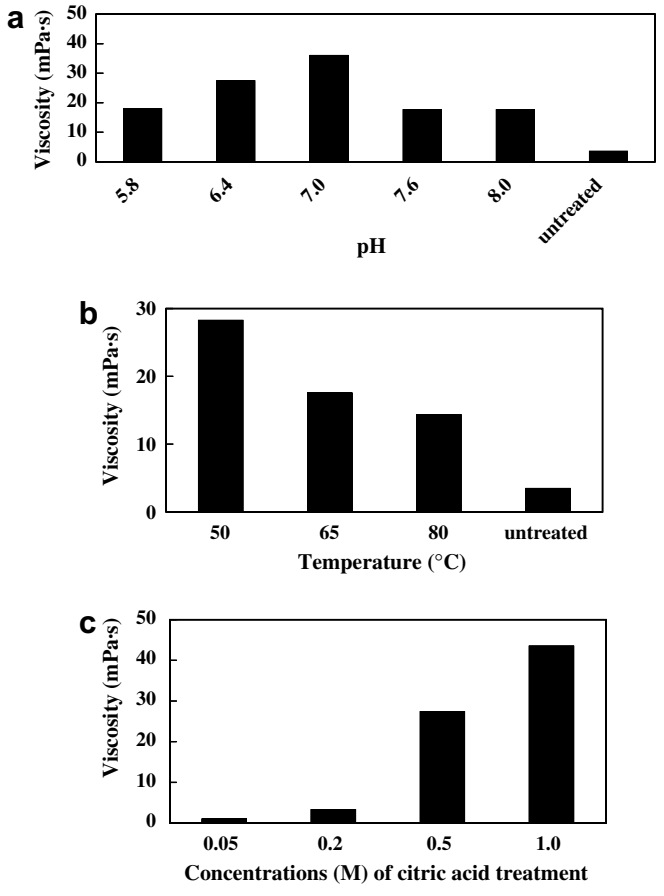

Fig. 1. Viscosity of citrus pectins (1% w/v) prepared with citric acid treatment. Different pH at 65 °C for 2 h (a), different temperature at pH 8.0 for 2 h (b), and different concentrations of citric acid at pH 8.0 at 65 °C for 2 h (c) were used. The viscosities of pectins which were solubilized with distilled water were measured at 25 °C. Data shown is from a typical experiment that was reproduced at least three times.

As the temperature increases, a β-elimination starts which results in chain cleavage of pectin (Van Buren, 1979). Just as expected, citric acid treatment at the high temperature showed the lowest viscosity. In every instance, pectin treated citric acid had higher viscosity than the untreated pectin (Fig. 1b). Considering that the experiment was performed at alkaline pH 8.0 which causes a greater degree of cleavage of pectin, the presence of citric acid seemed to inhibit the occurrence of β-elimination at high pH and temperature.

As the concentrations of citric acid increased, the viscosity increased (Fig. 1c). Citric acid is a tricarboxylic acid including a hydroxyl group. Both carboxyl groups and hydroxyl groups are acceptors of water molecules to form hydrogen bonding. Pectin molecules as well as citric acid contain both these functional groups so it is highly possible they attract each other as pectin produces a negative charge on the molecule at neutral pH. Thus, we have examined whether citric acid was bound to the extracted pectins with its treatment. Interestingly, significant correlation (R^2 = 0.978) between the viscosity and the bound amounts of citric acid to the pectins was observed (Fig. 2). The bound amounts were proportional to the concentrations of citric acid treatment (data not shown). As the bound amounts of citric acid to pectins increase, the viscosity correspondingly increases.

3.2. Pectin methylesterase activity

Citrus is known for its high amounts of pectin methylesterase (PME) (Arias & Burns, 2002; Do Amaral, Assis, & Oliveira, 2005). If the PME activity during the citric acid treatment were to be maintained or activated, the pectin would become less sensitive to β-elimination with decreasing DE. The result of the PME activities is shown in Table 1. Addition of citric acid more than 0.20 M caused the decrease of the PME activity although heat treatment at 65 °C increased by 185% in the absence of citric acid. However, the PME activity increased with increasing the temperature and was higher at neutral pH. The increase in PME activity was not, on the whole, significant during citric acid treatment at high temperature and high pH. As regards the deesterification in pectin, the remaining PME activity during the citric acid treatment would exert a greater influence on the pectin structure and let the pectin become less sensitive to β-elimination at high pH and temperature. Accordingly, the esterification degree of treated pectin is of great interest to what extent chemical and enzymatic deesterification can contribute in the extraction step. The analysis of the PME results obtained here and the satisfactory interpretation will be reported separately.

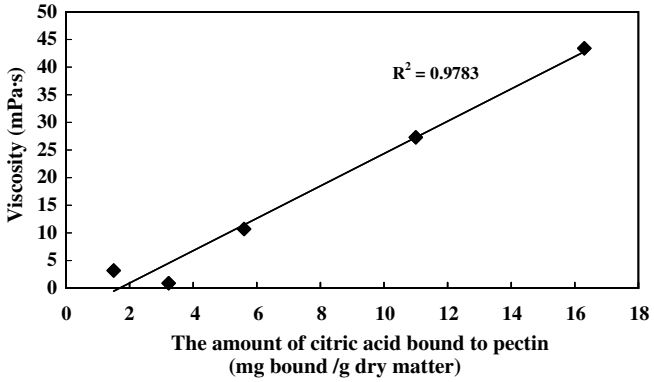

Fig. 2. Relationship between the viscosity and the bound amounts of citric acid to the citrus pectins. The citric acid bound to pectin was determined by HPLC after 6 N HCl hydrolysis.

Table 1
Specific activities of PME in crude extracts from dried peel powder of *Citrus unshiu* Marcovitch × *C. nobilis* Loureiro

Conditio			Specific activity (U/mg of protein)	
pH	Temp (°C)	Citric acid concentrations (M)		
Control				
6.5	4	0.00	4.90 ± 0.22	(100)
6.5	65	0.00	9.07 ± 0.34	(185)
6.5	65	0.05	10.9 ± 0.31	(222)
6.5	65	0.20	4.80 ± 0.19	(97)
6.5	65	0.50	4.34 ± 0.24	(88)
6.5	65	1.00	3.84 ± 0.25	(78)
5.8	65	0.50	3.57 ± 0.17	(72)
6.5	65	0.50	4.34 ± 0.24	(88)
7.0	65	0.50	5.70 ± 0.28	(116)
7.6	65	0.50	5.25 ± 0.16	(107)
8.0	65	0.50	5.01 ± 0.20	(102)
6.5	50	0.50	3.38 ± 0.11	(61)
6.5	65	0.50	4.34 ± 0.24	(88)
6.5	80	0.50	5.91 ± 0.36	(120)

Dried peel powder was constant for 2 h at each temperature, pH and citric acid concentrations. Then, crude extract was prepared by centrifugation and assayed. One unit of enzyme activity is the amount of enzyme producing 1 μmol of methanol per minute. The values are mean ± SD of duplicate tests with at least three measurement.

3.3. Chemical composition

As shown in Table 2, the treated pectin had a high content of citric acid (1.63% w/w) compared to untreated pectin (0.15% w/w). The yields were slightly lower under the condition with citric acid treatment at neutral pH. However, the purity of pectin treated with citric acid was higher than that of untreated pectin. In particular, the protein content of the pectin treated with citric acid was lower. Citric acid can react with amino groups in protein molecules to form amide. Previous study has shown that phosphorylation with polyphosphate was effective in removal of protein; as a result, the highly purified insoluble fiber could be fractionated (Yamazaki, Murakami, & Kurita, 2005). Incorporation of citric acid into the protein molecule might produce a more negative charge in the complex to solubilize the protein and then precipitate during heating at pH 2.2. In our experiment, the pectin treated with citric acid was extracted at same pH 2.2 as untreated pectin, though citrinylation from citric acid was identifiable in the treated pectin. The lower yields of treated pectin might be due to the higher purity.

Table 2
Chemical composition of the citrus pectins with or without citric acid treatment

Composition (%, w/w)	Untreated pectin	Treated pectin
Yields[a]	8.5 ± 0.08	7.4 ± 0.06
Moisture	9.6 ± 0.05	8.3 ± 0.35
Ash	3.7 ± 0.23	4.9 ± 0.10
Protein	5.4 ± 0.65	3.0 ± 0.54
Total sugar	81.1 ± 1.7	83.5 ± 1.9
Uronic acids	35.0 ± 3.1	27.2 ± 3.6
Glucuronic acid	0.7 ± 0.06	0.6 ± 0.05
Galacturonic acid	34.3 ± 3.2	26.6 ± 3.6
Neutral sugars	44.6 ± 4.1	53.2 ± 2.3
Rhamnose	6.4 ± 0.74	6.7 ± 0.36
Galactose	13.4 ± 1.0	24.7 ± 1.6
Mannose	0.2 ± 0.06	0.2 ± 0.08
Glucose	0.5 ± 0.35	0.8 ± 0.36
Arabinose	23.7 ± 2.9	19.4 ± 0.9
Fucose	Trace	0.8 ± 0.24
Xylose	0.4 ± 0.14	0.6 ± 0.29
Degree of methylation[b]	65.1 ± 3.6	8.4 ± 2.64
citric acid	0.15 ± 0.05	1.63 ± 0.12

The treated pectin was prepared with 0.5 M citric acid treatment at pH 7.0 at 65 °C for 2 h.
[a] Yield was expressed as percentage of 100 g dried citrus peel.
[b] Degree of methylation was calculated as molar ratio (%) of methanol to uronic acid. The values are mean ± SD of duplicate tests with at least three measurement.

There are two major differences between the treated and untreated pectins (Table 2). The first one is the degree of methylation. The treated pectin was a low-methoxy pectin (DM 8.4%) compared with the untreated pectin (DM 65.1%) even though determination of DM with incubation using PME was performed not to hydrolyze all methyl esters on the pectin chain. The treated pectin seemed to undergo deesterification during heating in the presence of citric acid at neutral pH by the remaining PME activity (Table 1) or extraction at acidic pH in boiling water because deesterification is observed at low pH-values, notably, at alkaline pH-values (Sriamornsak, 2003).

The second one is the ratio of neutral sugar to uronic acids. The neutral sugar contents of the treated pectin (53.2%) were much higher than that of untreated pectin (44.6%). In particular the contents of galactose which is rich in hairy regions of pectin (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996) were higher in the treated pectin. However, the contents of galacturonic acid in the treated pectin (26.6%) were considerably lower than that in the untreated pectin (34.3%). It seems that the presence of citric acid induced a breakdown in the smooth region composed of homogalacturonan. At low pH-values and elevated temperatures degradation due to hydrolysis of glycosidic linkages and the different sensitivities to acid hydrolysis (GalA–GalA > GalA–Rha > neutral sugar – neutral sugar) are observed (Thibault, Renard, Axelos, Roger, & Crépeau, 1993). The treated pectin was consistent with this observation. In addition, if the molar ratio of rhamnose to galacturonic acid is indicative of the degree of branching, then we would predict that the treated pectin is more branched (ratio 1:3.3) compared to the untreated pectin (ratio 1:4.5). Futhermore, the arabinose contents of treated pectin reduced by 82% to those of untreated pectin. Significant loss of arabinose occurs during dilute acid hydrolysis (Renard, Crépeau, & Thibault, 1995). During acid extraction at pH 2.2 in boiling water the presence of citric acid seemed to trigger more breakdowns of arabinan side chains. As the results, the treated pectin contains relatively more substituted pectin polymers (rhamnogalacturonan I and II), which render it less appropriate for its typical application: gelling additive. Additionally, citric acid treatment after heat extraction at acidic pH might achieve the pectin with high galacturonic acid content which is industrially relevant.

3.4. FT-IR

In order to confirm the presence of treated pectin by chemical analysis, both treated and untreated pectins were analyzed by FT-IR (Fig. 3). In the treated pectin, the weaker absorption at 1746 cm^{-1} corresponding to the C=O stretching vibration of methyl-esterified carboxyl groups (Chatjigakis et al., 1998) was assigned to be a low methoxy pectin. The differences between the treated and untreated pectins were observed in the characteristics absorption bands related to anion carboxylate (1603 and 1412 cm^{-1}) (Gonzaga, Ricardo, Heatley, & Soares, 2005). In the treated pectins, the intensities of the free carboxyl stretching band at 1412 cm^{-1} increased and those at 1628 cm^{-1} shifted to 1603 cm^{-1}. This represents the increase of carboxyl groups and the decrease of amide I by lower protein contents since amide band located around 1680 cm^{-1} was observed by Yang and Yen (2002). Additionally, the intensities of the absorbance of C—H stretching around 2900 cm^{-1} (Manrique & Lajolo, 2002) and C—H bending at 1371 cm^{-1} (Singthong, Ningsanond, Cui, & Goff, 2005) were weaker in the treated pectin than untreated pectin. This might be explained by further study addressing the question where citric acid is bound to pectin molecule.

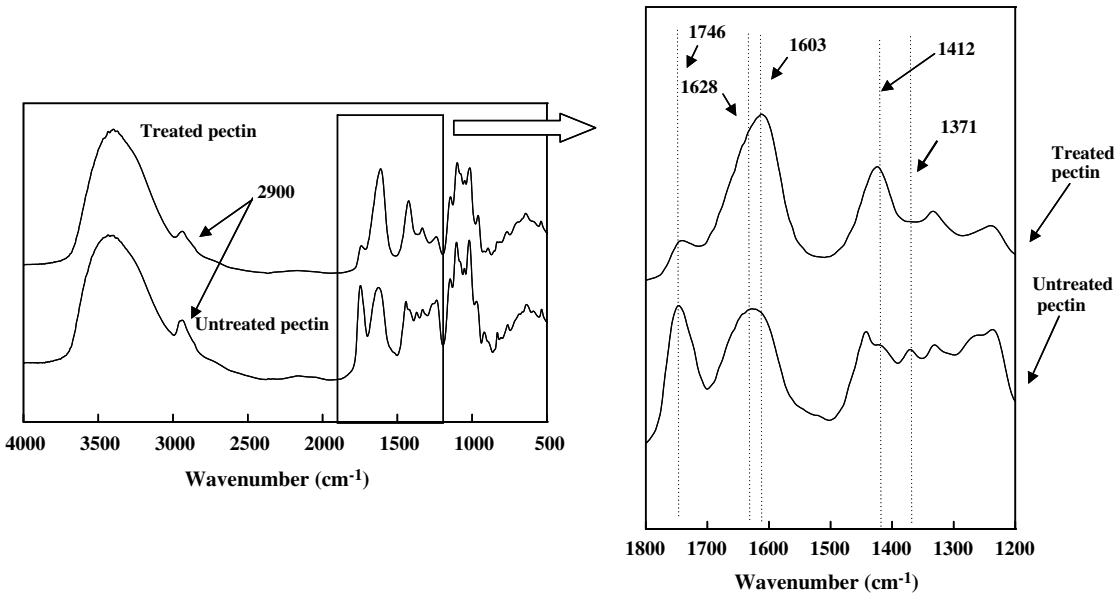

Fig. 3. Fourier transform infrared spectra of the citrus pectins with or without citric acid treatment. The treated sample was prepared with 0.5 M citric acid treatment at pH 7.0 at 65 °C for 2 h. Data shown is from a typical experiment that was reproduced at least three times.

3.5. Size-exclusion chromatography

The molecular weight range of the pectins was determined by size-exclusion chromatography on Sepharose CL-4B using dextrans as calibration standards (Fig. 4). The elution profile of the untreated pectin showed that it was significantly smaller with a molecular weight range below 70 kDa as shown by previous data (Owens, Lotzkar, Schultz, & MaClay, 1946) (Fig. 4b). In contrast, an increase in molecule weight of the treated pectin was more remarkable as the elution pattern with a peak at elution volume 22 mL (2000 kDa) was observed (Fig. 4a). The molecular weight range of the treated pectin was broad from 50 to 2000 kDa. The uronic acid content in the treated pectin was low through the broad peak with the weight ratios (uronic acid/total sugar) of 1:4.2 and 1:2.4 at elution volume 32 and 48 mL, respectively. The weight ratio in the untreated pectin was 1:1.6 on the peak at elution volume 51 mL. The remarkable increase of molecular weight in the treated pectin appeared to be due to polymerization mainly of neutral sugars. Considering the polymerization in the treated pectin together with the high amounts of citric acid bound to its molecule, it can at least be said that citric acid was regarded as a cross-linking agent between pectin molecules through its reaction with neutral sugars. Indeed, citrate induces polymerization of an inactive protomeric form of acetyl-CoA carboxylase into an active form into composed of 10–20 protomers (Beaty & Lane, 1983) and generates an inorganic polymer to allow immobilization of bacteria in silica matrices (Alvarez, Desimone, & Diaz, 2007). Otherwise stated, this work is the first observation of the pectin polymerization in the production process using citric acid. Finally, the higher molecular weight pectin treated with citric acid was reflected by increased viscosity. This might be due to strong association between the deesterified regions in the citric acid treated pectin which could involve small quantities of divalent cations. The viscosity in citric acid treated pectin did not change substantially in the presence of chelating agent, EDTA (data not shown). There exists a possibility that divalent cations strongly bind to the pectin molecules or that pectin polymerization induced by citric acid is superior to cross-linking by divalent cations between pectin molecules. Further studies to analyze the effect of divalent cations on pectin polymerization in treated pectin are required.

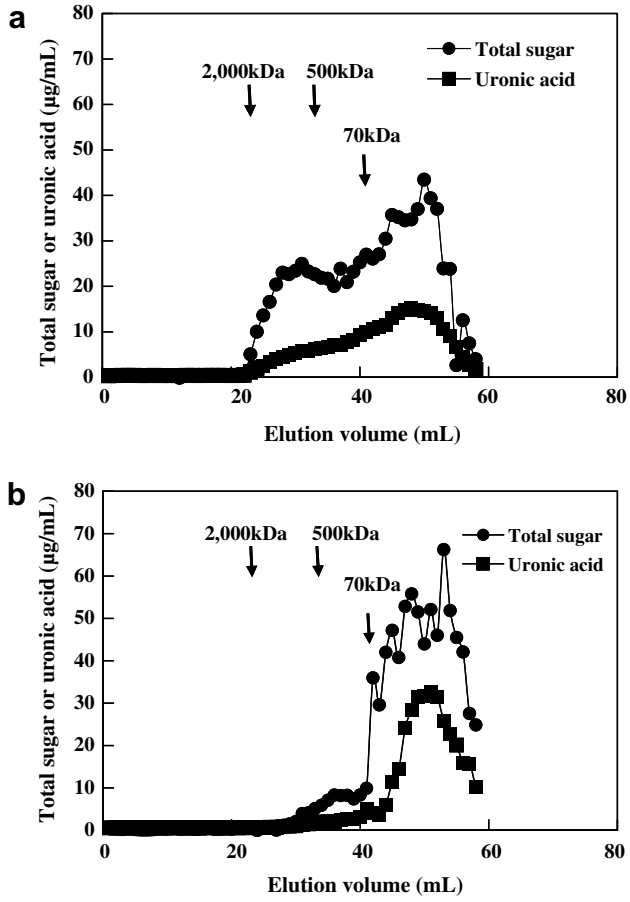

Fig. 4. Sepharose CL-4B size exclusion chromatograms of 0.1% (w/v) treated citrus pectin (a) and 0.1% (w/v) untreated pectin (b). The treated sample was prepared with 0.5 M citric acid treatment at pH 7.0 at 65 °C for 2 h. Eluent was 50 mM phosphate buffer (pH 6.8) containing 20 mM NaCl. Data shown is from a typical experiment that was reproduced at least three times.

4. Conclusions

Treatment with citric acid at neutral pH during extraction of pectin indicated that the isolated pectin had a higher molecular weight with higher contents of neutral sugars. The viscosity of pectin was proportional to the quantity of citric acid bound. This is the first report of chemically modified (low-methoxy) pectin without a significant loss of the molecular weight. The citric acid treatment preferred to polymerize rather than degrade the pectin molecule due to β-elimination at neutral pH and high temperature. The resulted pectins with a unique chemical composition of low methylation and high neutral sugar contents will satisfy the industrial demand for pectins with varying ability of gelling, thickening and stabilizing. Further study of citric acid treatment on pectin and its structural analysis in the application for gels will be of great interest.

References

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., & Voragen, A. G. J. (1996). Progress in biotechnology. In J. Visser & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 47–56). Amsterdam BV: Elsevier Science.

Alvarez, G. S., Desimone, M. F., & Diaz, L. E. (2007). Immobilization of bacteria in silica matrices using citric acid in the sol-gel process. *Applied Microbiology and Biotechnology, 73*, 1059–1064.

Arias, C. R., & Burns, J. K. (2002). A pectinmethylesterase gene associated with a heat-stable extract from citrus. *Journal of Agricultural and Food Chemistry, 50*, 3465–3472.

Beaty, N. B., & Lane, M. D. (1983). The polymerization of acetyl-CoA carboxylase. *The Journal of Biological Chemistry, 258*, 13051–13055.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*, 395–408.

Do Amaral, S. H., Assis, S. A. D., & Oliveira, O. M. M. D. F. (2005). Partial purification and characterization of pectin methylesterase from orange (*Citrus sinensis*) cv. *Pera-Rio, 29*, 367–380.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Gonzaga, M. LC., Ricardo, N. M. P. S., Heatley, F., & Soares, S. de. A. (2005). Isolation and characterization of polysaccharides from *Agaricus blazai* Murill. *Carbohydrate Polymers, 60*, 43–49.

Hotchkiss, A. T., Jr., Savary, B. J., Cameron, R. G., Chau, H. K., Brouillette, J., Luzio, G. A., et al. (2002). Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. *Journal of Agricultural and Food Chemistry, 50*, 2931–2937.

Klavons, J. A., & Bennett, R. D. (1986). Determination of methanol using alcohol oxidase and its application to methyl ester content of pectin. *Journal of Agricultural and Food Chemistry, 34*, 597–599.

Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology, 25*, 99–107.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*, 79–99.

Lee, C.-W., Wu, M.-C., Lee, B.-H., Jiang, C.-M., & Chang, H.-M. (2003). Changes in tomato and citrus pectinesterases as determined by gel permeation chromatography. *Journal of Agricultural and Food Chemistry, 51*, 5455–5461.

Owens, H. S., Lotzkar, H., Schultz, T. H., & MaClay, W. D. (1946). Shape and size of pectinic acid molecules deduced from viscometric measurements. *Journal of American Chemical Society, 68*, 1628–1632.

Ralet, M. C., Bonnin, E., & Thibault, J.-F. (2001a). Chromatographic study of highly methoxylated lime pectins deesterified by different pectin methyl-esterases. *Journal of Chromatography, 753*, 157–166.

Ralet, M.-C., Dronnet, V., Buchholt, H. C., & Thibault, J.-F. (2001b). Enzymatically and chemically de-esterified lime pectins: Characterisation, polyelectrolyte behaviour and calcium binding properties. *Carbohydrate Research, 336*, 117–125.

Renard, C. M. G. C., Crépeau, M.-J., & Thibault, J.-F. (1995). Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet, and citrus pectins. *Carbohydrate Research, 275*, 155–165.

Renard, C. M. G. C., & Thibault, J.-F. (1996). Pectins in mild alkaline conditions: β-Elimination and kinetics of demethylation. In J. Visser & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 603–608). New York: Elsevier.

Singthong, J., Ningsanond, S., Cui, S. W., & Goff, H. D. (2005). Extraction and physicochemical characterization of Krueo Ma Noy pectin. *Food hydrocolloids, 19*, 793–801.

Sriamornsak, P. (2003). Chemistry of pectin and its pharmaceutical uses: A review. *Silpakorn University International Journal, 3*, 206–228.

Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin-A review. *Critical Reviews in Food Science and Nutrition, 37*, 47–73.

Thibault, J. -F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., & Crépeau, M. -J. (1993). Studies of the length of homogalacturonic regions in pectin by acid hydrolysis. *Carbohydrate Research, 238*, 271–286.

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

Walter, W. M., Jr., Fleming, H. P., & MacFeeters, R. F. (1993). Base-mediated firmness retention of sweet potato products. *Journal of Food Science, 58*, 813–816.

Yamazaki, E., Murakami, K., & Kurita, O. (2005). Easy preparation of dietary fiber with the high water-holding capacity from food sources. *Plant Food for Human Nutrition, 60*, 17–23.

Yang, J., & Yen, H. E. (2002). Early salt stress effects on the changes in chemical composition in leaves of ice plant and Arabidopsis; A Fourier transform infrared spectroscopy study. *Plant physiology, 130*, 1032–1042.

Yasuno, S., Kokubo, K., & Kamei, M. (1999). New method for determining the sugar composition of glycoproteins, glycolipids, and oligosaccharides by high-performance liquid chromatography. *Bioscience, Biotechnology, and Biochemistry, 63*, 1353–1359.