JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Changes in Molecular Weight of Transacylated Pectin Catalyzed by Tomato and Citrus Pectinesterases As Determined by Gel Permeation Chromatography

CHING-WEN LEE,† MING-CHANG WU,‡ BOR-HON LEE,† CHII-MING JIANG,§ AND HUNG-MIN CHANG*,†

Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 10617, Taiwan, Department of Food Science, National Pingtung University of Science and Technology, Pingtung, 91207 Taiwan, and Department of Seafood Science, National Kaohsiung Institute of Marine Technology, Kaohsiung 811, Taiwan

The changes in molecular masses of pectin in 0.5% pectin−pectinesterase (PE) mixtures (2 units/mL) incubated at various temperatures, pH values, and NaCl levels for 30 min were observed by a Toyopearl TSK HW-65 (F) gel permeation chromatography. The molecular mass of pectin was remarkably increased from 103 to 266 kDa when the incubation temperature of pectin−tomato PE was increased from 25 to 45 °C. A further increase in molecular mass was observed when a pectin−citrus PE mixture was incubated at 65 °C. The values of pH and NaCl levels were also crucial to the transacylation activity of PEs. Reaction at pH 7.5 with tomato PE and citrus PE remarkably expanded the molecular mass of pectin to 410 and 670 kDa, respectively. The NaCl level of 0.3−0.5 and 0.3 M was favorable for the transacylation reaction of tomato PE and citrus PE, respectively. Only high methoxylpectin was the suitable substrate for PE to conduct the transacylation reaction.

KEYWORDS: Transacylation reaction; tomato PE; citrus PE; gel permeation chromatography

INTRODUCTION

Pectinesterases (pectin pectyl-hydrolase, EC 3.1.1.11) (PEs) are hydrolases, which release methanol from pectin molecules and convert pectin to pectic substances and polygalacturonic acids. Softening of the vegetable and fruit is enhanced in the presence of polygalacturonase during the maturation process.

The C_6 carboxyl groups in the pectin−PE complexes are transferred to adequate acceptors, such as water molecules, to fulfill the deesterification reaction. However, the hydroxyl groups of pectin molecules are occasionally acceptors of such a reaction and, therefore, form high molecular weight pectins (*1*−*3*). In vivo, such transacylation reactions may not occur due to the compartmentalization of pectin and PE in plant cells. However, some recent papers indicated the remarkable increase in the molecular weight of pectin in pectin−PE mixtures with in vitro. Citrus pectin (MW = 980 kDa) with a degree of esterification (DE) of 68% was increased in particle size (diameter) from about 100 to 400 nm after incubation with pea sprout PE at 30 °C, pH 6.6, for 16 h (*3*). In addition, a marked increase in the molecular weight of citrus pectin in a pectin−jelly fig PE mixture, incubated at 45 °C, pH 6.5, for 4 h, was observed by a gel permeation chromatography (*2*). An increase in the nonmethyl ester linkages by 40% during pectin gelling also supported the occurrence of the esterification reaction between C_6 carboxyl groups and hydroxyl groups in pectin molecules in the presence of PE (*4*).

The turbidity observation of pectin−PE mixtures was also effective in determining the progress of transacylation. It was interesting to note that tomato PE (2 units/mL) increased the turbidity (400 nm) of 1.5% citrus pectin in 0.25 M NaCl at pH 6.5 to about 1.4 after incubation for only 10 min (*5*). It suggests the remarkable formation of suspended particles of pectins with increased molecular weights.

As a series of studies on pectin polymerization and to develop the possible application of the transacylated pectin products, PEs from tomato and citrus, the vast fruit sources for food processing, were incubated with citrus pectins. Then, observations on chromatographic changes in molecular weight of pectin as a result of the transacylation reaction catalyzed by PEs under various temperatures, pH values, and NaCl levels were conducted. The DE of pectin on the molecular weight change was also discussed.

MATERIALS AND METHODS

Materials. Citrus pectin (Sigma Chemical Co., St. Louis, MO) with a DE of 31, 67, or 89% was used for the transacylation reaction, while pectin with a DE of 67% was used for the PE activity assay. Ions, especially divalent cations, possibly presented in pectin samples for transacylation reaction, were removed according to the method

* To whom correspondence should be addressed. Tel: +886-2-23630231 ext 2776. Fax: +886-2-23620849. E-mail: changhm@ccms.ntu.edu.tw.
† National Taiwan University.
‡ National Pingtung University of Science and Technology.
§ National Kaohsiung Institute of Marine Technology.

10.1021/jf021170t CCC: $25.00
Published on Web 07/24/2003

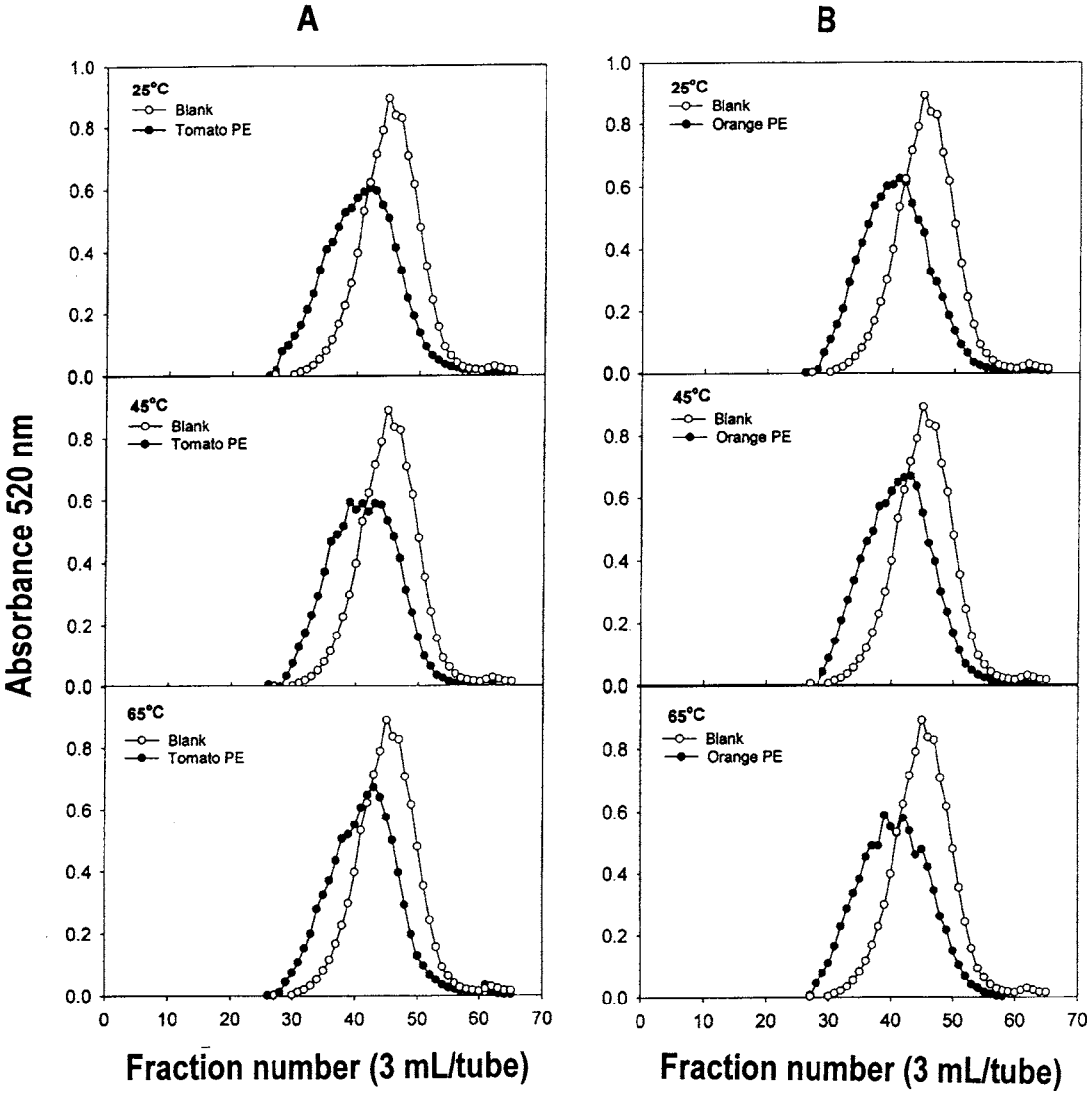

Figure 1. Toyopearl TSK HW-65 (F) gel permeation chromatograms of 0.5% citrus pectin–tomato pectinesterase (A) and 0.5% citrus pectin–citrus pectinesterase (B) (2 units/mL) mixture in 0.1 M NaCl at various temperatures and pH 6.5 for 30 min. Column, 1.6 × 100 cm; eluent, 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5); flow rate, 60 mL/h; fractionation, 3 mL/tube; detection, absorbance at 520 nm after *m*-hydroxydiphenyl reaction.

described by Jiang et al. (*2*). CM-Sepharose CL-6B was from Pharmacia (Uppsala, Sweden) while Toyopearl TSK HW-65 (F) was the product of Toyo Co. (Tokyo, Japan).

Preparation of PEs from Tomato and Citrus. The extraction of tomato PE was conducted following the methods described by Pressey and Avants (*6*). Edible portions of fruit were homogenized with four parts (1:4) 0.25 M NaCl/0.1 M phosphate buffer (pH 8.0) at 4 °C overnight. Supernatant by centrifugation (10 000*g*, 1 h, 4 °C) was added with ammonium sulfate to collect the precipitates of 0−80% saturation for CM-Sepharose CL-6B ion-exchange chromatography (eluent, 0−1 M NaCl/0.1 M phosphate buffer, pH 8.0) (*7*). Fractions with PE activity were pooled.

Citrus PE was extracted from edible portions of Sunkist from a local market with four parts (1:4) 1 M NaCl/0.3 M phosphate buffer for 2 h at 4 °C (*7*). Supernatant by centrifugation (12000*g*, 30 min, 4 °C) was treated with ammonium sulfate to collect the precipitates of 30−80% saturation for further purification by a CM-Sepharose CL-6B ion-exchange column, as described for the purification of tomato PE, to pool the fractions with PE activity.

After dialysis against 0.1 M NaCl/0.1 M phosphate buffer (pH 6.5) and membrane concentration by an Amicon concentrator (MWCO = 10 kDa) (Millipore, Boston, MA), pooled PE solutions were diluted with 0.1 M NaCl/0.1 M phosphate buffer (pH 6.5) to obtain a solution of 8 units/mL for the following transacylation reactions with pectin.

Gel Permeation Chromatography for Transacylation Determination. Three milliliters of 0.5% citrus pectin with a DE of 67% in 0.1 M NaCl/0.1 M phosphate buffer (pH 6.5) was mixed well and incubated with 1 mL of tomato PE or citrus PE (8 units/mL) for 30 min at the desired temperature (25, 45, or 65 °C). To investigate the effect of pH on the transacylation reaction, a pectin−PE mixture (2 unit/mL) was incubated in a 0.1 M NaCl/0.1 M phosphate buffer with pH 5.5, 6.5, or 7.5 at 25 °C for 30 min.

Similar procedures were conducted to investigate the effect of NaCl level (0−0.7 M) on the transacylation reaction except that the tomato PE activity in the pectin−PE mixture was reduced to I unit/mL and the incubation time for the pectin−citrus PE mixture (2 units/mL) was shortened to 5 min to avoid the severe turbidity occurrence upon the preparation of pectin−PE mixtures. Pectins with various DEs (31, 67, or 89%) were used to study the effect of DE on the transacylation reaction following the procedures described above. After incubation for the desired period of time (30 or 5 min), all of the mixtures were heated at 100 °C in a boiling water bath for 5 min to terminate the reaction.

After the mixture was filtered through a 0.45 μm membrane to remove the suspended particles in pectin−PE reaction mixtures, pectin samples were applied to a Toyopearl TSK HW-65 (F) column (1.6 × 100 cm), which was eluted with 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5) at a flow rate of 60 mL/h. A fractionation of 3 mL was collected. The pectin content in the eluate was quantified using the

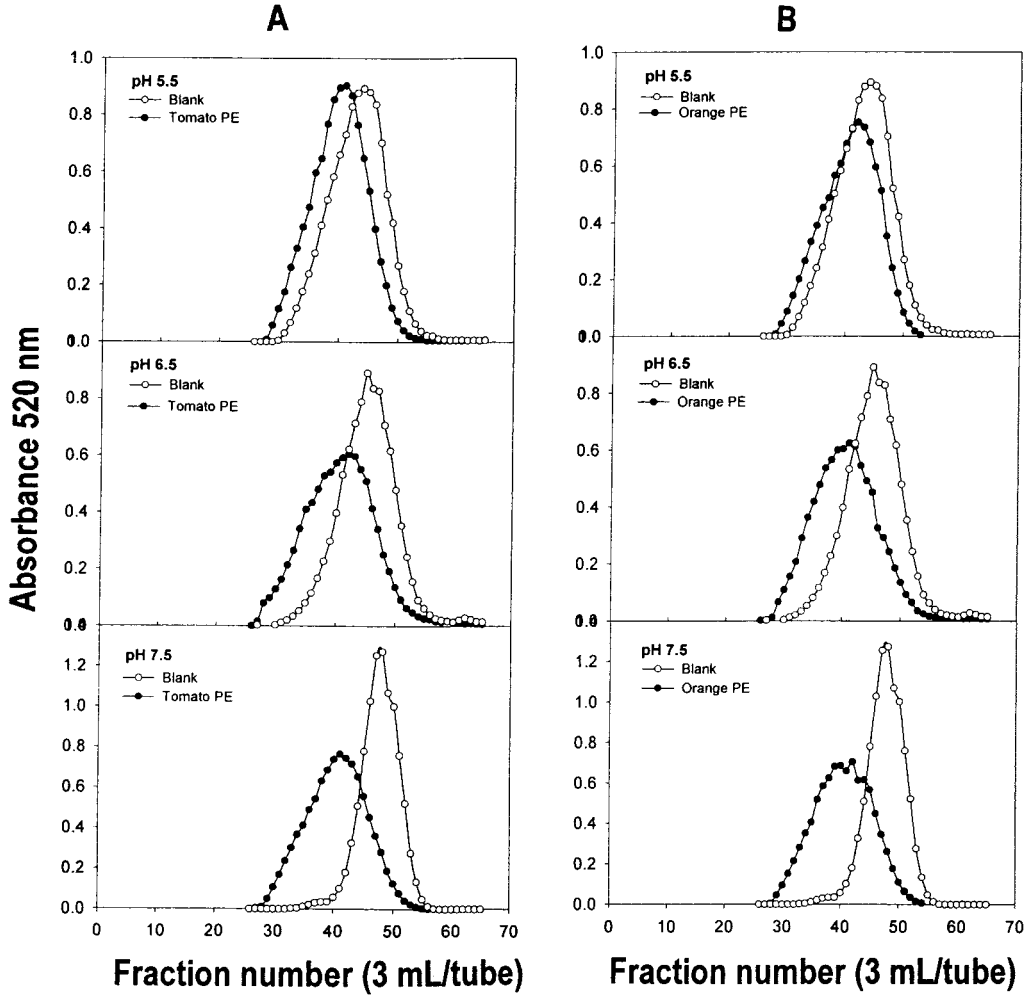

Figure 2. Toyopearl TSK HW-65 (F) gel permeation chromatograms of 0.5% citrus pectin–tomato pectinesterase (A) and 0.5% citrus pectin–citrus pectinesterase (B) (2 units/mL) mixture in 0.1 M NaCl at 25 °C and various pH values for 30 min. Column, 1.6 × 100 cm; eluent, 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5); flow rate, 60 mL/h; fractionation, 3 mL/tube; detection, absorbance at 520 nm after *m*-hydroxydiphenyl reaction.

method described below. Dextrans (77, 266, 410, and 670 kDa) (Fluka, Buchs, Sweden) were used to construct the standard curve ($y = -0.1927x + 1.4113$; x, log MW of Dextran kit; y, Kav; $r^2 = 0.9891$) for estimating the molecular weights of polymerized pectins as a result of the transacylation reaction.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). Gels containing 15% acrylamide were used to confirm the purity of the PE solution pooled from CM-Sepharose CL-6B ion-exchange chromatography using low molecular mass marker proteins (94, 67, 43, 30, 20.1, and 14.4 kDa) from Pharmacia Co.

PE Activity Assay. PE activity was determined according to the method described by Lee and MacMillan (*8*) with minor modifications. One milliliter of PE solution was added to 15 mL of 0.1 M NaCl/0.5% citrus pectin solution (substrate solution) (25 °C) with the pH adjusted to 6.5 immediately before assay. The activity of PE was measured by titrating (pH M83 Autocal pH meter, TTT 80 titrator, ABU80 autoburet, Radiometer Copenhagen Co., Copenhagen, Denmark) the free protons dissociated from the free carboxyl groups formed by the PE activity. The volumes (mL) of 0.01 N NaOH consumed to maintain a pH of 6.0 of the reaction solution at 25 °C (in a water bath) were recorded within 5 min. An activity unit represents 1 μequiv of the free carboxyl groups produced by the PE hydrolytic activity on the pectin substrate per min at 25 °C. An enzyme solution previously heated in boiling water for 5 min was used as a blank. Triplicate samples were each analyzed twice. Deionized water (Mili-Q System, Millipore, Tokyo, Japan) was used to prepare the substrate and enzyme solutions.

Determination of Pectin. Pectin was determined using the method described by Blumenkrantz and Asboe-Hansen (*9*). An adequate volume (0.5 mL) of pectin solution was well mixed with 3 mL of 0.0125 M Na−tetraborate solution (in concentrated sulfuric acid) and then heated in a boiling water bath for 5 min. After it was cooled in an iced water bath, the reaction mixture was uniformly mixed with 0.05 mL of 0.15% *m*-phenylphenol/0.5% NaOH solution; then it was allowed to stand for 5 min. The color that developed at 520 nm was recorded with a spectrophotometer. The standard curve ($r^2 = 0.975$) was constructed using different concentrations (0−100 μg/mL) of D-galacturonic acid (Sigma). This was used to provide the pectin content of the samples. Triplicate samples were analyzed twice each.

Determination of Protein. Protein content in solutions containing PE was determined by the Bradford (*10*) method using the Bio-Rad protein assay dye reagent. Bovine serum albumin (0.2−1.4 mg/mL) was used to construct the standard curve ($r^2 = 0.989$). Triplicate samples were analyzed twice each.

RESULTS AND DISCUSSION

Tomato PE purified by a CM-Sepharose CL-6B column chromatography was assayed to have an activity of 78 units/mg protein, whereas purified citrus PE was determined to have an activity of 56 units/mg protein (data not shown). Each obtained PE contains two PE isozymes (*11*, *12*), as were observed from the chromatograms and SDS−PAGE (data not shown) and were indicated by Versteeg et al. (*13*), Koner et al. (*14*), and Rillo et al. (*15*) using the different chromatographic systems. All of the fractions exhibiting PE activity were pooled for the following pectin−PE transacylation reaction.

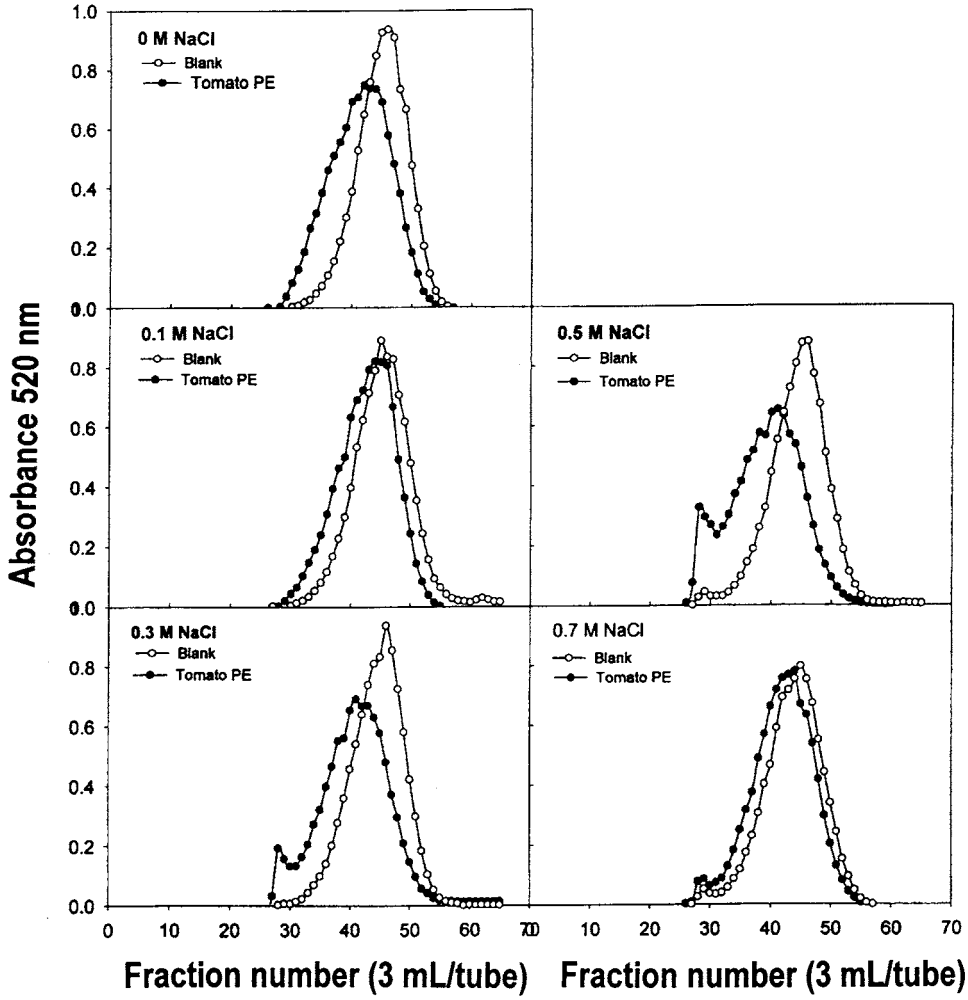

Figure 3. Toyopearl TSK HW-65 (F) gel permeation chromatograms of 0.5% citrus pectin–tomato pectinesterase (1 unit/mL) mixture in various NaCl levels at 25 °C and pH 6.5 for 30 min. Column, 1.6 × 100 cm; eluent, 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5); flow rate, 60 mL/h; fractionation, 3 mL/tube; detection, absorbance at 520 nm after *m*-hydroxydiphenyl reaction.

Effect of Reaction Temperature on Transacylation Reaction. The pectin−tomato PE mixture was incubated at 25, 45, or 65 °C for 30 min, and the elution pattern was shown in **Figure 1A**. Pectin solution alone was eluted with a peak at fraction number 45, revealing that the molecular mass of pectin used was about 103 kDa, as estimated with the standard curve constructed by dextrans. However, when tomato PE was incubated with the pectin solution at 25 °C, an elution pattern with a peak at fraction number 42 (266 kDa) was observed. An increase in pectin molecular weight (fraction number 39) was more remarkable when the pectin−tomato PE mixture reacted at 45 °C. A further increase in reaction temperature (65 °C) appeared to be unfavorable for the pectin−PE reaction as the elution pattern with a peak at fraction number 43 (209 kDa) was observed. Therefore, incubation at 45 °C appeared to be favorable for the transacylation reaction catalyzed by tomato PE. Similar results were reported by Jiang et al. using PE from jelly fig achenes as the enzyme source (*2*). However, tomato PE displayed the optimal temperature for a deesterification reaction at 65 °C (*5*), apparently higher than that for transacylation.

During the incubation of the pectin−tomato PE mixture, suspended particles were observed and the turbidity increased in response to the progress of the transacylation reaction (*5*). A previous paper (*3*) also indicated that isolated PE isozymes from pea sprouts increased the particle size of pectin from ∼50−70 to ∼250−350 nm after reaction at 30 °C, pH 6.5, for 4 h. Therefore, for convenience of gel permeation chromatography, filtration (0.45 μm) was conducted to remove the suspended particles of aggregated pectins before pectin−PE mixtures were applied to the column. As a result, a remarkable reduction in pectin quantity in the elution pattern of the pectin−PE mixture was observed (**Figure 1**). Urea (3 M) in eluent is effective in preventing transacylated pectin from aggregation during chromatographic analysis.

The reaction temperature also affected the pectin−citrus PE transacylation reaction (**Figure 1B**). At 25 °C, the molecular mass of pectin in incubated mixture was determined to be about 410 kDa (fraction number 41). Incubation of pectin−PE mixture at a higher temperature (65 °C) apparently facilitated the transacylation reaction, showing an elution pattern with a peak at fraction number 39. As compared to the molecular weight change in transacylated pectin catalyzed by citrus PE (**Figure 1B**), tomato PE appeared to be very potent in catalyzing the polymerization of pectin. This result was consistent with that observed by turbidity change (*5*).

Variance in PE sources displayed individual optimal transacylation conditions. However, it is interesting to note that tomato PE catalyzed two reactions with different optimal temperatures, while citrus PE showed the same optimal temperature for two reactions.

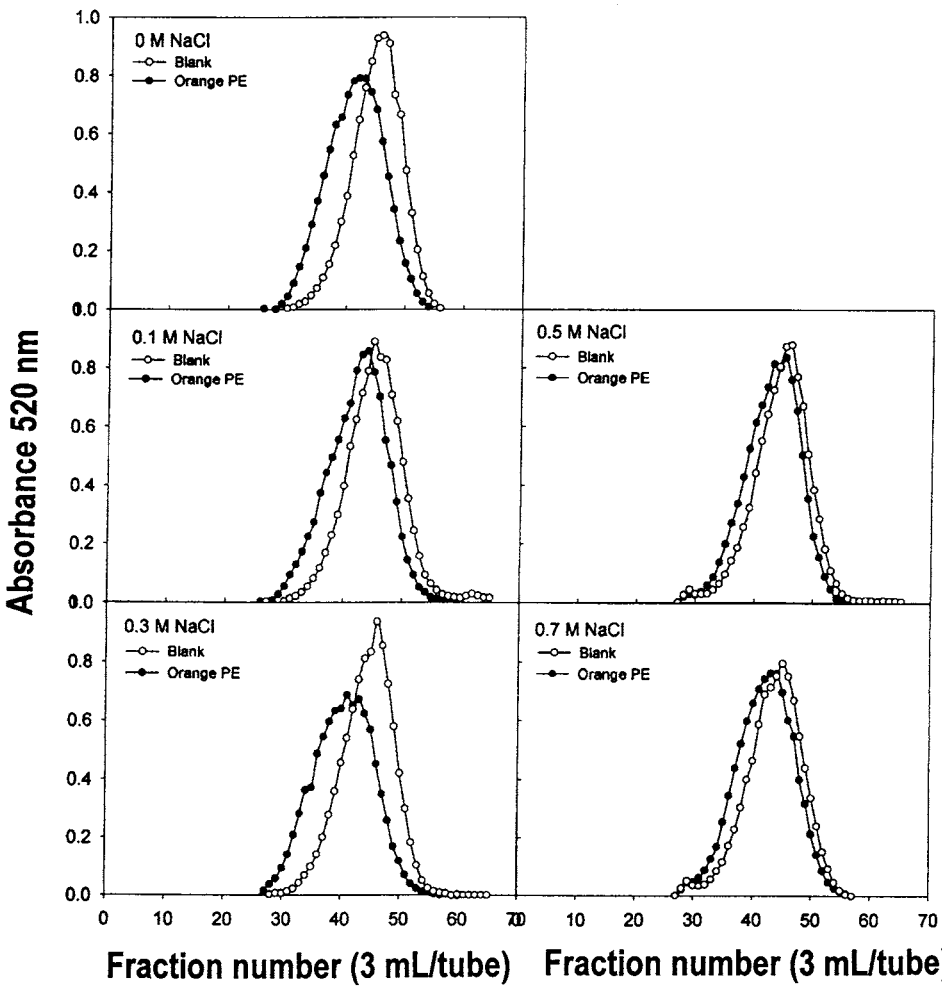

Figure 4. Toyopearl TSK HW-65 (F) gel permeation chromatograms of 0.5% citrus pectin–citrus pectinesterase (2 units/mL) mixture in various NaCl levels at 25 °C and pH 6.5 for 5 min. Column, 1.6 × 100 cm; eluent, 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5); flow rate, 60 mL/h; fractionation, 3 mL/tube; detection, absorbance at 520 nm after *m*-hydroxydiphenyl reaction.

Effect of Reaction pH on Transacylation Reaction. Figure 2 represents the elution patterns of incubated pectin−PE mixtures in 0.1 M NaCl solution at pH 5.5, 6.5, and 7.5 (at 25 °C). It was obvious that increases in pH value facilitated the polymerization of pectin. At pH 5.5, the molecular mass of pectin in the incubated mixture was determined to be about 410 kDa (fraction number 41). However, it was further increased to about 670 kDa (fraction number 40) at pH 7.5, showing the increased transacylation activity of tomato PE and citrus PE at higher pH (*5*). PEs from apple, carrot, banana, tomato (*5*, *16*), citrus, jelly fig (*2*), and papaya (*17*) sources showed optimal deesterification activity at weak alkaline pH values. Therefore, on the basis of the above findings, we concluded that PE catalyzed these two reactions simultaneously at pH ranges between neutral and weak alkaline. An apparent reduction in pectin quantity in the elution patterns of pectin−PE mixtures (**Figure 2**) was due to the removal of suspended pectin particles, as described in **Figure 1**.

In the pectin−PE mixture, carboxyl groups of pectin−PE complexes react with water molecules to conduct the deesterification reaction after the release of methoxyl groups from pectin molecules. However, at the same time, some of the carboxyl groups of pectin−PE complexes react with hydroxyl groups of other pectin molecules and, thus, ignite the transacylation reaction and lead to the increase in the pectin molecules (*1*). Therefore, nonmethyl ester linkages increased by about 40% during pectin gelling as a result of the esterification reaction in the presence of PE (*4*).

Effect of NaCl Level on Transacylation Reaction of PEs. The level of NaCl influences the charge of proteins and is crucial to the PE activity (*2*). The pectin−tomato PE mixture (I unit/mL) was incubated in 0−0.7 M NaCl/0.1 M phosphate buffer (pH 6.5) for 30 min, and the elution profiles of pectin were determined (**Figure 3**). An apparent increase in molecular mass of pectin (670 kDa, fraction number 40) in 0.3−0.5 M NaCl was observed. Similarly, 0.3 M NaCl was observed to facilitate the molecular mass (410 kDa) increase of pectin−citrus PE mixture (2 units/mL) after incubation at 25 °C for 5 min (**Figure 4**). A lower or higher NaCl level than in this range appeared to be unfavorable for the tomato and citrus PE transacylation.

The optimal NaCl level for tomato PE deesterification was reported to be 0.1 (*16*) or 0.3 M (*5*, *12*), which is almost the minimal value of the observed optimal level (0.3−0.5 M) for transacylation reaction in the present study. Also, the optimal NaCl level (0.3 M) for the citrus PE transacylation reaction was the maximal value of the optimal NaCl level (0.1−0.3 M) for the deesterification reaction (*5*). Therefore, the transacylation reaction catalyzed by those two PEs appeared to be favorable to occur at a slightly increased NaCl level than that for the deesterification reaction. The demand for extra NaCl could be closely related to the removal of methoxyl groups and to the subsequent esterification with hydroxyl groups from other pectin

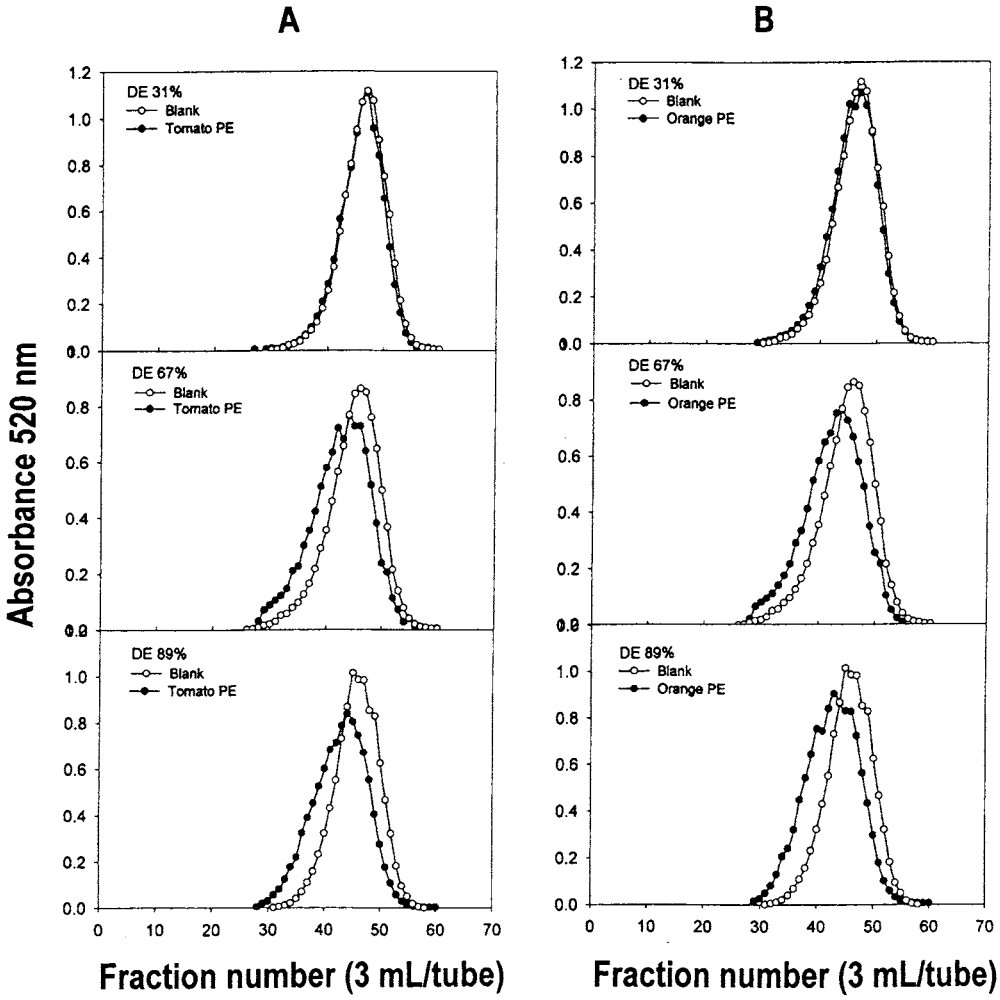

Figure 5. Toyopearl TSK HW-65 (F) gel permeation chromatograms of 0.5% citrus pectin (DE = 31, 67, or 89%)–tomato pectinesterase (A) and 0.5% citrus pectin–citrus pectinesterase (B) (2 units/mL) mixture in 0.1 M NaCl at 25 °C and pH 6.5 for 30 min. Column, 1.6 × 100 cm; eluent, 3 M urea/0.2 M NaCl/0.05 M acetate buffer (pH 5.5); flow rate, 60 mL/h; fractionation, 3 mL/tube; detection, absorbance at 520 nm after *m*-hydroxydiphenyl reaction.

molecules. However, the difference in intrinsic properties of PEs from different sources is relevant to the required NaCl level for catalyzing each of the two reactions (*2*, *5*).

Reductions of tomato PE activity by 50% (1 unit/mL mixture) and of pectin−citrus PE incubation times to 5 min in the investigation of NaCl level on transacylation reaction were to avoid the remarkable formations of pectin precipitates and suspended particles that were unfavorable for gel permeation chromatography.

Effect of Pectin DE on Transacylation Reaction. Figure 5 shows the elution patterns of pectin−PE mixtures (2 units/mL) in 0.1 M NaCl/0.1 M phosphate buffer (pH 6.5) at 25 °C for 30 min. It was obvious that both PEs did not catalyze the transacylation reaction when the pectic substance with a DE of 31% was used as substrate. However, molecular masses of pectin−PE mixtures apparently increased to about 209 kDa (fraction number 43), suggesting that the abundant presence of methoxyl groups in pectin was favorable for the transacylation reaction. Similar results were also observed when the turbidity method was applied to estimate the formation of transacylated pectin (*5*).

CONCLUSION

Mixtures of PE isozymes from tomato and citrus sources remarkably catalyzed the transacylation reaction to form polymerized pectin as observed by gel permeation chromatography. The optimal conditions of each of the PEs for the transacylation reaction were close to those for the deesterification reaction for the corresponding enzyme. Therefore, it was noteworthy that both reactions almost occurred simultaneously. A marked increase in the molecular weight of pectin−PE mixtures was observed in the present study; however, removed particles in pectin−PE mixtures before gel permeation chromatography were considered to be polymerized pectins with molecular weights far from determination by conventional gel permeation chromatography. Therefore, the determined optimal transacylation condition for each PE could be different with the real condition of the corresponding PE. Transacylated pectins with higher molecular weights are favorable for the gel preparation since pectin−PE mixtures are usually more viscous than pectin alone. Further studies on the application of transacylation reaction in pectin gels will be of great interest.

LITERATURE CITED

(1) Hou, W. C.; Chang, W. H. Pectinesterase-catalyzed firming effects during precooking of vegetables. *J. Food Biochem.* **1996**, *20*, 397−416.

(2) Jiang, C. M.; Lai, Y. J.; Lee, B. H.; Chang, W. H.; Chang, H. M. De-esterification and transacylation reaction of pectinesterase from jelly fig (*Ficus awkeotsang* Makino) achenes. *J. Food Sci.* **2001**, *66*, 810−815.

(3) Jiang, C. M.; Wu, M. C.; Chang, W. H.; Chang, H. M. Changes in particle size of pectin reacted with pectinesterase isozymes from pea pod (*Pisum sativum* L.) sprout. *J. Agric. Food Chem.* **2001**, *49*, 4383−4387.
(4) Jiang, C. M.; Lai, Y. J.; Lee, B. H.; Chang, W. H.; Wu, M. C.; Chang, H. M. Changes in physicochemical properties of pectin from jelly fig (*Ficus awkeotsang* Makino) seeds during extraction and gelling. *Food Res. Int.* **2002**, *35*, 31−35.
(5) Lee, C. W. Studies on the transacylation reaction catalyzed by pectinesterases from tomato and citrus. Master Thesis, Graduate Institute of Food Science and Technology, National Taiwan University, 2002.
(6) Pressey, R.; Avants, J. K. Multiple forms of pectinesterase in tomatoes. *Phytochemistry* **1972**, *11*, 3139−3142.
(7) Cameron, R. G.; Baker, R. A.; Grohmann, K. Multiple forms of pectin methylesterase from citrus peel and their effects on juice cloud stability. *J. Food Sci.* **1998**, *63*, 253−256.
(8) Lee, M.; MacMillan, J. D. Mode of action of pectin enzymes. I. Purification and certain properties of tomato pectinesterase. *Biochemistry* **1968**, *7*, 4005−4010.
(9) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.
(10) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(11) Pressey, R.; Avants, J. K. Solubilization of cell walls by tomato polygalacturonase: Effect of pectinesterase. *J. Food Biochem.* **1982**, *6*, 57−74.
(12) Tucker, G. A.; Robertson, N. G.; Grierson, D. Purification and changes in activities of tomato pectinesterase isozymes. *J. Sci. Food Agric.* **1982**, *33*, 396−400.
(13) Versteeg, C.; Rombouts, F. M.; Pilnik, W. Purification and some characteristics of two pectinesterase isozymes from orange. *Lebensm.-Wiss. Technol.* **1978**, *11*, 267−271.
(14) Koner, B.; Zimmermann, G.; Berk, Z. Orange pectinesterase—Purification, properties and effect on cloud stability. *J. Food Sci.* **1980**, *45*, 1203−1206.
(15) Rillo, L.; Castaldo, D.; Giovane, A.; Servillo, L.; Balestrieri, C.; Quagliuolo, L. Purification and properties of pectin methylesterase from Mandarin orange fruit. *J. Agric. Food Chem.* **1992**, *40*, 591−593.
(16) Nakagawa, H.; Yanagawa, Y.; Takehana, H. Studies on the pectolytic enzyme. Part V. Some properties of the purified tomato pectin methylesterase. *Agric. Biol. Chem.* **1970**, *34*, 998−1003.
(17) Lim, Y. M.; Chung, M. C. M. Isolation and characterization of pectin methylesterase from papaya. *Arch. Biochem. Biophys.* **1993**, *307*, 15−20.

Received for review November 27, 2002. Revised manuscript received May 26, 2003. Accepted June 5, 2003.

JF021170T