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Vacuum Infusion of Plant or Fungal Pectinmethylesterase and Calcium Affects the Texture and Structure of Eggplant

PANIDA BANJONGSINSIRI,† JOHN SHIELDS,§ AND LOUISE WICKER*,#

Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602; Institute of Science and Technology Research, Pathumthani, Thailand; and Center for Ultrastructural Research, University of Georgia, Athens, Georgia 30602

The effect of vacuum infusion on eggplant quality of a commercial fungal (*Aspergillus niger*) and citrus pectinmethylesterase (PME) with calcium chloride (4000 ppm) was investigated after processing and during storage. Firmness of infused eggplants using fungal or citrus PME was significantly increased compared to controls (fresh noninfused and water-infused control) after processing and during storage for 7 days at 4 °C. Activity of fungal PME-infused eggplant increased almost 32 times, whereas activity of eggplant infused with Marsh grapefruit PME increased 2-fold. Degree of esterification of pectin of eggplants infused with fungal or citrus PME decreased slightly. Cryo-SEM showed that samples treated with fungal PME/ $CaCl_2$ displayed more integrity among cells as compared with water-infused control. The change of pectin in the cell wall was visualized using monoclonal antibodies JIM5 (low-esterified pectin) and JIM7 (high-esterified pectin). JIM5 showed more binding than JIM7 with the cell walls of eggplant tissues from fungal PME/ $CaCl_2$ treatment.

KEYWORDS: Eggplant; pectinmethylesterase; degree of esterification; vacuum infusion; firmness

INTRODUCTION

Pectinmethylesterase (PME, EC 3.1.1.11) is widely distributed in plants and microorganisms and catalyzes the hydrolysis of methoxyl ester groups of polygalacturonic acids (*1*). PME plays an important role in the textural changes of fruits and vegetables. In fruit ripening, PME removes ester groups from the cell wall pectin, enhances polygalacturonase activity, and lowers intercellular adhesiveness and tissue rigidity (*2*). However, the firmness and quality of fruit and vegetable products can be enhanced by PME and formation of calcium pectate (*3*−*5*).

Differences occur in the mode of action, pH optima, and salt dependency between plant and fungal PMEs (*6*, *7*). The action pattern for apple PME at pH 7.5 results in blockwise distribution by a single-chain mechanism (*8*). The action pattern of apple PME at pH 4.5 also results in blockwise distribution, with shorter charged blocks and a multiple-chain mechanism. PMEs from *Aspergillus niger* act by a multiple-chain, single-attack mechanism (*9*). The optimum pH of plant PMEs is in the range between 6 and 9.5, whereas the optimal pH for *A. niger* PME activity is near pH 4.5 (*1*). Calcium gel strength of low-methoxyl pectin produced by blockwise-acting PMEs is weaker than that observed for randomly deesterified pectins (*10*−*12*). In addition, low-methoxyl pectin deesterified with a randomly acting *Aspergillus* PME produced strong calcium gels (*13*).

Vacuum infusion technology is used in many different processing applications to improve the quality of product structure by the active incorporation of functional ingredients (*14*−*16*). Vacuum infusion with plant and fungal PME with or without calcium chloride as a pretreatment increases the firmness of several fruit products. In canned peaches, vacuum infusion of Marsh grapefruit PME and calcium chloride increases firmness (*17*). The pretreatment of strawberries with commercial *Aspergillus* PME and calcium chloride in a vacuum also increases the firmness of strawberries in jam (*18*). Vacuum infusion pretreatment with plant or fungal PME and calcium chloride improves the firmness of strawberries (*19*, *20*) and mango (*21*). In a measure of sample volume deformation and infusion levels of different vegetables by vacuum infusion treatment (*22*), eggplants had a porosity of 60% compared to carrot (0.3%), mushroom (37%), and zucchini (18%). Gras et al. proposed that the wider pores of the eggplant enhanced infusion. On the basis of this information, eggplants were selected to determine the effect on physical and chemical properties by two types of plant PME and commercial fungal PME. Microstructural features were also determined using Cryo-SEM. Antibodies specific to low-ester pectin (JIM5) and high-ester pectin (JIM7) were used to investigate changes in the pectin of the cell wall of eggplant tissue after treatment.

MATERIALS AND METHODS

Sample Preparation. Fresh eggplants (*Solanum melongena* L. cv. Classic) were obtained from a local grocery and kept at 4 °C until use. Samples were sliced (1.0 cm thick) with a meat slicer (Hobart model 1612E, Hobart Corp., Troy, OH). To avoid seeds, cylindrical samples

* Corresponding author [telephone (706) 542-1055; fax (706) 542-1050; e-mail lwicker@uga.edu].
† Center for Ultrastructual Research, Univeristy of Georgia.
§ Institute of Science and Technology Research, Thailand.
# Department of Food Science and Technology, University of Georgia.

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(8 mm in diameter) were cut with a borer (no. 6) (Boekel Brass-plated cork borer, Fisher Scientific, Atlanta, GA) at the parenchyma cells close to the epidermis. To prevent the browning reaction of samples, ascorbic acid (200 ppm) was mixed with test solution before vacuum infusion of eggplant cylinders.

Microbial Pectinmethylesterase. A microbial pectinmethylesterase, with a pH optimum of 4.5 and derived from *A. niger*, was donated (Rohapect PME, Rohm Enzyme GmbH, Darmstadt, Germany).

Plant Pectinmethylesterase Extraction. Valencia orange pulp and Marsh grapefruit pulp were donated by Citrus World (Lake Wales, FL). PME was extracted as previously described (*23*). Crude extract was extracted from frozen pulp with 0.1 M NaCl and 0.25 M Tris buffer, pH 8.0, at a ratio of buffer to pulp of 3 to 1. The extract was homogenized (Pro 300A, Proscientific Inc., Monroe, CT) at 4 °C. The pH of the homogenate was adjusted to pH 8.0 with a few drops of 10 M NaOH to bring the pH close to 7.5 and with 0.1 M NaOH to bring the pH to 8.0 and maintain it there. The homogenate was filtered through Miracloth (CalBiochem, La Jolla, CA). The supernatant of a 30% ammonium sulfate cut was collected by centrifugation (Sorvall RC-5B centrifuge, DuPont Instruments, Doraville, GA) at 8000*g* at 4 °C for 20 min, dialyzed overnight against deionized water at 4 °C, and used for infusion. The dialysis tubing (Spectra/Por, MWCO 12000−14000, Fisher Scientific, Atlanta, GA) was boiled in 10% acetic acid for 5 min and rinsed with deionized water.

Pectinmethylesterase Activity. PME was extracted from a 5 g aliquot of infused eggplant with plant PME in 20 mL of 0.1 M NaCl and 0.25 M Tris, pH 8.0, or 0.25 M acetate and 0.1 M NaCl at pH 4.5 for citrus or fungal PME, respectively. After homogenization with a Sorvall Omni Mixer (DuPont, Newtown, CA) for 20 s at 4 °C, the homogenate was filtered through Miracloth and the PME activity in the filtrate was determined by a pH stat titrator (Brinkmann, Westbury, NY) at 30 °C in 1% high-methoxyl pectin (citrus pectin type CC104, Citrus Colloid Ltd., Hereford, U.K.) and 0.1 M NaCl. Set point pH values of 7.5 and 4.5 were used to assay plant and fungal PME, respectively. A unit of PME activity was defined as the microequivalent of ester hydrolyzed per minute at 30 °C.

Vacuum Infusion Procedures and Process Pretreatments. In the preliminary experiments, three infusion procedures were evaluated. The treatments were (A) vacuum infusion at 68 kPa for 15 min at 30 °C; (B) pulsed vacuum at 30 °C (85 kPa for 5 min, release vacuum to atmospheric pressure for 1 min, reapply vacuum for 5 min, and release again for 5 min at 30 °C; and (C) temperature gradient without vacuum from 30 to 4 °C and stored at 4 °C for 26 h.

To determine the effects of PME and calcium, 12 tests were performed using the three infusion methods (A−C): test 1 (RW), nontreated eggplant cylinders were used as nontreated control; test 2 (water), eggplant cylinders were infused in deionized water as water-infused control; test 3 (Ca), eggplant cylinders were infused with 4000 ppm of $CaCl_2 \cdot 2H_2O$; test 4 (Val PME), Valencia orange PME (25 units/mL); test 5 (fungal PME), eggplant cylinders were infused with fungal PME (10 units/mL); test 6 (MGF PME), eggplant cylinders were infused with Marsh grapefruit PME (10 units/mL); tests 7, 8, and 9, eggplant cylinders were infused with 4000 ppm of $CaCl_2 \cdot 2H_2O$ containing Val PME (Val PME + Ca), fungal PME (fungal PME + Ca), or MGF PME (MGF PME + Ca), respectively; test 10, eggplant cylinders were first infused in 4000 ppm of $CaCl_2 \cdot 2H_2O$ under a vacuum of 68 kPa for 5 min at 30 °C, followed by treatment A; test 11, eggplant cylinders were first infused in 4000 ppm of $CaCl_2 \cdot 2H_2O$ under a vacuum of 85 kPa for 5 min at 30 °C, followed by treatment B; test 12, eggplant cylinders were soaked in 4000 ppm of $CaCl_2 \cdot 2H_2O$ for 1 h at 4 °C, followed by treatment C.

Storage Study. Pulsed vacuum infusion was used in seven further studies, which included tests 1 (RW), 2 (water), 3 (Ca), 5 (fungal PME), 6 (MGF PME), 7 (fungal PME + Ca), and 8 (MGF PME + Ca). In the experimental procedure, eggplant cylinders were infused with test solutions under pulsed vacuum procedure. For storage study, samples were drained for 3 min, put in a zip-top bag, and stored at −34 °C or 4 °C for 1 week. Two replicates of the experiment were carried out.

To estimate yield and weight loss, the collective weights of six to seven eggplant cylinders were recorded before and after infusion. Frozen samples were thawed at 4 °C and equilibrated to 20 ± 1 °C prior to measurement. Yield and weight (wt) loss were calculated as follows:

$$\text{yield (\%)} = (\text{wt after process/initial wt}) \times 100$$

$$\text{wt loss (\%)} = \frac{(\text{wt after process} - \text{wt after storage})}{\text{wt after process}} \times 100$$

Textural Analysis. Eggplants were equilibrated to room temperature before textural analysis. Texture profile analysis (TPA) was used to evaluate eggplant texture using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY). TPA was determined using a cylindrical probe (35 mm diameter) with a 25 kg load cell (*21*). Samples were compressed in two consecutive cycles to 60% deformation from the initial sample height of 1.0 cm, at a compression speed of 2 mm/s. The textural parameters determined were hardness, cohesiveness, springiness, gumminess, and chewiness (*24*). Hardness, measured in newtons (N), is defined as the first maximum force necessary to compress the sample. Cohesiveness is assessed using the ratio of the area of work during the second compression divided by the area of work during the first compression. Adhesiveness (N·mm) consists of the negative force area for the first compression and represents the work required to overcome the attractive forces between the surface of a food and the surface of other materials. Springiness (mm) is defined as the distance the sample was compressed during the second compression to the peak force. Gumminess (N) is the product of hardness and cohesiveness, and chewiness (N·mm) is the product of gumminess and springiness. At least seven samples were analyzed for each treatment.

Alcohol Insoluble Solids (AIS). AIS was prepared as previously described (*21*) using a method initially described by Huber and Lee (*25*). A 20−50 g sample of eggplant sample was homogenized for 20 s in a Proscientific homogenizer, at a ratio of 1 part sample to 4 parts 95% ethanol. The homogenate was heated at boiling for 5 min and then cooled to room temperature. The residue was filtered through a medium-pore, sintered glass funnel (Fisher Scientific, Atlanta, GA). On the basis of the initial fresh weight, the residue was successively washed in 4 volumes of 95% ethanol and 6 volumes of acetone. AIS was dried under the hood overnight, weighed, and stored at −20 °C.

Degree of Esterification (DE). The DE was determined using a modified (*21*) titration method (*26*). An aliquot of 1 g of AIS was extracted with 90 mL of deionized water for 90 min at 60 °C. The extract was centrifuged at 8000*g*, for 20 min at 4 °C, and filtered through Miracloth. The supernatant was collected and analyzed for %DE. A volume of 20 mL of sample was titrated with 0.05 N NaOH, saponified with 20 mL of 0.05 N NaOH, and neutralized with an equivalent amount of 0.05 N HCl. The total carboxylic acid groups were estimated by titration with 0.05 N NaOH. The endpoint of titration was estimated in the presence of phenolphthalein, but was quantified to an endpoint pH between 8.0 and 9.6. The %DE was estimated as the mole ratio of free to total carboxylic acids.

Ion-Exchange Chromatography (IEX). An aliquot of dried AIS (50−175 mg) was suspended in 5 mL of 0.05 M sodium acetate buffer and 0.05 M EDTA (pH 5.0) and stirred for 12 h at room temperature. The extract was filtered with 0.45 μm syringe filters (Whatman, Clifton, NJ). The filtrate (0.5−1.5 mg/ mL of uronic acid) was applied to a 12 × 53 mm anion-exchange column of UNO Q-6 (Bio-Rad Laboratories, Richmond, CA). The column was equilibrated with 0.05 M acetate, pH 5.0, and operated at room temperature at a flow rate of 2.0 mL/min. After loading, pectin was eluted with a linear gradient from 0.05 to 1.3 M acetate, pH 5.0. Fractions of 2.5 mL were collected and assayed for uronic acids using the *m*-hydroxydiphenyl method (*27*).

Cryo-Scanning Electron Microscopy (Cryo-SEM). Eggplant structures from fresh and treatment samples were observed via a Cryo-SEM technique (*28*). Samples were cryo-fixed by plunging into nitrogen slush. The frozen sample was quickly transferred to an Alto 2500 cryo-preparation unit (Gatan Inc., Warrendale, PA) attached to a LEO 982 field emission (FE) SEM (LEO Electron Microscopy Inc., Thornwood, NY), fractured, and allowed to sublimate at a vacuum of 10^{-4} Pa and −80 °C for 15 min to remove frost on the surface. The sample was brought back to −100 °C before coating with gold palladium to a

Table 1. Firmness and Percent Yield of Eggplant Compared to Three Process Methods among Different Treatments[a]

	vacuum infusion (A)		pulsed vacuum (B)		temperature gradient (C)	
test ID[b]	hardness (N)	yield[c] (%)	hardness (N)	yield (%)	hardness (N)	yield (%)
1. RW	x 13.14 bcd		x 13.84 cde		x 13.68 abc	
2. water	x 10.94 d	180	x 10.44 e	214	x 9.71 c	168
3. Ca	x 14.87 abc	179	x 15.96 bcd	183	x 13.73 abc	155
4. Val PME	y 12.32 bcd	104	x 20.67 a	127	y 12.39 abc	100
5. fungal PME	x 11.58 dc	180	x 13.37 de	200	x 11.99 abc	150
6. MGF PME	x 15.62 ab	124	x 18.17 abc	157	y 9.72 c	129
7. Val PME + Ca	y 14.94 abc	104	x 20.61 a	114	y 14.58 ab	95
8. fungal PME + Ca	y 12.79 bcd	165	x 17.41 abcd	190	xy 15.53 a	133
9. MGF PME + Ca	xy 14.51 abcd	109	x 19.37 ab	124	y 11.52 abc	95
10. Ca_Val PME	x 14.72 ab	150	x 15.13 bcd	170	x 12.16 abc	111
11. Ca_fungal PME	xy 12.81 bcd	191	x 15.60 bcd	183	y 10.60 bc	145
12. Ca_MGF PME	xy 16.87 a	163	x 18.56 ab	177	y 11.82 abc	120

[a] Within columns, entries with the same letter (a–d) within each process method are not significantly different ($p \leq 0.05$). Within rows, entries with the same letter (x, y) within each treatment are not significantly different ($p \leq 0.05$). [b] RW, noninfused control; water, water-infused control; Ca, $CaCl_2 \cdot 2H_2O$ (4000 ppm); Val PME, Valencia orange PME (25 units/mL); fungal PME (10 units/mL); MGF PME, Marsh grapefruit PME (10 units/mL); Val PME (25 units/mL) + Ca (4000 ppm); fungal PME (10 units/mL) + Ca (4000 ppm); MGF PME (10 units/mL) + Ca (4000 ppm); Ca_Val PME, Ca (4000 ppm) infusion followed by Val PME (25 units/mL) infusion; Ca_fungal PME, Ca (4000 ppm) infusion followed by fungal PME (10 units/mL) infusion; Ca_ MGF PME, Ca (4000 ppm) infusion followed by Marsh grapefruit PME (10 units/mL) infusion. [c] Yield (%) = (wt after infusion/initial wt) × 100.

thickness of 30 nm. Once coated, the sample was transferred to a −100 °C cold stage in the FE-SEM for viewing.

Immunofluorescence Microscopy. Tissue fixation and microtomy (*29*) were performed on pieces of eggplant (8 mm in diameter, 1 cm in thickness) that were excised with a razor blade and immersed in freshly prepared 4% (w/v) formaldehyde in fixative buffer containing 100 mM piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] (PIPES), 4.0 mM $MgCl_2$, and 4.0 mM ethylene glycol bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) at pH 6.9, overnight. Pieces of tissue were washed in buffer for 15 min (three washes), embedded with Cryomatrix compound (Shandon, Pittsburgh, PA), and frozen at −20 °C. Samples of 60 μm thickness were cut at −20 °C using a Cryostat 2800 (Leica Microsystem Inc., Bannockburn, IL). Sections were collected on subbing slides coated with gelatin and chromic potassium sulfate and kept at 4 °C before further processing.

The slides were washed with deionized water to remove the Cryomatrix compounds. The sections were treated at room temperature for 1 h with a block solution of 3% w/v bovine serum albumin (BSA) (Fisher Scientific, Fair Lawn, NJ) in phosphate-buffered saline (PBS) at pH 7.35−7.45 to avoid nonspecific binding. The control and infused samples were incubated in a dilution (1:5) of primary antibodies [rat monoclonal antibody against JIM5 or JIM7 was provided by Dr. Paul Knox (University of Leeds, U.K.)] in 1% (w/v) BSA/PBS overnight, at 4 °C. Antibody control samples were incubated in 1% (w/v) BSA/PBS solution without primary antibodies. After overnight incubation, samples were washed in 1% (w/v) BSA/PBS for 15 min (three washes) and incubated in a dilution (1:50) of goat anti-rat IgG conjugated to Alexa Fluor 633 (Molecular Probes, Inc., Eugene, OR) with 1% (w/v) BSA/PBS for 4 h at 4 °C. Samples were washed with 1% (w/v) BSA in PBS solution for 15 min (three washes) and then mounted in a 1% (w/v) BSA in PBS solution containing a Citifluor anti-fade mountant pH 9.2 (Ted Pella, Inc., Redding, CA). Sections were observed with a confocal laser scanning microscopy (TCS NT SP2 Leica Confocal microscope, Leica Microsystems GmbH, Heidelberg, Germany). A helium−neon laser was used with excitation at 633 nm and emission set for 610−650 nm. Collected images were processed using Adobe Photoshop version 6.5 (Adobe Systems Inc.).

Statistical Analysis. A randomized complete block design was conducted. The analysis of variance was performed using Proc ANOVA and SAS statistical package V8.2, 2000 (SAS Institute Inc., Cary, NC). The least significant difference (LSD) was used for comparison between means at a significance level of $p \leq 0.05$. All treatments were carried out in duplicate.

RESULTS AND DISCUSSION

Infusion Method and Treatment. The infusion methods of vacuum, pulsed vacuum, and temperature gradient, as well as treatments that varied in PME source, presence of calcium chloride, and order of addition of infusion components, were compared for weight yield and hardness on eggplant cylinders. Yield was monitored as a rapid assessment of infusion efficiency, because earlier research (*22*) had correlated the efficiency of uptake of infusion broth with weight gain and textural parameters. Hardness was measured as an assessment of PME and calcium effect on texture. The data in **Table 1** summarize the yield and hardness of eggplant cylinders for the three infusion methods for the 12 PME/Ca treatments. No significant differences ($p \leq 0.05$) were observed in the no-treatment control (RW) among the infusion processes. Therefore, the initial raw material used in the experiments was consistent with respect to initial hardness.

A change in weight after infusion was observed depending on infusion method and/or treatment method (**Table 1**). Exceptions to an increase in yield were observed for temperature gradient infusion for treatments 7 and 9, Val PME + Ca and MGF PME + Ca. In general, yield was greatest using the pulsed vacuum method (B), compared to vacuum infusion (A) and temperature gradient (C) infusion. For the three infusion methods, yield from treatments that included fungal PME was higher and similar to that of water-infused control. In early studies, visible gels and lowered efficiency of PME uptake were observed using Val PME that was prepared without an ammonium sulfate cut (data not shown). Higher yields of fungal PME infused samples may be related to lower incidence of large pectin complexes in the fungal PME. This is presumptively substantiated by the higher yields observed when calcium was infused prior to PME infusion, treatments 7, 8, and 9 compared to treatments 10, 11, and 12, respectively. The range of change in yield was from 1.0- to 2.0-fold higher.

Infusion method influenced hardness values in the presence of plant PME and calcium (**Table 1**). Pulsed vacuum or vacuum infusion resulted in significantly higher hardness values than temperature gradient infusion in treatments 4 (Val PME), 6 (MGF PME), 7 (Val PME + Ca), 8 (fungal PME + Ca), 9 (MGF PME + Ca), 11 (Ca_fungal PME), and 12 (Ca_MGF PME). Pulsed vacuum infusion resulted in significantly higher hardness values when Val PME + Ca was infused. No significant differences in infusion method were observed for treatments 3 (Ca alone) or 5 (fungal PME) or if in treatment 10 calcium was infused before Val PME.

Table 2. Texture Profile Analysis of Eggplant (Day 0 and Day 7 at 4 and − 34 °C)[a]

test ID	hardness (N)	cohesiveness (unitless)	adhesiveness (N·mm)	springiness (mm)	gumminess (N)	chewiness (N·mm)
			Day 0			
RW	16.46 b	0.25 d	–0.58 abc	2.90 d	4.17 d	12.48 c
water	12.43 c	0.26 d	–2.30 d	3.45 cd	3.16 d	11.90 c
Ca	20.32 a	0.39 ab	–1.86 dc	4.13 ab	8.01 ab	36.90 a
fungal PME	16.70 b	0.36 bc	–1.91 dc	4.12 ab	6.01 c	26.14 b
MGF PME	17.47 b	0.34 bc	–0.45 ab	4.03 bc	6.00 c	24.87 b
fungal PME + Ca	20.89 a	0.44 a	–1.63 bcd	4.68 a	9.03 a	42.40 a
MGF PME + Ca	20.96 a	0.33 c	–0.118 a	3.43 cd	6.91 bc	24.00 b
			Day 7, 4 °C			
RW	13.89 b	0.28 bc	–0.56 ab	3.57 b	3.87 b	13.69 b
water	10.56 c	0.42 a	–0.14 a	4.86 a	4.83 b	24.89a
Ca	5.12 d	0.21 cd	–0.49 ab	2.82 c	1.29 c	5.04 c
fungal PME	2.41 d	0.33 ab	–0.42 ab	3.29 bc	0.81 c	3.23 c
MGF PME	4.62 d	0.18 d	–0.10 a	3.30 bc	0.86 c	2.85 c
fungal PME + Ca	17.48 a	0.36 ab	–1.11 b	4.48 a	6.29 a	28.81 a
MGF PME + Ca	17.57 a	0.28 bc	–0.10 a	3.06 bc	4.91 ab	15.12 b
			Day 7, –34 °C			
RW	8.22 a	0.36 ab	–0.070 c	2.31 b	3.12 a	7.18 a
water	2.13 b	0.28 c	–0.035 ab	3.29 a	0.58 b	1.85 b
Ca	2.06 b	0.34 bc	–0.032 ab	3.53 a	0.65 b	2.26 b
fungal PME	1.96 b	0.30 c	–0.025 a	3.55 a	0.56 b	2.00 b
MGF PME	3.26 b	0.29 c	–0.031 ab	3.44 a	0.92 b	3.01 b
fungal PME + Ca	1.63 b	0.35 b	–0.059 bc	3.47 a	0.51 b	1.74 b
MGF PME + Ca	1.86 b	0.41 a	–0.046 abc	3.51 a	0.75 b	2.60 b

[a] Within columns, entries with the same letters (a–d) within each day are not significantly different ($p \leq 0.05$). [b] RW, noninfused control; water, water-infused control; Ca, $CaCl_2 \cdot 2H_2O$ (4000 ppm); fungal PME (10 units/mL); MGF PME, Marsh grapefruit PME (10 units/mL); fungal PME (10 units/mL) + Ca (4000 ppm); MGF PME (10 units/mL) + Ca (4000 ppm).

On the basis of firmness, the pulsed vacuum technique yielded the greatest firmness values when significant differences were observed. Likewise, pulsed vacuum resulted in higher yield values for all treatments. This finding agrees with the earlier results (*30*) that after a single vacuum application and release of microcrystalline cellulose, ~20% of mushrooms remained floating. After the vacuum was drawn and released a second time, 100% of the mushrooms sank to the bottom of the liquid, indicating complete saturation. Pulsed pressure infusion facilitates pectinase infusion into fresh citrus fruits, which usually was blocked by collapsed tissues due to excessive pressure (*31*).

Effect of PME Infusion on Texture. In subsequent studies, the effect of storage time and temperature on changes in texture was considered. No difference in firmness of strawberry slices vacuum-infiltrated with polyamines or calcium immediately after vacuum infiltration was found, but an increase in firmness after 9 days at 1 °C was reported (*33*). Accordingly, the texture of infused eggplant cylinders was measured using TPA after infusion and after storage for 7 days at 4 or −34 °C. The pulsed vacuum infusion method was selected for further study because of the yield and hardness values. The treatments (5, 6, 8, and 9) using fungal PME and MGF PME with or without calcium chloride were selected on the basis of ease of use and higher hardness values.

The TPA values varied significantly ($p \leq 0.05$) by treatment after storage at 4 or −34 °C for 7 days (**Table 2**). The infused eggplants from fungal PME + Ca and MGF PME + Ca treatments had the greatest hardness values at day 0 and day 7 at 4 °C. In the absence of calcium or PME, a loss of hardness was observed during storage at 4 °C. There was a significant decrease in hardness values after storage at −34 °C regardless of infusion treatment, indicating that PME/calcium infusion was not able to prevent loss of quality of texture during freezing. Other TPA parameters such as cohesiveness, springiness, and gumminess were significantly higher after infusion on day 0, regardless of infusion method. However, after 7 days of storage at 4 °C, differences in cohesiveness, adhesiveness, and springiness were not readily apparent. For eggplant stored at 4 °C, an increase in gumminess for fungal PME + Ca and MGF PME + Ca was observed compared to the no-treatment control. In contrast, thawed eggplants from all treatments had lower hardness, gumminess, and chewiness than noninfused control after storage for 7 days at −34 °C. Springiness values of thawed eggplants from all treatments were higher than that of noninfused control at the same storage condition. The trend of cohesiveness and adhesiveness values in all treatments was not consistent after processing and at each storage time. Thus, the hardness value was an appropriate parameter to indicate the change of infused eggplant texture in this study.

Effect of Vacuum Infusion on Weight Loss during Storage. The effect of treatments on yield of eggplants was significant at storage temperatures of −34 and 4 °C for a storage time of 7 days (**Table 3**). Regardless of treatments, yield (1 and 2) increased if calcium, fungal PME, or MGF PME was present. Infused eggplants from the MGF PME + Ca treatment had a lower yield at −34 and 4 °C. No significant differences were found on the weight loss of infused eggplant for all treatments at 4 °C. At −34 °C, MGF PME + Ca infused samples had a significantly lower weight loss (17%) compared to the others, but not significantly different when compared to the weight loss of the fungal PME treatment. Thus, fungal PME + Ca and MGF PME + Ca pretreatment enhanced the yield of eggplant after treatment and also prevented weight loss during storage.

PME Activity and Degree of Esterification. Eggplants from each treatment were assayed for PME activity, as shown in **Table 4**. Due to the different optimal pH values from PME sources, pH 7.5 was set to assay PME activity of plant PME (MGF PME and MGF PME + Ca) including Ca, whereas pH 4.5 was set for fungal PME and fungal PME + Ca treatments. The activities of control treatments (RW and water) were assayed at pH 4.5 and 7.5. At pH 4.5, the activities of noninfused

Table 3. Percent Yield and Percent Weight Loss of Eggplant after Process and Storage Time at 4 and −34 °C for 7 Days[a]

treatment[b]	% yield (1)[c]	% wt loss[d]	% yield (2)[e]
	Day 7, 4 °C		
RW	100 d	4.15 a	95.92 d
water	226.00 a	4.43 a	216.00 a
Ca	213.05 ab	8.66 a	194.23 ab
fungal PME	218.00 ab	9.13 a	198.00 ab
MGF PME	197.26 bc	9.18 a	179.31 bc
fungal PME + Ca	200.32 b	3.00 a	194.23 ab
MGF PME + Ca	172.12 c	3.51 a	166.03 c
	Day 7, −34 °C		
RW	100 e	12.27 d	87.71 d
water	231.11 a	27.48a	167.48 a
Ca	198.08 c	24.77 ab	149.00 b
fungal PME	211.85 b	28.71 a	151.00 b
MGF PME	194.16 c	17.19 dc	160.77 a
fungal PME + Ca	196.53 c	22.09 bc	152.78 b
MGF PME + Ca	176.13 d	23.85 ab	134.14 c

[a] Within columns, entries with the same letters (a–e) within each storage condition are not significantly different ($p \leq 0.05$). [b] RW, noninfused control; water, water-infused control; Ca, $CaCl_2 \cdot 2H_2O$ (4000 ppm); fungal PME (10 units/mL); MGF PME, Marsh grapefruit PME (10 units/mL); fungal PME (10 units/mL) + Ca (4000 ppm); MGF PME (10 units/mL) + Ca (4000 ppm). [c] % yield (1) = (wt after infusion/initial wt) × 100. [d] % wt loss = (wt after infusion − wt after storage) × 100 ÷ (wt after infusion). [e] % yield (2) = (wt after storage time/initial wt) × 100.

Table 4. Enzyme Activity and %DE of Eggplant after Infusion[a]

treatment[b]	optimal pH	enzyme activity (units/g of dried wt)	%DE
RW	4.5	7.80 e	87 ab
	7.5	246.67 d	
water	4.5	26.76 e	92 a
	7.5	654.41 b	
Ca	7.5	468.54 c	74 cd
fungal PME	4.5	327.96 d	94 a
MGF PME	7.5	813.95 a	65 d
fungal PME + Ca	4.5	256.77 d	80 bc
MGF PME + Ca	7.5	562.42 bc	71 d

[a] Within columns, entries with the same letters (a–e) are not significantly different ($p \leq 0.05$). [b] RW, noninfused control; water, water-infused control; Ca, $CaCl_2 \cdot 2H_2O$ (4000 ppm); fungal PME (10 units/mL); MGF PME, Marsh grapefruit PME (10 units/mL); fungal PME (10 units/mL) + Ca (4000 ppm); MGF PME (10 units/mL) + Ca (4000 ppm).

(8.0 units/g of dried wt) and water-infused control (27 units/g of dried wt) were lower than activities measured at pH 7.5 (247 and 654 units/g of dried wt). This finding supports the fact that most plant PME activities have an optimal pH of 7.5−8.0, whereas optimal fungal PME activity is in the pH range of 4.0−5.2 (*1*). After vacuum infusion, the PME activity of infused eggplants from MGF PME (814 units/g of dried wt) increased significantly (3-fold) compared to RW (247 unit/g of dried wt) and by 1.2-fold compared to the water control (654 units/g of dried wt) at pH 7.5. However, the PME activity of MGF PME significantly decreased after the addition of Ca (4000 ppm) in MGF PME + Ca treatment (562 units/g of dried wt). In contrast, there were no significant differences between fungal PME and fungal PME + Ca treatment in PME activity. The activity of infused eggplant from fungal PME and fungal PME + Ca was increased 42- and 32-fold, respectively, when compared to RW at pH 4.5. In comparison to the activity with water control, fungal PME and Fungal PME + Ca had 12- and 10-fold activities, respectively, higher than the water control at pH 4.5. In Ca treatment, the PME activity of eggplant (469 units/g of dried wt) was higher than that of RW (pH 7.5), fungal PME (328 units/mL), and fungal PME + Ca (257 units/g of dried wt) but lower than that of the water control. The presence of 50−250 mM NaCl or 5−20 mM calcium chloride increases plant PME activity severalfold, but 1.5−2-fold in microbial PME (*1*). Without salt ions, PME may be trapped by carboxyl groups on pectin and activated by cations (*34*). At the optimal salt concentration, salt ions may interact with negatively charged groups, allowing the enzyme to interact with the ester bonds to be cleaved. However, at higher concentrations, the PME reaction is inhibited because some carboxyl groups adjacent to the ester bond to be cleaved are blocked by salt ions.

The %DE values of infused eggplants from each treatment are depicted in **Table 4**. The %DE value is high and did not decrease proportionately with increased PME activity. There was no significant difference in %DE of fresh noninfused (RW), water-infused control, and fungal PME treatment. The %DE of Ca (74%), MGF PME (65%), and MGF PME + Ca (71%) of eggplant pectin was lower when compared to control (RW and water), fungal PME (94%), and fungal PME + Ca (80%) treatments. In general, %DE of tissue pectins is in the range of 60−90%, depending on species, tissue, and plant maturity (*35*). The %DE has a bearing on the firmness and cohesion of plant tissue. Reductions in DE result in greater cohesion. The formation of free carboxyl groups increases the possibility and strength of calcium binding between pectin polymers. PME, present in most plant tissue, can decrease % DE by demethylation of pectin.

Ion-Exchange Chromatography. The pectin extracted from control and PME-treated eggplant was analyzed for separation charge by IEC (**Figure 1**). Separation of pectins by IEC depends primarily on charge (*36*). In the case of blockwise or random mechanism of de-esterification or of highly de-esterified pectin, pectin elution profiles differ (*37*). Variable peak height and number were observed with different treatments, indicating heterogeneity. Pectins extracted from untreated eggplant (RW) and from water-treated and calcium-treated eggplant (**Figure 1A**) showed two main components. The first and second peaks eluted approximately between fractions 13 and 25 and between fractions 25 and 33, respectively. Water treatment enhanced the recovery of both classes of pectins. Only the second peak was observed in pectins of calcium-treated eggplants. Pectins extracted from fungal or MGF PME treated eggplants are depicted in **Figure 1B**. The degree of binding, elution, and separation of pectin fractions was different for pectins treated by fungal or MGF PME. Pectins extracted from fungal PME + Ca or MGF PME + Ca are depicted in **Figure 1C**. Multiple peaks were observed, and most eluted after fraction 23, suggesting tighter binding to IEC. Pectins de-esterified by acid or alkaline appear to be homogeneous on IEC, whereas pectins de-esterified by plant PME elute in large fractions of various degrees of methoxylation (*37*). The recoveries of ion-exchange fractions of all treatments were around 25−34%, similar to earlier results reported for apple protopectin extracted by chemical means (*38*). Apple pectin extracted by alkaline (NaOH, hot and cold Na_2CO_3) solutions had a low percent recovery for uronic acid (24, 27, and 51%), but a high percent recovery for neutral sugars (108, 98, and 89%), which indicated that uronic acids mostly devoid of neutral sugars were retained on the column. In addition, some low-methoxylated pectin molecules can be precipitated inside the column if calcium ions are present. Nonionic adsorption via phenolic compounds and/or precipitation with multivalent cations may be responsible for incomplete recovery of galacturonic acid (*36*). With regard to fraction 2 or 3 (**Figure 1**), these fractions showed pink color after boiling,

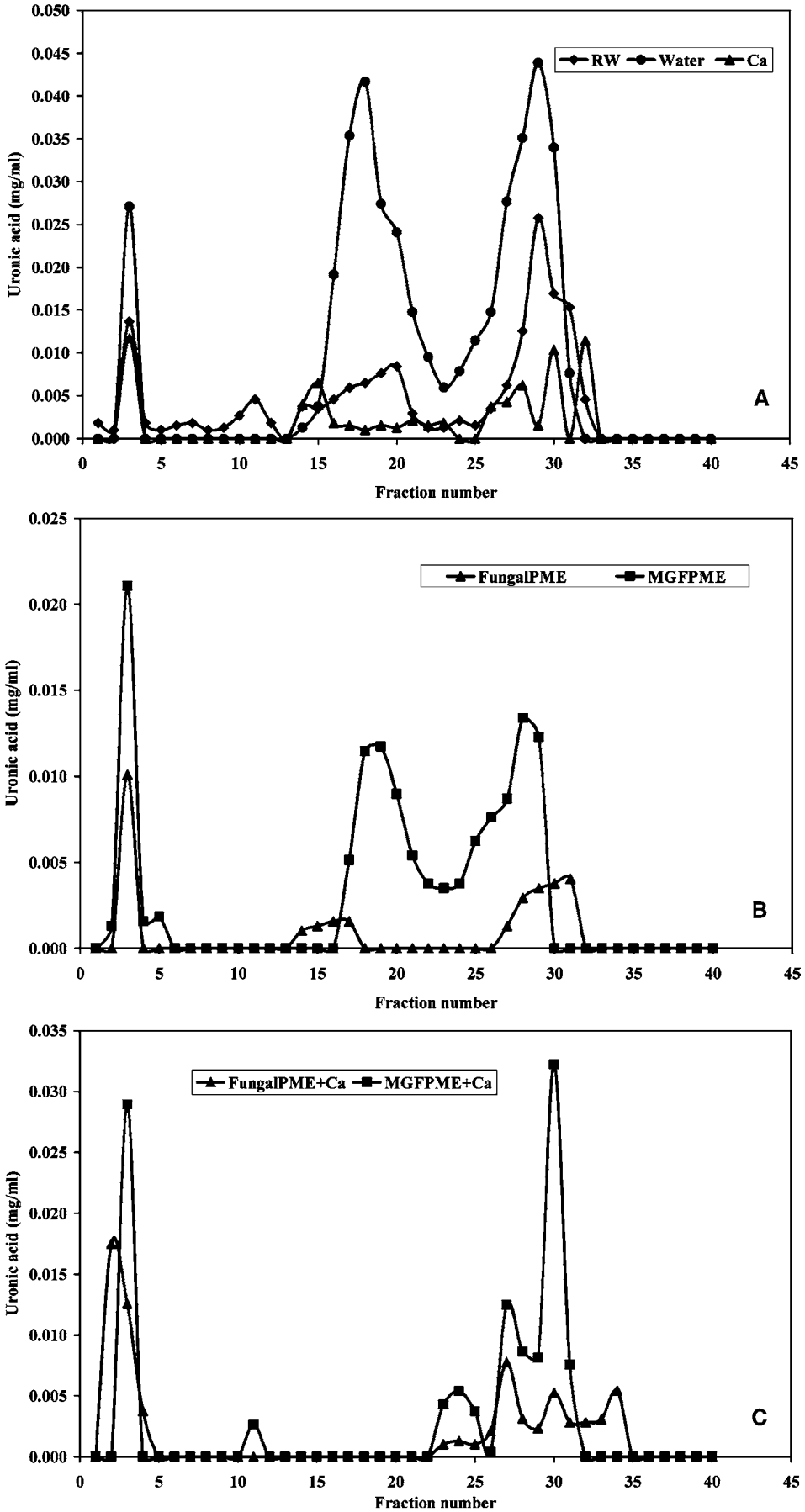

Figure 1. Elution of pectic substances extracted from eggplants: (**A**) RW, water, and Ca treatments; (**B**) fungal PME and MGF PME treatments; (**C**) fungal PME + Ca and MGF PME + Ca treatments.

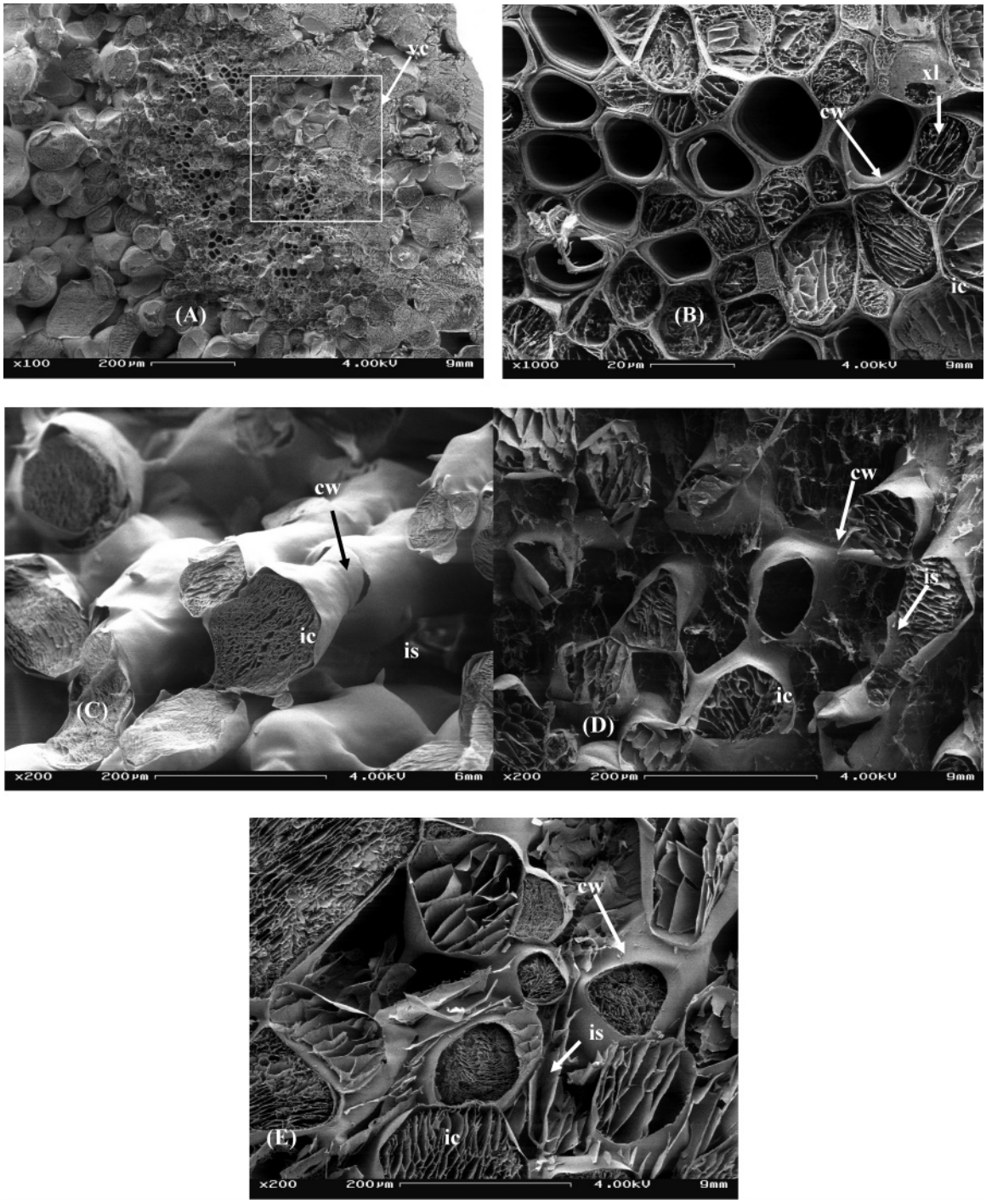

Figure 2. Cryo-SEM micrographs of eggplant: (**A**–**C**) RW (noninfused control); (**D**) water-infused control; (**E**) fungal PME + Ca treatment. Abbreviations: is, intercellular space in RW (**C**), space between cells located within the plant material; is with arrow (**D** and **E**), sheets of material left from ice crystal formation; cw, cell wall; ic, intracellular content; vc, vascular bundle; xl, xylem.

before the colorimetric assay for uronic acid. Because free neutral sugars and phenolic and proteinaceous compounds coeluted with pectin molecules at low ionic strength (*36*), these fractions might be free neutral sugars, because they eluted at low ionic strength.

Cryo-SEM. Noninfused eggplant (RW) displays parts of intercellular spaces between parenchyma cells and vascular bundle tissues (xylem and phloem tissues), as presented in **Figure 2A**−**C**. The regions in the micrographs are termed "solute-water glass" of the cell sap, cell membranes, and cell walls (*39*). Solute-water glass appears as a dentritic zone or sheets due to ice microcrystal sublimation and can be observed in the intracellular zone and in some intercellular spaces containing native liquid. Alternatively, intercellular spaces may appear to be completely empty (*40*). In the water treatment (**Figure 2D**), eggplant samples showed a deformation of cell structure and reticulated material from ice formation between cells, as compared to RW (**Figure 2C**). Eggplant treated with fungal PME + Ca (**Figure 2E**) also had deformed cells compared to RW, as well as larger sheets ("is" in **Figure 2E**) defined between cells, which differed from the material noted for the water treatment. Differences in ultrastructural features between atmospheric pressure (OD) and pulsed vacuum treatments (PVOD) of treated strawberry fruits were observed (*41*).

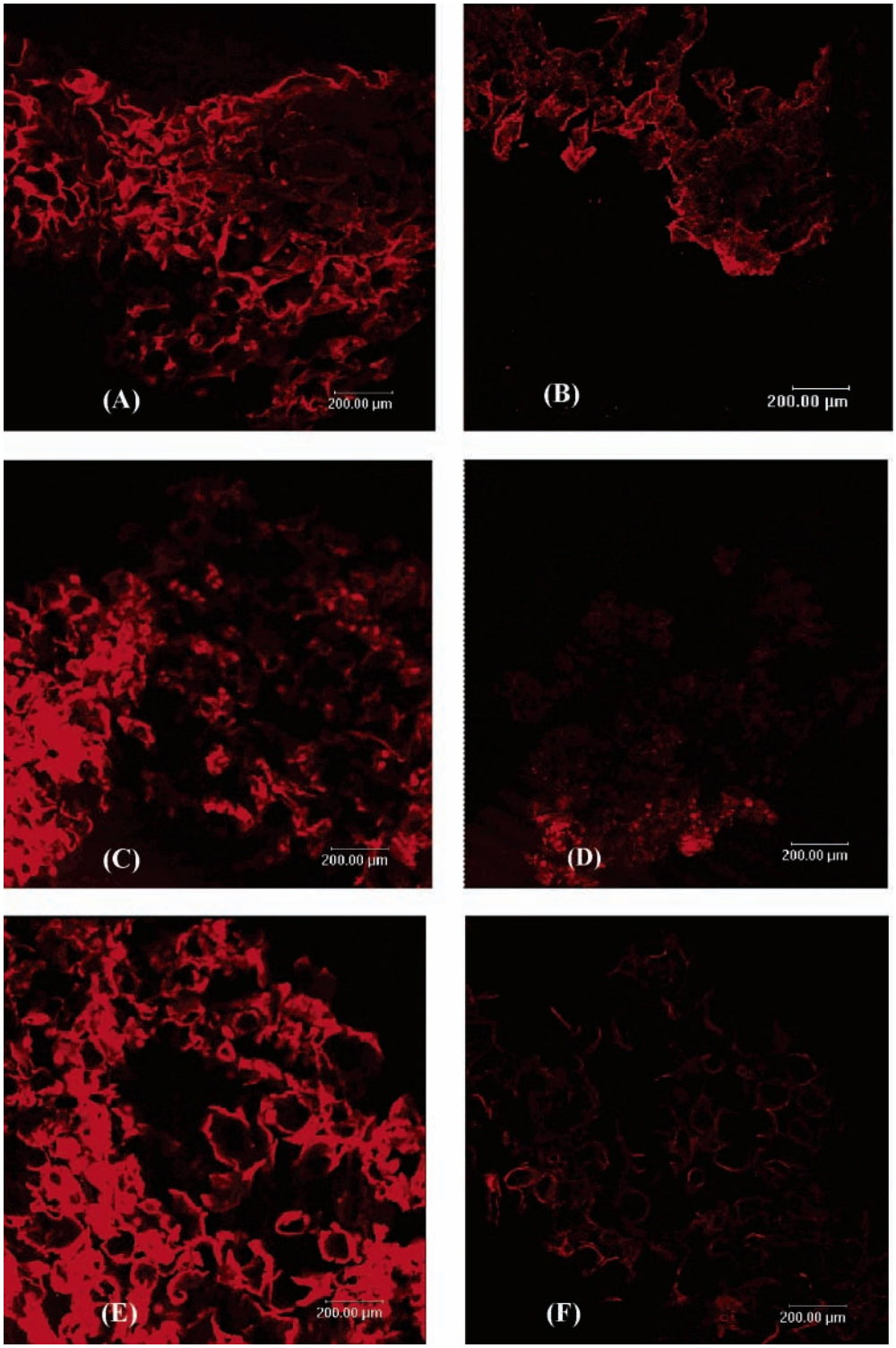

Figure 3. Immunofluorescence-labeled JIM5 (low-esterified pectin, **A**, **C**, and **E**) and JIM7 (high-esterified pectin, **B**, **D**, and **F**) binding to a cross section of eggplant tissues: (**A**, **B**) RW (noninfused control); (**C**, **D**) water-infused control; (**E**, **F**) fungal PME + Ca treatment.

The intercellular spaces of PVOD samples contained more compact dentritic structures compared to OD samples. Thus, a different aspect of the liquid phase of infused eggplant could be present in the intercellular spaces between water and fungal PME + Ca treatments. This liquid phase could be partially solubilized pectic substances of the middle lamella in the intercellular space, which could occur to a greater degree in fungal PME + Ca treatment (*41*). The differences seen with Cryo-SEM among the treatments were consistent with firmness measurements. The texture of fungal PME + Ca infused eggplants were firmer than that of RW and water treatment eggplants.

Immunofluorescence Labeling of Eggplant Tissues. JIM5 and JIM7 immunofluorescence of eggplant tissue cross sections is shown in **Figure 3**. JIM5 monoclonal antibody detects low-esterified pectin in a %DE range of 0−50%, and JIM7 detects high-methyl-esterified epitope of pectin (35−90% DE) in carrot root apex (*42*). Although JIM5 and JIM7 are widely used to

localize defined pectin within a single cell wall, the epitopes of JIM5 and JIM7 are not fully defined with respect to size or degree and patterns of methyl esterification. The structure of epitopes recognized by JIM5 and JIM7 antibodies was investigated (*43*). JIM5 binds weakly to completely de-esterified pectins, but its binding greatly increases with the presence of methyl-esterified pectin up to 40% DE and then decreases when %DE is >40%. JIM7 binding is strong in the range of 15−80% DE. In RW and water control, both JIM5 and JIM7 bound to the cell walls of eggplant tissues (**Figure 3A**−**D**), although the %DE value from our assay was in the range of 87−92%. This finding is similar to the pectin distribution in the root apex of carrot (*42*) and the inner surface of potato parenchyma cells (*44*). In water and fungal PME + Ca treatment, binding of JIM5 was greater in the cell walls (**Figure 3C**,**E**), whereas JIM7 binding was less (**Figure 3D**,**F**). Inconsistent binding of JIM5 and JIM7 with pectin epitopes was reported in kiwi (*45*) and blanched carrots (*46*). These researchers suggested that poor JIM7 binding with high-methyl-esterified pectin is caused by inaccessible pectin epitopes. Strong binding of JIM5 to pectin with high %DE might be from cell wall swelling (after vacuum infusion), which involves modification of pectic polymers and enhances accessibility of binding sites. More likely, the strong binding of JIM5 to pectin with high %DE may be related to the distribution of methyl-esterified and unesterified residues. JIM5 preferentially binds three or four adjacent unesterified residues that are bordered with methyl-esterified residues. On the other hand, JIM7 preferentially binds pectin when three or four contiguous methyl-esterified regions are bordered by unesterified residues and also binds if residues are alternating in methyl esterification (*47*).

Using the pulsed vacuum technique, higher eggplant yields of fungal PME, fungal PME + Ca, or Ca_fungal PME were observed. Yet the firmness of infused eggplant from fungal PME tests was lower than that of Val PME and MGF PME infused eggplant. The molecular size of fungal PME might be smaller than that of PME from Valencia orange and Marsh grapefruit, based on the weight gain after infusion. Although adding calcium in the fungal PME solution resulted in a decrease of infused eggplant yield compared to those without added calcium, the yield of these samples was higher than those with Val PME or MGF PME with calcium. The results suggest that fungal PME can enter eggplant tissue better than plant PME, due to the smaller size. With greater potential of PME activity, it was expected that the firmness of infused eggplant from fungal PME would be higher than that of plant PME. In contrast, infused eggplants from both Val PME and MGF PME had a higher firmness than fungal PME. This might be due to a different mechanism of de-esterification between plant PME and microbial PME. Plant PME de-esterified pectin linearly, creating blocks of free carboxyl groups, whereas the action of microbial PME from *A. niger* created a random de-esterification (*9*). Dependence of the binding of the counterions on the DE and the pattern (random and blockwise) have been investigated. It was found that the concentration of calcium salts has a profound influence on the calcium activity coefficient for the highest charged polymers or for plant enzyme-de-esterified pectins (*32*). The degree of binding of counterions with alkaline-de-esterified pectins (random distribution of free carboxyl groups) increased with increasing charge density, whereas the degree of binding of counterions was roughly independent of the DE for enzyme-de-esterified pectin (blockwise distribution of free carboxyl groups). Thus, there is the possibility that blockwise de-esterification yields much stronger bonds with calcium ions than that of carboxyl groups created randomly. Another reason might be that infused eggplant from MGF PME + Ca (562 units/g of dried wt) had a higher enzyme activity than that of fungal PME + Ca (257 units/g of dried wt) (**Table 4**). On the basis of the pattern and enzyme activity, the texture of plant PME infused eggplants is likely to be firmer than that eggplant infused with microbial PME.

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