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Pectinesterase Inhibitor from Jelly-Fig (*Ficus awkeotsang* Makino) Achenes Reduces Methanol Content in Carambola Wine

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Crude pectinesterase (PE) inhibitor (PEI) extracted from jelly-fig achenes (JFA) (*Ficus awakeosang* Makino) was added to carambola (*Averrhoa carambola* L.) puree to determine the change in methanol production during fermentation. Addition of pectin or microbial pectic enzyme to puree increased dose-dependently the methanol content in fermented products. Decreasing ratio (from 1:0 to 1:19, v:v) of pectic enzyme to diluted crude PEI solution in the puree−enzyme mixture decreased the PE activity remarkably. Except for transmittance (%T), addition of crude PEI to puree did not affect apparently the physical and chemical properties of wine; however, it reduced methanol content in the control from 256 to 58 ppm. The degree of esterification (DE) of pectin in starting puree was ∼70%. It decreased to ∼27% in the control group and reduced slightly to ∼67% in fermented puree with crude PEI added after 14 days of fermentation. This reveals that crude PEI solution was potent in inhibiting intrinsic carambola PE activity and appeared to be a potential alternative for methanol reduction in wines.

KEYWORDS: Jelly-fig achenes; methanol; pectinesterase inhibitor; carambola wine

INTRODUCTION

Methanol in wines and liquors is derived from pectin, which is hydrolyzed by pectinesterase (PE) (pectin pectyl-hydrolase) (EC 3. 1. 1. 11) (*1*−*3*) during fermentation. Lao and Lopez-Tamames (*4*) indicated that the level of methanol, released from crushed red grapes, varied with the harvest year as well as cultivars and species of grapes. In fresh juices of Sunkist, watermelon, carrot, tomato, papaya, and coconut, a low content (7−42 ppm) of methanol is assayed when they are rested at 30 °C for 60 min due to the conversion of pectin by PE into low-methoxyl pectin (LMP).

Commercial pectic enzymes, containing PE, polygalacturonase, and pectin lyase, have been used in wine industries to increase the yield and quality, including pigment, flavor, transmittance, and viscosity (*4*−*6*). In the juice industry, pectic enzymes have also been proved to be contributory to the increase in yield (*7*) and, in addition, to the decrease in transmittance (*8*). However, use of pectic enzymes suffers from the major drawback of a higher methanol content in wine products (*9*).

Jelly-fig is a native woody vine in Taiwan. Pectin jelly curd prepared from jelly-fig achenes (JFA) by repeated extraction with tap water is a popular summer drink in Taiwan. PE activity increased gradually to ∼12 units/mL when enzyme was extracted with tap water from intact achenes at a ratio of 1:10 (w/v); however, PE activity in crushed achene suspension was maintained at ∼0.2−0.3 unit/mL throughout the 8 h extraction process, suggesting the presence of substances from crushed seeds that exhibited PE inhibition (*10*). Characterization of PE inhibitor (PEI) in JFA revealed that PEI was thermally stable polypeptides (3.5−4.5 kDa), containing 57% of basic amino acids and competitively inhibited citrus PE and JFA PE (*11*). On the other hand, PEI reduced the activities of PEs from tomato, apple, asparagus, and guava and remarkably prevented cloud loss of fruit juices (*12*).

In an effort to reduce the methanol content in wines, some basic investigations on the inhibition of JFA PEI on commercial pectic enzymes and carambola PE in carambola puree or in a model system were done in the first phase of the present study. Then, JFA PEI was added to purees, in the presence or absence of pectic enzymes, to compare its effects on the physical and chemical properties including specific gravity, total soluble

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solids (TSS), ethanol content, pH change, titratable acidity, transmittance (%T), and methanol content of carambola wines during the 14 day fermentation.

MATERIALS AND METHODS

Raw Materials. Fresh carambola (*Averrhoa carambola* L.) from a local supermarket in Pingtung County, Taiwan, was used. Solar-dried fresh JFA (*F. awkeotsang* Makino) were kindly provided by Mr. Li, a local jelly-fig wholesaler in Pingtung County, Taiwan. Liquid commercial pectic enzyme (530 PE units/mL) from microbial source was the product of Lallemand Australia Pty. Ltd., North Adelaide, Australia. Commercial dried bread yeast (*Saccharomyces cerevisiae*) was from Wako Co. (Tokyo, Japan). Citrus pectin with a degree of esterification (DE) of 68%, methanol (HPLC grade), acetonitrile (HPLC grade), and potassium pyrosulfite ($K_2S_2O_5$) were all from Sigma (St. Louis, MO). Ethanol of 95% was from Taiwan Tobacco and Liquor Corp., Taipei, Taiwan.

Preparation of Crude JFA PEI. Crude PEI was prepared according to the method described by Jiang et al. (*11*). Achenes were repeatedly rinsed in 20 volumes (w/v) of 4% NaCl to deplete most of the pectin and PE, followed by homogenization with a cycle blender for 2 min and extraction with 15 parts (1/15, w/v) of distilled water for 5 h. Centrifugation (10000*g*, 10 min, 4 °C) was conducted to obtain supernatant, which was heated in a boiling water bath for 30 min to denature residual PE. Concentration of crude PEI solution was performed by a rotary evaporator at reduced pressure (<100 mmHg). PEI activity (131.1 units/mL) of crude PEI solution was assayed with the method described below. Triplicate samples each were analyzed twice.

Determination of PE and PEI Activities. PE activity was determined according to the method described by Lee and MacMillan (*13*). Fifteen milliliters of 0.1 M NaCl/0.5% citrus pectin solution (25 °C) (substrate solution), with the pH adjusted to 6.5 immediately before assay, was mixed well with 1 mL of PE solution. PE activity was determined by titrating (pH M83 Autocal pH-meter, TTT 80 titrator, ABU 80 autoburet; Radiometer, Copenhagen, Denmark) the free protons dissociated from the free carboxyl groups formed by the PE activity, and the volume of 0.01 N NaOH required to maintain a pH of 6.5 of the reaction solution at 25 °C in a water bath was recorded within 5 min. An enzyme activity unit represents 1 μequiv of the free carboxyl groups produced by the PE hydrolytic activity on the pectin substrate per minute at 25 °C. Heated enzyme solution in a boiling water bath for 5 min was treated as the blank. Deionized water (Milli-Q System, Millipore, Tokyo, Japan) was used to prepare the substrate and enzyme solutions.

For the PEI activity assay, similar procedures were performed except that 1 mL of 200-fold diluted crude PEI solution, heated in a water bath for 15 min to denature residual PE (*11*), was incubated previously with 1 mL of citrus PE solution (1 unit/mL) for 10 min before it was mixed with 15 mL of substrate solution. The reduction in PE activity was referred to PEI activity: PEI activity (units/mL) = dilution fold × (activity of PE − residual activity of PE in PE−PEI mixture). In the determination of the PEI activity versus pH values, the PE−PEI mixture was incubated at pH values between 3.5 and 8.0, which was adjusted by 0.01 N HCl or NaOH prior to PEI activity assay. Triplicate samples each were analyzed twice.

Preparation of Carambola Wine. One kilogram of clean carambola was blended with 10 mL of crude PEI solution (131.1 units/mL) to make puree, followed by the addition of sucrose and sodium pyrosulfite to reach 24 °Brix and 100 ppm (as SO_2), respectively, before inoculation with yeast. Commercial bread yeast (0.25 g), activated previously in warm water (40−43 °C) for 10 min, was added to 1 kg of the above puree. Fermentation was conducted at room temperature (25 ± 2 °C) for 14 days. Carambola puree without the addition of PEI was used as control. Meanwhile, pectic enzyme (53 PE units/kg of puree)-added puree was treated as positive control.

Aging was subsequently conducted at 16 °C for 1 month to determine the changes in methanol and ethanol contents by the methods described below. During fermentation, sampling was conducted every 2 days to determine the changes in the physical and chemical properties after centrifugation (13000*g*, 20 min, 4 °C).

Similar fermentation procedures, except the addition of 0.1−0.5% (w/v) pectin and/or 0.1 mL (530 PE units/mL) of commercial pectic enzyme solution to 1 kg of puree, were conducted to investigate the effects of pectin level and pectic enzyme on methanol formation.

To observe the formation of methanol by yeast in a model system, similar fermentation procedures were performed except that the solution of 1 L of 24% sucrose/0.1% pectin/0.1% ammonium diphosphate was used. Sampling was conducted every 2 days for methanol determination during fermentation. Triplicate samples each were analyzed twice.

Effect of Pectic Enzymes on Methanol Formation in a Model System. To 1 L of 24% sucrose/12% ethanol/0.1% pectin was added 0.1 mL of commercial pectic enzyme (530 PE units/mL), and incubation was then conducted at room temperature in a dark place for up to 14 days. Distilled water was used to prepare the control group. Sampling was conducted every 2 days during incubation. Triplicate samples each were analyzed twice.

Effect of Crude PEI on PE Activity of Pectic Enzymes. Commercial pectic enzyme solution (53 PE units/mL) diluted 10-fold with distilled water was mixed well with 1−19 parts (1:1, 1:4, 1:9, 1:19) (v/v) of diluted crude PEI solution (13.1 units/mL) for 10 min at pH 4.3 (the natural pH of puree) before the PE activity assay. The PE activity of the commercial pectic enzyme solution in the starting solution was treated as 100% to calculate the relative residual PE activity. Triplicate samples each were analyzed twice.

TSS. A hand-held refract-meter (N-1E, Atago, Tokyo, Japan) was used to determine the TSS (as °Brix) of semiproducts and wines. The refract-meter was adjusted with distilled water each time before use. Triplicate samples each were analyzed twice.

Value of pH. Changes in the pH value of semiproducts during fermentation were determined by a pH meter (pH M82, standard pH meter, Radiometer, Copenhagen, Denmark) at room temperature (27 ± 2 °C). Triplicate samples each were analyzed twice.

Specific Gravity. The specific gravity of semiproducts and final products was determined according to the method described by Zoecklein et al. (*14*) at 20 °C. Triplicate samples each were analyzed twice.

Titratable Acidity. To 10 mL of supernatants of semiproducts was added 20 mL of distilled water, and the pH value was then brought to 8.1 by adding 0.1 N NaOH. The volume (milliliters) required to bring the pH to 8.1 by a pH-stat was recorded. The acidity was calculated and expressed as citric acid. Triplicate samples each were analyzed twice.

Color. Color changes of supernatants of semiproducts during fermentation were monitored, for Hunter's *L* (brightness), *a* (redness−greenness), and *b* (yellowness−blueness) by a Spectro-Colorimeter (JP7100F, JUKI Co., Tokyo, Japan) using a comparator block: *X*, 93.80; *Y*, 95.64; *Z*, 113.70. Triplicate samples each were analyzed twice.

%T. A spectrophotometer (U-2001, Hitachi, Ltd., Tokyo, Japan) was used to determine the transmittance of wine and semiproducts at a wavelength of 660 nm. Samples were all centrifuged (13000*g*, 20 min) before determination. Triplicate samples each were analyzed twice.

Ethanol Content (Percent, v/v). Distillation and hydrometric analysis of ethanol in wine were conducted (*15*). Calcium hydroxide and pumice were added to 200 mL of wine, which was previously centrifuged (13000*g*, 20 min) to remove suspended particles, followed by distillation. A volume of 198−199 mL of distillate was collected in a 200 mL volumetric flask in a stream-dragging apparatus and filled to 200 mL with water. The ethanol content in the distillate was determined by density measurements at 20 °C. Triplicate samples each were analyzed twice.

Determination of Pectin. Pectin content was determined according to the method of Blumenkrantz and Asboe-Hansen (*16*). An adequate volume (1.0 mL) of pectin solutions was mixed well with 6 mL of 0.0125 M sodium tetraborate solution (in sulfuric acid) in an ice bath and then heated in a boiling water bath for 5 min. After being cooled in an ice bath, the reaction mixture was mixed well with 0.05 mL of 0.15% *m*-phenylphenol/0.5% NaOH solution, followed by resting for 5 min. Absorbance at 520 nm was recorded. Different levels (0−100 μg/mL) of d-galacturonic acid were used to construct the standard curve

($r^2 = 0.9856$) for the calculation of pectin content in samples. Triplicate samples each were analyzed twice.

Author: Please correct "*m*-phenyl[henol" in the above paragraph (what is the "[" supposed to be?).

Determination of Pectin DE. Pectin DE was determined according to the method described by Mizote et al. (*17*) and Jiang et al. (*10*). Briefly, a pectin sample (2 g) was mixed well in a mixture of 90 mL of distilled water, 10 mL of sulfuric acid, and 100 mL of 2-propanol with slight stirring for 15 min. Then, the mixture was filtered with a Whatman no. 2 filter paper to obtain pectin residues, which were rinsed first with 300 mL of 65% 2-propanol, followed by 200 mL of 2-propanol and 50 mL of acetone. Subsequently, the rinsed residues were dried at room temperature in a hood overnight to obtain pectin powder. The powder (0.5 g) thus obtained was moistened first with 65% 2-propanol and then completely dissolved in 100 mL of distilled water with slight stirring. The volume (*a* mL) of 0.1 N NaOH required to bring the pH value to 7.5 was recorded, followed by thorough mixing with 30 mL of 0.1 N NaOH. After resting for 30 min, 30 mL of 0.1 N sulfuric acid was added to the pectin solution, and the volume (*b* mL) of 0.1 N NaOH was then measured during the titration of pectin solution to pH 7.5. The DE of pectin was determined by the following equation: DE (%) = $(b/a + b) \times 100\%$. Triplicate samples each were analyzed twice.

Gas Chromatography. Semiproducts and wine were centrifuged (10000*g*, 15 min, 4 °C) and then pressed through a 0.45 μm membrane filter for a GC analysis (Varian GC 3800, Varian, Palo Alto, CA). Experimental conditions were as follows: column, CP-Wax (length, 6 m; inner diameter, 0.53 mm); carrier gas, N_2; flow rate, 5.0 mL/min; split ratio, 10:1; injection volume, 20 μL; detector, flame ionization detector (FID); column temperature, 50 °C/12 min, 40 °C/min to 230 °C; injector temperature, 180 °C; detector temperature, 220 °C. Methanol solution (0−100 ppm) was injected into the GC column to construct the standard curve ($r^2 = 0.9986$) for the determination of methanol content in samples and for the identification of methanol peak by comparison of the retention time. Dilution, if needed, of fermented semiproducts was conducted with distilled water to <100 ppm during methanol quantification. Triplicate samples each were analyzed twice.

RESULTS AND DISCUSSION

Effect of Pectin, Pectic Enzyme, and PEI on Methanol Formation. Star fruit, with a production of ~30000 tons per year, is an important fruit for winemaking in Taiwan. To reduce the methanol content in wine, star fruit was used to prepare puree, and the effects of pectin, pectic enzyme, and PEI on methanol formation were studied.

To the fruit puree was added 0.1, 0.25, or 0.5% pectin or microbial pectic enzymes to observe the methanol formation during fermentation. Methanol content (average of three determinations) increased gradually to about 240 and 400 ppm in the 0.1% pectin group during fermentation for 6 and 14 days, respectively, compared with only 256 ppm in final products of the control (**Figure 1**). Higher levels (0.25 and 0.5%) of pectin in puree resulted in higher methanol contents during fermentation, and a higher methanol content of ~600 ppm was reached in final products. This suggests that methanol in wine products is mainly derived from pectin in the presence of intrinsic PE (1.5 units/g of puree) in fruit juices or purees during fermentation. This observation was in agreement with those reported by Nicolini et al. (*1*) and Frenkel et al. (*3*).

On the other hand, the addition of pectic enzyme (53 PE units/kg of puree) to puree enhanced the methanol formation in wine (**Figure 1**). Puree added with 0.5% pectin and pectic enzyme (0.5% pectin enzyme) increased sharply the methanol level to ~800 ppm in 2 days during fermentation and reached ~1200 ppm in the final products. A similar trend was also observed in the 0.1% pectin and 0.25% pectin-enzyme groups with lower methanol content in both semiproducts and final products during fermentation. Removal of methoxyl groups at C6 positions of galacturonic acid residue on pectin molecules by PE is responsible for the methanol formation during fermentation. Bartolome and Hoff (*18*) indicated that an increase (0.02, 0.01, 0.005, and 0.0025%) in PE level of a model system containing 1% pectin resulted in an elevated amount of methanol. They reported that the addition of 0.0025 and 0.02% PE resulted in the formation of 40 and 150 μequiv of methanol/mL, respectively. An additional amount of PE in grape also enhanced the level of methanol in wines (*9*).

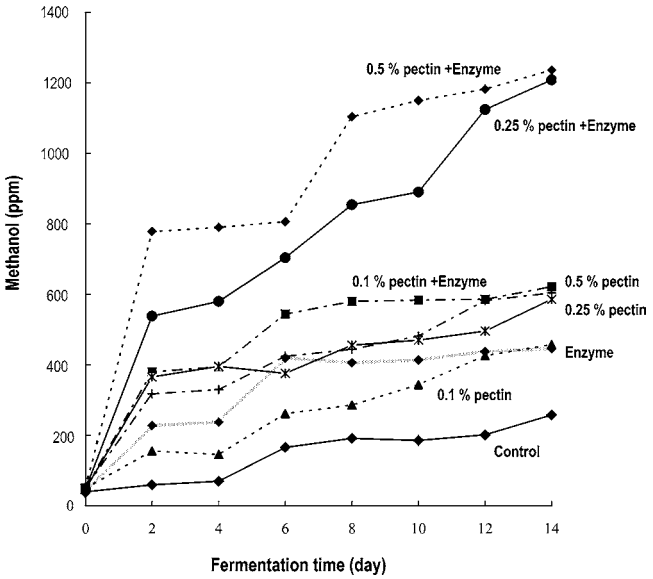

Figure 1. Effect of 0.1–0.5% citrus pectin (DE = 68%) on methanol formation in the presence or absence of commercial microbial pectic enzyme (53 PE units/kg of puree) during fermentation of carambola puree. Each value is the average of three determinations.

Formation of Methanol in a Model System. To confirm the role of yeast in methanol formation during fermentation, yeast in a 24% sucrose/0.1% pectin solution was prepared, and the change in methanol formation was detected by GC (**Figure 2A**). Methanol was found at 15 and 17 ppm in day 3 and day 14 samples, respectively, suggesting the rapid formation of methanol at an early stage of fermentation. The growth of yeast is strongly influenced by fermentation conditions, ethanol content, carbon dioxide level, and acetic acid content. During wine fermentation, the growth of yeast was inhibited when the ethanol content reached 12% at day 6 (data not shown). In addition, the results in **Figure 2B** also supported the hypothesis that PE was the main factor in producing methanol, which converted pectin into LMP. Pectic enzymes (53 PE units/L) in 24% sucrose/12% ethanol/0.1% pectin solution in a model system enhanced methanol formation, revealing that the presence of 12% ethanol was irrelevant to methanol formation. Variance in yeast strain might result in different methanol levels in wine products (*4*, *9*); however, according to the results in **Figure 2**, we concluded that the high methanol content in **Figure 1** was mainly attributed to the demethoxylation of pectin by pectic enzymes.

Reduction in PE Activity by JFA PEI. To 1 mL of 10-fold diluted PEI solution (13.1 units/mL) was added equal volumes of commercial pectic enzyme solution (53 units/mL), and the homogeneous mixture then was incubated at room temperature for 10 min before reaction with 15 mL of pectin solution (**Figure 3A**). Jelly-fig peptide extracts displayed maximal (~90%) PE activity inhibition at pH 4.5. Increase or decrease in pH value of the PEI−PE mixture reduced the PEI activity, and no apparent

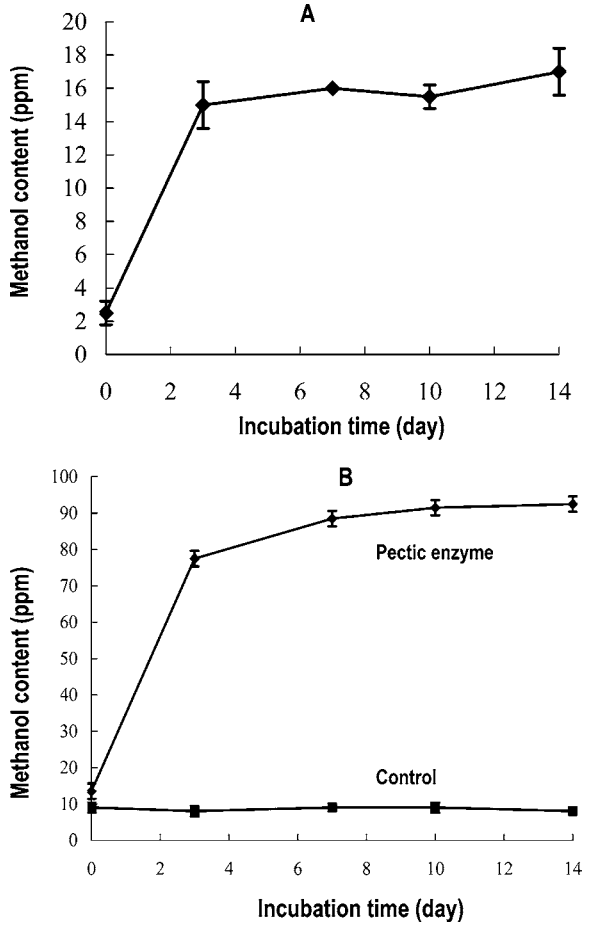

Figure 2. Methanol formation by yeast in 24% sucrose/0.1% pectin/0.1% ammonium diphosphate solution (**A**) and by commercial microbial pectic enzyme (53 PE units/L) in 24% sucrose/12% ethanol/0.1% pectin solution (**B**). Distilled water is used as control. Each value is the average of three determinations.

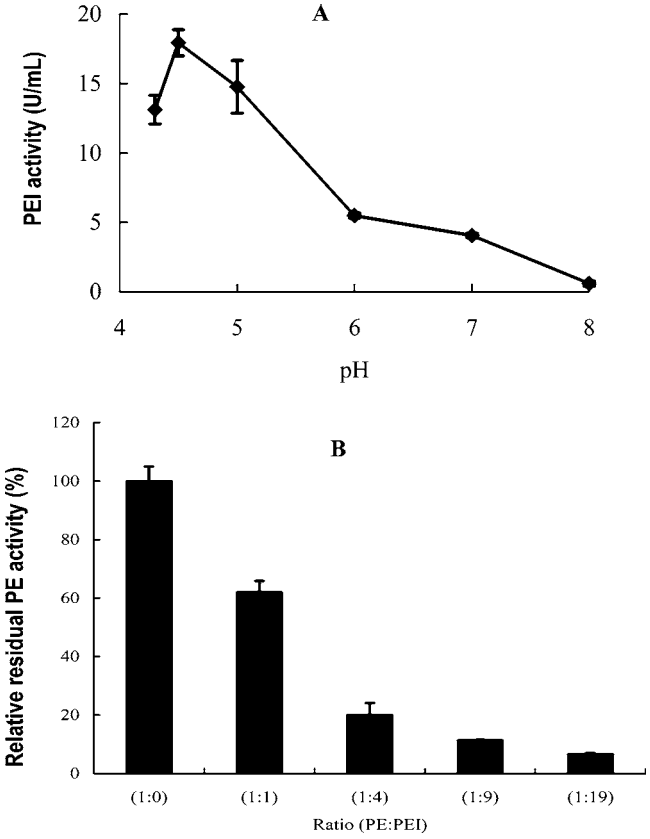

Figure 3. Changes in jelly-fig achene PEI activity versus pH value (**A**) and changes in relative residual PE activity versus ratio (v/v) of pectic enzyme to PEI at pH 4.3 (**B**). Each value is the average of three determinations. Diluted crude PEI solution, 13.1 units/mL; commercial microbial pectic enzyme solution, 53 PE units/mL.

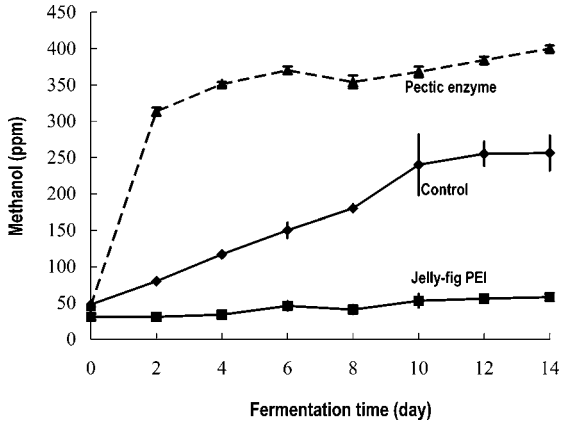

Figure 4. Changes in methanol content of carambola wines in the presence of microbial pectic enzyme (53 PE units/kg puree) or PEI (131 units/kg puree) during fermentation. Methanol content in starting puree is 48 ppm. Each value is the average of three determinations.

PEI activity was observed at pH 8.0. Similar results were reported by Jiang et al. (*11*) when observing the effect of JFA PEI on citrus PE activity versus pH value. Thus, it was clear that PEI displayed a marked inhibitory effect on PE activity of commercial pectic enzymes at acidic pH values, between pH 4.3 and 5.0, very close to the natural pH value of 4.3 of carambola puree.

For further investigation of PE activity inhibition by PEI, PE (53 units/mL) was incubated previously with different volume ratios of 10-fold diluted PEI solution (13.1 units/mL) before PE activity assay. It was noteworthy that PE activity decreased to about 62 and 6.6% when PE was added with equal volumes and 19 volumes of PEI solution, respectively, suggesting the dose-dependent inhibitory effect of PEI on PE (**Figure 3B**). However, no complete inhibition of PE activity by JFA PEI was observed under the present experimental conditions, similar to the results reported by Jiang et al. (*10*) in determining the mixing ratio (v/v) of pea pod PE (10 units/mL) solution to JFA PEI solution. In addition, cloud loss of tomato juices, apple juice, and papaya juice was greatly inhibited by JFA PEI during 12 weeks of storage due to the strong inhibition of PEs (*12*).

Inhibition of Methanol Formation by PEI. Figure 4 represents the change in methanol content during fermentation in the presence of microbial pectic enzymes (53 units/kg of puree) or PEI (131 units/kg of puree) in comparison with that of the control. It was obvious that methanol formation was strongly inhibited in the presence of PEI, and the methanol level in the final wine product was only 58 ppm, slightly increased from the original 48 ppm in puree, due to the strong inhibition of PEI on carambola PE activity during fermentation. The methanol content with reference to the ethanol level was calculated to be 483 mg/L. In the control group, the methanol content increased almost linearly during fermentation to ~256 ppm, ~4 times higher than that of the PEI group. Therefore, it was confirmed that JFA PEI was effective in reducing the methanol content by inhibiting the intrinsic PE activity of carambola.

Table 1. Physicochemical Properties of Carambola Wines in the Presence of Microbial Pectic Enzymes (53 PE Units per Kilogram of Puree) or Jelly-Fig Achene PEI (131 Units per Kilogram of Puree) Prepared from Carambola Puree

	puree	control	puree + jelly-fig achene PEI	puree + pectic enzymes
TSS[a] (°Brix)	24	6	6	7
specific gravity	1.10	0.99	0.99	0.99
ethanol (%)	0	12	12	12
pH	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.1 ± 0.1
titratable acidity (%)	0.14	0.32 ± 0.01	0.28 ± 0.01	0.30 ± 0.01
%T[b]	0	36.15 ± 0.80	24.33 ± 1.72	94.40 ± 0.65
L	–[c]	46.94 ± 0.28	36.07 ± 2.75	91.39 ± 1.03
a	–	–1.98 ± 0.09	4.03 ± 0.27	–3.08 ± 0.03
b	–	11.64 ± 0.48	19.27 ± 1.68	9.77 ± 0.08
DE[d] (%)	69.83 ± 1.30	27.22 ± 1.19	67.01 ± 1.82	17.80 ± 1.48

[a] Total soluble solids. [b] Transmittance. [c] Not determined. [d] Degree of esterification of pectin in puree.

Comparison of Physical and Chemical Properties. Ethanol content in each wine product prepared in the presence or absence (control) of PEI or in the presence of commercial microbial pectic enzymes was ~12% (**Table 1**), suggesting that PEI or pectic enzymes did not interfere with the fermentation process. The pH value of the PEI group was 4.4, slightly higher than the values of 4.3 and 4.1 in the control and pectic enzyme group, respectively. This could be due to the strong inhibition of PEI on demethoxylation of pectin and the formation of free protons dissociated from the free carboxyl groups in galacturonic acids. Similarly, titratable acidity was only 0.28%, lower than the 0.32% in the control and the 0.30% in the pectic enzyme group. Faquembergue and Grassin (*19*) and Hohn (*20*) reported that the use of pectic enzymes in wine preparation facilitated the formation of polygalacturonic acids and titratable acid.

Of note, the transmittance (%T) of wine in the PEI group was ~24.33, whereas that of the control and PE group was 36.15 and 94.40, respectively. It could be due to the decrease in PE activity in the presence of PEI and the resulting decrease in cloud loss during fermentation. Jiang et al. (*12*) reported that cloud loss of fresh tomato juice, apple juice, and papaya juice was greatly inhibited by PEI during 12 weeks of storage as a result of the PE inhibition by PEI. Castaldo et al. (*21*) found that cloud loss of out-back juice was effectively prevented in the presence of kiwi PEI during long storage.

In color, the pectic enzyme group exhibited the highest *L* value of ~91, followed by 47 for the control and 36 for the PEI group, revealing that the decrease in PE activity resulted in loss of brightness. These results were consistent with those in transmittance. The *a* value (4.03) of the PEI group was higher than that of the control (−1.98) and the PE group (−3.08). It was mainly due to the slight reddishness of the PEI crude extracts used in the experiment. Partial purification of the crude PEI extract to remove reddishness would be helpful in improving the appearance of wine products.

Changes in the DE of pectin in the control, PEI, and PE groups appeared to be of interest. The DE of pectin in the starting material was ~70; however, it decreased to about 27% in the control and 18% in the pectic enzyme group, revealing the remarkable conversion of high-methoxyl pectin into low-methoxyl pectin during fermentation. However, no apparent difference in DE of pectin between raw puree (70%) and the PEI group (67%) was observed. This result strongly supported the assumption that the decreased level of methanol in wine of the PEI group was due to the PE activity suppression by PEI.

Table 2. Changes in Ethanol and Methanol Contents of Carambola Wine in the Presence or Absence (Control) of Jelly-Fig Achene PEI (131 Units per Kilogram of Puree) during Aging at 16 °C for 1 Month

	control[a]		puree + jelly-fig achene PEI[b]	
	0 days	1 month	0 days	1 month
ethanol (%)	12.1 ± 0.2	13.1 ± 0.3	12.4 ± 0.3	12.3 ± 0.2
methanol (ppm)	256.0 ± 24.3	261.5 ± 23.0	58.1 ± 8.6	65.2 ± 3.5

[a] Puree without PEI. [b] PEI is added to puree to prepare wine that undergoes aging.

With the exception of methanol content, use of JFA PEI in wine fermentation resulted in physical and chemical properties similar to those of the control group.

Formation of Methanol during Aging. The intrinsic PE in carambola wine products increased methanol content by ~5 ppm during aging at room temperature for 1 month (**Table 2**). Methanol content in the PEI group during aging increased by 7 ppm, from about 58 to 65 ppm; however, it was still very low compared with that of the control group. This suggests that the interactions between PEI and PE are stable during storage and/or aging.

In conclusion, the activity of PE in fruits contributed mainly to the formation of methanol in wine preparation. JFA PEI is a side-product of the jelly curd business and is effective in reducing the methanol content in wine products. However, use of JFA PEI in wine production may suffer from some minor drawbacks such as loss of transmittance and brightness.

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