JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pineapple Juice and Its Fractions in Enzymatic Browning Inhibition of Banana [*Musa* (AAA Group) Gros Michel]

CHITSUDA CHAISAKDANUGULL,[†] CHOCKCHAI THEERAKULKAIT,*[,†] AND RONALD E. WROLSTAD[‡]

Department of Food Science and Technology, Faculty of Agro-industry, Kasetsart University, Bangkok 10900, Thailand, and Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

The effectiveness of pineapple juice in enzymatic browning inhibition was evaluated on the cut surface of banana slices. After storage of banana slices at 15 °C for 3 days, pineapple juice showed browning inhibition to a similar extent as 8 mM ascorbic acid but less than 4 mM sodium metabisulfite. Fractionation of pineapple juice by a solid-phase C_{18} cartridge revealed that the directly eluted fraction (DE fraction) inhibited banana polyphenol oxidase (PPO) about 100% when compared to the control. The DE fraction also showed more inhibitory effect than 8 mM ascorbic acid in enzymatic browning inhibition of banana puree during storage at 5 °C for 24 h. Further identification of the DE fraction by fractionation with ion exchange chromatography and confirmation using model systems indicated that malic acid and citric acid play an important role in the enzymatic browning inhibition of banana PPO.

KEYWORDS: Polyphenol oxidase; enzymatic browning; browning inhibition; pineapple juice; banana

INTRODUCTION

Many types of fruits, especially bananas, brown rapidly when their tissues are cut or bruised. The brown color is developed due to the enzymatic oxidation of phenols to quinones by polyphenol oxidase (PPO) in the presence of oxygen. Subsequently, these quinones condense and react nonenzymatically with other substances such as phenolic compounds and amino acids to produce complex brown polymers. PPO (1.14.18.1) is also known as tyrosinase, *o*-diphenol oxidase, or catechol oxidase (*1*). Enzymatic browning impairs not only the color of fresh fruits but also the flavor and the nutritional quality (*2*). To prevent browning, sulfite is used extensively because of its effectiveness as a browning inhibitor. Sulfites act as PPO inhibitors by serving as reducing agents and also react with intermediates to prevent pigment formation. However, sulfites are known to induce adverse allergenic effects in certain sensitive individuals such as asthmatics (*3*). Various chemicals are known to be able to reduce enzymatic browning, including PPO inhibitors, reducing agents, acidulants, and chelating agents; however, they have always been found to be less effective than sulfites (*4*).

Nowadays, consumers are concerned about the possible dangers of synthetic food additives. This consumer awareness has stimulated the search for natural and safe antibrowning agents. Oszmianski and Lee (*5*) reported that honey can inhibit enzymatic browning in apple slices, grape juice, and model systems. The compound responsible for this inhibitory effect of honey appeared to be a small peptide with an approximate molecular mass of 600 Da. In contrast, Martyniuk reported that the PPO inhibitors in honey were *t*-cinnamic and *p*-coumaric acids, which acted as strong, noncompetitive inhibitors of mushroom PPO (*6*).

Labuza et al. (*7*) investigated the effect of proteolytic enzymes on enzymatic browning inhibition. They found that ficin and papain were as effective as sulfite in preventing browning in potatoes and apples; however, bromelain was effective only in apples during storage at 4 °C. McEvily (*8*) reported that ficin-free extract prepared from the fig latex can inhibit enzymatic browning in apple and shrimp. The component that was responsible for browning inhibition was 4-substituted resorcinol such as 4-hexyl-, 4-dodecyl-, and 4-cyclohexylresorcinol with I_{50} values of 0.5, 0.3, and 0.2 μM, respectively (*9*). Monsalve-Gonzalez et al. (*10*) showed that 4-hexylresorcinol (4-HR) was useful in inhibiting browning reactions in stored apples; however, hexylresorcinol beyond 0.03% might result in undesirable flavor effects. Subsequent research has demonstrated that commercial papain preparations (from papaya latex) contain some substances that act as "quinone-trapping" compounds. The compounds were identified as cysteine and a dipeptide of cysteine-glutamic acid, commercially available as γ-Glu-Cys. These two thiol compounds block the *o*-quinone by forming additional compounds, which show competitive properties toward endive PPO; however, there were some additional

* To whom correspondence should be addressed. Tel: +66-2-562-5032. Fax: +66-2-562-5021. E-mail: fagicct@ku.ac.th.
[†] Kasetsart University.
[‡] Oregon State University.

10.1021/jf0705724 CCC: $37.00
Published on Web 04/18/2007

efficient PPO-inactivating agents whose structure and inactivation mechanism remain to be elucidated (*11*).

Pineapple [*Ananas comosus* (L.) Merr.] is an important fruit crop in many tropical and subtropical countries that is consumed fresh or in various processed forms (*12*). Pineapple juice is a popular product due to its very pleasant aroma and flavor (*13*). The use of pineapple juice to inhibit enzymatic browning was investigated by Lozano-de-Gonzalez et al. (*14*) who found that pineapple juice and ion-exchanged pineapple juice were as effective as sulfite in enzymatic browning inhibition of fresh and dried apple rings. After pineapple juice was fractionated using various size and charge separation methods, the best results were achieved with a cation-exchanged fraction. Wen and Wrolstad (*15*) reported that a nonvolatile organic acid in pineapple juice was the major inhibitor of enzymatic browning in apple products.

There are no published reports, however, on the effectiveness of pineapple juice as an inhibitor of enzymatic browning in bananas. The objectives of this study were to fractionate pineapple juice, conduct compositional analyses, and identify the fraction that was most effective for banana PPO inhibition and preventing enzymatic browning in banana puree.

MATERIALS AND METHODS

Plant Materials. Bananas [*Musa* (AAA Group) Gros Michel] grown in Thailand were chosen at ripening stage 5 (yellow peel with green tip) according to the peel color index. Pineapples (Smooth Cayenne variety) were obtained from Dole Inc.'s farm, Thailand. They were harvested at maturation stage 4 (shell color is on-half to three-fourths gold) as Dole's fresh pineapple color standard guide.

Pineapple Juice Preparation. Pineapples were peeled and crushed to juice with a hydraulic press. The juice was then centrifuged at 19000*g* for 30 min. The clear juice was lyophilized in powder form by a freeze dryer (Heto FD 2.5, Heto Lab Equipment, Denmark) for use throughout the study. The lyophilized pineapple juice was dissolved in distilled water to obtain 12 °Brix as fresh pineapple juice before use.

Banana PPO Preparation. The crude banana PPO was extracted by a modified method of Galeazzi et al. (*16*). Banana pulp (25 g) was homogenized with 50 mL of a cold 0.1 M sodium phosphate buffer, pH 6.5, containing 1% (w/v) polyvinylpyrrolidone and 0.5% Triton X-100. The homogenate was centrifuged for 30 min (4 °C) at 29000*g* (Sorvall RC 5 C Refrigerated Centrifuge, DuPont, Newtown, CT). The crude enzyme was then partially purified by fractionation with 80% ammonium sulfate saturation followed by conventional column chromatography on Sephacryl S-200 HR (Pharmacia, Uppsala, Sweden) as a modified method of Ngalani et al. (*17*). The fractions containing at least 30% of the PPO activity of the most active fraction from Sephacryl S-200 HR column were pooled, lyophilized, and used as a source of banana PPO. The PPO activity was determineded by measuring the increase in absorbance at 475 nm, following the method of Jayaraman et al. (*18*) using dopamine as the substrate.

Effectiveness of Pineapple Juice in Enzymatic Browning Inhibition of Banana. Bananas with similar sizes and no external defects were selected. They were peeled and cross-sectionally cut with a stainless knife at the head and the end of fruits and then longitudinally sliced into pieces approximately 3 mm thick and 7.5 cm long. Banana slices were treated by immersion in distilled water (control), pineapple juice, and various inhibiting solutions (H_2O, pH 4.0, 4 mM sodium metabisulfite, 8 mM ascorbic acid, and 100 mM 4-HR). Treated banana slices were drained and packed in a plastic bag for storage at 15 °C. The *L** (lightness), *a** (red to green color dimension), and *b** (yellow to blue color dimension) values of the cut surfaces were measured by UltraScan XE (Hunter Associates Laboratory, Inc., Reston, VA) to evaluate the browning inhibition of each treatment. They were measured just after immersion (0 h) and after storage at 15 °C for 6, 12, 24, 48, and 72 h.

Pineapple Juice Fractionation. The pineapple juice was centrifuged at 10000*g* (4 °C) for 30 min. The supernatant was filtered through a 0.45 μm membrane filter, and the filtrate was ultrafiltered through a 10000 Da molecular mass cutoff ultrafiltration membrane with an ultrafiltration cell (Amicon Inc., Beverly, MA). The permeate was then further fractionated by solid-phase extraction using a C_{18} cartridge (total volume of 20 mL, Alltech Associates Inc., Deerfield, IL) into three fractions following the method of Jaworski and Lee (*19*); first, the cartridge was preconditioned for neutral phenolic adsorption by sequentially passing 50 mL of methanol and 50 mL of Milli Q water with a resistivity of 18 MΩ cm as ASTM standard specification for reagent water type I (Millipore Corp., Bedford, MA); for acidic phenolic adsorption, the other cartridge was preconditioned by passing 50 mL of 0.01 N HCI instead of water. Then, 100 mL of juice permeate was adjusted to pH 7.0 with 0.1 N NaOH and passed through the preconditioned neutral C_{18} cartridge to adsorb the neutral phenolic compounds. The effluent portion was then adjusted to pH 2.5 with 0.1 N HCl and passed through the second acidic C_{18} cartridge to adsorb acidic phenolic compounds. The effluent from the preconditioned acidic C_{18} cartridge was defined as the directly eluted fraction (DE fraction). The neutral phenolics and acidic phenolics from each C_{18} cartridge were then eluted with 100 mL of methanol and were defined as the NP fraction and the AP fraction. The three fractions were then rotary evaporated at 35 °C to dryness, and the residue was dissolved in 20 mL of water to test for enzymatic inhibition.

Fractionation by Ion Exchange Chromatography. Because the DE fractions from solid-phase extraction showed the highest inhibition, this fraction was further fractionated by ion exchange chromatography technique. Cation exchange resin (SP Sephadex C 25, Sigma) was preconditioned in water, pH 2.0, adjusted with 1 N HCl and was filled in a polyprep column (Bio-Rad Laboratories, Richmond, CA) for a total volume of 6 mL. Three milliliters of the DE fraction was adjusted to pH 2.0 with 6 N HCl and applied to the column. The cationic substances were retained. The elution was continued with 20 mL of water, pH 2.0, adjusted with 1 N HCl, and then, cationic substances were eluted with water, pH 7.8, adjusted with 0.1 N NaOH. This fraction was defined as F1. The first effluent was rotary evaporated at 35 °C to 3 mL and adjusted to pH 7.0 with 0.1 N NaOH and then applied to the anion exchange resin (DEAE Sephadex A 25, Sigma, preconditioned in water, pH 7.0, and filled in the polyprep column for a total volume of 6 mL). Organic acids and anionic substances were retained. The elution was continued with 20 mL of water, pH 7.0. This fraction was defined as F2. Then, organic acids and other anionic substances were eluted with 20 mL of 1% formic acid in methanol. This fraction was defined as F3. Each of the three fractions was rotary evaporated at 35 °C to dryness, and the residue was dissolved in 3 mL of water.

Inhibition Study. To test the relative inhibition caused by each fraction, 10 mg of lyophilized banana PPO (0.06 mg protein/mg) from Sephacryl-S 200 HR was dissolved in 2 mL of 20 mM sodium phosphate buffer, pH 7.0, to provide a PPO activity of 20−30 units/mL of enzyme. Twenty microliter aliquots of the enzyme were added to the mixture containing 2.5 mL of 5 mM dopamine (Sigma Chemical Co., St. Louis, MO) substrate solution in 20 mM sodium phosphate buffer, pH 7.0, and 0.5 mL of each tested fraction (AP, DE, and NP fractions and F1, F2, and F3 fractions) at 25 °C. The change in absorbance at 475 nm was recorded for 1 min. As a control, 0.5 mL of water was mixed with the same substrate solution before adding the enzyme. One unit of enzyme activity was defined as the amount of enzyme responsible for a change of 1 absorbance unit at 475 nm/min at 25 °C, pH 7.0 (*18*). The percent inhibition was calculated as follows:

$$\% \text{ inhibition} = [(\text{activity of control} - \text{activity of treatment})/\text{activity of control}] \times 100 \quad (1)$$

All determinations were performed in triplicate.

Chemical Analysis. The bromelain activity was determined in the pineapple juice, permeate, and retentate of pineapple juice after ultrafiltration following the method of Ota et al. (*20*) using casein as the substrate. One unit of enzyme (casein digestion unit, CDU) was defined as μg of tyrosine produced per min at 37 °C, pH 7.6. Total phenols were analyzed using the modified method of Slinkard and Singleton (*21*). Sulfhydryl contents were determined by the method of Ewart (*22*). l-Ascorbic acid, sugar, and organic acid compositions were

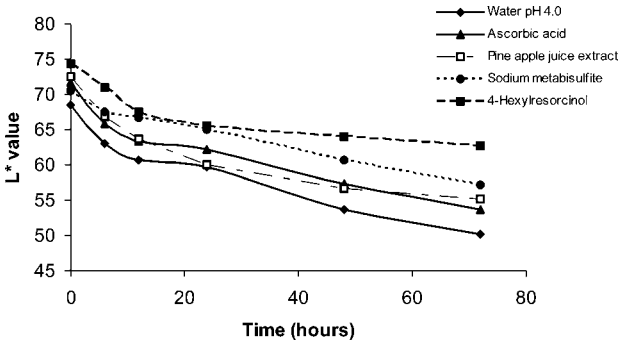

Figure 1. *L** value of banana slices treated with pineapple juice and various inhibitors during storage at 15 °C for 3 days.

determined by high-performance liquid chromatography (HPLC) using the methods of Wen and Wrolstad (*23*). For L-ascorbic acid and organic acids analyses, columns ODS-2 and ODS-1 (250 mm × 4.6 mm i.d., 5 μm particle size; Alltech Associates Inc.) were connected in series to a Dynamax model SD-300 pump and a HP 1040 diode array detector. For L-ascorbic acid, the mobile phase was 0.5% potassium dihydrogen phosphate, pH 2.5, with 0.1% dithiothreitol at a flow rate of 0.7 mL/min, and detection was measured at 245 nm. For organic acids, isocratic elution with 0.1 M potassium dihydrogen phosphate, pH 2.4, was used at a flow rate of 0.7 mL/mm, and detection was measured at 214 nm. Malic and citric acid contents were determined and reported as g total acids/100 mL. For sugar analysis, the HPLC system consisted of a 4100 LDO pump and a RI 4 LDC detector. The column used was Zorbax-NH_2 (250 mm × 4.6 mm id). Isocratic elution with 82% (v/v) acetonitrile in water was used at a flow rate of 0.2 mL/mm. Glucose, fructose, and sucrose were determined, summed, and reported as g total sugars/100 mL. Free amino acids were determined by derivatization method (*24*) using the Waters Acc Q. Tag Chemistry Package (Waters, Milford, MA), which consisted of Waters Acc Q. Tag reagents and a C_{18} column. Phenolic profiles were determined using the method described by Wen and Wrolstad (*23*).

Effectiveness of DE Fraction on Enzymatic Browning Inhibition of Banana Puree. Banana puree was prepared by blending 30 g of banana pulp for 30 s with 20 mL of water (control) and with 20 mL of the following test solutions: water with the pH adjusted to 2.0 (as the same pH of DE fraction), 8 mM ascorbic acid, 4 mM sodium metabisulfite, and DE fraction. The puree was then filled in plastic containers and the *L**, *a**, and *b** values of the puree were measured at 0 (initial condition), 1, 3, 6, 12, and 24 h of storage time at 5 °C. The purees were left to reach room temperature before measurement.

Statistical Analysis. The SPSS (Chicago, IL) statistical analysis system was used for analysis of the data. The statistical significance difference was assessed by one-way analysis of variance. Significant differences ($P \leq 0.05$) among treatments were detected using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effectiveness of Pineapple Juice in Enzymatic Browning Inhibition of Banana. The effectiveness of pineapple juice in enzymatic browning inhibition on the cut surface of banana slice was evaluated and compared to various inhibitors (**Figure 1**). Because controlled banana slices and banana slices treated with distilled water, pH 4.0, browned to a similar extent, only the *L** values of banana slices treated with distilled water, pH 4.0, are shown. The results indicated that *L** values of banana slices for all treatments decreased with time. However, browning of banana slices treated with 100 mM 4-HR and 4 mM sodium metabisulfite were lower than other treatments. Banana slices treated with 8 mM ascorbic acid as well as those treated with pineapple juice showed similar decreases in *L** values. This indicated that pineapple juice was as effective as 8 mM ascorbic acid in inhibiting browning of banana slices stored at 15 °C for 3 days. With respect to banana slices treated with 100 mM 4-HR, they exhibited a turbid white appearance and had an unusual odor. Monsalve et al. (*10*) also found that treatment of apple slices with 4-HR at concentrations beyond 0.03% (w/v) influenced apple flavor due to the residual content in the apple tissue.

Table 1. Effect of Pineapple Juice and Ultrafiltration-Treated Juice on Banana PPO Inhibition[a]

	pH	°Brix	Bromelain activity (CDU/g PJ powder)	% inhibition
pineapple juice	3.9	12.0	1.04	52.3 a
permeate	3.9	11.6	0.15	49.1 a
retentate	4.0	12.4	11.78	27.7 b

[a] Means within the same column with different letters are significantly different ($p \leq 0.05$).

Table 2. Effect of Fractions Separated by Solid-Phase C_{18} Cartridge on Banana PPO Inhibition[a]

	pH	°Brix	total phenols (ppm)	SH content (μM)	% inhibition
AP	2.4	3.5	122	7.5	20.7 a
DE	2.2	40.2	349	22.0	99.9 b
NP	7.1	5.4	923	3.6	65.4 c

[a] Means within the same column with different letters are significantly different ($p \leq 0.05$). AP, acidic phenolic fraction; and NP, neutral phenolic fraction.

Effectiveness of Fractions from Pineapple Juice. Ultrafiltration was used to remove compounds greater than 10000 Da. The ultrafiltration was performed for only a short time (3−4 h); therefore, total soluble solids of the retentate was a little higher than that for the pineapple juice and permeate. As the molecular mass of bromelain is more than 30000 Da (*25*), the permeate of pineapple juice, not surprisingly, had the lowest level of bromelain activity as shown in **Table 1**. Nevertheless, it still inhibited PPO (c.a. 50%) to the same extent as pineapple juice. Moreover, the retentate that contained the highest bromelain activity (11.78 CDU/g of pineapple juice powder) showed the lowest value in %PPO inhibition (27.7%). Therefore, it can be concluded that the protease activity or bromelain in pineapple juice was not involved in PPO inhibition. Labuza et al. (*7*) also reported that bromelain did not inhibit mushroom PPO activity in an aqueous model system; however, it was effective in the browning inhibition of refrigerated apple. McEvily (*8*) confirmed that ficin from fig latex did not affect the activity of mushroom PPO but the protein-free composition inhibited the enzymatic and nonenzymatic browning of foods and beverages susceptible to browning.

The juice permeate was further fractionated into three fractions by solid-phase extraction using C_{18} cartridges. Each fraction was concentrated five times as compared to the juice permeate. The DE fraction had significantly more inhibition than other fractions and resulted in 100% PPO inhibition as compared to the control (**Table 2**). The DE fraction contained higher amounts of total soluble solids and sulfhydryl content than the NP and AP fractions. The NP fraction, which contained low amounts of total soluble solids (5.4 °Brix), had the highest concentration of total phenols and gave about 65% PPO inhibition. This indicated that the major polyphenolics in pineapple juice were neutral phenolic compounds that could inhibit enzymatic browning to some extent. The AP fraction had a higher sulfhydryl content than the NP fraction but seemed to have no inhibition effect on PPO. The DE fraction was further fractionated by ion exchange chromatography, and the level of

Table 3. Effect of Fractions Separated by Ion Exchange Column on Banana PPO Inhibition[a]

	pH	total[b] acid (g/100 mL)	total amino acid (g/ mL)	Cys (g/mL)	Tyr (g/mL)	ascorbic acid (mg/100 mL)	total[c] sugars (g/100 mL)	total phenols (ppm)	SH content (μM)	% inhibition
F1	8.3	0	87.1	0	0.4	0	0	1	0	3.7 a
F2	5.2	0.5	1550.6	41	51.1	0.4	27.2	104	11.2	21.0 b
F3	2.2	2.8	193.1	0	3.1	0	0	12	3.7	36.0 c

[a] Means within the same column with different letters are significantly different ($p \leq 0.05$). F1, F2, and F3, fractions obtained from the pineapple juice DE fraction. [b] Total acid is the sum of organic acids (malic acid and citric acid) determined by HPLC. [c] Total sugar is the sum of sugars (glucose, sucrose, and fructose) determined by HPLC.

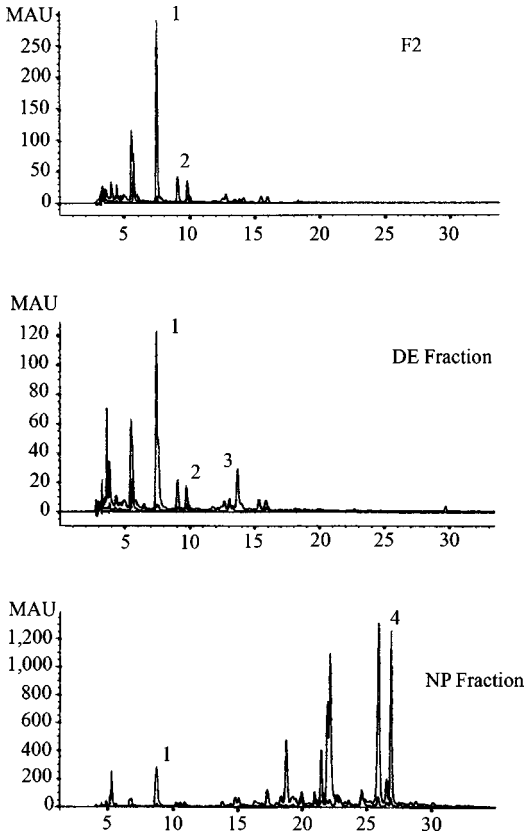

Figure 2. Chromatograms of phenolic compounds from pineapple juice fractionation tentatively identified by HPLC: 1, tyrosine; 2, serotonin; 3, DMHF (D-methylhydroxylfuranone); 4, *p*-coumaric acid; F2, fraction from pineapple juice; and NP, neutral phenolic fraction.

PPO inhibition achieved by the fractions and their compositions are shown in **Table 3**. The fraction F1 consisted largely of positively charged compounds. The lowest level of PPO inhibition of F1 indicated that positively charged compounds did not effectively contribute to pineapple juice enzymatic browning inhibition.

The F2 fraction, which was directly eluted from anion exchange resin, consisted of negatively charged compounds. Organic acids were present in low amounts in this fraction. The phenolic compounds in F2 were composed of tyrosine and serotonin as shown in **Figure 2**. The F2 fraction contained more sugars, total phenolics, sulfhydryl compounds, ascorbic acid, and total amino acids than the other fractions. However, the level of PPO inhibition of F2 was lower than F3, which consisted mainly of organic acids (malic and citric acids) as shown in **Table 4**. Ascorbic acid is known to inhibit PPO, but no ascorbic acid and only a trace amount of sulfhydryl compounds were found in F3. These results indicate that the major components of pineapple juice that are responsible for enzymatic browning inhibition are organic acids.

Table 4. Organic Acid Contents of Fractions Obtained from Ion Exchange Column

	concentration (g/100 mL)		
organic acids	F1	F2	F3
malic acid	0	0.13	1.80
citric acid	0	0.41	1.03
total	0	0.54	2.83

Table 5. Effect of Various Compounds Found in the Separated Fractions by Ion Exchange Chromatography on Banana PPO Inhibition[a]

solution	% inhibition
27.2 g % Su (F2)	1.5 a
2.8 g % Org acid	54.1 d
27.2 g % Su + 0.5 g % Org acid (F2)	14.6 c
27.2 g % Su + 0.5 g % Org acid + 51.1 (g/mL) Tyr (F2)	12.4 c
27.2 g % Su + 0.5 g % Org acid + 41.0 (g/mL) Cys (F2)	16.0 c
27.2 g % Su+ 0.5 g % Org acid + 51.1 (g/mL) Tyr + 41.0 (g/mL) Cys (F2)	14.0 c
51.1 (g/mL) Tyr (F2)	0.6 a
41.0 (g/mL) Cys (F2)	6.8 b

[a] Means within the same column with different letters are significantly different ($p \leq 0.05$). Su, sugar (6.7% glucose, 20% sucrose, and 0.6% fructose); Org acid, organic acid (0.1% malic acid and 0.4% citric acid); Tyr, tryrosine; Cys, cysteine; and F2, fraction from pineapple juice.

Amino acids are known to affect PPO activity in at least two ways: by reacting with the *o*-quinones and by chelating the essential copper at the active site of PPO. Of the amino acids, L-cysteine was shown to be the most effective inhibitor of browning in avocado and banana tissue as well as of mushroom tyrosinase (*26*). It is generally accepted that thiol compounds block the enzymatic browning reaction by forming colorless addition compounds with *o*-quinones (*27*). Citric acid is also known as a phenolase Cu-chelating agent, and the inhibition of PPO was attributed to the chelating action (*28*). Therefore, the inhibitory effect of F2 may be due to the combined effect of organic acids, cysteine, and other sulfhydryl compounds.

To further test the effect of the possible inhibitors on PPO activity, model system experiments were conducted as shown in **Table 5**. The compounds, which were detected in F2 and F3 and expected to be inhibitors of PPO, were prepared at the same concentration of F2 except that the organic acid solution (malic acid and citric acid) was prepared at the same concentration of F3. The results show that sugars and tyrosine (major peak in phenolic profile of F2) were not effective inhibitors of PPO. Cysteine alone resulted in 6.8% inhibition. This suggests a minor role for cysteine in PPO inhibition of F2. Combinations of sugars and organic acids, a mixture of sugars, organic acids, and

Table 6. *L** Values and Color Acceptable Score of Banana Puree Treated with DE Fraction and Various Antibrowning Solutions after Storage at 5 °C for 3 h[a]

	H_2O pH 2.0	8 mM ascorbic acid	4 mM sulfite	DE fraction
*L** values	62.9 b	63.4 b	70.1 a	64.9 c
color acceptable score	3.3 a	4.3 b	6.2 c	7.7 d

[a] Means within the same row with different letters are significantly different ($p \leq 0.05$). Color acceptable scores: 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; and 9, like extremely.

cysteine, and a mixture of sugars, organic acids, cysteine, and tyrosine inhibited PPO to the same extent, about 14% inhibition, which is less than the 21.0% inhibition of F2. Therefore, compounds in addition to organic acids and cysteine may be present in F2 that could inhibit PPO activity. The highest level of inhibition (54.1%) was shown by the organic acid fraction having the same concentration as F3 (**Table 5**). This confirms the important role of organic acids in enzymatic browning inhibition of pineapple juice. Wen and Wrolsad (*15*) reported similar results in that most of the browning inhibition activity in pineapple juice was caused by a nonvolatile organic acid whose structure was not completely characterized.

Effectiveness of DE Fraction on Enzymatic Browning Inhibition of Banana Puree. The preliminary experiment showed that the water at pH 2.0 did not exert a browning inhibitory effect; therefore, the low pH of the DE fraction is not believed to be responsible for browning inhibition. As shown in Table 6, *L** values of banana puree treated with the DE fraction were higher than for those treated with 8 mM ascorbic acid but lower than those treated with 4 mM sodium metabisulfite. While banana puree treated with sulfite had a higher *L** value (lighter color) after 3 h at 5 °C than puree treated with the DE fraction, the color acceptability score of the samples treated with sulfite was lower than for those treated with the DE fraction (**Table 6**).

In conclusion, our results have shown that pineapple juice was as effective as 8 mM ascorbic acid in enzymatic browning inhibition of banana slices stored at 15 °C for 3 days. The inhibitory effect was not caused by the bromelain activity. The DE fraction, which contained polar compounds, gave the highest percent PPO inhibition and was more effective than ascorbic acid in enzymatic browning inhibition of banana puree. Further fractionation of the DE fraction by ion exchange chromatography along with model system experiments indicated that the main organic acids (malic and citric acids) in pineapple juice played an important role in PPO inhibition. The fraction that contained cysteine and other sulfhydryl compounds also contributed to the effectiveness of pineapple juice in enzymatic browning inhibition.

ACKNOWLEDGMENT

We thank Bob Durst and the staff at the Department of Food Science and Technology, Oregon State University, for their help, guidance, and friendship. Special thanks go to Dole Inc., Thailand, for providing pineapples for the research.

LITERATURE CITED

(1) McEvily, A. J.; Iyengar, R.; Steven, W. S. Inhibition of enzymatic browning in foods and beverages. *Crit. Rev. Food Sci. Nutr*. **1992**, *32* (3), 253−273.

(2) Rigal, D.; Cerny, M.; Richard-Forget, F.; Varoquaux, P. Polyphenoloxidase by a Carica papaya latex preparation. *Int. J. Food Sci. Technol*. **2001**, *36*, 677−684.

(3) Sapers, G. M. Browning of food: Control by sulfites, antioxidants and other means. *Food Technol.* **1993**, *47*, 75−84.

(4) Janovitz-Klapp, A.; Richard, F.; Nicolas, J. Polyphenoloxidase from apple, partial purification and some properties. *Phytochemistry* **1989**, *28*, 2903−2907.

(5) Oszmianski, J.; Lee, C. Y. Inhibition of polyphenoloxidase activity and browning by honey. *J. Agric. Food Chem.* **1990**, *38*, 1892−1895.

(6) Martyniuk, S. Some inhibitors of polyphenoloxidase in honey. *Pszczelinicze Zesz. Nauk.* **1994**, *38*, 67−73.

(7) Labuza, T. P.; Lillemo, J. H.; Taoukis, P. S. Inhibition of polyphenol oxidase by proteolytic enzymes "Killer Enzymes". Symposium on The 20th International Symposium International Federation of Fruit Juice Producers, Paris, 1990; p 8.

(8) McEvily, A. J. U.S. Patent 4,981,708, 1991.

(9) McEvily, A. J.; Iyengar, R.; Gross, A. T. Inhibition of polyphenol oxidase by phenolic compounds. In *Phenolic Compounds in Food and Their Effect on Health*; Ho, C. T., Lee, C. Y., Huang, M. T., Eds.; John Wiley and Sons Inc.: New York, 1992; pp 235−340, 435.

(10) Monsalve-Gonzalez, A.; Barbosa-Canovas, G. V.; Canalieri, R. P.; McEvily, A. J.; lyengar, R. Control of browning during storage of apple slices preserved by combined method, 4-hexylresorcinol as an antibrowning agent. *J. Food Sci.* **1993**, *58*, 797−800.

(11) Richard-Forget, F. M.; Cerny, M.; Fayad, N.; Saunier, T.; Varoquaux, P. Isolation and characterization of a 'quinone-trapping' substance from a crude Carica papaya protein preparation. *Int. J. Food Sci. Technol*. **1998**, *33*, 285−296.

(12) Avallone, S.; Guiraud, J. P.; Brillouet, J. M.; Teisson, C. Enzymatic browning and biochemical alterations in black spots of pineapple [*Ananas comosus* (L.) Merr.]. *Curr. Microbiol.* **2003**, *47*, 113−118.

(13) Rattanathanalerk, M.; Chiewchan, N.; Srichumpoung, W. Effect of thermal processing on the quality loss of pineapple juice. *J. Food Eng.* **2005**, *66*, 259−265.

(14) Lozano-de-Gonzalez, P. G.; Barrett, D. M.; Wrolstad, R. E.; Durst, R. W. Enzymatic browning inhibited in fresh and dried apple rings by pineapples juice. *J. Food Sci.* **1993**, *58*, 399−404.

(15) Wen, L.; Wrolstad, R. E. *Abstr. Pap. 1999 IFT Annu. Meeting* **1999b**, Abstract No. 11A−15A.

(16) Galeazzi, M. A. M.; Sgarbieri, V. C.; Constantinides, S. M. Isolation, purification and physicochemical characterization of polyphenoloxidase (PPO) from a Dwarf variety of banana (*Musa Cavendishii*, L). *J. Food Sci.* **1981**, *46*, 50−155.

(17) Ngalani, J. A.; Signoret, A.; Gouzet, J. Partial purification and properties of plantain polyphenol oxidase. *Food Chem.* **1993**, *48*, 341−347.

(18) Jayaraman, K. S.; Ramanuja, M. N.; Dhakne, Y. S.; Vijavaraghavan, P. K. Enzymatic browning in some banana varieties as related to polyphenol oxidase activity and other endogenous factors. *J. Food Sci. Technol*. **1982**, *19*, 181−186.

(19) Jaworski, A.; Lee, C. Y. Fractionation and HPLC determination of grape phenolics. *J. Agric. Food Chem*. **1987**, *35*, 257−259.

(20) Ota, S.; Moore, S.; Stein, W. H. Preparation and chemical properties of purified stem and fruit brimelains. *Biochemistry* **1961**, *3*, 182−185.

(21) Slinkard, K.; Singleton, V. L. Total phenol analysis. Automation and comparison with manual methods. *Am. J. Enol. Vitic.* **1977**, *28*, 49−55.

(22) Ewart, J. A. D. Studies on disulfide bonds in glutenin. *Cereal Chem.* **1988**, *65*, 95−100.

(23) Wen, L.; Wrolstad, R. E. Phenolic composition of pineapple juice concentrate. Research Report, Oregon State University, Corvallis, 1999a.

(24) Cohen, S. A.; Michau, D. P. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of the hydrolysate amino acid via high-performance liquid chromatography. *Anal. Biochem.* **1993**, *211*, 279−287.

(25) Murachi, T.; Yasui, M.; Yasuda, Y. Purification and physical characterization of stem bromelain. *Biochemistry* **1994**, *3*, 48−52.

(26) Kahn, V. Effect of protein, protein hydrolysates and amino acids on o-dihydroxyphenolase activity of mushroom, avocado and banana. *J. Food Sci.* **1985**, *50*, 111−115.

(27) Richard-Forget, F. M.; Goupy, P. M.; Nicolas, J. J. Cysteine as an inhibitor of enzymatic browning II. Kinetic studies. *J. Agric. Food Chem.* **1992**, *40*, 2108−2112.

(28) Jiang, Y. M.; Fu, J. R.; Zauberman, G.; Fuchs, Y. Purification of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. *J. Sci. Food Agric.* **1999**, *79*, 950−954.

Received for review February 27, 2007. Revised manuscript received March 14, 2007. Accepted March 16, 2007. We express sincere gratitude to the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Thailand, and Southeast Asian Ministers of Education Organization Regional Center for Graduate Study and Research in Agriculture (SEARCA) for the financial support.

JF0705724