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Purification and Characterization of Polyphenol Oxidase from Jackfruit (*Artocarpus heterophyllus*) Bulbs

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Supporting Information

ABSTRACT: Polyphenol oxidase (PPO) from jackfruit bulb was purified through acetone precipitation, ion-exchange column, and gel filtration column. PPO was a dimer with the molecular weight of 130 kDa determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and gel filtration. The K_m was 8.3 and 18.2 mM using catechol and 4-methylcatechol as substrates, respectively. The optimum pH was 7.0 (catechol as the substrate) or 6.5 (4-methylcatechol as the substrate). The optimum temperature was 8 °C. The enzyme was stable below 40 °C. The activation energy (E_a) of heat inactivation was estimated to be 103.30 kJ/mol. The PPO activity was activated by Mn^{2+}, SDS, Tween-20, Triton X-100, citric acid, and malic acid but inhibited by K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, cetyl trimethyl ammonium bromide (CTAB), kojic acid, tropolone, glutathione (GSH), cysteine (Cys), and ascorbic acid (AA). Cys and AA were effective to reduce browning of jackfruit bulbs during the storage at 8 °C for 15 days.

KEYWORDS: *polyphenol oxidase, jackfruit, purification, inhibition, antibrowning*

INTRODUCTION

Polyphenol oxidase (PPO) is a widely distributed copper-containing enzyme, which is responsible for enzymatic browning in fruits. PPO catalyzes the hydroxylation of monophenols (such as tyrosine) to *o*-diphenols (cresolase or monophenolase activity, EC 1.14.18.1) and the oxidation of *o*-diphenols to *o*-quinones (catecholase or diphenolase activity, EC 1.10.3.2). Quinones are highly reactive electrophilic molecules that can react further with other quinones, amino acids, or proteins, forming red, brown, or black pigments that cause food deterioration and food value loss.[1]

PPO has received much attention from researchers in the fields of food science because of its involvement in the enzymatic browning. PPO has been isolated from various fruits and vegetables, such as pineapple,[2] snake fruit,[3] iceberg lettuce,[4] and broccoli.[5]

Jackfruit (*Artocarpus heterophyllus*) is the largest fruit in the world, mainly cultivated in tropical or close to tropical areas. Jackfruit contains many vitamins and minerals and offers numerous health benefits.[6,7] Because of its large size, jackfruit is usually sold in minimal processing forms, for example, fresh cut. Enzymatic browning of fruits after minimal processing is one major cause of quality loss and is also the major practical limitation of jackfruit storage. However, no work has been carried out on PPO from jackfruit. Therefore, the objective of this study was to isolate, purify, and characterize PPO. The results presented here could help us to develop effective methods to control the enzymatic browning of jackfruit.

MATERIALS AND METHODS

Materials and Reagents. Jackfruits were purchased from a local market on July and August. DEAE Sepharose Fast Flow and Sephacryl S-200 HR were GE products. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) protein ladder was from Thermo. All other reagents were local products of analytical grade.

Purification of PPO. The following process was repeated in its entirety several times to generate enough material for all experiments and studies described here. Jackfruit bulbs (20 g) were washed and homogenized in 100 mL of 100 mM sodium phosphate buffer (PBS, pH 6.0) containing 1% (w/v) soluble polyvinyl pyrrolidone (PVP). The homogenate was laid overnight at 4 °C and then centrifuged at 10000*g* for 20 min at 4 °C. The precipitate (fruit residues) was discarded, and the supernatant (crude enzyme solution) was collected for acetone precipitation.

A total of 4 times the sample volume of cold acetone (−20 °C) was added to centrifuge tubes for precipitation of the enzyme. This process was conducted in an ice−salt bath, and the temperature was −15 °C. After incubation for 2 h at −20 °C, the samples were centrifuged at 10000*g* for 20 min at 4 °C. Finally, the supernatant was discarded, and the protein pellets were redissolved in a small amount of 50 mM Tris-HCl buffer (pH 7.8).

The above enzyme solution was placed on a DEAE Sepharose Fast Flow column (2 × 32 cm, $\Phi \times h$). Equilibration and elution were performed first with 50 mM Tris-HCl (pH 7.8) buffer to remove unbound proteins and then with a linear salt gradient from 0.1 to 0.4 M NaCl. The active fractions were combined and applied onto a Sephacryl S-200 HR column (2 × 32 cm, $\Phi \times h$). Elution of the enzyme was carried out with 50 mM NaAc buffer (pH 5.8, containing 0.1 M NaCl). The enzyme fractions were pooled and used in the study.

Enzyme Activity Assay. PPO activity was determined by measuring the initial rate of quinone formation, as indicated by an increment in absorbance at 420 nm, using catechol as the substrate. The enzyme reaction solution contained 50 μL of the sample and 200 μL of 50 mM catechol (dissolved in 50 mM PBS buffer at pH 7.0). The absorbance was read from a microplate reader (Thermo Fisher, model 1510). A total of 1 enzyme unit (U) was defined as a change in absorbance of 0.001 in 1 min.

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Electrophoresis. SDS−PAGE was performed using a discontinuous buffer system with a stacking gel of 6% acrylamide and a separating gel of 10% acrylamide. The samples were run at a constant voltage of 200 V in a Bio-Rad Mini-Protein Tetra electrophoresis system. Gels were stained for protein using a silver nitrate staining method.[8]

Molecular Weight. For molecular weight determination, the enzyme was rechromatographed on the Sephacryl S-200 column calibrated with blue dextran 2000 and protein markers: lysozyme (14.3 kDa), papain (21 kDa), ovalbumin (OB, 43 kDa), bovine serum albumin (BSA, 67 kDa), and calf intestine alkaline phosphatase (CIP, 140 kDa). The logarithm of molecular weight was plotted against the partition coefficient K_{av} that was calculated as follows: $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of each protein, V_0 is the elution volume of blue dextran, and V_t is the total column volume.[9] Molecular weights of subunits were estimated on a SDS−PAGE gel.

Enzyme Kinetics and Substrate Specificity. For substrate specificity, PPO activity was determined using 2 mM L-tyrosine, 20 mM catechol, 20 mM 4-methylcatechol, 20 mM pyrogallol, and 20 mM gallic acid as substrates. To test the potential monophenolase, 0.01, 0.05, 0.1, and 0.2% SDS, cetyl trimethyl ammonium bromide (CTAB), Tween-20, and Triton X-100 (w/v) were added in the reaction mixture as activators.[10] For determination of the Michaelis constant (K_m) and maximum velocity (V_{max}), PPO activities were measured with catechol and 4-methylcatechol at various concentrations. K_m and V_{max} values of PPO were calculated from a plot of $1/v_0$ versus $1/[S]$ by the method of Lineweaver−Burk.[11]

Optimum pH and pH Stability. The optimum pH was determined by measuring the enzyme activities as described above, using catechol and 4-methylcatechol as substrates, at the different pH values (pH 3.5−9.0) at 8 °C. The maximum activity detected at optimum pH was defined as 100%. The pH stability of the enzyme was monitored by incubating the enzyme with different pH buffers (pH 3.5−9.0) for 12 h at 4 °C. Then, 50 μL of treated enzyme was assayed at pH 7.0 and 8 °C. The highest residual activity was defined as 100%. The following buffers were used: citric acid−sodium citrate buffer (pH 3.5−4.0), HAc−NaAc buffer (pH 4.5−5.5), PBS buffer (pH 6.0−7.5), and Tris−HCl buffer (pH 8.0−9.0).

Optimum Temperature, Thermal Stability, and Heat Inactivation. The optimum temperature of the enzyme was determined by measuring the activity as described above, using catechol or 4-methylcatechol as substrates, at various temperatures (4−40 °C) at pH 7.0. The maximum activity detected at optimum temperature was defined as 100%. The thermal stability of the enzyme was monitored by incubating the enzyme at various temperatures (10−70 °C) for 30 min. After preincubation, 50 μL of treated enzyme was immediately cooled on ice for 5 min and then assayed at pH 7.0 and 8 °C. The highest residual activity was defined as 100%. For heat inactivation, the enzyme was incubated at 40−90 °C up to 25 min. The residual activity was measured as described above. The activity of the enzyme without preincubation was defined as 100%.

Effects of Metal Ions, Food Additives, Surfactants, and Inhibitors on Enzyme Activity. Experiments were conducted in the standard assay system described above with different concentrations of various reagents. Enzyme activity determined in the absence of these reagents was defined as 100%. The inhibition type was assayed by the Lineweaver−Burk plot. The dissociation constants of the inhibitor and enzyme (K_i) and the inhibitor and enzyme−substrate complex (K_i') were determined by the apparent $K_{m,app}$ and $V_{m,app}$.[12]

Protein Estimation. The protein content was determined according to the dye-binding method of Bradford[13] using BSA as the standard.

Dip Treatment with Antibrowning Solutions. Jackfruit edible bulbs were cut manually into slices (approximately 2 × 2 × 0.5 cm) and then randomly selected for the treatments. The sliced bulbs were dipped into antibrowning solution for 20 min. The antibrowning solution was prepared by dissolving ascorbic acid (AA), cysteine, or reduced glutathione (GSH) in deionized water. Each solution contained 0.1% (w/v) sodium dehydroacetate to prohibit the growth of bacterial and fungal microbes. Finally, the slices were drained and distributed in nonvented plastic clamshell containers of 50 g each and stored at 8 °C in an incubator.

Browning Index (BI). BI was estimated, by the method of Saxena et al.[14] with some modifications, by extracting 0.5 g of sample in 10 mL of ethanol−formic acid solution (containing 5 mL of absolute alcohol and 5 mL of 85% formic acid) for 30 min. The homogenates were centrifuged at 10000*g* for 30 min. The absorbance of the resulting supernatant was measured immediately at 420 nm. Higher values of absorbance correspond to higher browning.

Determination of Total Phenols. Total phenolic content was measured using the Folin−Ciocalteau reagent. The supernatant for BI determination was used to measure the phenol content. Aliquots (20 μL) of the supernatant were diluted in 80 μL of deionized water, and 100 μL of Folin−Ciocalteau reagent was added to 100 μL of the resulting solution. After 10 min, 1 mL of 10% sodium carbonate solution was added. After 40 min at room temperature, the absorbance of the solution was measured at 760 nm.[15]

Assay for PPO Activity. A total of 0.5 g of sample was homogenized in 3 mL of 100 mM sodium phosphate buffer (pH 6.0) containing 1% PVP. The homogenate was laid overnight at 4 °C and then centrifuged at 10000*g* for 20 min at 4 °C. The supernatant was used to measure PPO activity. The enzyme unit (U) was defined as mentioned earlier. The enzyme activity was expressed per gram of fresh weight (U/g of FW).

RESULTS AND DISCUSSION

Purification of PPO. PPO from the jackfruit bulb was purified by cold acetone precipitation, an anion-exchange DEAE Sepharose Fast Flow column, and a Sephacryl S-200 HR column. The elution profile of PPO on the DEAE Sepharose Fast Flow column was shown in Figure 1A. The proteins with PPO activity appeared in the form of two peaks: one major peak (I) and one minor peak (II). The major peak was observed in fractions 37−39, while the minor peak was observed in fractions 45−47. The fractions of the minor peak showed low enzyme activity and was therefore discarded, and those of the major peak were pooled, concentrated, and further purified on the Sephacryl S-200 HR column. For the gel filtration column, the proteins with PPO activity were found in peak III (Figure 1B). The active fractions 8−10 were pooled for characterization. The specific activity of the purified PPO was determined to be 18 731 U/mg, with a purification fold of 33.5 and a recovery of 18.8%. The purification steps were summarized in Table 1. The SDS−PAGE of the purified sample was shown in Figure 2.

Molecular Weight Determination. The molecular weight of the whole enzyme was 132 kDa according to the result from gel filtration on the Sephacryl S-200 HR column (Figure 3). SDS−PAGE (Figure 2) indicated one dominant band with a molecular weight of 65 kDa. It can be concluded the PPO isolated from jackfruit was a dimer, composed of two subunits. PPOs isolated from Barbados cherry[16] and rape flower[17] were also dimers, while PPO isolated from snake fruit[3] was reported to be a monomer. In a few cases, PPOs were tetramers (from pineapple[2] or iceberg lettuce[4]) or a multimer (from potato tubers).[18]

Kinetic Parameters and Substrate Specificity. To study the substrate specificity, the PPO activities toward L-tyrosine, catechol, 4-methylcatechol, pyrogallol, and gallic acid were determined with regard to its monophenolase (tyrosine), diphenol (catechol and 4-methylcatechol), and triphenol oxidase (pyrogallol and gallic acid) activities. PPO from jackfruit showed diphenolase activity toward catechol and 4-methylcatechol rather than monophenolase toward tyrosine and triphenolase activity toward pyrogallol and gallic acid

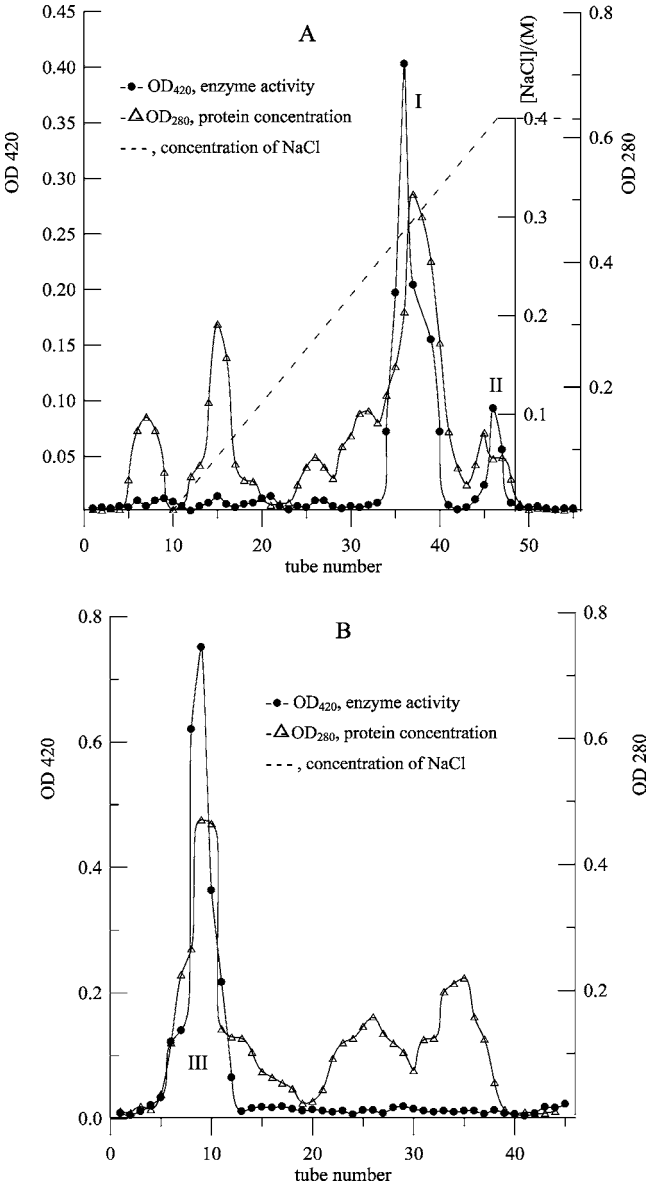

Figure 1. Chromatography of PPO from jackfruit bulb: (A) DEAE Sepharose Fast Flow and (B) Sephacryl S-200 HR.

(Table 2), corresponding to PPO from Henry chestnuts.[19] In contrast, PPOs from nettle leaf[20] and peppermint[21] showed monophenolase toward tyrosine. Potential tyrosinase of jackfruit PPO activated by surfactants was not detected.

The kinetics behavior of PPO during the oxidation of catechol and 4-methylcatechol was determined by varying the substrate concentration. The hydrolysis of both substrates by PPO followed Michaelis–Menten kinetics. The Lineweaver–Burk plot analysis of PPO showed that the K_m and V_{max} values were 8.2 mM and 109.9 U/min for catechol and 18.2 mM and 82.1 U/min for 4-methylcatechol, respectively (Table 2).

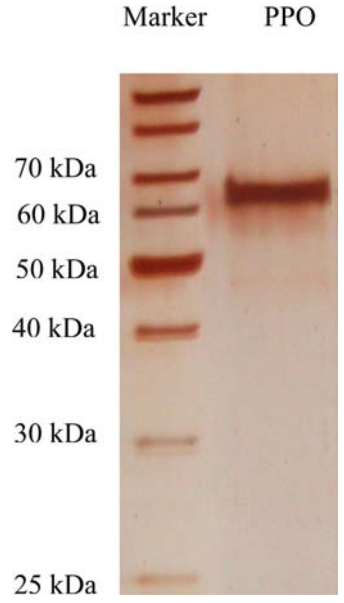

Figure 2. SDS–PAGE of jackfruit PPO. The polyacrylamide gel (10%) was stained with silver nitrate solution.

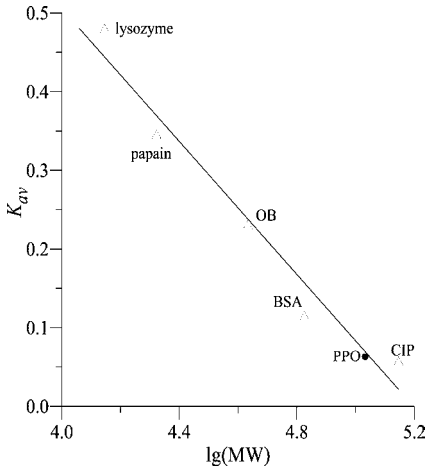

Figure 3. Calibration plot for the molecular weight determination of PPO from jackfruit by gel filtration. The standard proteins used were lysozyme (14.3 kDa), papain (21 kDa), OB (43 kDa), BSA (67 kDa), and CIP (140 kDa). PPO was estimated to be 132 kDa.

Table 2. Kinetic Parameters of Jackfruit PPO

substrate	K_m (mM)	V_{max} (U/min)	V_{max}/K_m (U min^{-1} mM^{-1})
catechol	8.2	109.9	13.4
4-methylcatechol	18.2	82.1	4.5
tyrosine/pyrogallol/gallic acid	nd[a]	nd	nd

[a]nd = not detected.

Table 1. Purification Steps and Folds of PPO from Jackfruit

steps	volume (mL)	total activity (U)	protein (mg)	specific activity (U/mg)	recovery (%)	fold
crude enzyme	106	196100	351	558.7	100	1
acetone precipitation	8	136000	99.4	1368.2	69.4	2.5
DEAE SFF	4.5	50400	7.79	6469.8	25.7	11.6
Sephacryl	4.5	36900	1.97	18731.0	18.8	33.5

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K_m obtained with catechol was similar to that of aubergine (eggplant) (8.7−9.3 mM)[22] and artichoke (10.7 mM)[16] but higher than that of tobacco (1.2 mM)[23] and lower than that of mamey (44 mM).[24] When using 4-methylcatechol as the substrate for PPO, the K_m value was similar to that of artichoke (11.6 mM)[12] but higher than that of Barbados cherry (6.9 mM).[16]

To evaluate the substrate specificity, the catalytic power (V_{max}/K_m ratio) was taken into account. The ratios were calculated to be 13.4 U min^{-1} mM^{-1} for catechol and 4.5 U min^{-1} mM^{-1} for 4-methylcatechol. It can be concluded that catechol was the most suitable substrate for jackfruit PPO.

pH and Temperature Dependency. The effect of pH on PPO activity for the oxidation of catechol and 4-methylcatechol was determined at 8 °C. The optimum pH of PPO was found to be pH 7.0 using catechol as the substrate and pH 6.5 using 4-methylcatechol as the substrate (Figure 4). At acidic pH <4.5 or alkaline pH >8.0, less than 50% activity was retained. PPO was stable at pH 6.0−7.0 (12 h at 4 °C) (Figure 4). Most plants show maximum PPO activity at pH 6−8. For example, the optimum pH values of PPO from tobacco,[23] artichoke,[12] and Barbados cherry[16] have been reported to be 6.0, 7.0, and 7.2, respectively, using catechol as the substrate.

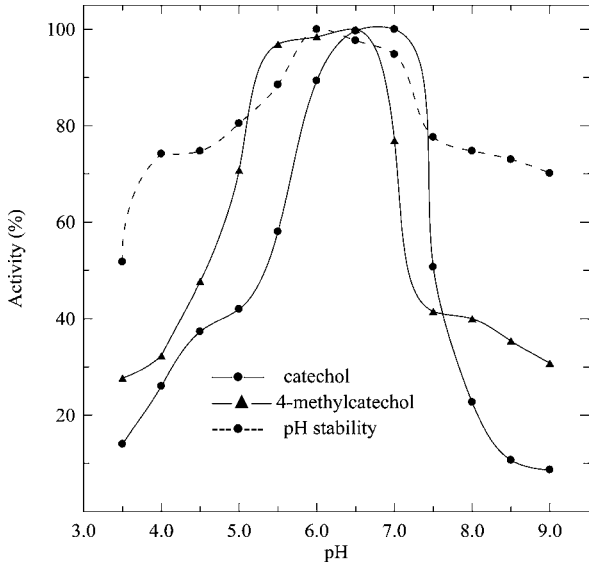

Figure 4. Effects of pH on PPO activity and pH stability. The enzyme activity was assayed at 8 °C, using catechol or 4-methylcatechol as substrates. The maximum activity detected at optimum pH was defined as 100%. For pH stability of PPO, the enzyme was incubated at different pH buffers for 12 h at 4 °C and then 50 μL of the mixture was taken for activity assay at pH 7.0 and 8 °C using catechol as the substrate. The highest residual activity was defined as 100%.

At pH 7.0, PPO activities were measured at various temperatures to determine the optimum temperature. The result showed that the optimum temperature for PPO was 8 °C (Figure 5) using catechol or 4-methylcatechol as substrates. The optimum temperature of PPO for most plants is at 30−40 °C, for example, aubergine (30 °C),[22] mamey (35 °C),[24] and artichoke (40 °C),[12] using catechol as the substrate. In contrast, PPO from jackfruit showed a low optimum temperature. The results were similar to that of dill (10 °C)[25] and ferula (12 °C).[26]

Thermal Stability and Heat Inactivation. The thermal stability profile of PPO from jackfruit, presented as the remaining activity after preincubation at various temperatures for 30 min, was shown in Figure 5. PPO was stable below 40 °C, while at temperatures of >50 °C, the PPO activity rapidly decreased. The process of heat inactivation was presented in Figure 6A. The semi-log plots of the remaining activity of PPO versus heating time were linear at 40−90 °C, indicating a simple first-order process. The inactivation rate constants k were calculated from the slopes of these lines, and the k values were plotted in an Arrhenius plot (Figure 6B). The activation energy (E_a) for heat inactivation of PPO was estimated to be 103.30 kJ/mol by the Arrhenius equation. The E_a value for jackfruit PPO was similar to that for Elsanta and Madame Moutot strawberry PPO[27] or Amarillo melon PPO[28] but half of that for Crimson seedless grape[29] and 5 times that for Charantais melon PPO.[28] These results indicated that jackfruit PPO was more thermostable than Charantai melon PPO but less thermostable than Crimson seedless grape PPO.

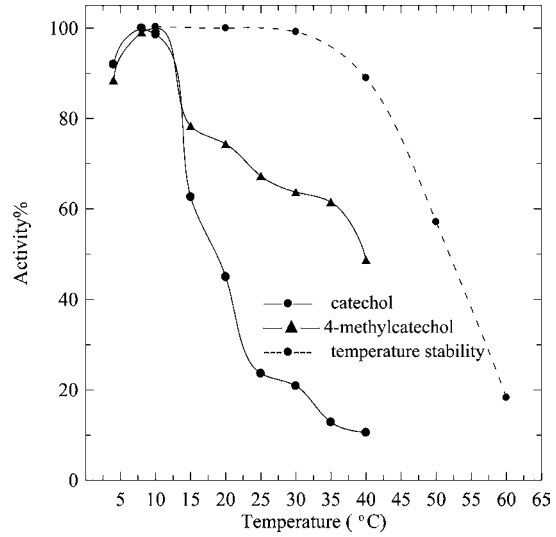

Figure 5. Effects of the temperature on PPO activity and thermal stability. The enzyme activity was measured at various temperatures using catechol or 4-methylcatechol as substrates. The maximum activity detected at optimum temperature was defined as 100%. For thermal stability of PPO, the enzyme was incubated at different temperatures for 30 min in 50 mM PBS buffer (pH 7.0) and then 50 μL of the mixture was immediately cooled on ice for 5 min and then assayed at pH 7.0 and 8 °C using catechol as the substrate. The highest residual activity was defined as 100%.

Effect of Metal Ions on PPO Activity. Some metal ions were assayed for their effects on PPO activity. The results are shown in Table 3. Mn^{2+} (4 mM) activated the enzyme activity, while K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, and Ba^{2+} (4 mM) showed various degrees of inhibitory effects.

Effect of Surfactants on PPO Activity. In some cases, PPOs in plants were in an inactive or latent form that could be activated by surfactants.[30−33] For this reason, we investigated the effects of four surfactants, SDS, CTAB, Tween 20, and Triton X-100, on PPO activity (Table 3). Cationic surfactant CTAB showed obvious inhibition, similar to the results from red Swiss chard.[30] Anionic surfactant SDS at 0.2% level slightly enhanced the enzymatic activity. The activation of PPO activity by SDS was reported in many fruits and vegetables, such as Persimmon fruit[31] and broad bean.[32] This might be due to the conformational change of PPO caused by the binding of SDS to the enzymatic active site.[31,32] Non-ionic surfactants, both Triton X-100 and Tween 20, enhanced the enzyme activity.

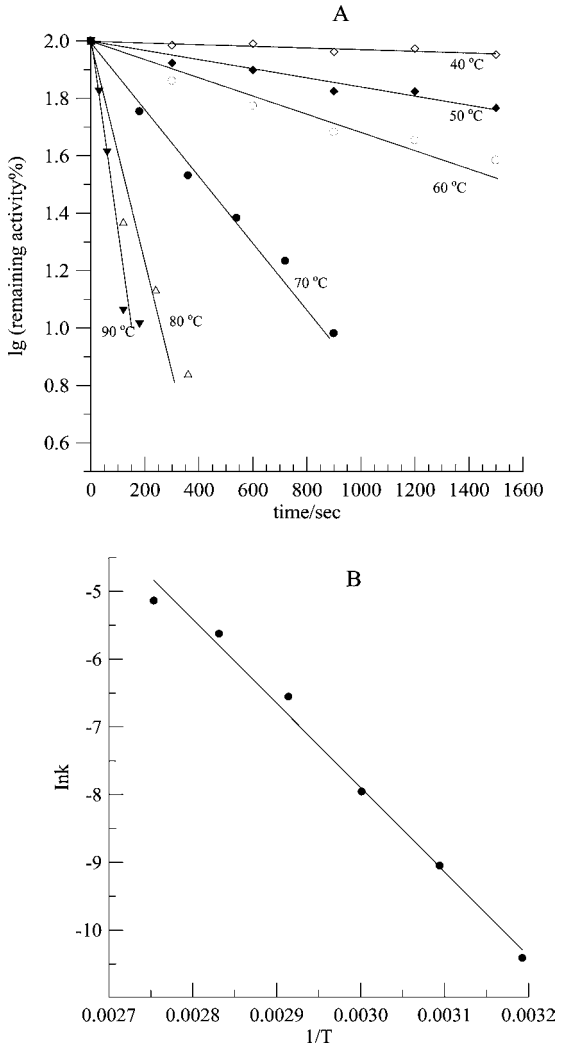

Figure 6. (A) Heat inactivation of jackfruit PPO. The enzyme was incubated at 40−90 °C up to 25 min. After preincubation, 50 μL of treated enzyme was immediately cooled on ice for 5 min and then assayed at pH 7.0 and 8 °C. The activity of the enzyme without preincubation was defined as 100%. (B) Arrhenius plot of heat inactivation rates of jackfruit PPO.

Similar activation of PPO activity by Triton X-100 and Tween 20 was found in PPO from spiderflower.[33]

Effect of Inhibitors and Food Additives on PPO Activity. Several inhibitors and food additives, tropolone, kojic acid, AA, malic acid, citric acid, sodium benzoate, L-cysteine (Cys), D-sorbitol, GSH (reduced form), EDTA·Na_2, and β-cyclodextrin, were chosen to test their inhibition effects on PPO activity from jackfruit. The results were presented in Table 3. D-Sorbitol and sodium benzoate at all tested concentrations showed no significant effects on PPO activity. Citric and malic acids at 4 and 10 mM showed activation. EDTA·Na_2 at 1 mM inhibited 14% of PPO activity, while EDTA·Na_2 at 10 mM activated the PPO activity. Similar activation of PPO by citric acid and EDTA was also observed in mamey fruit.[24] The reasons were not clear. β-Cyclodextrin (4 and 10 mM) showed an inhibition effect on PPO. β-Cyclodextrin may form a complex with the PPO substrates to prevent them from enzymatic oxidation.[34] Kojic acid, AA, Cys, and GSH at 1 mM caused a high degree of inhibition. Tropolone (1 mM) brought about total inhibition of PPO activity. The IC_{50} values of these four inhibitors were shown in Table 4.

Table 3. Effects of Metal Ions, Surfactants, Food Additives, and Inhibitors on PPO Activity[a]

chemicals	remaining activity (%)		
metal ions	0.1 mM	1 mM	4 mM
K^+	102.2	89.2[b]	83.3[b]
Zn^{2+}	102.0	86.3[b]	61.3[b]
Mg^{2+}	103.1	90.7[b]	92.8[b]
Ca^{2+}	101.2	98.0	94.7[b]
Ba^{2+}	98.8	101.3	93.7[b]
Mn^{2+}	99.1	106.2[b]	111.1[b]
surfactants	0.05% (w/v)	0.1% (w/v)	0.2% (w/v)
SDS	98.0	100.5	108.6[b]
CTAB	52.4[b]	47.6[b]	38.2[b]
Tween-20	104.7[b]	105.5[b]	114.4[b]
Triton X-100	121.7[b]	122.7[b]	123.7[b]
food additives and inhibitors	1 mM	4 mM	10 mM
D-sorbitol	99.2	102.2	101.8
citric acid	96.1	122.3[b]	127.5[b]
malic acid	100.1	123.9[b]	133.6[b]
β-cyclodextrin	97.3	83.5[b]	78.7[b]
sodium benzoate	102.1	99.5	95.8
EDTA·Na_2	85.9	97.9	122.8[b]
kojic acid	11.2[b]	nd[c]	nd
GSH	16.2[b]	nd	nd
ascorbic acid	10.9[b]	nd	nd
L-cysteine	17.0[b]	nd	nd
tropolone	nd	nd	nd

[a]Values represent means of three replicates. [b]A statistically significant difference between the treatment and the control means ($p < 0.05$). [c]nd = not detected.

Kojic acid,[35] AA,[36] and GSH[37] are capable of reducing *o*-quinones to diphenols to prevent the pigment formation. AA[38] may not only chelate the copper ions at the active center of PPO to reduce them from Cu^{2+} to Cu^+ but also react with the histidine residues at the active site to inhibit the enzyme activity.[39] Cys was proposed to form stable complexes with the copper ions at the active center of PPO and, thus, directly inhibit the enzyme activity.[20] Tropolone was a substrate structural analogue and copper chelator.[40]

The mechanisms of inhibition were further studied. Three types of inhibition were observed in our research (Table 4). In competitive inhibition, the inhibitor can only bind the enzyme if the substrate has not already been bound. In noncompetitive inhibition, the inhibitor may bind to the enzyme or the enzyme−substrate complex and the values of the dissociation constants of the inhibitor and enzyme (K_i) and the inhibitor and enzyme−substrate complex (K_i') are equal. When $K_i \neq K_i'$, it is mixed inhibition.[11]

The type of inhibition depends upon the structural and functional properties of the PPOs from different species. AA was a mixed type inhibitor to PPO from jackfruit. In contrast to our results, some authors reported that AA was a noncompetitive inhibitor for PPO from broccoli[5] or a competitive inhibitor for PPO from peppermint.[21]

GSH was a competitive inhibitor toward PPO from jackfruit, similar to the results from Thymus herb[41] and Dog-rose.[42] In contrast, Dogan et al.[12] found GSH to be a noncompetitive inhibitor toward PPO from artichoke.

Table 4. IC_{50}, K_i, and Inhibition Types of Inhibitors for Jackfruit PPO

inhibitors	inhibition type	concentration (mM)	K_i (mM)	K_i' (mM)	inhibition (%)	IC_{50} (mM)
GSH	competitive	0.20	0.309		14	0.45
		0.40	0.168		43	
		0.80	0.148		78	
AA	mixed	0.20	0.444	1.818	24	0.30
		0.40	0.204	0.299	64	
		0.80	0.133	0.513	80	
Cys	noncompetitive	0.20	0.364		50	0.19
		0.40	0.449		59	
		0.80	0.300		78	
kojic acid	noncompetitive	0.10	0.300		31	0.23
		0.20	0.299		48	
		0.50	0.225		74	
tropolone	noncompetitive	0.08	0.119		59	0.073
		0.10	0.094		68	
		0.16	0.090		79	

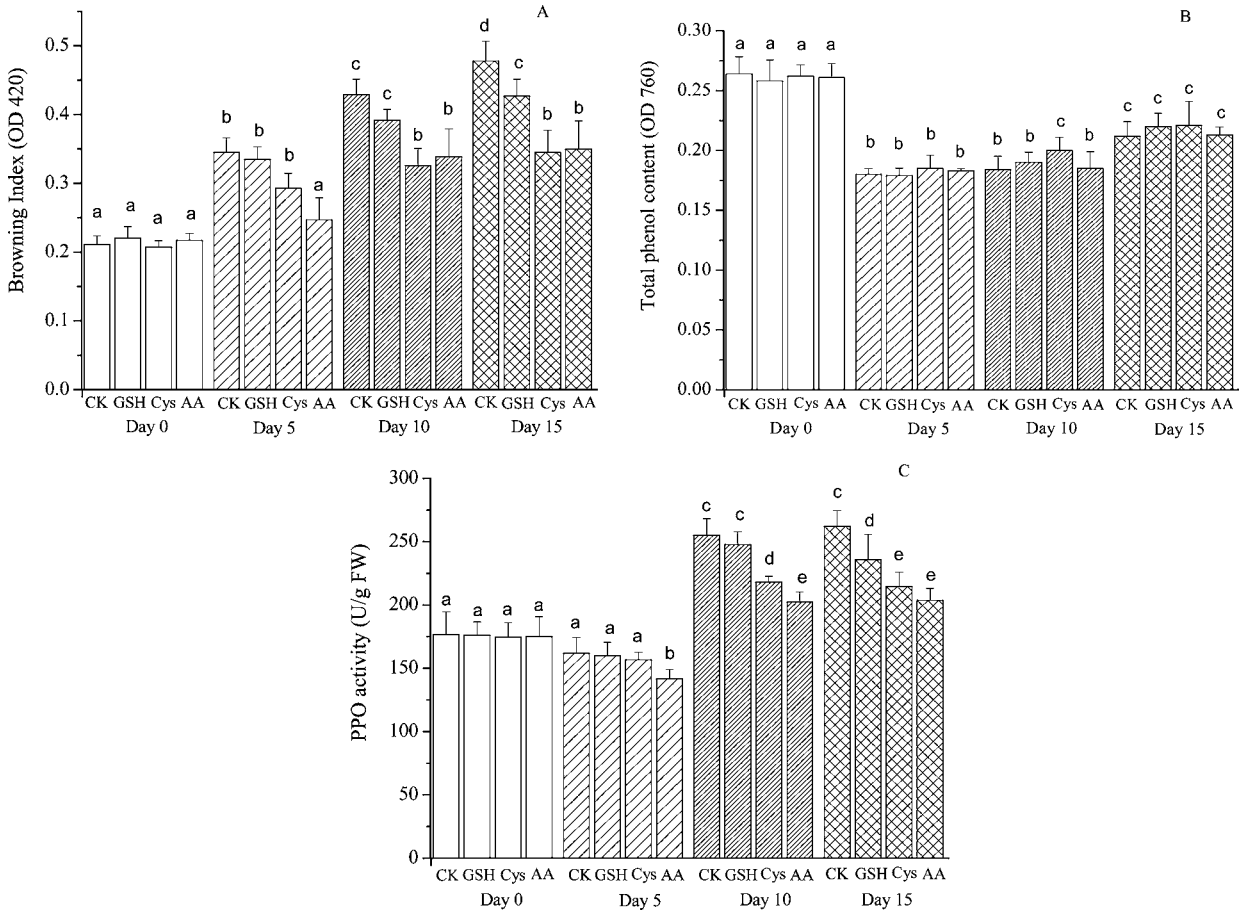

Figure 7. (A) BI, (B) total phenol content, and (C) PPO activity of jackfruit slices after treatments. The slices were treated with 0.02% GSH, 0.02% Cys, and 0.02% AA or without solutions (CK). Slices were stored at 8 °C. Values are means ± standard error (n = 5). Different letters indicate statistically significant differences ($p < 0.05$).

Cys was a noncompetitive inhibitor toward PPO from jackfruit, similar to the results obtained by Dogan et al.[12] Kojic acid and tropolone also showed noncompetitive inhibitions toward PPO from jackfruit. Tropolone, being a structural analogue, was a very effective inhibitor to PPO. Competitive inhibitions with tropolone were found in PPOs from field bean[43] and artichoke.[12] A mixed inhibition with tropolone was reported for potato PPO.[44]

BI, Total Phenol Content, and PPO Activity after Treatment with Antibrowning Solutions. AA, Cys, and GSH are nontoxic and nutritional and may be useful in practice to control enzymatic browning of jackfruit during processing. To test their practical antibrowning effects, 0.02% GSH, 0.02% Cys, and 0.02% AA solutions were prepared. After dip treatments, the jackfruit slices were kept at 8 °C for 15 days. The BI, total phenol content, and PPO activity were measured every 5 days.

The BI indicates the proportion of oxidized phenols during storage. On the 15th day, the BI of control (0.478) and GSH-treated samples (0.427) showed higher values, while the BI of Cys (0.345) and AA (0.350) treatments showed lower values, indicating that Cys and AA were effective to reduce overall browning (Figure 7A). The Cys- and AA-treated samples maintained surface brightness and good visual appearance. However, the addition of Cys should be a small amount in process of dip treatment because of its unpleasant odor.

Figure 7B showed the changes in total phenolics in the samples after treatments. The total phenol content of all samples decreased significantly on the 5th day and remained at low levels thereafter. During the storage, the changes between the control and treatments were not remarkable.

Figure 7C presented the changes in PPO activity in the samples after treatments. On the 5th day, only AA-treated samples showed a significant decrease ($p < 0.05$) in PPO activity compared to the control and other treatments. After 10 days of storage, the PPO activity in all samples significantly increased in comparison to days 0 and 5. This might be the results of postharvest physiological changes and responses to peeling and cutting.[45] In comparison to the control and other treatments, 0.02% AA showed the best inhibition toward PPO activity on the 10th and 15th days ($p < 0.05$). Moderate correlation was observed between BI and PPO activity (Pearson correlation; $r = 0.790$; $p < 0.01$).

■ ASSOCIATED CONTENT

Supporting Information

PPO activity assay, determination of IC_{50}, inhibition type, and K_i. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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