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Inhibitory Effects of Propyl Gallate on Tyrosinase and Its Application in Controlling Pericarp Browning of Harvested Longan Fruits

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ABSTRACT: Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a key enzyme in pigment biosynthesis of organisms. The inhibitory effects of propyl gallate on the activity of mushroom tyrosinase and effects of propyl gallate on pericarp browning of harvested longan fruits in relation to phenolic metabolism were investigated. The results showed that propyl gallate could potently inhibit diphenolase activity of tyrosinase. The inhibitor concentration leading to 50% activity lost (IC_{50}) was determined to be 0.685 mM. Kinetic analyses showed that propyl gallate was a reversible and mixed type inhibitor on this enzyme. The inhibition constants (K_{IS} and K_I) were determined to be 2.135 and 0.661 mM, respectively. Furthermore, the results also showed that propyl gallate treatment inhibited activities of PPO and POD in pericarp of harvested longan fruits, and maintained higher contents of total phenol and flavonoid of longan pericarp. Moreover, propyl gallate treatment also delayed the increases of browning index and browning degree in pericarp of harvested longan fruits. Therefore, application of propyl gallate may be a promising method for inhibiting tyrosinase activity, controlling pericarp browning, and extending shelf life of harvested longan fruits.

KEYWORDS: *tyrosinase, propyl gallate, diphenolase activity, inhibition kinetics, longan (Dimocarpus longan Lour.), pericarp browning, phenolic metabolism*

■ INTRODUCTION

Longan (*Dimocarpus longan* Lour.) is a famous subtropical fruit in the world. Longan is grown commercially in many countries, including China, Thailand, Vietnam, India, Australia, and some tropical and subtropical regions in the U.S., which account for most of the world production. Longan is one of the most economically important fruits in the south of China. The longan industry has undergone rapid expansion since 1990 in China, the largest cultivation and production nation in the world, with 73.6% cultivated area and 59.7% output. Because longan fruits mature under high temperature and humidity, the fruits deteriorate rapidly after harvest due to pericarp browning, aril breakdown, and fruit decay, among which pericarp browning is the most important factor affecting the shelf life of longan fruit.[1−5] Previous studies showed that low temperature,[1,6] controlled atmosphere,[7] nitric oxide,[3] chitosan coating,[8] hydrochloric acid,[9] and ozone combined with organic acid[10] could delay pericarp browning and fruit deterioration, maintain higher quality, and prolong the storage life of harvested longan fruits. The cause of pericarp browning of harvested longan fruits has been mainly attributed to the accumulation of free radical as well as the oxidation of phenolic by polyphenol oxidase (PPO) and peroxidase (POD).[3,4,7−10] Therefore, increasing free radical scavenging capacity or delaying enzymatic browning should be a vital way to maintain quality and extend storage life of harvested longan fruits.

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing multifunctional oxidase. The biosynthetic pathway of melanin includes two steps: the hydroxylation of monophenol to *o*-diphenol, and the oxidation of *o*-diphenol to *o*-quinones,[11] which is responsible for the formation of mammalian melanin pigments and enzymatic browning of fruits and vegetables.[12−16] Therefore, tyrosinase inhibitors have attracted great attention recently, the numbers of which have already been reported so far. Many tyrosinase inhibitors, such as cardol triene,[15] bibenzyl glycosides,[17] naphthols,[18] chlorobenzaldehyde thiosemicarbazones,[19] benzaldehyde thiosemicarbazones,[20] thymol,[21] mimosine tetrapeptides,[22] hydroxyphenolic acids, and its esters,[23] are composed of compounds that are structurally analogous to phenolic substrate.

Gallic acid is a phenolic compound and an important substrate in the synthesis of propyl gallate in the food industry.[24] Recently, there have been many reports concerning gallic acid derivatives, such as dodecyl gallate, epigallocatechin, epigallocatechin gallate, epicatechin, epicatechin gallate, and epigallocatechin gallate, and their antityrosinase-activity effects.[25−29] Also, many of them have been applied in medicine with various biological and pharmaceutical activities including free radical scavenging effect and antimicrobial activity.[27−29] Propyl gallate, with a molecular structure composed of a phenolic ring and a alkyl chain, is one of the most widely used phenolic antioxidants in food containing dehydrated foodstuffs,

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edible fats, and oils.[30,31] Also, propyl gallate has been reported to have free radical scavenging capacity and antibrowning property.[31−34] However, little information is available on the effects of propyl gallate on tyrosinase and pericarp browning of harvested longan fruits. In this Article, propyl gallate kinetic parameters and its inhibitory effects on mushroom tyrosinase and pericarp browning of harvested longan fruits were studied. The objective of this study was to further examine the potential role of browning inhibitors.

■ MATERIALS AND METHODS

Chemicals. Methyl gallate, ethyl gallate, and propyl gallate were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). L-3,4-Dihydroxyphenylalanine (L-DOPA) and dimethylsulfoxide (DMSO) were obtained from Aldrich (U.S.). Tyrosinase (EC 1.14.18.1) from mushroom was the product of the Sigma Chemical Co. Ltd. (U.S.). The specific activity of the enzyme was 6680 U/mg. All other reagents were homemade and analytical grade. The water used was redistilled and ion-free.

Assay of the Diphenolase Activity. The diphenolase activity assay was performed as previously reported.[18] The reaction media (3 mL) for activity assay contained 0.5 mM L-DOPA in 50 mM Na_2HPO_4−NaH_2PO_4 buffer (pH 6.8) and 0.1 mL of different concentrations of inhibitor (dissolved in DMSO previously), with L-DOPA used as the substrate. 0.1 mL of the aqueous solution of mushroom tyrosinase was added to the mixture. The final concentration of mushroom tyrosinase was 6.67 μg/mL for diphenolase activity. The enzyme activity was determined by following the increasing absorbance at 475 nm, accompanying the oxidation of the substrates with a molar absorption coefficient of 3700 ($M^{-1}\cdot cm^{-1}$) by using a Beckman UV-800 spectrophotometer. The temperature was controlled at 30 °C. The inhibitor was first dissolved in DMSO and then diluted 30 times. The final concentration of DMSO in the test solution was 3.3%. The extent of inhibition by adding the sample was expressed as the percentage necessary for 50% inhibition (IC_{50}). Controls, without inhibitor but containing 3.3% DMSO, were routinely carried out. The inhibition type was assayed by the Lineweaver−Burk plot, and the inhibition constant was determined by the second plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the inhibitor. Michaelis−Menten equation would change to be $(1/v) = (K_m/V_m)\cdot(1/[S]) + (1/V_m)$. While in the experiment, enzyme initial velocity (v) was determined at different substrate concentrations ([S]), and their reciprocal was calculated, which is expressed as $1/[S]$ and $1/v$, and $1/v$ versus $1/[S]$ is a straight line. The vertical axis intercept is $1/V_m$, the slope is K_m/V_m, and the horizontal axis intercept is $-(1/K_m)$, so that we can obtain the value of K_m and V_m.[18]

Plant Materials and Treatments. Fruits of longan (*Dimocarpus longan* Lour.) cv. "Fuyan", at commercially mature stage, were harvested from an orchard located in Anxi County of Fujian province, China, and transported under ambient conditions within 3 h to the laboratory of Institute of Postharvest Technology of Agricultural Products, Fujian Agriculture and Forestry University in Fuzhou, Fujian Province, China. Fruits were selected on the basis of the uniformity of maturity, color, shape, and size. Any blemished or diseased fruits were excluded. Preliminary study showed that, as compared to methyl gallate and ethyl gallate, propyl gallate most effectively inhibited the activity of tyrosinase, and at a concentration range of 0.05−1.0 mM, treatment with propyl gallate at 0.5 mM most effectively reduced pericarp browning of longan fruits held for 8 days at 15 °C. Therefore, 0.5 mM of propyl gallate was used in this study. The selected fruits were dipped in 0.5 mM propyl gallate solution for 20 min at 15 °C and air-dried, and then packed in 0.015 mm thick polyethylene bags (50 fruits per bag) and stored at (15 ± 1) °C and 80% relative humidity. The fruits dipped in distilled water were used as control. Samples were taken initially and at 2-days interval during storage for quality evaluation and following analyses.

Evaluation of Pericarp Browning. The method of Lin et al.[4] was employed to evaluate pericarp browning of longan fruit. Pericarp browning was assessed by measuring the extent of the total browned area of inner pericarp of 50 individual fruits according to the following visual appearance scale: (1) no browning, (2) <1/4 browning, (3) 1/4−1/2 browning, (4) 1/2−3/4 browning, (5) >3/4 browning, and (6) all browning. The browning index was calculated as $\sum$(browning scale × proportion of corresponding fruits within each class).

1.0 g of longan pericarp from 10 fruits with various browning indices was ground with 5 mL of 60% methanol in 0.1 M sodium phosphate buffer (pH 6.8) and 0.2 g of polyvinyl pyrrolidone (PVP). After centrifugation at 15 000*g* for 20 min, the supernatant was collected and then diluted to 20 mL with 0.1 M sodium phosphate buffer (pH 6.8). The absorbance of solution was measured spectrophotometrically at 450 nm according to the method of Jiang.[35] The results were recorded with $OD_{450}\cdot g^{-1}$ FW.

Determination of Total Phenols and Flavonoid Contents. Total phenolic compounds content was determined by the method of Folin−Ciocalteu reaction[36,37] with some modifications. Longan pericarp (2 g from 10 fruits) was homogenized with 10 mL of methanol containing 1% HCl. The mixture was incubated at 4 °C for 24 h and then centrifuged at 15 000*g* for 20 min. The mixture made of 1 mL of the supernatant, 1 mL of 1% HCl−methanol, 5 mL of distilled water, and 0.5 mL of 50% Folin−Ciocalteu phenol reagent was incubated for 10 min at room temperature. After that, 1 mL of 5% (w/v) sodium carbonate was added, and the mixture was vortexed briefly. The absorbance of the solution was assayed in spectrophotometer at 725 nm after incubation for 2 h in the dark at room temperature, and phenols were quantified by comparison to a standard curve of gallic acid. Total phenolic compounds content was calculated and expressed as gallic acid equivalents in milligrams on fresh weight (FW) basis.

Flavonoid content was determined using a colorimetric assay taking a slightly modified method of Meng et al.[37] Longan pericarp tissue (2 g from 10 fruits) was homogenized with 10 mL of methanol containing 1% HCl, and then centrifuged at 15 000*g* for 20 min. One milliliter of the supernatant was mixed with 0.3 mL of 10% aluminum chloride reagent, followed by the addition of 0.3 mL of aluminum nitrate reagent, and 2 mL of 1 M sodium hydroxide solution was added to terminate the reaction 5 min later. The absorbance of the mixture was determined at 325 nm after 10 min. Total flavonoid concentration was expressed as $OD_{325}\cdot g^{-1}$ FW.

Extraction and Assay of PPO and POD Activities. Polyphenoloxidase (PPO) was extracted according to the modified methods of Sun et al.[38] and Lin et al.[39] Longan pericarp (2 g from 10 fruits) was homogenized in 10 mL of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.2% (w/v) polyvinyl pyrrolidone at 4 °C. The homogenate was centrifuged for 20 min at 15 000*g* and 4 °C. The supernatant was collected for the determination of PPO activity. PPO activity was assayed with pyrocatechol as substrate according to the spectrophotometric procedure. The enzyme solution (0.1 mL) was rapidly added to 2.9 mL with 10 mM pyrocatechol prepared in 0.01 M sodium phosphate buffer (pH 5.5). The increase in absorbance at 400 nm was recorded for 3 min at 25 °C. One unit of PPO activity was defined as the amount of the enzyme that caused a change of 0.01 in the absorbance per minute.

Peroxidase (POD) were extracted and assayed according to the modified methods of Sun et al.[38] and Lin et al.[39] Longan pericarp (2 g from 10 fruits) was homogenized in 10 mL of 0.1 M sodium phosphate buffer (pH 7.0) at 4 °C. The homogenate then was centrifuged for 20 min at 15 000*g* and 4 °C. The supernatant was collected for the determinations of POD activities. The assaying mixture for determining POD activity consisted of 2.5 mL of 0.05 M sodium phosphate buffer (pH 5.5), 0.2 mL of 2% (v/v) H_2O_2, 0.2 mL of 0.05 mM guaiacol, and 0.1 mL of enzyme extract. The increase in absorbance at 470 nm was recorded for 3 min at 25 °C. One unit of POD activity was defined as the amount of enzyme that caused a change of 0.01 in absorbance per minute. Protein content was determined according to the method of Lin et al.,[39] with bovine serum as standard.

Statistical Analysis. The experiments were arranged in a completely randomized design, and each was comprised of three replicates. Data were tested by analysis of variance, using SPSS version 17.0. Least significant differences (LSD) were calculated to compare significant effects at the 5% level.

RESULTS

Effects of Methyl Gallate, Ethyl Gallate, and Propyl Gallate on the Diphenolase Activity of Mushroom Tyrosinase. The effects on the enzymatic oxidation of L-DOPA of propyl gallate at different concentrations were assayed, as well as methyl gallate and ethyl gallate. When L-DOPA was taken as substrate for the diphenolase activity of tyrosinase assay, the reaction course immediately reached a steady-state rate. Meanwhile, the effect of inhibitory concentration on the diphenolase activity of mushroom tyrosinase was assayed, with methyl gallate, ethyl gallate, and propyl gallate as inhibitors. As shown in Figure 1, all three inhibitors could inhibit the diphenolase activity, which decreased with increasing inhibitor concentrations. The IC_{50} values of methyl gallate, ethyl gallate, and propyl gallate were estimated to be 1.188, 0.927, and 0.685 mM, respectively. Thus, the inhibition capacity was listed in this order: methyl gallate < ethyl gallate < propyl gallate.

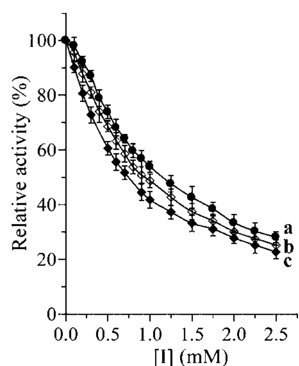

Figure 1. Effects of methyl gallate (a), ethyl gallate (b), and propyl gallate (c) on the diphenolase activity of mushroom tyrosinase.

Inhibition Mechanism of Propyl Gallate on the Diphenolase Activity of Tyrosinase. The inhibition mechanism of propyl gallate against the enzyme for the oxidation of L-DOPA was studied. Figure 2 showed the relationship between enzyme activity and different propyl gallate concentrations. The plots of the remaining enzyme activity versus the concentrations in the presence of different concentrations of propyl gallate gave a family of straight lines that all passed through the origin. Increase in inhibitor concentration resulted in a descent of the line slope, indicating that the inhibition caused by propyl gallate on diphenolase activity of mushroom tyrosinase was reversible. The presence of the inhibitor did not bring down the amount of the efficient enzyme, except inhibiting enzyme activity. In general, propyl gallate was a reversible inhibitor of mushroom tyrosinase for oxidation of L-DOPA.

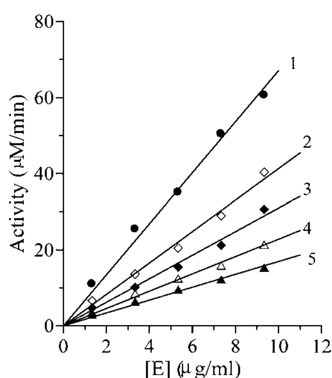

Figure 2. Determination of the inhibitory mechanism of propyl gallate on mushroom tyrosinase. Concentrations of propyl gallate for curves 1−5 were 0, 0.25, 0.5, 0.75, and 1.0 mM, respectively.

Inhibition Type of Propyl Gallate on Diphenolase Activity of Tyrosinase. Inhibition type of propyl gallate on the diphenolase activity of tyrosinase was investigated, and the results were illustrated in Figure 3. With increasing concentration of propyl gallate, K_m increased, while V_{max} decreased. Double-reciprocal plots yielded a family of straight lines intersecting at the second quadrant. Thus, propyl gallate belongs to mixed type inhibitor, indicating that propyl gallate inhibited the enzyme activity not only by binding with the free enzyme but also with the enzyme−substrate complex. The equilibrium constants of inhibitor binding with the free enzyme (K_I) and with enzymesubstrate complex (K_{IS}) were obtained from the slope or the vertical intercept versus the inhibitor concentration, respectively. From Figure 3II and III, the values of K_{IS} and K_I of propyl gallate were determined to be 2.135 and 0.661 mM, respectively.

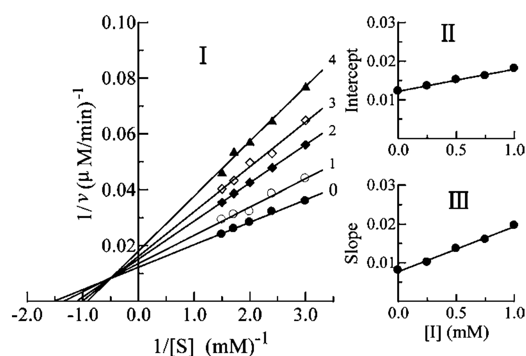

Figure 3. Determination of the inhibitory type and inhibition constants of cefodizime propyl gallate on mushroom tyrosinase. Lineweaver−Burk plot for the determination of the inhibitory mechanism of propyl gallate on mushroom tyrosinase (I). The concentrations of propyl gallate for curves 0−4 were 0, 0.25, 0.5, 0.75, and 1 mM, respectively. (II) and (III) represent the plot of intercept and slope versus the concentration of propyl gallate for determining the inhibition constants K_{IS} and K_I, respectively.

Effects of Propyl Gallate on Pericarp Browning Index and Browning Degree of Harvested Longan Fruits. As shown in Figure 4A−C, propyl gallate treatment retarded pericarp browning of harvested longan fruit. Figure 4D showed that the browning index in pericarp of harvested longan fruits increased with increasing storage time. For the control fruits, the pericarp browning index increased slightly in the first 2 days of storage, and then rapidly, while the pericarp browning index in propyl gallate-treated fruits increased slightly during the whole storage. Statistical analysis showed that browning index in pericarp of propyl gallate-treated fruits was significantly ($P < 0.05$) lower than that of the control fruits from day 2 to day 8 of storage time (Figure 4D).

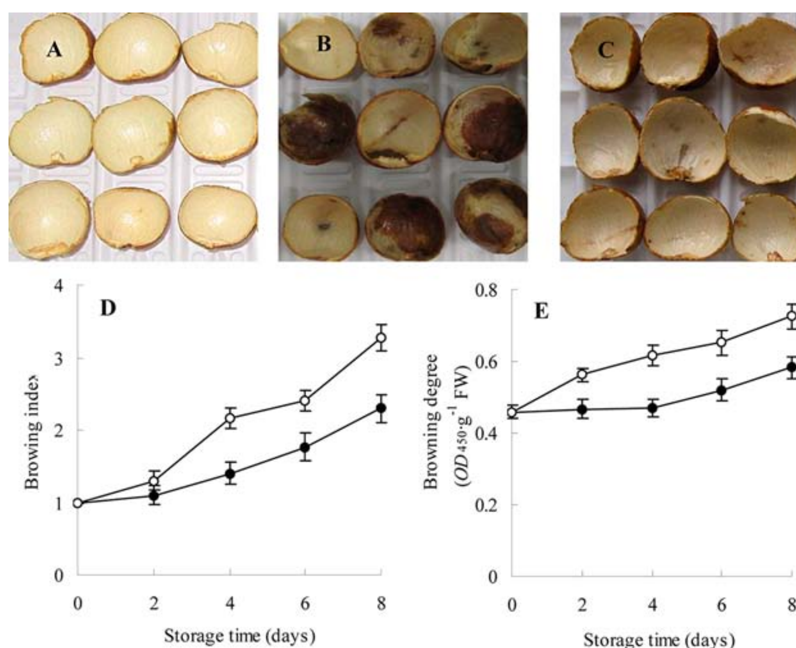

Figure 4. (A) Pericarp of longan fruit at harvesting day; (B) pericarp of the control longan fruit storage at (15 ± 1) °C for 8 days; and (C) longan pericarp of propyl gallate treatment fruit storage at (15 ± 1) °C for 8 days. Effects of propyl gallate treatment on browning index (D) and browning degree (E) in pericarp of harvested longan fruits during storage at (15 ± 1) °C for 8 days. Vertical bars represent standard errors of means, n = 3. ○, control; ●, propyl gallate treatment.

Pericarp browning degree showed coincident tendency similar to that of the pericarp browning index (Figure 4D,E). Generally, the browning degree in pericarp of harvested longan fruits increased with increasing storage time. As shown in Figure 4E, the browning degree in pericarp of the control fruits increased sharply before the fourth day of storage, but slightly increased afterward, while the browning degree in pericarp of propyl gallate-treated fruits increased slightly during the whole storage time. Statistical analysis indicated that browning degree in pericarp of propyl gallate-treated fruits was significantly (P < 0.05) lower than that of the control fruits during storage from day 2 to day 8 of storage time.

These results indicated that propyl gallate treatment could significantly reduce pericarp browning of harvested longan fruits.

Effects of Propyl Gallate on Contents of Total Phenolics and Flavonoid in Pericarp of Harvested Longan Fruits. Figure 5A showed that the contents of total phenolics in pericarp of harvested longan fruits decreased as storage time increased. The contents of total phenolics in pericarp of the control fruits declined sharply during the whole storage time. As compared to the control fruits, there were significantly (P < 0.05) higher contents of total phenolics in pericarp of propyl gallate-treated longan fruits.

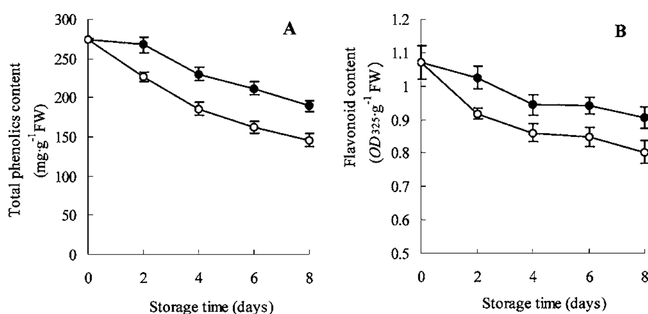

Figure 5. Effects of propyl gallate treatment on contents of total phenolics (A) and flavonoid (B) in pericarp of harvested longan fruits during storage at (15 ± 1) °C for 8 days. Vertical bars represent standard errors of means, n = 3. ○, control; ●, propyl gallate treatment.

As shown in Figure 5B, for both control and propyl gallate-treated longan fruits, flavonoid content decreased rapidly in the first 4 days of storage, followed by a slowly decrease after that. Statistical analysis showed that the flavonoid contents in pericarp of propyl gallate-treated fruits were significantly (P < 0.05) higher than those of the control fruits during the whole storage time.

The above results indicated that propyl gallate treatment could significantly reduce the degradation of total phenolics and flavonoid in pericarp of harvested longan fruits.

Effects of Propyl Gallate Treatment on Activities of PPO and POD in Pericarp of Harvested Longan Fruits. PPO and POD are associated with phenolic metabolism. As shown in Figure 6A, PPO activity in pericarp of the control longan fruits rapidly increased with increasing storage time, while there were little changes in PPO activity in pericarp of the propyl gallate-treated longan fruits throughout the storage time. Statistical analysis showed that PPO activities in propyl gallate-treated fruits were significantly (P < 0.05) lower than those of the control fruits. For example, when fruits were stored for 6 days and 8 days, PPO activities in pericarp of propyl gallate-treated fruits were 48% and 49% of the control fruits, respectively.

Figure 6B showed that POD activity in pericarp of the control longan fruits increased sharply during the whole storage time, while there was a slight increase of POD activity in pericarp of the propyl gallate-treated longan fruits. Further comparison indicated that POD activities in propyl gallate-treated fruits were significantly (P < 0.05) lower than those of

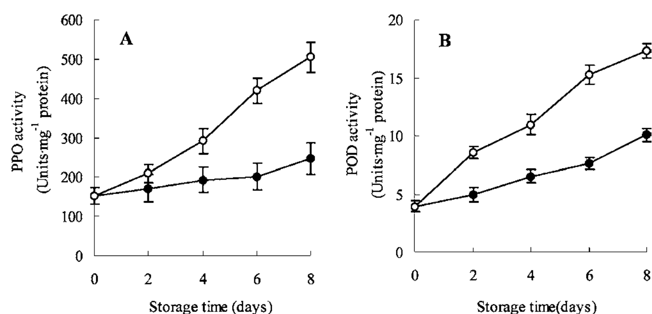

Figure 6. Effects of propyl gallate treatment on activities of PPO (A) and POD (B) in pericarp of harvested longan fruits during storage at (15 ± 1) °C for 8 days. Vertical bars represent standard errors of means, $n = 3$. ○, control; ●, propyl gallate treatmemt.

the control fruits. For instance, at days 4, 6, and 8, POD activities in pericarp of propyl gallate-treated fruits were 60%, 50%, and 58% of the control fruits, respectively.

The results indicated that propyl gallate treatment could significantly inhibit activities of PPO and POD in pericarp of harvested longan fruits.

DISCUSSION

Tyrosinase has both monophenolase activity and diphenolase activity, which can catalyze the hydroxylation of a monophenol, as well as the conversion of *o*-diphenol to the corresponding *o*-quinone.[18−20] In this effort, L-DOPA was used as substrate to determine the diphenolase activity of the enzyme. The results showed that methyl gallate, ethyl gallate, and propyl gallate could strongly inhibit the diphenolase activity of tyrosinase. As shown in Figure 1, it was clear that propyl gallate had a stronger inhibition effect on diphenolase activity than did methyl gallate and ethyl gallate, and the IC_{50} of propyl gallate for diphenolase activity was 1.73× and 1.35× that of methyl gallate and ethyl gallate, respectively. With respect to diphenolase activity, propyl gallate was a reversible competitive−uncompetitive mixed type inhibitor. Therefore, the presence of the inhibitor did not bring down the amount of the efficient enzyme, except inhibiting enzyme activity. Furthermore, the value of K_{IS} is 2.23 times that of K_I, indicating that the affinity of the inhibitor for the free enzyme is stronger than that for the enzyme−substrate complex.

Application of propyl gallate was found to delay ripening and senescence of some horticultural crops by scavenging free radical or inhibiting production of brown polymer.[31−34] Jiang et al. reported that propyl gallate could inhibit the activities of PPO and POD, and then reduce enzymatic browning of banana fruits at low temperature.[34] Additionally, propyl gallate may act as a free radical scavenger to scavenge active oxygen and to decelerate the accumulation of active oxygen, and then delay senescence of plant tissues.[32] Senescence and deterioration of harvested longan fruit are characterized by increased pericarp browning and aril breakdown, as well as reduced flavor and nutritive quality. In this study, propyl gallate significantly reduced the increases of pericarp browning index (Figure 4D) and pericarp browning degree (Figure 4E), which indicated that propyl gallate delayed the senescence of longan fruit during storage.

PPO and POD are crucial enzymes closely related to enzymatic browning of harvested longan fruit.[3,8−10] PPO can catalyze the oxidation of phenolic compounds to quinones and then condense tannins to brown polymers; however, with the presence of hydrogen peroxide, POD can catalytic the oxidation and polymerization of phenolic compounds as well, thus accelerating fruit and vegetable tissue browning process.[3,40] Under normal circumstances, phenolic compounds locate in the vacuole, while PPO and POD located in cell membrane, cytoplasm, chloroplasts, and other plastids; this kind of cellular compartmentalized distribution can prevent phenolase from contacting phenolic compounds, which result in avoiding occurrence of enzymatic browning in normal fruit and vegetable tissue.[41] Duan et al. found that treatment with sodium nitroprusside, a nitric oxide donor, could inhibit activities of PPO, POD, and PAL to maintain a high total phenol content, and then delay pericarp browning of longan fruit during storage.[3] In our work, the results showed that activities of PPO and POD of longan pericarp increased (Figure 6A), while total phenol content decreased (Figure 5A), and browning index increased (Figure 4D) during storage. Correlation analysis showed that there was a negative correlation between browning index and total phenol content (correlation coefficient $r = -0.9501$, $P < 0.05$), a negative correlation between total phenol content and PPO activity ($r = -0.9491$, $P < 0.05$), a negative correlation between total phenol content and PPO activity ($r = -0.9846$, $P < 0.05$), a significant positive correlation between browning index and PPO activity ($r = 0.9744$, $P < 0.01$), and a significant positive correlation between browning index and POD activity ($r = 0.9541$, $P < 0.01$). Therefore, the rapid increase in activities of PPO and POD (Figure 6) possibly accelerated the oxidation of polyphenols (Figure 5), and thus led to rapid pericarp browning of longan fruit (Figure 4). Furthermore, propyl gallate treatment inhibited activities of PPO and POD (Figure 6) in association with high contents of total phenolic and flavonoid (Figure 5), which may account for the inhibition of the pericarp browning (Figure 4).

In conclusion, propyl gallate could inhibit diphenolase activity of tyrosinase. Also, it was a reversible, mixed type inhibitor. Furthermore, treatment with propyl gallate effectively inhibited pericarp browning index, pericarp browning degree, and activities of PPO and POD of longan fruits during storage. The results suggest that the inhibition of the pericarp browning by propyl gallate may be due to an increase in activities of reactive oxygen scavenging enzymes and amounts of endogenous antioxidant substances, and a decrease in accumulation of active oxygen and membrane lipid peroxidation, to maintain the integrity of cellular membrane structure (material to be published), which, in turn, may postpone cellular decompartmentation, resulting in preventing PPO and POD located in plastid and other organelles, from coming into contact with phenolic and flavonoid substrates located in vacuole, to form brown polymers. Therefore, it is suggested that application of propyl gallate may be a promising method for inhibiting tyrosinase, controlling pericarp browning, and extending shelf life of longan fruits.

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Author Contributions

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; L-DOPA, L-3,4-dihydroxyphenylalanine; IC_{50}, the inhibitor concentration leading to 50% activity lost; K_I, the equilibrium constant of the inhibitor combining with the free enzyme; K_{IS}, equilibrium constant of the inhibitor combining with the enzyme−substrate complex; PPO, polyphenol oxidase; POD, peroxidase

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