Purification and Properties of Polyphenol Oxidase from Loquat Fruit

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Polyphenol oxidase (PPO) was purified to homogeneity from loquat (*Eriobotrya japonica* Lindl. cv. Mogi) fruit. The enzyme was purified 422-fold with a total yield of 35.6%. The molecular weight was estimated to be about 58 000 and 55 000 by SDS−PAGE and FPLC gel filtration chromatography, respectively, indicating that PPO is a monomer. The optimum pH and temperature of the enzyme activity were found to be pH 4.5 and 30 °C, respectively, and the enzyme was stable in the range of pH 4−8. In substrate specificity, a maximum activity was shown with epicatechin, followed by chlorogenic acid, neochlorogenic acid, 4-methylcathechol, and pyrocatechol, and no activity was apparent toward monophenol and *p*-diphenol. The K_m values for chlorogenic and neochlorogenic acids were 0.105 and 0.425 mM, respectively. The enzyme activity was markedly inhibited by sodium ascorbate, diethyldithiocarbamate, metabisulfide, dithiothreitol, mercaptoethanol, NaF, NaN₃, L-cysteine, and reduced glutathione.

Keywords: *Polyphenol oxidase; loquat fruit; Eriobotrya japonica Lindl.; polyphenols; browning*

INTRODUCTION

Browning of damaged tissues of fruits and vegetables occurs from the oxidation of phenolic compounds and contributes significantly to quality loss. The primary enzyme responsible for the browning reaction is polyphenol oxidase (PPO) (Mayer and Harel, 1979). This copper enzyme catalyzes the oxidation of *o*-diphenols to *o*-quinones (diphenolase, catecholase activity) in the presence of oxygen, and the final polymerized product is the undesirable brown, red, or black pigments (Mason, 1955). PPO has been studied in many fruits and vegetables including apples (Murata et al., 1992), peaches (Luh and Phithakpool, 1972), grapes (Wissemann and Lee, 1981), pears (Tono et al., 1986), avocado (Kahn, 1976, 1977; Espin et al., 1997), eggplant (Fujita and Tono, 1988), oil bean (Chilaka et al., 1993), and cabbage (Fujita et al., 1995), but not loquat. PPO from different plant tissues shows different substrate specificities and susceptibility toward inhibitors (Mayer and Harel, 1979). Avocado PPO has an activity toward both mono- and dihydroxyphenols (Espin et al., 1997), while PPO extracted from other sources utilized only dihydroxyphenols (Cash et al., 1976).

Loquat (*Eriobotrya japonica* Lindl.) originated in the regions of southwestern China (Zhang et al., 1990) and is widely cultivated in the subtropical regions of southern China, Japan, northern India, Israel, and the Mediterranean area. Loquat is consumed mainly as fresh fruit, some as canned products, and some as jams, juices, jellies, and other processed products (Shaw, 1980). Maximum use of loquat for processing is limited because it browns after it is peeled or crushed (Ding et al., 1998). Browning also occurred on the surface and internal tissue of fresh loquat during storage (Ding et

al., 1999). Potential use of loquat can be expanded if the browning can be prevented or inhibited.

Loquats have a relatively high concentration of polyphenols, of which about 60% of it in ripe fruit is chlorogenic and neochlorogenic acids (Ding et al., 1999). Very little is known about the properties of PPO in loquat, in particular the substrate specificities and inhibitor of reactions. We characterized, as described in this paper, the properties of PPO in loquat, which should be helpful in developing methods to inhibit or retard the browning.

MATERIALS AND METHODS

Materials. Loquats (*E. japonica* Lindl. cv. Mogi) fruits were harvested at the ripe commercial stage (light orange) from trees grown on the farm of the College of Agriculture, Osaka Prefecture University, Osaka, Japan, and PPO was extracted immediately.

Neochlorogenic acid was prepared according to the method described previously (Ding et al., 1999). Toyopearl HW 55F and DEAE-Toyopearl were obtained from Toyo Soda Co. Ltd. (Tokyo, Japan). Other reagents were obtained from Wako Pure Chemical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO).

Enzyme Extraction. In extraction of PPO, endogenous phenolic compounds react with the enzyme and inactivate PPO, thus initially, a procedure was developed to absorb the phenolic compounds during extraction. Polyclar SB-100 (an insoluble absorbent of polyphenolics; Wako), sodium ascorbate (10, 20, 30, and 50 mM), and a combination of Polyclar SB-100 and sodium ascorbate were evaluated as a means to absorb the phenolic compounds and reduce quinones to phenolic substrates during extraction. When only Polyclar SB-100 was used did the extracted solution become brown to black overnight at 4 °C. Increasing Polyclar SB-100 cannot inhibit the browning effectively. This indicated that all phenolic compounds were not absorbed. Several concentrations of sodium ascorbate combined with Polyclar SB-100 were tested to find the proper sodium ascorbate content. Of the different concentrations of sodium ascorbate, 30 or 50 mM in combination with the Polyclar SB-100 was the most effective (Table 1).

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Table 1. Activities of Loquat PPO Extracted by Different Buffers

compound in buffers	PPO activity,[a] unit/g fw	absorbance at 400 nm[b]	yield, %
10% PSB[c]	4.5	2.50	16.6
10% PSB + 10 mM AA[d]	18.2	1.20	67.2
10% PSB + 20 mM AA	21.8	0.80	80.4
10% PSB + 30 mM AA	26.5	0.51	97.8
10% PSB + 50 mM AA	27.1	0.50	100
50 mM AA	21.2	0.62	79.8

[a] PPO activity was assayed from the precipitate of 40−85% $(NH_4)_2SO_4$ saturation. [b] Absorbance was assayed from the supernatant of 40−85% $(NH_4)_2SO_4$ saturation. [c] PSB, Polyclar SB-100. [d] AA, ascorbic acid (sodium salt).

Extraction of the enzyme with neutral detergents such as Triton X-100 failed to improve enzyme activity (data not shown).

All the following steps were carried out at 4 °C. Peeled loquat pulp was homogenized with 1.5-fold of the weight of 0.1 M sodium phosphate buffer (pH 7.2), 30 mM sodium ascorbate, and 10% Polyclar SB-100. The homogenate was filtered through a double layer of cotton gauze, the filtrate centrifuged at 10000*g* for 30 min, and ammonium sulfate added to the supernatant. The protein fraction, which precipitated between 40% and 85% saturation with ammonium sulfate, was dissolved in a small volume of 10 mM sodium phosphate buffer, pH 7.2, and dialyzed overnight against the same buffer. The dialyzed solution was added to a Toyopearl HW 55F column (gel filtration, 80 × 1.5 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.2, and eluted with the same buffer (Murata et al., 1992). The active fractions (6 mL/tube) of PPO were pooled and dialyzed against 10 mM sodium phosphate buffer, pH 7.2. The dialyzed solution was added to a DEAE-Toyopearl column (ion exchange, 30 × 2.2 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.2, washing with 120 mL of the same buffer, and then eluted with a linear gradient of sodium phosphate buffer concentration (0.01−0.3 M, pH 7.2). The PPO active fractions (6 mL/tube) were pooled, dialyzed, further added to a Mono-Q column (HR 5/5, Pharmacia Biotech) equilibrated with 20 mM sodium phosphate buffer, pH 7.2, and then eluted with a linear gradient of NaCl concentration (0.02−0.7 M) in sodium phosphate buffer (0.02 M, pH 7.2). The PPO active fractions (1 mL/tube) were pooled and dialyzed against 10 mM sodium phosphate buffer, pH 7.2. The dialyzed solution was used as purified PPO.

Polyacrylamide Gel Electrophoresis (PAGE). PAGE was performed using the method described by Davis (1964), and sodium dodecyl sulfate (SDS)−PAGE was performed using the Laemmli method (1970). The samples were denatured by heating in boiling water for 3 min with 2% SDS and 5% β-mercaptoethanol for SDS−PAGE. Electrophoresis was performed at a constant current of 150 V/slab gel (130 × 140 × 2 mm). The gel was stained with Coomassie brilliant blue R-250. The markers used for molecular weight calibration were lactalbumin (14.4 kDa), soybean trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), ovalbumin (43.0 kDa), bovine serum albumin (67.0 kDa), and phosphorylase *b* (94.0 kDa) (LWM calibration kit, Pharmacia Biotech). For native PAGE, the gel was also stained for PPO activity with 1 mM chlorogenic acid and 1 mM (+)-catechin in McIlvaine buffer (pH 4.5) by incubating for 30 min at 30 °C.

Molecular Weight Estimation. The molecular weight of the enzyme was estimated by SDS−PAGE and fast protein liquid chromatography (FPLC). Superdex 75 FPLC column (HR 10/30, Pharmacia Biotech) was eluted with a 20 mM sodium phosphate buffer, pH 7.2. The markers used for molecular weight calibration on FPLC were bovine serum albumin (67 kDa), ovalbumin (45 kDa), and chymotrypsinogen (24 kDa).

Enzyme Activity and Protein Assay. PPO activity, except for substrate specificity, was measured by the spectro-photometric method at 325 nm to detect the decrease in absorption of the reacting solution, which resulted as the substrate, chlorogenic acid, became oxidized (Fujita and Tono, 1988). This measures the initial polyphenol oxidation catalyzed by PPO and is more sensitive than absorbance measurement at 420 nm to estimate the browning products. The reaction solution for enzyme activity consisted of 0.85 mL of McIlvaine buffer (pH 4.5), 0.1 mL of 1 mM chlorogenic acid and 0.05 mL of the enzyme solution. Absorption at 325 nm (maximum absorbance of chlorogenic acid) of the solution decreased as chlorogenic acid, was oxidized (Fujita and Tono, 1988); the absorption at 325 nm decreases, so the activity was based on the rate of decrease in absorption at 325 nm with time. A decrease in absorbance of 0.01 per min at 30 °C is defined as 1 unit of PPO activity.

To determine the K_m, the concentrations of chlorogenic acid and neochlorogenic acid were varied from 0.025 to 0.1 mM (final concentration) at pH 4.5. Data were plotted as 1/activity vs 1/substrate concentration according to the method of Lineweaver and Burk (1934).

Protein was assayed by Bradford's method (1976) with bovine serum albumin as the standard.

Substrate Specificity. Epicatechin, chlorogenic acid, neochlorogenic acid, 4-methylcatechol, pyrocatechol, pyrogallol, D-(+)-catechin, dopamine, protocatechuic acid, L-DOPA, hydroquinone, phloroglucinol, resorcinol, coumaric acid, and cresol were used to study their specificity at a concentration of 10 mM. Caffeic acid and tyrosine were 3 mM because of their poor solubility. The rate of the reaction was measured in terms of the increase in absorbance at the optimum absorbance wavelength of browning products for each substrate (Zhou et al., 1993).

Thermal Activity and Stability. The optimum temperature of the PPO−chlorogenic acid reaction was determined for 2 min at various temperatures ranging from 10 to 80 °C. To determine the thermal stability, enzyme solution was incubated from 2 up to 30 min at different temperatures (30−85 °C). The remaining activity was assayed at different time intervals under standard conditions.

pH Optimum and Stability. PPO activity as a function of pH was determined using chlorogenic acid as substrate in McIlvaine buffer (pH 2.2−8.0). The pH stability was determined by incubating the enzyme in the above buffer for 24 h at 4 °C. The activity was assayed under standard conditions with chlorogenic acid as substrate after incubating for 24 h. The optimum pH obtained from this procedure was used in other studies (substrate specificity, temperature properties, etc.).

Inhibitor Studies. The following compounds were evaluated for their effectiveness as an inhibitor of PPO activity: thiourea, L-cysteine, 2-mercaptoethanol, glutathione (reduced), dithiothreitol, sodium diethyldithiocarbamate, L-ascorbic acid (sodium salt), $Na_2S_2O_5$, NaCl, $CuSO_4$, NaF, and NaN_3. An aliquot (0.1 mL) of each inhibitor (final concentration of 0.1, 1.0, and 10.0 mM) was added to the standard reaction solution (total 1.0 mL). A final concentration of 100 mM was added in NaCl. The enzyme activity was determined by the decrease in absorbance at 325 nm under standard conditions.

RESULTS AND DISCUSSION

Extraction and Purification of PPO. A summary of loquat fruit PPO extraction and purification is shown in Table 2. A 422-fold purification of the sample PPO with a total yield of 35.6% was achieved.

Following ammonium sulfate precipitation, the dialyzed enzyme extract was applied to a Toyopearl HW 55F column yielding fractions 12−18 with PPO activity (Figure 1). The active fractions were further applied to a DEAE-Toyopearl column yielding three peaks with enzyme activity (Figure 2). Peak 1 (F−I) corresponded to a peak of protein and had strong activity, while the second and third peaks were not accompanied by a peak

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Table 2. Purification of Polyphenol Oxidase from Loquat Fruit

purification step	total volume, mL	enzyme activity, U/mL	total activity, U	total protein, mg	specific activity, U/mg	purification, fold	yield, %
crude extract	600	7.6	4560	296	15.4	1.0	100.0
$(NH_4)_2SO_4$ (40–80%)	15.6	242	3775	64.0	59.0	3.8	83.0
Toyopearl HW 55F	22.3	155	3450	14.2	243	15.8	76.0
DEAE-Toyopearl (F–I)	22.0	92	2020	0.84	2410	157	44.4
Mono Q (FPLC)	28.5	57	1630	0.25	6500	422	35.6
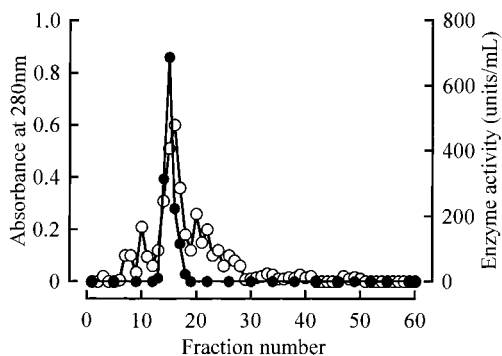
Figure 1. Elution pattern of PPO activity on a Toyopearl HW 55F column: ○, absorbance at 280 nm; ●, enzyme activity.
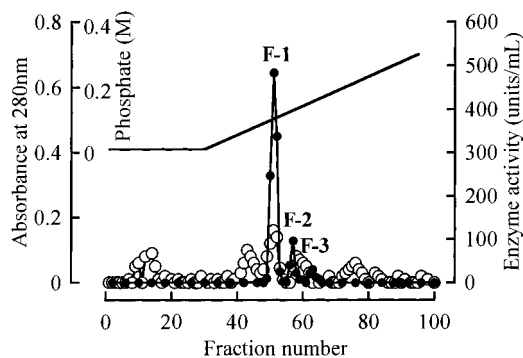
Figure 2. Elution pattern of PPO activity on a DEAE-Toyopearl column. F-1, F-2, and F-3 represent peaks of PPO activity: ○, absorbance at 280 nm; ●, enzyme activity; —, phosphate buffer.
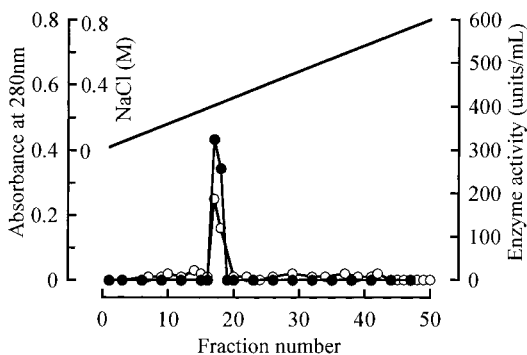
Figure 3. Elution pattern of PPO activity (F-1) on an FPLC Mono-Q column: ○, absorbance at 280 nm; ●, enzyme activity; —, NaCl concentration.

of absorbance at 280 nm and had weak activities. Fractions from peak 1 (F–I) had 90% of total activity, and when it was passed through the Mono-Q column (FPLC), only one peak had enzyme activity (Figure 3). The enzyme sample obtained from Mono-Q (FPLC) was used in the characterization of the PPO.

Molecular Weight. The purified enzyme migrated as a single band on the SDS–PAGE (Figure 4), which corresponded to a molecular weight of 58 kDa (Figure 5A). The FPLC Superdex 75 gel filtration of purified
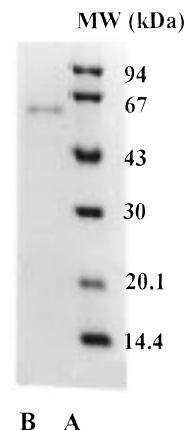
Figure 4. SDS–PAGE of the purified enzyme: A, molecular weight markers lactalbumin (14.4 kDa), soybean trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), ovalbumin (43.0 kDa), bovine serum albumin (67.0 kDa), and phosphorylase *b* (94.0 kDa) (LWM calibration kit, Pharmacia Biotech); B, purified PPO.
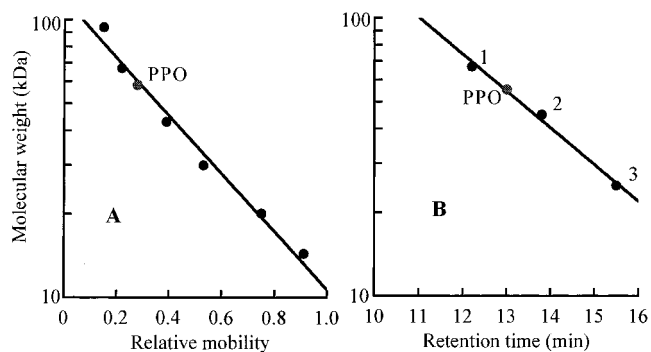
Figure 5. Estimation of molecular weight of loquat PPO by SDS–PAGE (A) and FPLC Superdex 75 (B). Molecular weight markers: A, same as in Figure 4; B, 1 bovine serum albumin (67.0 kDa); 2, ovalbumin (45.0 kDa); 3, chymotrypsinogen (25.0 kDa); PPO, purified enzyme.
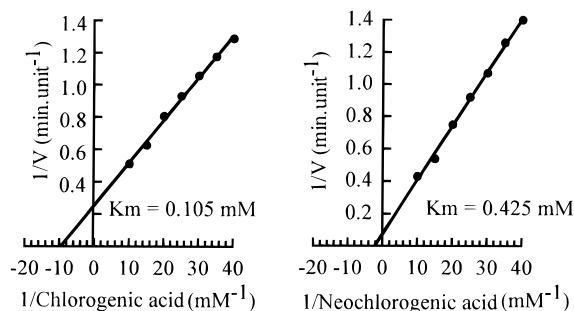
enzyme also resulted in a weight of 55 kDa (Figure 5B). The results showed that the enzyme was a monomer.

Substrate Specificity. The relative activity of PPO, based on absorption at the wavelength maximum of the product, was compared with the activity of chlorogenic acid (189.75 units/mL of PPO solution) as 100% (Table 3). Epicatechin and chlorogenic and neochlorogenic acids were oxidized significantly by the enzyme, followed by 4-methylcatechol, pyrocatechol, pyrogallol, caffeic acid, D-catechin, and dopamine. The activity was weak with protocatechuic acid and L-DOPA as substrates. No activity was noted with monophenols and *p*-diphenols. The loquat PPO had a stronger specificity for chlorogenic and neochlorogenic acids than with 4-methylcatechol. This is different from most reported plant PPO, which has a stronger affinity for 4-methylcatechol (Siddiq et al., 1992). The purified loquat PPO probably is an *o*-diphenol oxidase (EC 1.10.3.1) which also is known as catecholase. Chlorogenic and neochlorogenic acids are the main compounds in ripe loquat (Ding et

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Table 3. Substrate Specificity of Loquat Fruit (PPO)

substrate	concentration, mM	wavelength, nm	relative activity, %[a]
epicatechin	10	400	186.2
chlorogenic acid	10	400	100.0
neochlorogenic acid	10	400	97.6
4-methylcatechol	10	400	87.6
pyrocatechol	10	400	83.8
pyrogallol	10	334	59.3
caffeic acid	3	400	47.6
D-(+)-catechin	10	400	43.5
dopamine	10	400	39.5
protocatechuic acid	10	400	14.4
L-DOPA	10	460	13.0
hydroquinone	10	400	0.0
phloroglucinol	10	400	0.0
resorcinol	10	400	0.0
coumaric acid	10	400	0.0
tyrosine	3	472	0.0
cresol	10	400	0.0

[a] Relative activity calculated taking chlorogenic acid to be 100%. The enzyme activity was measured by the increase of absorbance at the optimum wavelength of their products.
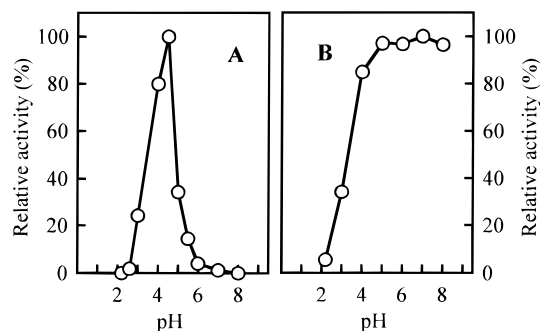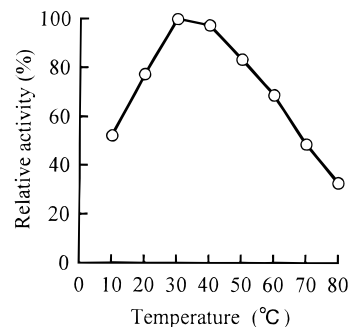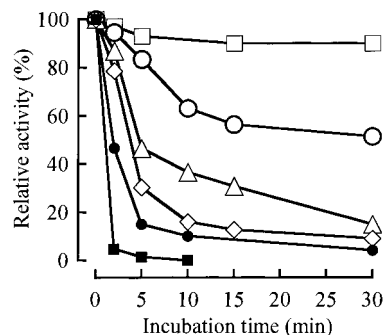

Figure 6. Lineweaver−Burk plot of loquat PPO.

Figure 7. Optimum pH (A) and pH stability (B) of loquat PPO.

Figure 8. Optimum temperature of loquat PPO.

Figure 9. Thermal stability of loquat PPO: □, 30 °C; ○, 45 °C; △, 55 °C, ◇, 65 °C; ●, 75 °C; ■, 85 °C.

al., 1999), and since they are good substrates for loquat PPO, the two phenols may be responsible for the rapid browning of loquats. Since the results of PPO activity in substrate specificity measured by spectrophotometry for color development and polarography for oxygen uptake are varied due to the effects of the secondary reaction products formed from condensation of quinones (Janovitz-Klapp et al., 1990), we plan to compare the substrate specificity by polarography.

The Lineweaver−Burk plots of chlorogenic and neochlorogenic acids oxidation by the purified enzyme are shown in Figure 6. The choice of chlorogenic and neochlorogenic acids as substrates resulted from their dominant presence of the two compounds in ripe loquat fruit. The Michaelis constant (K_m) of the enzyme was 0.105 mM for chlorogenic acid and 0.425 mM for neochlorogenic acid as substrates. These results are similar to that of apple reported by Murata et al. (1992) but lower than that reported for grapes (Cash et al., 1976) and plums (Siddiq et al., 1992).

pH Optimum and Stability. The loquat PPO had maximum activity at pH 4.5 with a rapid decrease in activity between 4.5 and 5.0 (Figure 7A). The activity at pH 5.0 was only 34% of that at pH 4.5. The optimum pH for PPO activity differs among fruits. It is around pH 7 for PPO of kiwifruit, cherry, and pineapple, for satsuma mandarin it was around 7 (Benjamin and Montgomery, 1973; Das et al., 1997; Fujita and Tono, 1981), and it is around pH 4−5 for apple, eggplant, potatoes, pears, and olives (Murata et al., 1992; Fujita and Tono, 1988; Tono et al., 1986).

The pH stability curve of PPO activity is shown in Figure 7B. The loquat PPO was fairly stable between pH 4 and 8 and unstable at below pH 3. The pH of juice in ripe fruits is between 4.3 and 4.6 which is in the optimum pH of loquat PPO. Those results indicated that the pH condition is favored for PPO in ripe fruit.

Optimum Temperature and Stability. The optimum temperature of activity for loquat PPO was 30 °C (Figure 8). The enzyme retained most of its activity (70% of the maximum) over a wide temperature range (20−50 °C). Above 50 °C, the PPO activity declined rapidly as the temperature increased but the enzyme was not completely inactivated even at 80 °C. At the lower temperature of 10 °C, the activity did not decline below 50% of the maximum. The optimum temperatures for PPO of plum (Siddiq, 1992), Concord grape (Cash et al., 1976), Koshu grape (Nakamura et al., 1983) and peach (Jen and Kahler, 1974) were 20, 25, 30, and 37 °C, respectively.

The loquat PPO was stable for 30 min at 30 °C and moderately stable for 5 min at 55 °C (Figure 9). At a higher temperature of 85 °C, heat denaturation of the enzyme occurred rapidly within 2 min of incubation. PPO is not a very heat-stable enzyme compared with other enzymes responsible for food-quality degradation (Amiot et al., 1997). Thus although loquat PPO activity

Table 4. Effect of Various Inhibitors on the Oxidation of Chlorogenic Acid by Loquat PPO

| | relative PPO activity, % | | |
inhibitor	0.1 mM[a]	1.0 mM[a]	10.0 mM[a]
none	100	100	100
thiourea	30	21	12
L-cysteine	18	13	4
2-mercaptoethanol	14	5	1
glutathione (reduced)	17	14	6
(±)-dithiothreitol	15	2	0
DDC[c]	3	0	0
L-ascorbic acid	0	0	0
$Na_2S_2O_5$	7	0	0
NaCl[b]	100	78	22
$CuSO_4$	98	95	*
NaF	61	8	0
NaN_3	78	10	0

[a] Final concentration of inhibitor. [b] No PPO activity was observed when the final concentration of NaCl increased to 100 mM. [c] DDC, sodium diethyldithiocarbamate. *Blue solution at this concentration.

and the subsequent browning cannot be reduced by low temperature, a high temperature of 85 °C is effective for inactivation.

Effect of Inhibitors. Among the different inhibitors tested, sodium L-ascorbic acid (sodium salt), sodium diethyl dithiocarbamate (DDC), and sodium metabisulfite ($Na_2S_2O_5$) exhibited nearly complete inhibition of loquat PPO at the range of 0.1–1.0 mM (Table 4). The enzyme also was sensitive to 2-mercaptoethanol, glutathione, and L-cysteine, as 80% or more inhibition occurred at the concentration of 0.1 mM. Among the thiol compounds, many researchers reported that the strongest inhibition of PPO occurred with thiourea (Zhou et al., 1993), but it is not the case with loquat PPO. The loquat PPO also was strongly inhibited by sodium fluoride and sodium azide (10 mM). NaCl at a concentration of 0.1–10 mM was not effective, but at 100 mM, the PPO activity was inhibited completely. Copper sulfate was a poor inhibitor of the loquat PPO enzyme, which was also noted with eggplant PPO (Fujita and Tono, 1988). Those results confirmed that the traditional inhibitor, sulfur dioxide (SO_2), still was effective to prevent PPO activity, and it has been one of the most effective inhibitors and has been used for many years (Mayer et al., 1964). However, the use of sulfites becomes more and more restricted due to potential hazards (Langdon, 1987; FDA, 1990; Sapers, 1993). Therefore, many researchers have been devoted to finding an alternative to SO_2. Recent studies showed that sulfhydryl (SH or thiol) compounds (Dudley and Hotchkiss, 1989; Richard et al., 1991; Friedman and Bautista, 1995) and ascorbic acid (Santerre et al., 1988; Sapers et al., 1989) were good inhibitors of the enzyme PPO. Our research showed that ascorbic acid, L-cysteine, and the combination of those two compounds appear to be potent inhibitors of loquat PPO and need further study with intact fruit as well as loquat juice.

CONCLUSIONS

Loquat fruits have a relatively high concentration of polyphenols, and chlorogenic and neochlorogenic acids were the main compounds in ripe fruits. These two compounds were good substrates for loquat PPO. Moreover, the pH of juice in ripe fruits is between 4.3 and 4.6, which agrees with the optimum pH of loquat PPO. These contributed to the fact that loquats turned brown

rapidly. On the other hand, loquat PPO was completely inactivated by high temperature (85 °C) after 2 min. Ascorbic acid showed strong inhibition to loquat PPO, and L-cysteine also appears to be a potent inhibitor of loquat PPO.

LITERATURE CITED

Amiot, M. J.; Fleuriet, A.; Cheynier, V.; Nicolas, J. Phenolic compounds and oxidative mechanism in fruit and vegetables. In *Phytochemistry of Fruit and Vegetables*; Tomas-Barberan, F. A., Robins, R. J., Eds.; Oxford Science Publications: London, 1997.

Benjamin, N. D.; Montgomery, M. W. Polyphenol oxidase of Royal Ann cherries: purification and characterization. *J. Food Sci.* **1973**, *38*, 799–806.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

Cash, J. N.; Sistrunk, W. A.; Stutte, C. A. Characteristics of concord grape polyphenol oxidase involved in juice color loss. *J. Food Sci.* **1976**, *41*, 1398–1402.

Chilaka, F. C.; Amosike, E. O.; Egubuna, P. C. Purification and properties of polyphenol oxidase from oil bean (*Pentaclethra macrophylla* Benth) seeds. *J. Sci. Food Agric.* **1993**, *61*, 125–127.

Das, J. R.; Santhoor, G.; Gowda, L. R. Purification and characterization of a polyphenol oxidase from Kew cultivar of Indian pineapple fruit. *J. Agric. Food Chem.* **1997**, *45*, 2031–2035.

Davis, B. J. Disc electrophoresis: method and application to human serum protein. *Ann. N.Y Acad. Sci.* **1964**, *121*, 404–427.

Ding, C.-K.; Chachin, K.; Ueda, Y.; Mochioka, R. Changes in polyphenol concentrations and polyphenol oxidase activity of loquat (*Eriobotrya japonica* Lindl.) fruits in relation to browning. *J. Jpn. Soc. Hortic. Sci.* **1998**, *67*, 360–366.

Ding, C.-K.; Chachin, K.; Ueda, Y.; Imahori, Y. Effects of high CO_2 concentration on browning injury and phenolic metabolism in loquat fruits. *J. Jpn. Soc. Hortic. Sci.* **1999**, *68*, in press.

Dudley, E. D.; Hotchkiss, J. H. Cysteine as an inhibitor of polyphenol oxidase. *J. Food Biochem.* **1989**, *13*, 65–75.

Espin, J. C.; Trujano, M. F.; Tudela, J.; Garcia-Canovas, F. Monophenolase activity of polyphenol oxidase from Haas avocado. *J. Agric. Food Chem.* **1997**, *45*, 1091–1096.

FDA. Sulfiting agents; Revocation of GRAS status for use on "fresh" potatoes served or sold unpackaged or unlabeled to consumers. *Fed. Regist.* **1990**, *55*, 9826–9833.

Friedman, M.; Bautista, F. F. Inhibition of polyphenol oxidase by thiols in the absence and presence of potato tissue suspensions. *J. Agric. Food Chem.* **1995**, *43*, 69–76.

Fujita, S.; Tono, T. Enzymatic browning of immature fruit of satsuma mandarin and its chlorogenic acid oxidase activity. *Nippon Shokuhin Kogyo Gakkaishi* **1981**, *28*, 600–605 (in Japanese with English summary).

Fujita, S.; Tono, T. Purification and some properties of polyphenol oxidase in eggplant (*Solanum melongena*). *J. Sci. Food Agric.* **1988**, *46*, 115–123.

Fujita, S.; Saari, N. B.; Maegawa, M.; Tetsuka, T.; Hayashi, N.; Tono, T. Purification and properties of polyphenol oxidase from cabbage (*Brassica oleracea* L.). *J. Agric. Food Chem.* **1995**, *43*, 1138–1142.

Janovitz-Klapp, A. H.; Richard, F. C.; Goupy, P. M.; Nocolas, J. J. Kinetic studies on apple polyphenol oxidase. *J. Agric. Food Chem.* **1990**, *38*, 1437–1441.

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Jen, J. J.; Kahler, K. R. Characterization of polyphenol oxidase in peaches grown in the Southeast. *Hortscience* **1974**, *9*, 590−591.

Kahn, V. Polyphenol oxidase in avocado. *Phytochemistry* **1976**, *15*, 267−272.

Kahn, V. Some biochemical properties of polyphenoloxidase from two avocado varieties differing in their browning rate. *J. Food Sci.* **1977**, *42*, 38−43.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head Bacteriofage T4. *Nature* **1970**, *227*, 680−685.

Langdon, T. T. Preventing of browning in fresh prepared potatoes without the use of sulfiting agents. *Food Technol.* **1987**, *41*, 64−67.

Lineweaver, H.; Burk, D. The determination of enzyme dissociation constants. *J. Am. Chem. Sci.* **1934**, *56*, 685−666.

Luh, B. S.; Phithakpol, B. Characteristics of polyphenoloxidase related to browning in cling peach. *J. Food Sci.* **1972**, *27*, 264−268.

Mason, H. Comparative biochemistry of the phenolase complex. *Adv. Enzymol.* **1955**, *16*, 105−184.

Mayer, A. M.; Harel, E. Polyphenol oxidases in plants. *Phytochemistry* **1979**, *18*, 193−215.

Mayer, A. M.; Harel, E.; Shain, Y. 2,3-Naphthalenediol, a specific competitive in inhibitor for phenolase. *Phytochemistry* **1964**, *3*, 447−451.

Murata, M.; Kurokami, C.; Homma, S. Purification and properties of chlorogenic acid oxidase from apple (*Malus pumula*). *Biosci., Biotechnol., Biochem.* **1992**, *56*, 1705−1710.

Nakamura, K.; Amano, Y.; Kagami, M. Purification and some properties of polyphenol oxidase from Koshu grapes. *Am. J. Enol. Vitic.* **1983**, *34*, 122−127.

Richard, F. C.; Goupy, P. M.; Nicolas, J. J.; Lacombe, J.-M.; Pavia, A. A. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *J. Agric. Food Chem.* **1991**, *39*, 841−847.

Santerre, C. R.; Cash, J. N.; VanNorman, D. J. Ascorbic acid/ citric acid combinations in the processing of frozen apple slices. *J. Food Sci.* **1988**, *53*, 1713−1716.

Sapers, G. M. Browning of foods: control by sulfites, antioxidants and other means. *Food Technol.* **1993**, *47* (10), 75−84.

Sapers, G. M.; Hicks, K. B.; Phillips, J. G.; Garzarella, L.; Pondih, D. L.; Matulaitis, R. M.; McCormack, T. J.; Sondy, S. M.; Seib, P. R.; El-Ataway, Y. S. Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors and complexing agents. *J. Food Sci.* **1989**, *54*, 997−1002.

Shaw, P. E. Loquat. In *Tropical and Subtropical Fruits;* Nagy, S., Shaw, P. E., Eds.; AVI Publishing: Westport, CT, 1980.

Siddiq, M.; Sinha, K.; Cash, J. N. Characterization of polyphenoloxidase from Stanley plums. *J. Food Sci.* **1992**, *57*, 1177−1179.

Tono, T.; Fujita, S.; Kawasaki, H.; Li, Z.-F. Purification and high L-epicatechinoxidase activity in polyphenoloxidases of Japanese pears. *Nippon Nogeikagaku Kaishi* **1986**, *60*, 705−712 (in Japanese with English summary).

Wissemann, K. W.; Lee, C. Y. Characterization of polyphenoloxidase from Rovat 51 and Niagara grapes. *J. Food Sci.* **1981**, *46*, 506−508.

Zhang, H.; Peng, S.; Cai, L.; Fang, D. The germplasm resources of the genus Eriobotrya with special reference on the origin of E. japonica Lindl. *Acta Horticulturae Sinica* **1990**, *17*, 5−12 (in Chinese with English abstract).

Zhou, P.; Smith, N. L.; Lee, C. Y. Potential purification and some properties of Monroe apple peel polyphenol oxidase. *J. Agric. Food Chem.* **1993**.