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Characterisation of Polyphenol Oxidase and Peroxidase and the Role in Browning of Loquat Fruit

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Abstract

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Polyphenol oxidase (PPO) and peroxidase (POD) were extracted from a new loquat fruit cultivar (Ninghaibai) and characterised using reliable spectrophotometric methods. In both cases, the optimum pH for PPO was 4.5 and 5 for POD, and their optimum temperatures were 30 and 35°C, respectively. Both enzymes followed Michaelis-Menten kinetics, showing K_m= 47.11 mmol/l for PPO with catechol as the substrate and K_m = 153.00 mmol/l for POD with guaiacol as the substrate. PPO was much more thermolabile than POD, losing more than 40% of relative activity after 30 min of heating at 40°C. PPO activation energy was much lower than POD activation energy ($\Delta E^{\#}$ = 39.74 and 94.65 kJ/mol for PPO and POD, respectively): Both enzymes activities showed decreasing patterns as the compound concentration in the assay medium increased. 4-hexylresorcinol (4-HR), oxalic acid, and L-cysteine showed strongly inhibitive effects on the enzymes. Changes in L^*, a^*, and b^* values were chosen to describe the browning of loquat pulp. Only PPO displayed a higher negative correlation with L^* values, which indicated that PPO plays an important role in the browning of stored loquat cv. Ninghaibai.

Keywords: *Eriobotrya japonica*; PPO; POD

Loquat (*Eriobotrya japonica* Lindl.), which is very popular among global consumers, originates from southwestern China and is widely cultivated throughout Japan, India, Israel, Turkey and the Mediterranean region (Ding *et al*. 1998a), while China, with more than 100 000 ha of the dedicated cultivation area and 380 000 t the annual production, is the leading producer (Ding *et al*. 2006). According to the colour of the pulp, loquats can be divided into red and white-fleshed fruits (Zhou *et al*. 2007). The loquat cv. Ninghaibai is a new cultivar that has been developed by a systematic selection method. It is characterised by its white flesh, and its total soluble solid content is 14.0% (Feng *et al*. 2006). This loquat has undergone great commercial development in recent years due to its soft, juicy taste and high nutritional value.

Loquat fruit is consumed not only as fresh fruit, but is also processed into juices, jams, jellies, canned and other products. However, its consumption is limited due browning that occurs after mechanical damage, during long term storage and long-distance transportation, or when it is peeled and crushed in processing (Ding *et al*. 1998b).Severe browning, caused by the oxidation of phenolic compounds, is frequently observed in the loquat cv. Ninghaibai and leads to a significant reduction in quality (Ding *et al*. 1998b).

Generally, the main enzyme responsible for browning is polyphenol oxidase (PPO): PPO catalyses the oxidation of polyphenols to *o*-quinones in the presence of oxygen, and *o*-quinones polymerise into undesirable brown, red, or black pigments (Hs 1955). These pigments can affect the sensory and nutritional qualities of loquat fruit or products, which depreciate their commodity values (Sun *et al*. 2012). In plants, PPO is located in the chloroplasts, and its phenolic substrates are mainly located in the vacuoles. However,

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upon any cell-damaging treatment, the enzyme may become exposed to substrates, leading to rapid oxidation of phenols (Chazarra *et al*. 2001). There have been numerous studies on PPO from various sources, such as olives (Ünal *et al*. 2011), bananas (Ünal 2007), melon (Chisari *et al*. 2007a), and strawberries (Chisari *et al*. 2007b). However, there are few studies regarding the role of loquat PPO (Ding *et al*. 1998a; Selles-Marchart *et al*. 2006; Şener et al. 2011ab), and no specific studies regarding the loquat cv. Ninghaibai.

Peroxidase (POD) is another enzyme involved in enzymatic browning, as it may reduce diphenols (Robinson & Eskin 1991) and is also involved in the production of lignin (Cai *et al*. 2006a). Although POD activity is limited by the absence of electron compounds such as superoxide radicals, hydrogen peroxide, and lipid peroxides, its involvement in the browning of various fruits and vegetables has been reported (González-Barrio *et al*. 2005; Spagna *et al*. 2005). The role of POD has been studied in many fruits and vegetables including olives (Saraiva *et al*. 2007), tomatoes (Loukili *et al*. 1999) and loquats cv. Zaozhong 6 (Lin *et al*. 2007); however, no report on this new cultivar of loquat was found.

There are many reports on the characterisation of oxidative enzymes from various fruits and vegetables, such as bananas and olives (Ünal 2007; Ünal *et al*. 2011), but only a few of them have found a clear correlation between the oxidative enzymes and browning of the samples (Chisari *et al*. 2007b). And also, there is a lack of data that concerning the direct influence of the processing and storage conditions on PPO and POD activities in loquats cv. Ninghaibai. The objective of this experiment was to extract and characterise PPO and POD from loquats cv. Ninghaibai to determine this kinetic parameters, pH, and temperature optima, and also the inhibitory effects of various compounds. Furthermore, storage tests of loquat fruit at 5 and 25°C were performed to evaluate the influence of enzyme activities on pulp browning during storage. These results will help to optimise the post-harvest procedures for loquats cv. Ninghaibai, which are important for the loquat storage and product development. In order to allow the results to find a better application to the processing and storage industry at the industrial level, we used crude enzyme solution from the fresh loquat cv. Ninghaibai (Liu *et al*. 2012; Goyeneche *et al*. 2013; Taticchi *et al*. 2013). These results were also compared with PPO and POD characterisation obtained with plants studied previously.

MATERIAL AND METHODS

Plant materials. Loquats (*Eriobotrya japonica* Lindl.) cv. Ninghaibai were obtained from a local producer in Ninghai, Zhengjiang Province, China. The fruit was harvested at commercial maturity, selected on the basis of size, and immediately transported to the laboratory (avoiding all physical damage) and stored at 5°C prior to processing.

PPO and POD extraction and assay. 20 g of fruit pulp was homogenised with 100 ml 0.1 mol/l sodium phosphate buffer (pH 6.5) containing 1% polyvinyl-polypyrrolidone, then centrifuged at 10 000 *g* at 4°C for 10 minutes. The supernatant was collected for the enzyme assay.

PPO activity was determined by the method of Liu et al (Liu *et al*. 2005). The standard reaction mixture contained 1.5 ml of 40 mmol/l catechol and 2.3 ml of 0.1 mol/l phosphate buffer (pH 6.5) in a 10 ml test tube, and was placed in a 25°C water bath for 5 minutes. Then, 0.2 ml of crude enzyme was added to the test tube and mixed thoroughly. Immediately, the increase in absorbance was measured at 420 nm with a UV-spectrophotometer (Mapada UV1600; Mapada, Shanghai, China). The reaction time for PPO was 2 min, and the activity was expressed in units – one unit = $0.001\Delta A_{420}$/min/g fresh weight (FW).

For the determination of POD activity, 3 ml of reaction solution was added (50 ml pH 6.0 0.1 mol/l phosphate buffer, 19 µl Guaiacol and 28 µl 30% H_2O_2) to a 10-ml test tube and placed in a 25°C water bath for 5 minutes. Then, 0.05 ml of crude enzyme was added to the test tube and mixed thoroughly. Immediately, by measuring the increase in absorbance for 2 min at 470 nm with UV-spectrophotometer, the POD activity was expressed in units – one unit= $0.001\Delta A_{470}$/min/g FW.

Optimum pH and temperature. PPO and POD activities were determined in a pH range of 3.0–8.0 in 100 mmol/l citrate-phosphate buffer, using catechol and guaiacol as substrates, respectively. The residual activity at different pH values was compared with that obtained at optimal pH (%). Additional tests were carried out to determine optimal temperature. PPO and POD activities were assayed at various reaction temperatures (15–50°C) controlled by a circulation water bath. The residual activity (%) at different temperatures was compared with that obtained at optimal temperature.

Thermal stability. Enzyme solutions were incubated in a water bath at four different temperatures (40, 50, 60, and 70°C for PPO; 50, 60, 70, and 80°C for

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POD) are for different time intervals (2, 4, 6, 8, 10, 20, 30 min). PPO activity was determined at 25°C and pH 6.5, using catechol as the substrate. POD activity was determined at 25°C and pH 6.0, using guaiacol as the substrate. The percentage of residual activity was calculated by comparison with unheated enzyme.

Kinetic properties. PPO and POD activities were assayed with their specific substrates: catechol and guaiacol (0–80 mmol/l). The enzyme reactions proceeded at pH 6.5 and 25°C for PPO and pH 6.0 and 25°C for POD. The enzyme behaviour was explained by the Michaelis-Menten equation, and kinetic parameters (K_m and V_{max}) were calculated by Linewaver-Burk plot (Lineweaver & Burk 1934).

Inhibition test. Oxalic acid (0.005–0.025 mmol/l), citric acid (0.005–0.025 mmol/l), L-cysteine (0.010 to 0.050 mmol/l), ascorbic acid (0.010–0.050 mmol/l) and 4-hexylresorcinol (4-HR, 0.010–0.050 mmol/l) were dissolved in the assay medium. The activity was measured at 25°C and pH 6.5 for PPO and at pH 6.0 for POD to determine the effect of the above compounds on enzyme activity. Percentage inhibition was determined using the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_t)/A_0] \times 100$$

where: A_0 – initial PPO or POD activity (without inhibitor); A_t – PPO or POD activity with inhibitor

Colour, PPO and POD activities of loquat samples during storage at different temperatures. Loquat fruits were selected for uniform size and colour, randomly divided into 2 groups; one group were stored at 5°C for 28 days, and the other group at 25°C for 4 days. Loquat samples were taken during storage for the measurements of pulp colour index and PPO and POD activities at 7-day intervals storage at 5°C or daily storage at 25°C. Three replicates were used, each with 3 kg of loquat per treatment, and the experiment was conducted twice.

The samples were peeled and the fresh pulp was incubated at 25°C for 10 minutes. The pulp colour was determined with a compacted tristimulus chromameter (Minolta CR-400; Minolta, Tokyo, Japan): The readings were expressed as L^*, a^*, and b^* parameters. The PPO and POD extraction and assays were performed as described above.

Statistical analysis. All measurements were conducted in triplicates and all experiments were repeated twice. The data obtained were subjected to statistical analysis using SPSS 20.0 software (IBM, Armonk, USA). In this study, the data were presented as mean values ± standard deviation (SD), the significant differences of storage days were tested using the analysis of the Student-Neuman-Keuls (S-N-K) test ($P = 0.05$) .

RESULTS AND DISCUSSION

pH optimum. As seen in Figure 1A, the PPO activity curve had two activity peaks at pH 4.5 and 6.5, using catechol as the substrate. PPO activity was higher in acidic pH than in neutral pH. This result may be explained as an effect caused by the presence of isoenzymes, since two pH optima have been reported in other loquat cultivars, e.g. about pH 4.0 and pH 6.5 for Algerie loquat (Selles-Marchart *et al*. 2006), and pH 4.5 and pH 6.8 for Goldnugget loquat (Şener *et al*. 2011a). However, only one pH optimum of 4.5 was found in the Mogi loquat (Ding *et al*. 1998a). It was concluded that the optimal pH of PPO in loquat varies depending on the cultivar. We found the physiological pH of the loquat cv. Ninghaibai at commercial maturity to be 4.0, at which PPO relative activity was about 80%. A more

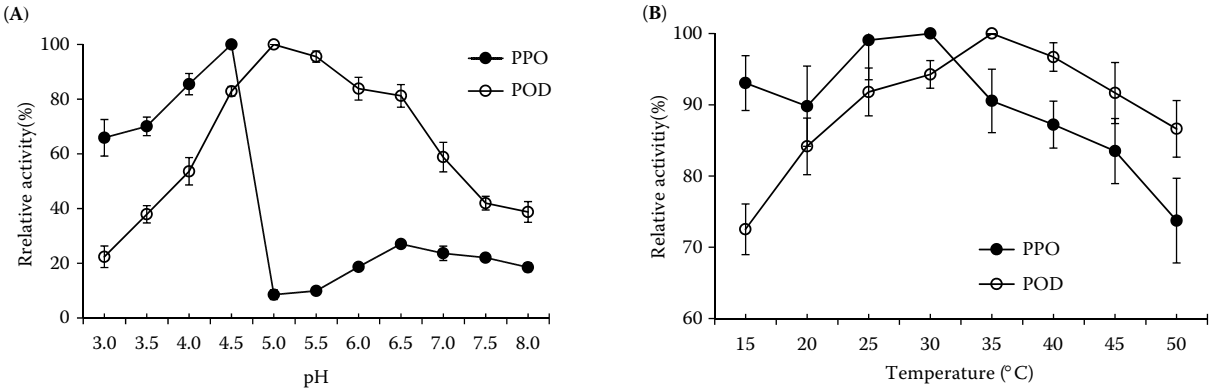

Figure 1. Effect of pH on loquat PPO and POD relative activities (**A**) and effect of temperature on loquat PPO and POD relative activities (**B**)

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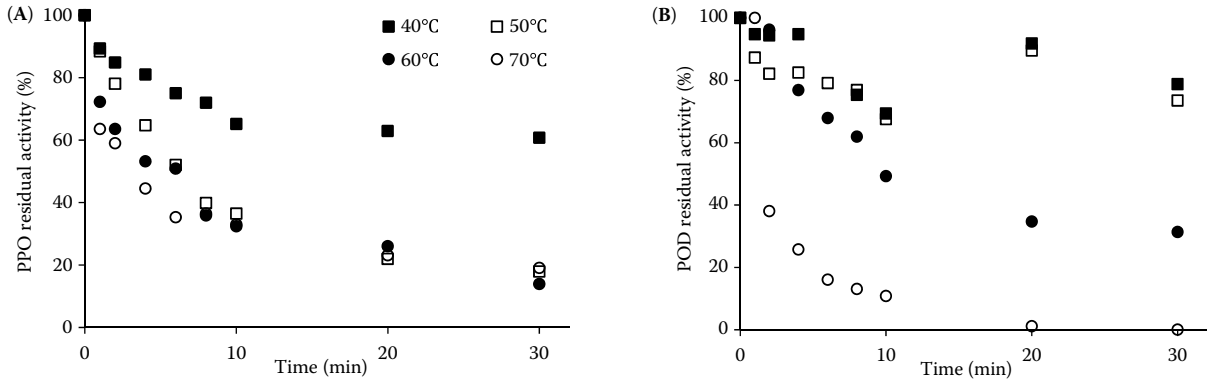

Figure 2. Thermal stability of loquat PPO (**A**) and POD (**B**)

effective PPO activity in the processing or due to mechanical damage of loquat is predictable, because there is an optimal pH environment in this loquat.

In the case of POD, maximum activity was found at pH 5.0 by using guaiacol as the substrate (Figure 1a), retaining > 50% residual activity over a wide pH range (4.0–7.0). This result is similar to that with the loquat cv. Zaozhong 6 at pH 5.0 (Lin *et al.* 2007), but is slightly lower than that obtained with *Moringa oleifera* L. leaves (Khatun *et al.* 2012). At the physiological pH 4.0, the residual activity of POD in this loquat was about 50%, indicating that the commercial maturity seems to have a significant effect on the expression of POD activity.

Optimum temperature. PPO showed maximum activity at 30°C in the temperature range of 15–50°C (Figure 1B). This value was the same as those obtained with Mogi (Ding *et al.* 1998a), Algerie (Selles-Marchart *et al.* 2006), and gold nugget (Şener *et al.* 2011a,b) loquats. POD was found to have a temperature optimum at 35°C (Figure 1B): The same temperature optimum was detected for POD from loquat cv. Zaozhong 6 (Lin *et al.* 2007). PPO and POD retained > 70% residual activity in a temperature range of 15–50°C, indicating that the ambient temperature does not seem to affect negatively the expression of these enzymes.

Thermal stability. The residual activities of PPO and POD were studied as a function of temperature to determine their thermodynamic properties. As seen in Figure 2, the activities of PPO and POD decreased with increasing temperature and treatment time. PPO is not a very heat-stable enzyme as seen in Figure 2A. After the treatment at 40°C for 10 min, PPO retained 65% of its activity, whereas at 50, 60, and 70°C for the same time interval, it retained less than 36% of its activity. The residual activity showed changes of less than 20% although the time interval increased. The above results indicate that PPO is stable at a temperature of 40°C, whereas it is partly denatured in the temperature range of 50–70°C. This may be due to the tertiary structure changes at higher temperatures. POD showed greater stability than PPO in a temperature range of 50–70°C, displaying 78 and 30% of residual activity after 30 min of incubation at 50 and 70°C, respectively (Figure 2B), POD activity was found to be completely inactive after 20 min of incubation at 80°C (98% loss of activity). The data indicated that high temperature blanching is necessary to control the enzymatic browning caused by PPO and POD in loquat cv. Ninghaibai.

The rate constants for the kinetic inactivation k were calculated from the slope of the curve at each temperature. The temperature dependence of k was evaluated using the Arrhenius equation:

$$\ln k = \ln A - \Delta E^{\#}/RT$$

where: A – Arrhenius constant; $\Delta E^{\#}$ – activation energy (J/mol); R – universal gas constant (8.314 J/mol); T (K) – absolute temperature in Kelvin

Table 1. Transition state parameters for the heat inactivation of crude loquat PPO and POD

Enzymatic extract	$\Delta E^{\#}$ (kJ/mol)	$\Delta G^{\#}$ (kJ/mol)	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J/mol/K)
PPO	39.74	362.02 ± 15.90	37.01 ± 0.11	–990.12 ± 9.93
POD	94.65	371.41 ± 17.95	91.84 ± 0.11	–826.12 ± 21.91

$\Delta E^{\#}$ – activation energy for crude PPO and POD heat inactivation; $\Delta G^{\#}$ – Gibbs free energy for enzyme inactivation; $\Delta H^{\#}$ – enthalpy change; $\Delta S^{\#}$ – entropy change

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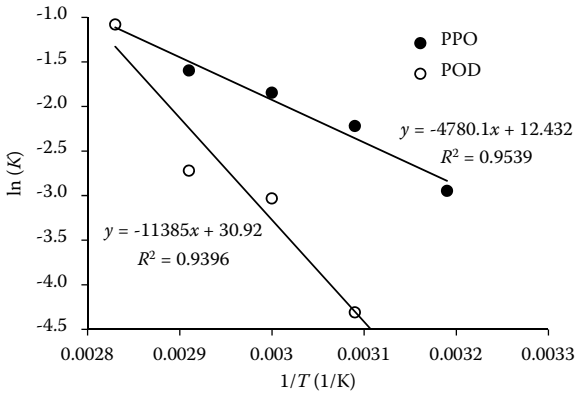

Figure 3. Arrhenius plot for heat inactivation of loquat PPO and POD

The activation energy ($\Delta E^{\#}$) was calculated from the slope of the straight line in Figure 3. Other activation parameters, namely, $\Delta G^{\#}$ (Gibbs free energy for enzyme inactivation), $\Delta H^{\#}$ (enthalpy change, a measure of the number of non-covalent bonds broken), and $\Delta S^{\#}$ (entropy change, a measure of net enzyme and solvent disorder), were calculated using the relationships given below as described previously (Forsyth *et al.* 1999)

$$\Delta G^{\#} = RT \ln(kT/K_B h)$$
$$\Delta H^{\#} = \Delta E^{\#} - RT$$
$$\Delta S^{\#} = (\Delta H^{\#} - \Delta G^{\#})/T$$

where: $\Delta E^{\#}$ – activation energy for crude PPO and POD heat inactivation; $\Delta G^{\#}$ – Gibbs free energy for enzyme inactivation; $\Delta H^{\#}$ – enthalpy change; $\Delta S^{\#}$ – entropy change; K_B – Boltzman constant (1.3806×10^{-23} J/K); h – Planck constant (6.6261×10^{-34} J·s)

In summary, $\Delta E^{\#}$ and $\Delta H^{\#}$ for PPO were lower than those for POD. Then considering the lower values of $\Delta E^{\#}$ and $\Delta H^{\#}$ for PPO in comparison with those for POD, it is possible to state that loquat cv. Ninghaibai PPO is less stable than POD (Table 1). There are no literature reports regarding loquat thermostability difference between PPO and POD. The results are similar to those for melons, showing that POD is more thermostable than PPO (Chisari *et al.* 2007a), but different from those for strawberries (Chisari *et al.* 2007b).

Kinetic properties. The effect of catechol concentration on PPO activity and guaiacol concentration on POD activity were investigated, and the results are presented in Figure 4. As seen in Figure 4A, when catechol concentration was low, the activity of PPO increased in a dose-dependent manner. However, when the concentration exceeded 60 mmol/l, PPO activity increased very little with further increases in concentration. This is qualitatively similar to the results obtained with strawberries (Dalmadi *et al.* 2006). By using the Lineweaver-Burk plot, the

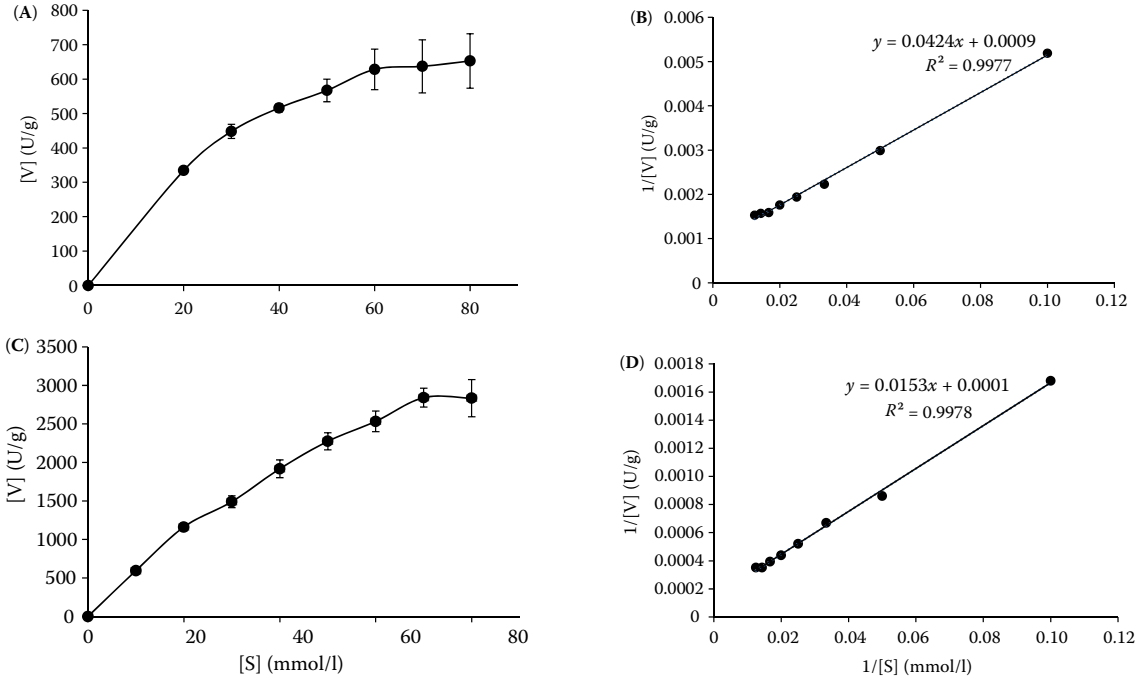

Figure 4. Activity of loquat cv. Ninghaibai PPO as a function of catechol concentration (**A**) and POD as a function of guaiacol concentration (**C**), Lineweaver-Burk plot for loquat PPO (**B**) and POD (**D**)

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apparent K_m for catechol was calculated. The value obtained was 47.11 mmol/l, which is smaller than those for the isoenzyme A of Akko loquats (Şener *et al*. 2011b), Algerie loquats (Selles-Marchart *et al*. 2006), and Thompson seedless grapes (Zheng *et al*. 2012). The data indicated that the loquat cv. Ninghaibai PPO has a greater affinity when compared to those of the above plants.

As seen in Figure 4C, POD activity displays the same trend with the changes in guaiacol concentration, and the K_m value from Michaelis-Menten kinetics was 153.00 mmol/l. This guaiacol K_m value is higher than that reported for the POD from loquat cv. Zaozhong 6 (Lin *et al*. 2007), suggesting that POD of cv. Ninghaibai has a low apparent affinity toward guaiacol in comparison to that of loquat cv. Zaozhong 6.

Compounds inhibition. The effects of inhibitors on loquat cv. Ninghaibai PPO and POD activities were studied at five concentrations with oxalic acid, citric acid, L-cysteine, ascorbic acid, and 4-HR, using catechol and guaiacol as substrates, respectively. The results are shown as percentage inhibition in Table 2. With the increase in inhibitors concentration PPO activity was decreased. 4-HR was the most effective of the inhibitors tested for PPO and POD activities, followed by oxalic acid, and L-cysteine and ascorbic acid. Citric acid had only weak inhibitory effects on PPO and POD activities.

There are several types of mechanisms that inhibit the activities of PPO and POD. First, some inhibitors such as citric acid and oxalic acid chelate metallic ions, copper ions for PPO and ferric ions for POD (Sun *et al.* 2012). Second, some compounds compete with the substrates and combine so that fewer amounts of the substrates are catalysed, such as L-cysteine (Zheng *et al.* 2012). Third, reducing agents, such as ascorbic acid, reduce the block enzymatic browning by interacting with *o*-quinone and other substances (Gómez-López 2002). Fourth, when the concentrations of acidic substances increase, the pH changes, far from the optimum pH of PPO and POD (Sun *et al.* 2012). Citric acid not only changes the pH value, but also acts as a chelator to chelate copper ions and ferric ions. 4-HR can directly interact with the PPO and POD (Arias *et al.* 2007) and, therefore, 4-HR is the most effective on these enzymes. In short, different inhibitors have different influences on PPO and POD and therefore particular measures are required to control certain systems. These results are beneficial for understanding the best applicable methods to the browning control during the processing of loquat cv. Ninghaibai.

Table 2. Effects of various compounds on PPO and POD activities

Compounds	Concentration (mmol/l)	Inhibition (%)	
		PPO	POD
Oxalic acid	0.005	5.37 ± 3.23	46.74 ± 2.63
	0.010	59.14 ± 1.08	91.47 ± 0.41
	0.015	58.06 ± 1.08	98.82 ± 0.70
	0.020	97.85 ± 0.36	99.70 ± 0.34
	0.025	99.64 ± 0.00	99.80 ± 0.40
Citric acid	0.005	6.00 ± 5.38	10.87 ± 9.04
	0.010	9.25 ± 5.39	11.20 ± 7.01
	0.015	20.80 ± 7.47	24.26 ± 6.63
	0.020	28.50 ± 8.87	57.77 ± 6.25
	0.025	37.89 ± 6.53	69.09 ± 4.36
L-Cysteine	0.010	53.70 ± 1.61	90.96 ± 3.18
	0.020	85.30 ± 3.89	95.82 ± 1.68
	0.030	96.93 ± 0.83	98.02 ± 0.22
	0.040	97.81 ± 0.52	98.92 ± 0.07
	0.050	98.99 ± 0.32	99.25 ± 0.12
Ascorbic acid	0.010	11.75 ± 5.61	17.31 ± 13.30
	0.020	22.06 ± 0.36	61.55 ± 14.67
	0.030	58.04 ± 18.81	62.61 ± 15.20
	0.040	70.62 ± 20.40	95.95 ± 3.98
	0.050	75.90 ± 3.60	99.65 ± 0.30
4-HR	0.010	59.25 ± 26.21	58.14 ± 12.19
	0.020	86.53 ± 0.90	71.46 ± 10.42
	0.030	100.00 ± 0.00	83.79 ± 4.81
	0.040	100.00 ± 0.00	87.90 ± 2.93
	0.050	100.00 ± 0.00	92.01 ± 1.06

Loquat pulp browning. Polyphenols were oxidased to *o*-quinones in the presence of oxygen, and the final polymerised product was an undesirable brown, red, or black pigment displaying to what extent the colour can be affected by enzymatic browning (López-Nicolás *et al*. 2007). As seen in Table 3, the changes in L^*, a^*, and b^* values were chosen to describe the loquat pulp browning during storage at 5 and 25°C. Under the protection of loquat skin, the pulp colour in storage was mainly associated with the enzyme activity of the sample and the pulp changed irregularly. The variations in PPO and POD activities are shown in Figure 5. PPO activity at 5°C decreased during the first 7 days, increased in the following 14 days, and then proceeded to decline. POD activity at 5°C decreased after a rise in the first 7 days as seen in Figure 5B, PPO activity at 25°C continued declining, then increased on the third

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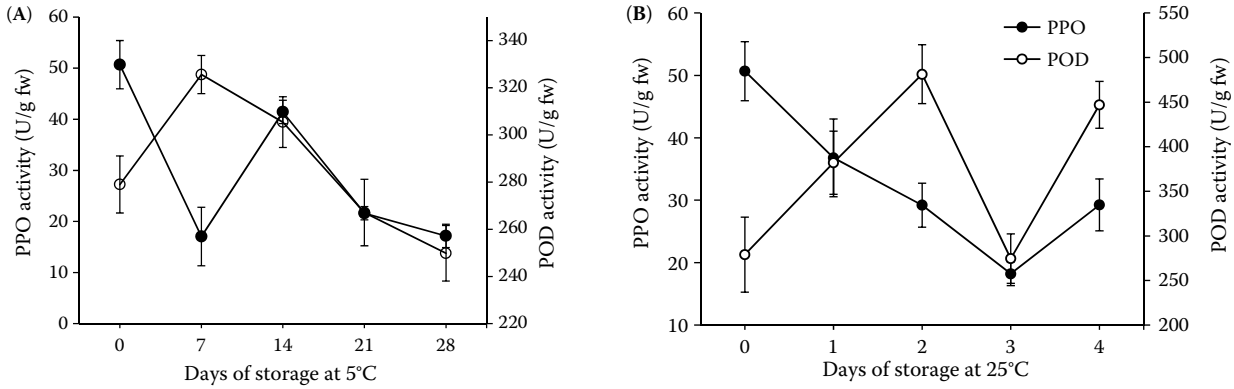

Figure 5. Activity of crude loquat PPO and POD during storage at 5°C (**A**) and 25°C (**B**)

day, while POD activity increased in the first 2 days, decreased the next day, then continued increasing. Parameters L^* showed a significant negative correlation with PPO activity ($r = -0.810$, $P < 0.05$), however, the relationship between L^* and POD activity was not significant ($P > 0.05$). The parameters a^* and b^* showed no significant relationships with PPO and POD activities ($P > 0.05$). The change in colour was mostly due to the changes in the lightness (L^*) value, whereas green–red (a^*) and blue–yellow (b^*) did not have any significant influence on those parameters (Chisari *et al*. 2007b). Therefore, PPO is more correlated with loquat browning than POD. On the other hand, POD did not seem to reveal correlations with the loquat browning. The results, similar to those shown with loquat cv. Luoyangqing flesh browning, positively correlated with PPO activity during the chilling injury (Cai *et al*. 2006b). The role of PPO in the browning of fruits and vegetables has been reported in several studies regarding various products, such as tomatoes (Spagna *et al*. 2005) and water caltrop pericarp (Ciou *et al*. 2011). In previous studies, we found a clear correlation between PPO and browning. However, because of the low hydrogen peroxide content in fruits and vegetables, the role of POD in enzymatic browning was also under question (Chisari *et al*. 2007b). These results suggested an important role played by PPO in enzymatic browning of stored loquat cv. Ninghaibai.

Table 3. The colour change of pulp of loquat after storage at 5°C and 25°C

Temperature	Days of storage	L^*	a^*	b^*
5°C	0	68.69 ± 2.24^{b}	−2.78 ± 0.33^{ab}	22.39 ± 0.22^{ab}
	1	70.62 ± 2.72^{ab}	−2.75 ± 0.14^{ab}	25.57 ± 2.31^{a}
	2	71.34 ± 2.10^{ab}	−3.05 ± 0.14^{b}	23.65 ± 2.13^{ab}
	3	72.62 ± 0.57^{a}	−2.31 ± 0.81^{a}	24.30 ± 1.31^{ab}
	4	71.44 ± 2.26^{ab}	−2.78 ± 0.13^{ab}	21.74 ± 2.78^{b}
25°C	0	68.69 ± 2.24^{b}	−2.78 ± 0.33^{a}	22.39 ± 0.22^{ab}
	7	69.15 ± 2.94^{ab}	−2.46 ± 0.26^{a}	22.69 ± 1.39^{a}
	14	71.63 ± 0.99^{ab}	−2.26 ± 0.42^{a}	25.01 ± 2.44^{a}
	21	71.81 ± 1.69^{a}	−2.06 ± 0.75^{a}	24.56 ± 3.00^{a}
	28	72.14 ± 0.44^{a}	−2.37 ± 0.36^{a}	23.91 ± 2.12^{a}

^{a,b}means in a column followed by the same letter are not significantly different at the $P \leq 0.05$ level according to S-N-K test

CONCLUSION

This study, for the first time, reports the characterisation of PPO and POD and their roles in the browning of the loquat cv. Ninghaibai. The data show that PPO and POD have maximum activity at pH 4.5 and 5.0, respectively, while optimum temperature is 30°C for PPO and 35°C for POD, respectively. PPO was much more thermolabile than POD, losing more than 40% of activity after 30 min of heating at 40°C. PPO and POD activation energy were 39.74 and 94.65 kJ/mol, respectively. K_m values were 47.11 mmol/l for PPO with catechol as the substrate and 153.00 mmol/l for POD with guaiacol as the substrate. Additionally, 4-HR, oxalic acid, and L-cysteine showed strong inhibitive effects on PPO and POD. Especially in the stored loquat fruit, PPO showed a significant negative correlation with the pulp browning. Overall, the results obtained in this study indicate that PPO and POD characteristics are variable and cultivar dependent, and that PPO of cv. Ninghaibai plays an important role in browning, which is an item of knowledge that will substantially aid in controlling the enzymatic browning taking place during processing and storage of loquat cv. Ninghaibai.

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