Partial Characterization of Peroxidase and Polyphenol Oxidase Activities in Blackberry Fruits

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A partial characterization of peroxidase (POD) and polyphenol oxidase (PPO) activities in blackberry fruits is described. Two cultivars of blackberry (Wild and Thornless) were analyzed for POD and PPO activities. Stable and highly active POD and PPO extracts were obtained using insoluble poly(vinylpyrrolidone) and Triton X-100 in 0.05 M sodium phosphate, pH 7.5, buffer. Blackberry POD and PPO activities have a pH optimum of 6.5, in a reaction mixture of 0.2 M sodium phosphate. Optimal POD activity was found with 3% *o*-dianisidine. Maximum PPO activity was found with catechol (catecholase activity) followed by 4-methylcatechol. Polyacrylamide gel electrophoresis of blackberry extracts under non-denaturing conditions resolved in various bands. In the POD extracts of Wild fruits, there was only one band with a mobility of 0.12. In the Thornless POD extracts there were three well-resolved bands, with R_f values of 0.63, 0.36, and 0.09. Both the Wild and Thornless blackberry cultivars produced a single band of PPO, with R_f values of 0.1 for Wild and 0.06 for Thornless.

Keywords: *Blackberry; peroxidase; polyphenol oxidase; characterization; native PAGE*

INTRODUCTION

The postharvest life of blackberry fruit is relatively short due to its extremely fragile structure and high rate of fruit respiration. Precooling treatment of blackberries to −0.6 to 0 °C and 90−95% relative humidity is essential, especially when berries are destined to be shipped to distant markets (Skrede, 1996). The fruits cannot be stored for >2−3 days because longer storage results in loss of quality. Morris et al. (1981) found that mechanically harvested blackberries had raw and processed quality compared to hand-picked fruits regardless of berry temperature. Storage of machine-harvested fruit in 20 and 40% CO_2 at 20 °C for up to 48 h maintained their raw and processed quality. Also, these authors stated that the use of high-CO_2 storage atmospheres with blackberries held at 20 °C partially offset the need for refrigeration to reduce the postharvest quality loss.

Peroxidase (POD, EC 1.11.1.7, donor:hydrogen-peroxide oxidoreductase) and polyphenol oxidase (PPO, EC 1.14.18.1; monophenol dihydroxyphenylalanine:oxygen oxidoreductase), together with lipoxygenase (EC 1.13.1.13) and catalase (EC 1.11.1.6) have been considered the principal enzymes responsible for quality deterioration in most fruits and vegetables (Whitaker, 1985). POD can contribute to adverse changes in the flavor, color, texture, or nutrient value of raw and processed fruits (Fils et al., 1985). Generally, the enzyme is found in a glycosylated form and associated to membranes, although soluble isoenzymes were also encountered in banana fruit (Haard and Tobin, 1971) and tomato fruit (Thomas et al., 1981). Miesle et al. (1991) observed that peroxidase has various functions in the ripening process, including changes in cell wall plasticity and anthocyanin breakdown (Yokotsuka and Singleton, 1997). The enzyme participates in the later stages of the lignin-forming process (Mäder and Füssi, 1982; Wakamatsu and Takahama, 1993) and in the protection of tissues damaged by, or infected with, pathogenic microorganisms (Wakamatsu and Takahama, 1993; Biles and Martyn, 1993).

The presence of PPO in fruit and vegetable tissues is of concern to food processors and researchers. PPO catalyzes the formation of highly active quinones that react with amino or sulfhydryl groups in proteins or enzymes. These reactions lead to changes in physical, chemical, or nutritional characteristics of proteins and, in many cases, to the inactivation of enzymes including PPO (Mayer and Harel, 1979). Quinones also lead to polymerization and condensantion reactions between proteins and polyphenols, forming brown pigments (Lee, 1991; Mathew and Parpia, 1971). PPO activity may also be responsible for the loss of the red color of some fruits through the degradation of anthocyanin pigments (Markakis, 1974).

POD and PPO activities in other berry fruits such as raspberry (Gonzalez et al., 1999), strawberry (Wesche-Ebeling and Montgomery, 1990a,b; Marcos et al., 1995), and blueberry (Kader et al., 1997; Miesley et al., 1991) have been reported. However, little is known about the POD and PPO in blackberry fruits and their involvement in anthocyanin degradation. The purpose of the present study was to develop a procedure for the extraction of POD and PPO from blackberry fruit tissues, their partial characterization, and the study of differences among blackberrry cultivars (Wild and Thornless) in terms of POD and PPO enzymes.

MATERIALS AND METHODS

Plant Material. Blackberry fruits of two cultivars (Wild and Thornless) were obtained from commercial producers in the region of Valle del Jerte (Cáceres, Spain) and transported

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Table 1. Physicochemical Characteristics and PPO and POD Activities of Blackberry Cultivars[a]

characteristic	blackberry cultivar	
	Wild	Thornless
pH	4.45 ± 0.01a	3.64 ± 0.02b
titratable acidity (g of citric acid/100 g of fw)	0.37 ± 0.01a	1.16 ± 0.00b
soluble solids (°Brix)	15.52 ± 0.07a	11.96 ± 0.12b
moisture content (% at 20 °C)	77.75 ± 1.12a	83.10 ± 2.25a
total solids (% at 20 °C)	22.25 ± 0.7a	16.90 ± 0.9b
soluble proteins (mg/100 g of fw)	221.51 ± 3.45a	181.43 ± 1.77a
PPO activity (ΔOD/min/g of fw)	1.30 ± 0.1a	1.85 ± 0.1b
POD activity (ΔOD/min/g of fw)	0.73 ± 0.01a	0.63 ± 0.04b

[a] Different letters in the same row indicate significant differences ($p \leq 0.05$); fw, fresh weight; values are the mean (±SD) of three determinations.

under refrigeration to Instituto del Frío (Madrid, Spain) within 12 h after harvest. On arrival, undamaged fruits were selected with the characteristics shown in Table 1. One kilogram of each cultivar was homogenized using a blender (Osterizer), and the pulps obtained were used immediately for physical and physicochemical determinations. Another 2 kg of each cultivar was immediately frozen in liquid nitrogen and stored at −70 °C until biochemical analyses were performed.

pH and Titratable Acidity. Ten grams of blackberry pulp was minced and blended with 40 mL of deionized water in a Sorvall Omnimixer. The pH was measured at this temperature with a Crison pH-meter. After determination of the pH, the solution was titrated with 0.1 N NaOH up to pH 8.1. Results were expressed as citric acid percentage [grams of citric acid per 100 g of fresh weight (fw)] (AOAC, 1984).

Soluble Solids. Soluble solids were measured with an Atago digital refractometer dbx-30 at 20 °C. Results were reported as degrees Brix.

Total Solids and Moisture Content. The AOAC (1984) vacuum oven method was modified, using a microwave oven operating at 200 W for 25−30 min, as described in Cano et al. (1990).

Enzyme Extraction. Enzymatic extracts were prepared so that POD and PPO activities were determined at the highest level (Table 2). In all assays, 10 g of pulverized liquid nitrogen frozen pulp was homogenized and mixed with 40 mL of extraction buffer for 2 h at 4 °C in the dark. The homogenates were centrifuged in a Sorvall model RC-5B refrigerated superspeed centrifuge for 30 min at 18000*g* and 4 °C until assayed for POD and PPO activities.

Peroxidase Activity. POD activities were determined at 25 °C by measuring the initial rate of increase in absorbance at 485 nm. The assay mixture contained 0.3 mL of extracts, 2.4 mL of 0.2 M sodium phosphate buffer, pH 6.5, 0.1 mL of hydrogen peroxide 20% (v/v), freshly prepared [2.9 mL of H_2O_2 (33%) and 2.1 mL of deionized water], and 0.2 mL of 3% (w/v) *o*-dianisidine. The enzyme activity was determined by measuring the slope of the reaction line at zero time (initial rate). The enzyme activity unit was defined as the change in absorbance per minute per milligram of protein extracted (specific activity) or the change in absorbance per minute per gram of tissue.

Polyphenol Oxidase Activity. PPO activities were determined at 25 °C by measuring the initial rate of increase in absorbance at 420 nm. Unless otherwise stated, activity was assayed in 3 mL of reaction mixture, consisting of 2.7 mL of 0.1 M cathecol in 0.2 M sodium phosphate buffer, pH 6.5, plus 0.3 mL of prepared enzyme, with a Perkin-Elmer spectrophotometer model Lambda 15. The enzyme activity was determined by measuring the slope of the reaction line at zero time (initial rate). The enzyme activity unit was defined as the change in absorbance per minute per milligram of protein extracted (specific activity) or the change in absorbance per minute per gram of tissue.

Protein Determination. Protein concentrations of the extracts were measured according to the Bradford (1976) method, measuring optical density (OD) at 595 nm, with bovine serum albumin as a standard.

Substrate Specificity. The substrates used for the specificity study are listed in Tables 4 and 5. All compounds were prepared in 0.2 M sodium phosphate buffer, pH 6.5.

Effect of pH. A study was made on the effect of pH on the *o*-dianisidine and catechol oxidation by blackberry POD and PPO. Enzyme activity was determined in 0.2 M sodium phosphate buffer at different pH values, ranging from 5.0 to 8.0.

Effect of Substrate Concentration. Solutions of catechol and 4-methylcatechol varying in concentration from 6 mM to 0.1 M for catechol and from 10 mM to 0.8 M for 4-methylcatechol were employed to study the effect of substrate concentration in Wild and Thornless blackberry extracts. In a cuvette, 0.3 mL of enzyme solution was mixed with 2.7 mL of catechol or 4-methylcatechol at different concentrations in 0.2 M sodium phosphate buffer at pH 6.5. Michaelis constants (K_m) and maximum velocities (V_{max}) of PPO were calculated from a plot of 1/activity versus 1/substrate concentration according to the method of Lineweaver and Burk (1934).

Polyacrylamide Gel Electrophoresis (PAGE). Electrophoresis was performed on a Miniprotean II dual slab cell unit (Bio-Rad). Runs were performed at constant current intensity (35 mA per plate), with cooling to 4 °C for 30 min. Polyacryalmide gels (10%) were prepared according to the method of Laemmli (1970) without SDS (native conditions). After running, the gels were incubated at 10 g/L in a 0.8 M catechol solution (for the PPO samples) or in *o*-dianisidine and H_2O_2 (20% v/v) (for the POD samples) in 0.2 M sodium phosphate buffer, pH 6.5, for 15 min.

Data Analysis. Values are the average of three independent determinations. These results were analyzed for variance (ANOVA) and statistical significance by *t* test with Statgraphics and InStat software packages.

RESULTS AND DISCUSSION

Characteristics of Blackberry Fruits. Table 1 shows the physicochemical characteristics of the blackberry fruits studied, Wild and Thornless cultivars. Thornless fruits showed the lower pH (3.64 ± 0.02) and the higher titratable acidity (0.37 ± 0.01 g of citric acid/100 g of fw). Soluble solids were significantly higher in Wild blackberries, indicating that these fruits were sweetest and less acidic. These sensorial properties are characteristics of these blackberries. With regard to the presence of oxidative enzymes, PPO activity was significantly higher in Wild fruits (1.85 ± 0.1 ΔOD/min/g of fw), whereas Thornless fruits had an enzyme activity of 1.30 ± 0.1 ΔOD/min/g of fw. POD activities in blackberry fruits were also significantly different between cultivars but to a minor magnitude compared with the differences found in PPO activities. Previous studies in PPO activity in other berry fruits such as raspberry showed values ranging from 0.64 to 1.21 ΔOD/min/g of fw (González et al., 1999). In this way blackberry fruits showed higher PPO activity than raspberries, indicating that the quality problems related to oxidative pathways could be more severe in blackberries. However, other factors can be taken into account such as phenol composition and content, metals, ions, and ascorbic acid content (Wrolstrad, 1983).

Selections of Conditions for the Enzyme Assay. Several buffer compositions were employed to select the most suitable to extract PPO and POD from blackberry tissues (Table 2). Increases in the molar concentration of sodium phosphate buffer did not affect the extraction rates of PPO or POD activity. It was observed that the addition of 0.5% (w/v) Triton-X 100 caused a 3-fold increase in the extraction of PPO and POD in Wild

Table 2. Influence of Extraction Buffer Composition on Blackberry Cultivar PPO and POD Activities

	blackberry cultivar			
	Wild		Thornless	
buffer composition	PPO activity[a] (ΔOD/min/g of fw)	POD activity (ΔOD/min/g of fw)	PPO activity (ΔOD/min/g of fw)	POD activity (ΔOD/min/g of fw)
0.05 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP	0.365 ± 0.06	0.245 ± 0.1	0.215 ± 0.001	0.168 ± 0.002
0.05 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP + 0.5% (w/v) Triton X-100	1.01 ± 0.04	0.784 ± 0.1	1.35 ± 0.04	0.536 ± 0.03
0.05 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP + 0.1% (w/v) NaCl	0.342 ± 0.02	0.210 ± 0.03	0.210 ± 0.03	0.120 ± 0.06
0.05 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP + 0.5% (w/v) Triton X-100 + 0.1% (w/v) NaCl	0.856 ± 0.06	0.575 ± 0.01	1.10 ± 0.06	0.458 ± 0.08
0.1 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP + 0.5% (w/v) Triton X-100	1.08 ± 0.03	0.695 ± 0.02	1.08 ± 0.04	0.480 ± 0.01
0.2 M sodium phosphate (pH 7.0) + 4% (w/v) insoluble PVP + 0.5% (w/v) Triton X-100	1.16 ± 0.08	0.740 ± 0.08	0.983 ± 0.01	0.469 ± 0.01

[a] Activity values are the average of three independent determinations ± standard deviation; fw, fresh weight.

Table 3. Effect of Reaction Mixture Buffer Molarity on Blackberry PPO and POD Activities

	blackberry cultivar			
	Wild		Thornless	
buffer composition	PPO activity[a] (ΔOD/min/g of fw)	POD activity (ΔOD/min/g of fw)	PPO activity (ΔOD/min/g of fw)	POD activity (ΔOD/min/g of fw)
0.2 M sodium phosphate (pH 7.5)[b]	1.30 ± 0.1	0.650 ± 0.01	1.85 ± 0.1	0.610 ± 0.002
0.1 M sodium phosphate (pH 7.5)	1.38 ± 0.03	0.523 ± 0.1	0.930 ± 0.03	0.630 ± 0.01
0.05 M sodium phosphate (pH 7.5)	1.45 ± 0.1	0.562 ± 0.05	0.965 ± 0.08	0.690 ± 0.002

[a] Values are the average of three independent determinations ± standard deviation; fw, fresh weight. [b] pH 7.5 was selected by a previous pH study of optimal conditions for spectrophotometric PPO and POD assays.

Table 4. Effect of Substrate on Blackberry POD Activity[a]

	blackberry cultivar			
	Wild		Thornless	
substrate[a]	POD activity (ΔOD/min/g of fw)	POD activity (ΔOD/min/mg of protein)	POD activity (ΔOD/min/g of fw)	POD activity (ΔOD/min/mg of protein)
p-phenylenediamine[b]	0.65 ± 0.01a	4.63 ± 0.2a	0.61 ± 0.02a	4.35 ± 0.03a
o-dianisidine	0.73 ± 0.01b	5.21 ± 0.3b	0.63 ± 0.04a	4.49 ± 0.02a

[a] Concentration of the substrates = 3% (w/v). [b] Different letters in the same column indicate significant differences; fw, fresh weight; values are the mean (±SD) of three determinations.

Table 5. Effect of Substrate on Blackberry PPO Activity[a]

	blackberry cultivar			
	Wild		Thornless	
substrate (0.003 M)[a]	PPO activity	PPO activity (ΔOD/min/mg of protein)	PPO activity (ΔOD/min/g of fw)	PPO activity (ΔOD/min/mg of protein)
4-methylcatechol	0.60 ± 0.03	4.28 ± 0.25	0.24 ± 0.0	0.88 ± 0.04
protocatechin	0.28 ± 0.04	2.00 ± 0.25	0.06 ± 0.00	0.23 ± 0.02
catechol	0.69 ± 0.02	4.94 ± 0.26	0.51 ± 0.05	1.84 ± 0.16
catechin	0.23 ± 0.00	1.64 ± 0.8	0.11 ± 0.01	0.39 ± 0.02
caffeic acid	0.20 ± 0.01	1.42 ± 0.08	0.27 ± 0.01	0.97 ± 0.06
chlorogenic acid	0.39 ± 0.00	2.76 ± 0.13	0.40 ± 0.01	1.44 ± 0.06
L-DOPA	0.26 ± 0.01	1.76 ± 0.09	ND	ND
p-coumaric acid	0.25 ± 0.01	1.80 ± 0.08	ND	ND

[a] Values are the average of three independent determinations ± standard deviation; fw, weight; ND, not detectable.

blackberries (0.856 and 0.575 ΔOD/min/g of fw, respectively) and a 5-fold increase in the extraction of PPO and POD in Thornless ones (1.10 and 0.458 ΔOD/min/g of fw, respectively). The increase of ionic strength by the addition of sodium chloride did not increase enzyme activity (Table 2). Therefore, a 0.05 M sodium phosphate buffer containing 4% (w/v) insoluble poly(vinylpyrrolidone) (PVP) and 0.5% (w/v) Triton X-100 was employed for all enzyme assays. The use of PVP has been reported to produce good results in POD and PPO extractions from different plant tissues, such as banana (Galeazzi et al., 1981), strawberry (Wesche-Ebeling and Montgomery, 1990a,b), papaya (Cano et al., 1996), and raspberry (González et al., 1999).

Tanning reactions during enzyme extraction can cause partial inactivation of the enzymes. To avoid these reactions, reducing agents are often added during extraction but must be removed before the assay. Alternatively, phenolic substrates must be removed prior to the assay with insoluble PVP (Mayer and Harel, 1979). In the present work, the use of insoluble PVP alone was not enough to remove all of the phenols and, consequently, extracted PPO and POD activities were very low. However, the use of a detergent (Triton X-100), together with insoluble PVP, improved the extraction by combining polar and nonpolar binding capacities.

pH Optimization for Extraction and Activity. The pH stability was measured by extracting the PPO

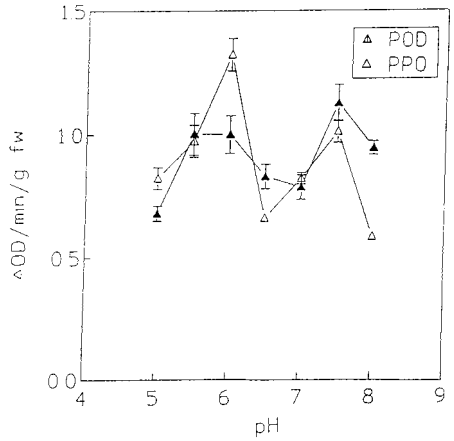

Figure 1. Effect of buffer extraction of spectrophotometric assay on the enzymatic activities of Wild blackberry POD and PPO.

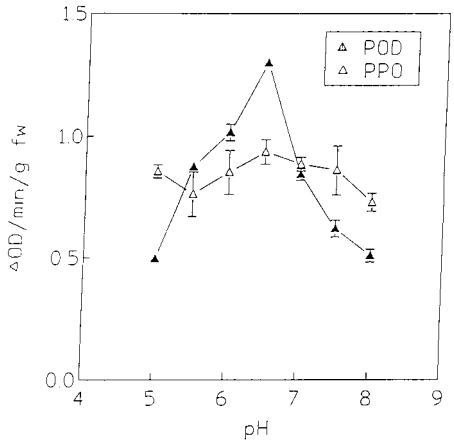

Figure 2. Effect of reaction mixture pH of spectrophotometric assay on the enzymatic activities of Wild blackberry POD and PPO.

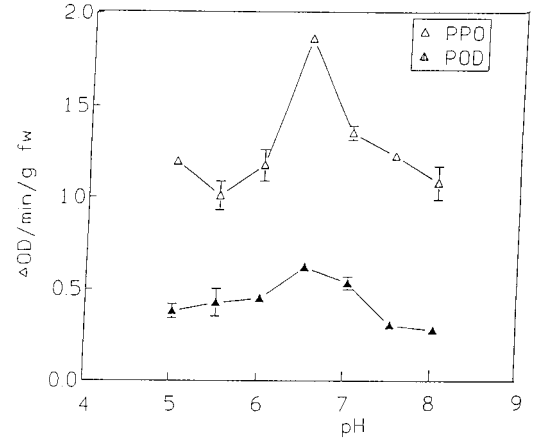

Figure 3. Effect of reaction mixture pH of spectrophotometric assay on the enzymatic activities of Thornless blackberry POD and PPO.

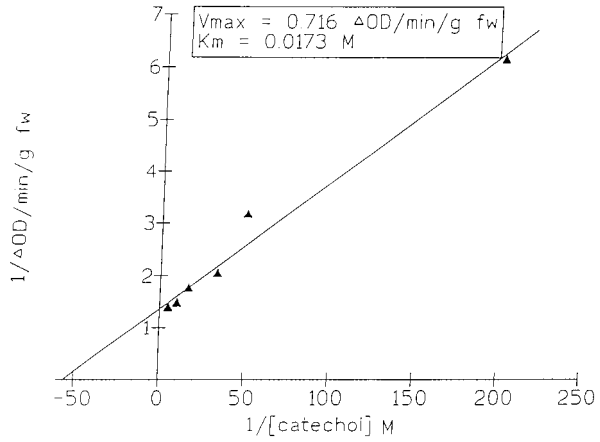

Figure 4. Effect of substrate concentration (catechol) on Wild blackberry PPO activity (Lineweaver−Burk plot).

or POD enzymes from Wild blackberries in buffers ranging from pH 5.0 to 8.0 (Figure 1). The optimal pH values obtained from this study were subsequently utilized in the Thornless samples. Maximum POD activity (1.10 ΔOD/min/g of fw) was extracted at pH 7.5 for Wild cultivars, with catechol as substrate. There were two maximum PPO values, at pH levels of 6.0 and 7.5. No extractions were attemped at pH values <5.0. However, in this work all of the enzyme assays were conducted at pH 7.5 due to the best solubilization of enzyme extract aliquots in the reaction mixture.

Optimization of the Reaction Mixture. Using the optimized extraction conditions with a 0.05 M sodium phosphate buffer containing 4% (w/v) insoluble PVP and 0.5% (w/v) Triton X-100, pH 7.5, a study was performed to determine the optimal pH values for the reaction mixture for both the Wild and Thornless cultivars (Figures 2 and 3). POD and PPO activities were highest at pH 6.5 in both samples. It was determined that a 0.2 M concentration of sodium phosphate produced increases in the activity values for the PPO of the Wild blackberries (1.30 ΔOD/min/g of fw) and the POD of the Thornless fruits (0.61 ΔOD/min/g of fw) (Table 3).

Substrate Specificity. To determine the POD activity, only two substrates were studied (Table 4). The two substrates gave similar values, ranging between 0.65 and 0.73 ΔOD/min/g of fw for Wild fruits and between 0.61 and 0.63 ΔOD/min/g of fw for Thornless blackberries. For this reason *o*-dianisidine was the substrate of choice due to its higher workability. Wild blackberry PPO activity was more active than Thornless with 4-methylcatechol and catechol as a substrate, using a concentration of 0.003 M (Table 5). Nevertheless, the Thornless blackberry PPO activity was the most active with catechol and chlorogenic acid as a substrate (0.51 and 0.50 ΔOD/min/g of fw, respectively), although 4-methylcatechol also produced a relatively high value (0.24 ΔOD/min/g of fw). No activity was detected toward L-DOPA or *p*-coumaric acid in the Thornless cultivars. All of these substrates are naturally occurring phenolic compounds in blackberry tissues, and the results point toward 4-methylcatechol and catechol as the substrates in blackberries that have a great potential for enzymatic browning during postharvest handling or processing and storage of blackberry-based products.

Effects of Substrate Concentration. The effects of catechol concentrations ranging from 6 mM to 0.1 M and 4-methylcatechol concentrations ranging from 10 mM to 0.8 M on the PPO activity of Wild and Thornless blackberries were investigated (Figures 4−7). The K_m and V_{max} values for the PPO were determined from Lineweaver−Burk plots (Figures 4 and 5) for Wild and Thornless blackberries, respectively, using catechol as a substrate. Figures 6 and 7 showed similar plots using 4-methylcatechol. The K_m values for catechol were 17.3 mM for Wild extracts and 196.4 mM for Thornless. When 4-methylcatechol was used for the enzymatic

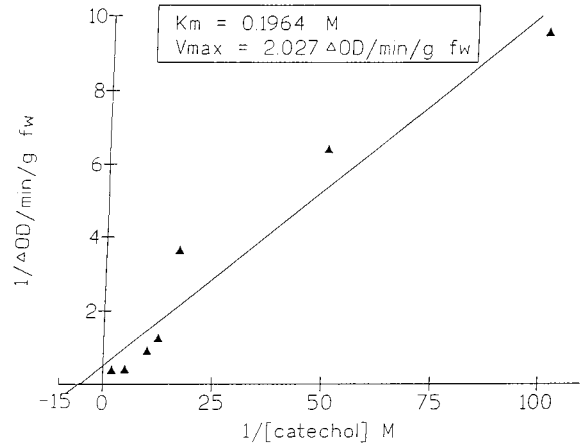

Figure 5. Effect of substrate concentration (catechol) on Thornless blackberry PPO activity (Lineweaver−Burk plot).

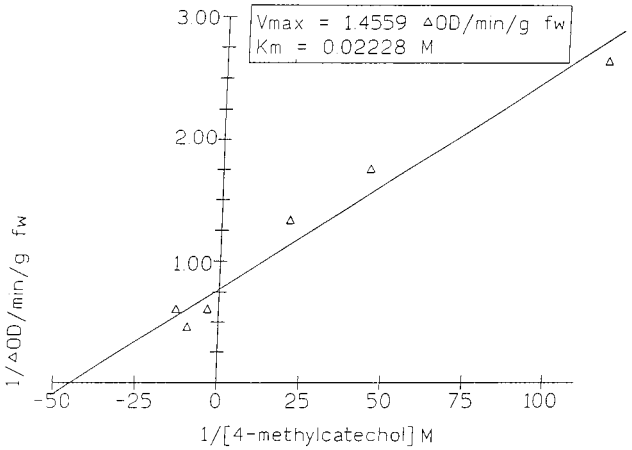

Figure 6. Effect of substrate concentration (4-methylcatechol) on Wild blackberry PPO activity (Lineweaver−Burk plot).

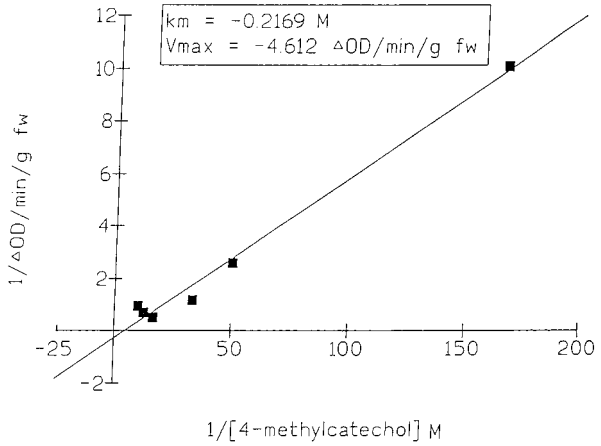

Figure 7. Effect of substrate concentration (4-methylcatechol) on Thornless blackberry PPO activity (Lineweaver−Burk plot).

assays, the K_m values were 22.3 mM for the Wild blackberries and 216.9 mM for the Thornless blackberries. The V_{max} values were 0.716 and 2.02 ΔOD/min/g of fw for Wild and Thornless, respectively, using catechol for the PPO reaction. Moreover, the V_{max} values were 1.45 and 4.62 ΔOD/min/g of fw for these two cultivars in the same order, using 4-methylcatechol as the substrate.

POD and PPO Activities on Blackberry Cultivars. The values of PPO activity were higher in Thornless than in Wild cultivars (Table 1) ($p \leq 0.05$), 1.85 and

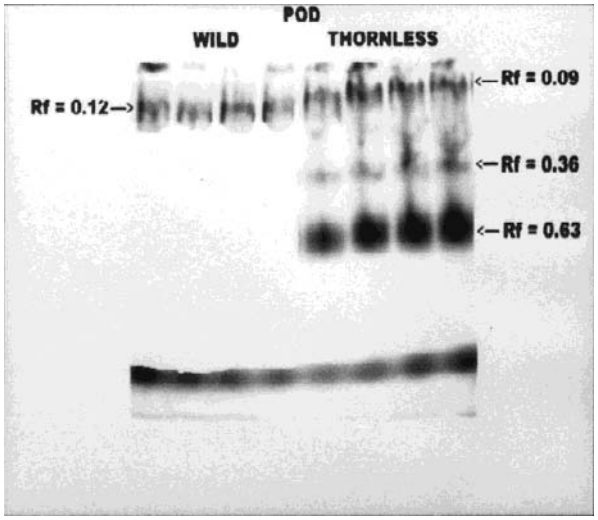

Figure 8. Electrophoretic pattern of soluble POD activity from Wild and Thornless blackberries. Staining gel was 3% (w/v) *o*-dianisidine.

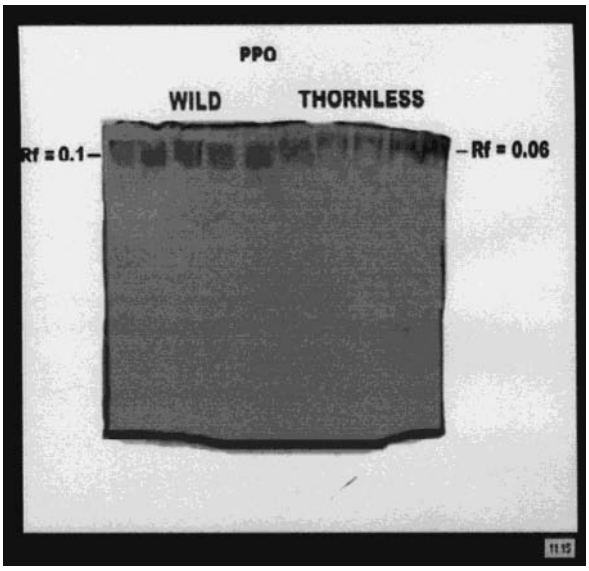

Figure 9. Electrophoretic pattern of soluble PPO activity from Wild and Thornless blackberries. Staining gel was 0.8 M catechol.

1.30 ΔOD/min/g of fw, respectively. However, the values of POD activity were higher in Wild than in Thornless blackberries ($p \leq 0.05$), 0.73 and 0.63 ΔOD/min/g of fw, respectively. The maturity index of Wild blackberries was higher than that of the Thornless cultivar. A similar pattern was found in previous studies dealing with raspberry fruits (González et al., 1999) in which the Ceva cultivar had the highest maturity index and lowest PPO activity values. We were not able to compare the POD activities values of blackberry and raspberry because POD activity was not found in any of the four raspberry cultivars studied.

Electrophoresis. PAGE on 10% gels under native conditions resolved crude POD and PPO extracts in various bands (Figures 8 and 9). To determine the active isoenzyme of POD and PPO, solutions of different substrates were assayed according to the procedures described for POD and PPO substrate affinity. To visualize POD bands, *p*-phenylenediamine and *p*-dianisidine were used in concentrations of 3−10% in 0.2 M sodium phosphate buffer, pH 6.5, and H_2O_2 (20% v/v). The gels were stained for 15 min, and various bands

were visualized. In the Wild POD samples, there was one band with a mobility of 0.12. In the Thornless POD samples, there were three distinct bands, with R_f values of 0.63, 0.36, and 0.09 (Figure 8). The darkest band was at the R_f level of 0.63. To visualize the PPO bands, two different solutions of catechol and 4-methylcatechol were utilized. It was found that 0.1 M catechol provided the best resolution of the band in the gel. Both the Wild and Thornless cultivars produced a single band, with R_f values of 0.1 for Wild and 0.06 for Thornless. According to a study by González et al. (1999) in four Spanish raspberry cultivars, only one band was resolved for a the PPO extracts, and its mobility was 0.25 for all four cultivars. Duplicate gels were also washed several times with ethanol to produce a partial gel dehydration to bring out other possible POD and PPO bands, following a protocol reported by Galeazzi et al. (1981) and employed in other studies with papaya PPO (Cano et al., 1996). However, in the present work the use of ethanol did not improve the staining of blackberry POD and PPO.

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