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Characterization and Role of Polyphenol Oxidase and Peroxidase in Browning of Fresh-Cut Melon

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Polyphenol oxidase (PPO) and peroxidase (POD) were extracted from two different varieties of melon (*Cucumis melo* L. *cantalupensis* cv. Charentais and *C. melo* L. *inodorus* cv. Amarillo) and characterized using reliable spectrophotometric methods. In both cases the enzymes followed Michaelis–Menten kinetics, showing different values of kinetics parameters between the two cultivars: $K_m = 7.18 \pm 0.70$ mM ('Charentais') and 6.66 ± 0.20 mM ('Amarillo') mM; $V_{max} = 7.93 \pm 0.35$ units/min ('Charentais') and 13.82 ± 0.37 units/min ('Amarillo'), relative to PPO; $K_m = 24.0 \pm 2.10$ mM ('Charentais') and 5.05 ± 0.19 mM ('Amarillo') mM; $V_{max} = 344.83 \pm 10.32$ units/min ('Charentais') and 80.64 ± 2.01 units/min ('Amarillo'), relative to POD. Optimum pH for PPO was 7.0 for 'Charentais' and 7.5 for 'Amarillo, whereas it was 4.5 for both cultivars relative to POD. Melon PPO had maximum activity at 60 °C in both 'Charentais' and 'Amarillo' cultivars, whereas POD maximum activity was found at 45 °C in 'Charentais' and at 25 °C in 'Amarillo'. POD from both cultivars showed higher thermolability compared with PPO, losing >90% of relative activity after only 5 min of incubation at 70 °C. POD's activation energy was much higher than that of PPO ($\Delta E^{\#} = 86.3$ and 160.6 kJ mol^{-1} for 'Charentais' and 'Amarillo', respectively). PPO and POD activities from both cultivars showed a decreasing pattern as sugar concentration in the assay medium increased, except in POD extract from 'Charentais', which maintained its activity in the presence of high D-glucose concentration (up to 5 M). Changes in L^*, a^*, b^*, chroma, and hue angle values were chosen to describe the browning development in the samples during storage at 5 °C. A slight decrease in L^* value and a more marked reduction of a^* value were noted in both cultivars above all at the end of storage period. POD activity during storage at 5 °C was highly correlated with changes of parameters a^*, b^*, chroma, and hue angle (r^2 from 0.82 to 0.97) for cultivar 'Charentais'. According to these results, only POD activity seemed to be involved in browning of minimally processed melon.

KEYWORDS: Melon; polyphenol oxidase; peroxidase; browning; fresh-cut melon

INTRODUCTION

Minimally processed fruits and vegetables with pH >4.6 and water activity (a_w) >0.85 are considered to be highly perishable when they are not subjected to preservation processes that delay undesirable biological and biochemical changes (*1*). This group includes some fresh-cut processed fruits such as melons (*Cucumis melo* L.). In addition to the increased respiration rate caused by fresh-cut processing, water, microbial, and enzyme activities are accelerated (*2, 3*). These increased reaction rates lead to an acceleration of softening and browning phenomena and consequently to a shelf-life reduction.

Browning of damaged tissues of fresh fruits and vegetables mainly occurs from the oxidation of phenolic compounds and contributes significantly to quality loss (*4, 5*). In general, the major enzyme responsible for the browning reaction is polyphenol oxidase (PPO; EC 1.14.18.1) (*6*). In the presence of oxygen, this copper enzyme catalyzes the hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the oxidation of *o*-diphenols to their corresponding *o*-quinones (catecholase activity) (*7*). These, in turn, are polymerized to undesirable brown, red, or black pigments (*8*). In plants, PPO is predominantly located in the chloroplast thylakoid membranes, and its phenolic substrates are mainly located in the vacuoles, but upon any cell-damaging treatment, the enzyme and substrates may come into contact, leading to rapid oxidation of phenols (*9*).

Peroxidase (POD; EC 1.11.1.7) is another oxidoreductase enzyme involved in enzymatic browning, because diphenols may function as reducing substrates in its reaction (*10*). The involvement of POD in browning is reported by many researchers (*11, 12*), although it is limited by the availability of electron acceptor compounds such as superoxide radicals, hydrogen peroxide, and lipid peroxides. Furthermore, it has been proposed that POD catalyzes the cross-linking between the ferulic acid substituents of pectins (*13*), and a clear correlation

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has been found between its activity and the synthesis of lignin and suberin polymers (*14*).

Characterization of oxidative enzymes from various minimally processed fruit and vegetables, such as lettuce (*15*), apples (*16*), and pears (*17*), is widely reported in the literature, but only a few works found a clear correlation between the degradative activities and browning of samples (*18*).

Although microbial spoilage and loss of firmness are considered to be the main causes of quality loss in fresh-cut melon stored in air at chilling temperatures (*19*), discoloration and browning of the pulp surface are also considered to be negative reactions to be avoided during the shelf life of such products (*19, 20*).

The objectives of this work were to extract and characterize polyphenol oxidase and peroxidase from two cultivars of melon to determine kinetic parameters, optimum conditions of pH and temperature, thermal stability, and inhibition effects by D-glucose and D-fructose. Furthermore, storage tests at 5 °C were performed on minimally processed fruit to evaluate the variations of physicochemical properties and to evaluate the influence of enzyme activities on browning during cold storage.

MATERIALS AND METHODS

Plant Materials. Melons (*Cucumis melo* L.) belonging to groups *inodorus* (cv. 'Amarillo') and *cantalupensis* (cv. 'Charentais') were obtained on the day of harvest from local producers in the area of Catania (Sicily, Italy). Fruits were harvested at commercial maturity (35–37 days after anthesis for 'Charentais' and 43–45 days for 'Amarillo') during August of 2006. They were sorted on the basis of size and absence of physical damage and then divided randomly into three replicates of 10 fruits from each group. The fruits were transported to the laboratory and stored overnight at 10 °C prior to processing. The unprocessed fruits were surface disinfected with 80% ethanol and placed in a laminar flow hood that was also sanitized with ethanol. All materials used for cutting and handling of the fruits were continually disinfected, and metallic surfaces were heat-sterilized at regular intervals. Fruits were sliced horizontally into halves with a sharp knife. Seeds were removed, and the fruit cavity was cleaned. Each half was cut at the exposed end into four equal slices. The skins were then removed, and each slice was cut into approximately 3 cm cubes. The melon cubes were stored at 5 °C and 95% relative humidity in transparent polystyrene baskets with a capacity of 500 g, in the same way that they are usually exposed in supermarket displays. Ten fruits of each species were sampled for physicochemical analyses and enzymatic assays at days 0, 3, 7, and 10. Five hundred grams of fruits from each species was used for enzyme characterization.

PPO and POD Extraction and Assay. A 200 g melon sample was passed through a juice centrifuge and homogenized using an Ultra-Turrax T25 homogenizer (Janke & Kunkel, Staufen, Germany) for 60 s. Twenty grams of homogenate was added to 40 mL of cold acetone (−20 °C) and continuously stirred for 10 min. The homogenate was filtered through Whatman no. 42 paper under vacuum on a Buchner funnel; the acetone powder, after elimination of the acetone under vacuum, was collected and suspended in 30 mL of 0.1 M citrate phosphate buffer (pH 7.5) and kept overnight at 4 °C, before again being filtered through Whatman no. 42 paper under vacuum on a Buchner funnel. The clear solution was ultrafiltered in a Millipore stirred cell with a 10 kDa membrane (Millipore 8050, Milan, Italy) and utilized as crude enzymatic extract.

The enzymatic assay was performed according to a reliable spectrophotometric method, using 3-methyl-2-benzothiazolinone hydrazone (MBTH) to trap the enzyme-generated *o*-quinone (*21, 22*). PPO activity was assayed spectrophotometrically at 505 nm using 3,4-dihydroxyphenylacetic acid as phenolic substrate with MBTH. The standard reaction mixture contained 0.9 mL of 40 mM phenolic substrate, 0.1 mL of 2% (w/v) MBTH in methanol, 0.05 mL of dimethylformamide (DMF), 1.5 mL of 50 mM sodium acetate buffer (pH 7.0), and 0.5 mL of enzyme extract. Reaction was stopped at different times with 0.5 mL of 5% H_2SO_4. The blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. One unit of PPO activity was defined as the amount of enzyme that produces 1 μmol of MBTH−DOPAC adduct ($\varepsilon = 8861\ M^{-1}\ cm^{-1}$) per minute at 25 °C under the conditions previously described.

POD activity was determined spectrophotometrically as the change in absorbance at 470 nm. The reaction mixture contained 2 mL of 0.01 M citrate phosphate buffer (pH 7.0) containing 1.0% (v/v) guaiacol, 0.25 mL of 32 mM H_2O_2, and 0.1 mL of enzyme extract (*23*). The blank was prepared without the addition of the enzyme extract. One guaiacol unit is defined as the amount of enzyme that oxidizes 1 μmol of guaiacol per minute at 25 °C and pH 7.0 under the conditions above-described.

Kinetics Properties. PPO and POD activities were assayed with their specific substrates [3,4-dihydroxyphenylacetic acid (DOPAC) and H_2O_2, respectively] to increase molarity (up to 80 mM for DOPAC and 256 mM for H_2O_2). Enzyme behavior (at pH 7.0 and 25 °C) was explained by the Michaelis–Menten equation, whereas kinetic parameters (K_m and V_{max}) were calculated by Linewaver−Burk plot (*24*).

Optimum pH and Temperature. PPO and POD activities were determined in a pH range of 4.0–9.0 in 50 mM citrate−phospate buffer, using DOPAC (40 mM) and hydrogen peroxide (32 mM) as substrates; residual activity at different pH values was compared with that obtained at optimal pH (100%). Then, tests were carried out at the pH producing maximum activity to find the optimal temperature; PPO and POD activities were assayed at various reaction temperatures as controlled by a circulation water bath. The temperature was varied over the range of 5–80 ± 0.1 °C.

Thermal Stability. The enzyme solutions were incubated in Eppendorf tubes in a water bath at four different temperatures (40, 45, 50, and 60 °C for PPO; 40, 50, 60, and 70 °C for POD) for different times, up to 60 min for POD and up to 120 min for PPO. PPO activity was determined at 25 °C and pH 7.0, using DOPAC (40 mM) as phenolic substrate. POD activity was determined at 25 °C and pH 7.0, using hydrogen peroxide (32 mM) as substrate The percentage of residual enzymatic activity was calculated by comparison with unheated enzyme. The temperature dependence of the kinetic inactivation constant (k) was evaluated using the Arrhenius equation (*25*).

Inhibition Tests. D-Glucose and D-fructose at different concentrations (0.02, 0.06, 0.1, 0.5, 1, 2, 3, 4, and 5 M) were dissolved in the assay medium, and PPO and POD activities were measured at 25 °C and pH 7.0 to determine inhibitor effects of sugars on enzymatic activities.

Physicochemical Properties of Melon Samples. Pulp samples were blended with a standard blender (Solac 850, Vitoria, Spain), and the obtained juice was used for chemical analysis. Titratable acidity (TA) was quantified by titrating 10 mL of melon juice with 0.1 N NaOH to an end point of pH 8.1 (Metrohm 716 DMS titrator, Swiss) and expressed as grams of citric acid per 100 g (*26*). The total soluble solids (TSS) content was measured with a digital refractometer (Abbe 1S, Milan, Italy) and expressed as °Brix at 20 °C. The pH was measured with the same equipment used for measuring TA. Total phenolics content was determined according to the Folin−Ciocalteau method (*27*), using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalents per gram of fresh weight. The juice color was determined with a compact tristimulus chromameter (Minolta CR-300, Ramsey, NJ) with an 8 mm ∅ viewing aperture and white plate reference ($Y = 94.3$; $x = 0.3142$; $y = 0.3211$), and C illuminant (CIE, 2° observer) was used. Readings were expressed as L^*, a^*, and b^* parameters. Chroma [$(a^{*2} + b^{*2})^{1/2}$] and hue angle [$\tan^{-1}(b^*/a^*)$] were calculated.

Statistical Analysis. All determinations were done in triplicate, unless noted otherwise. Analysis of variance (ANOVA) of the data was evaluated by the Statistical Analysis System (SAS ver. 9.0). Duncan's multiple-range test was employed to determine the statistical significance of the differences between the means ($p \leq 0.05$).

RESULTS AND DISCUSSION

Kinetics Properties. Relative to polyphenol oxidase, in both cultivars the enzyme followed Michaelis–Menten kinetics and

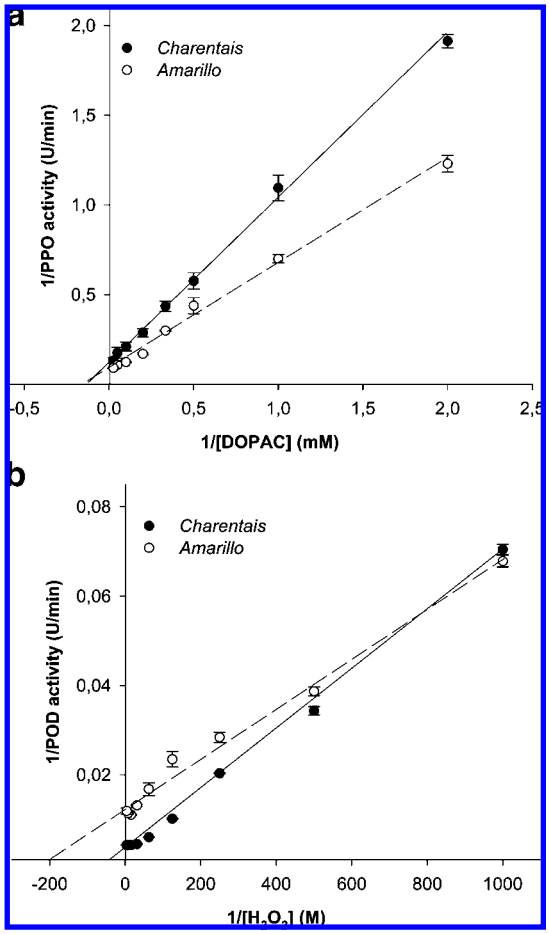

Figure 1. (**a**) Lineweaver–Burk plot for melon PPO: (●) 'Charentais' (V_{max} = 7.93 ± 0.35 units/min; K_m = 7.18 ± 0.7 mM); (○) 'Amarillo' (V_{max} = 13.82 ± 0.37 units/min; K_m = 6.66 ± 0.20 mM). (**b**) Lineweaver–Burk plot for melon POD: (●) 'Charentais' (V_{max} = 344.83 ± 10.32 units/min; K_m = 0.024 ± 0.005 M); (○) 'Amarillo' (V_{max} = 80.64 ± 2.01 units/min; K_m = 0.005 ± 0.0001 M).

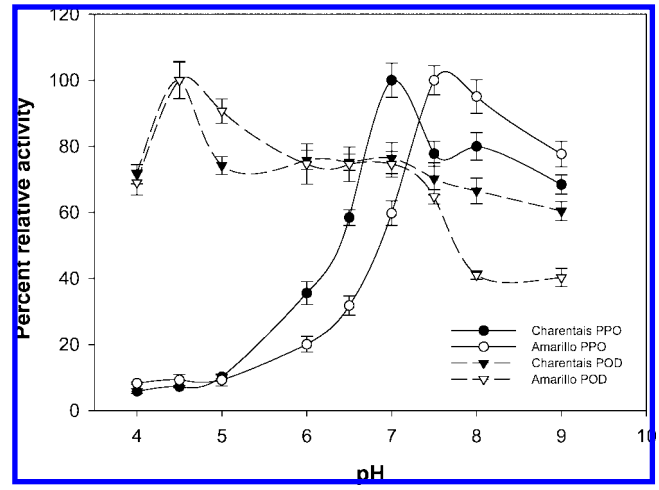

Figure 2. Effect of pH on melon PPO and POD relative activities.

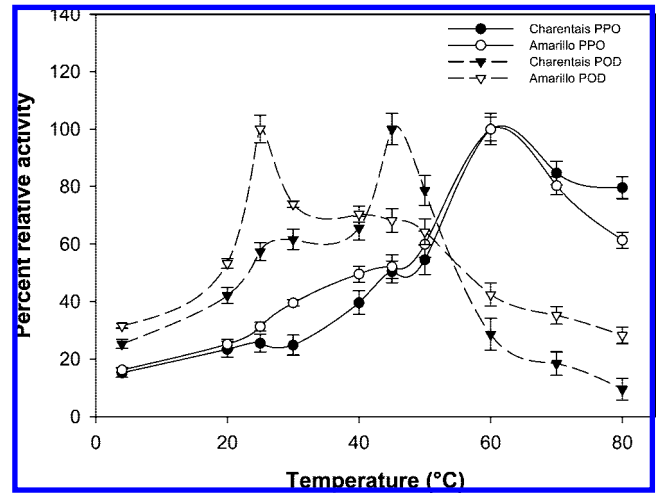

Figure 3. Effect of temperature on melon PPO and POD relative activities.

the Lineweaver–Burk plot resulted linear (**Figure 1a**), with the following K_m and V_{max} values for DOPAC substrate: K_m = 7.18 ± 0.7 mM ('Charentais') and 6.66 ± 0.2 mM ('Amarillo'); V_{max} = 7.93 ± 0.35 units/min ('Charentais') and 13.82 ± 0.37 units/min ('Amarillo'). Results indicated that DOPAC saturation was similar in PPO from both melon cultivars, whereas the enzyme extracted from *inodorus* type showed a maximum velocity 74% higher than that in *cantalupensis* type. Also, peroxidase extracted from melon samples showed Michaelis–Menten kinetics, with values of kinetics parameters significantly higher in the 'Charentais' cultivar: K_m = 24.0 ± 2.1 mM ('Charentais') and 5.05 ± 0.19 mM ('Amarillo'); V_{max} = 344.83 ± 10.32 units/min ('Charentais') and 80.64 ± 2.01 units/min ('Amarillo') (**Figure 1b**).

pH Optimum. The melon PPO had maximum activity at pH 7.0 in cultivar 'Charentais' and at pH 7.5 in cultivar 'Amarillo' (**Figure 2**), rapidly decreasing in both cases at more acidic pH values to 5% residual activity at pH 4.0. The physiological pH in fruits at commercial maturity condition was 7.0 in cultivar 'Charentais' and 6.0 in cultivar 'Amarillo', in which conditions PPO relative activity was 100% in 'Charentais' and 20% in 'Amarillo'. Then, a more effective PPO activity in *cantalupensis* is predictable, because there is an optimal pH environment in comparison with the *inodorus* type. In the case of peroxidase, maximum activity was found at pH 4.5 in both cultivars (**Figure 2**), retaining >60% residual activity over a wide pH range (4.0–7.5). At melon physiological pH values (7.0 for 'Charentais' and 6.0 for 'Amarillo'), the residual activities were very similar between the cultivars (76% in 'Charentais' and 74% in 'Amarillo'), indicating that commercial maturity condition did not seem to negatively affect the expression of peroxidase in either cultivar.

Optimum Temperature. Melon PPO had maximum activity at 60 °C in both 'Charentais' and 'Amarillo' cultivars (**Figure 3**) and showed very similar courses over the temperature range of 4–80 °C. Results agree with those reported in the literature relative to PPO extracted from different sources (*28–30*). Furthermore, PPO retained >50% residual activity at temperatures >45 °C, but was found to be strongly inhibited at 5 °C (15 and 16% of the maximum in 'Charentais' and 'Amarillo', respectively). With regard to peroxidase, maximum activity was found at 45 °C in 'Charentais' and at 25 °C in 'Amarillo' (**Figure 3**). In both cases, temperatures >60 °C in the assay medium caused a progressive inactivation, resulting in 91 and 72% losses of activity at 80 °C in 'Charentais' and 'Amarillo', respectively. A cold storage temperature (5 °C) also produced a good inactivation, leading to 25 and 31% POD residual activity in 'Charentais' and 'Amarillo', respectively .

Thermal Stability. The thermostability profiles of PPO extracts from 'Charentais' and 'Amarillo' are shown in **Figure 4**. PPO from 'Charentais' showed a good stability in a temperature range from 40 to 60 °C, showing 78 and 52% of residual activity after 120 min of incubation at 40 and 60 °C,

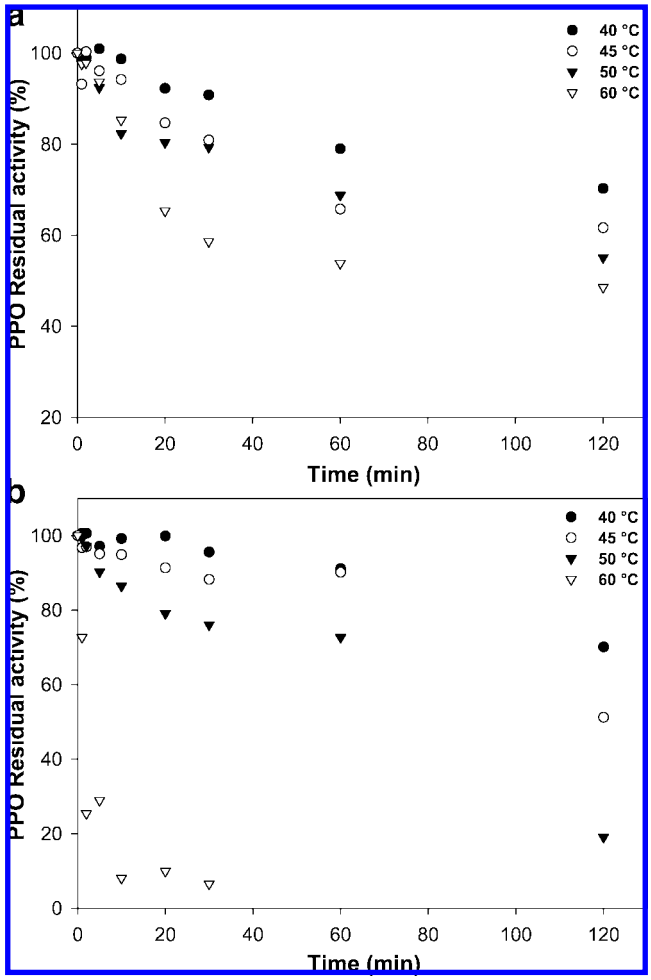

Figure 4. Thermal stability of (**a**) 'Charentais' PPO and (**b**) 'Amarillo' PPO.

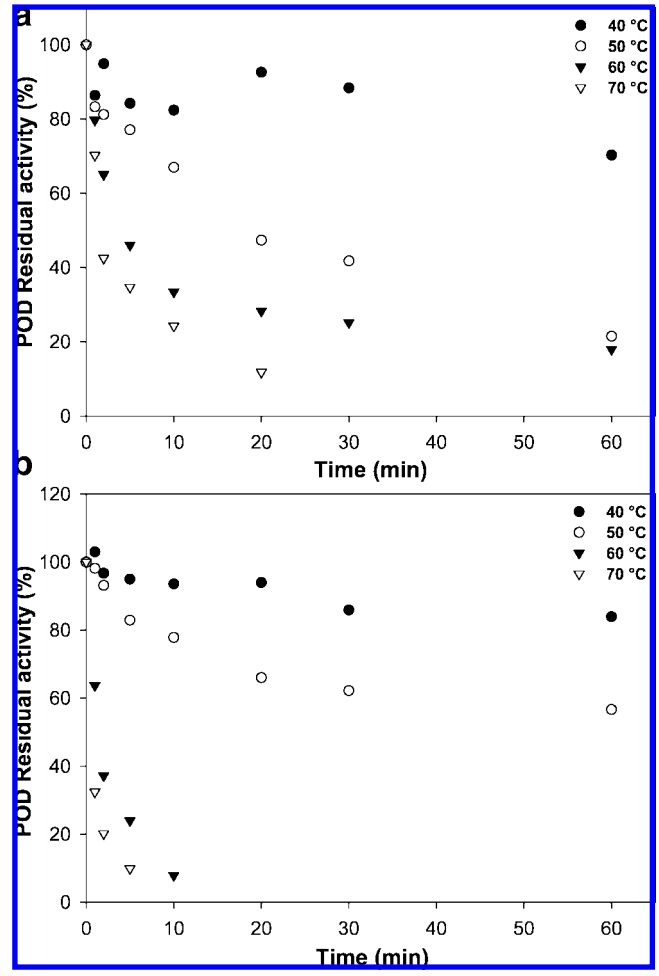

Figure 5. Thermal stability of (**a**) 'Charentais' POD and (**b**) 'Amarillo' POD.

respectively. 'Amarillo' PPO was more thermostable than 'Charentais' after 60 min at temperatures up to 45 °C, but was found to be quite completely inactivated after 30 min of incubation at 60 °C (94% loss of activity). Although PPO is not a very heat-stable enzyme compared to other enzymes responsible for quality degradation of fruits and vegetables (*31*), melon PPO showed high resistance at temperatures ranging from 40 to 60 °C, much higher than those used for storage of fresh-cut produce (from 4 to 8 °C).

Thermostability profiles of POD from 'Charentais' and 'Amarillo' are shown in panels **a** and **b**, respectively, of **Figure 5**. POD from both cultivars was more thermolabile than PPO, also showing sharp activity variations among temperatures in the range of 40–70 °C. POD from 'Charentais' retained >70% activity after 60 min of incubation at 40 °C while losing 90% of relative activity after only 20 min at 70 °C. The profile of POD from 'Amarillo' cultivar was similar to that from 'Charentais', but with more drastic inactivation at high temperatures (losses of 93 and 91% of activity after 10 min at 60 °C and 5 min at 70 °C, respectively).

The temperature dependence of the kinetic inactivation constant (k) was evaluated using the Arrhenius equation

$$\ln\ k = \ln k_0 - \Delta E^{\#}/RT \qquad (1)$$

as shown in **Figure 6**. The activation energy ($\Delta E^{\#}$) for crude 'Charentais' and 'Amarillo' PPO and POD heat inactivation was determined from results in **Figure 6**. Other activation parameters, namely, $\Delta G^{\#}$ (Gibbs free energy for enzyme inactivation),

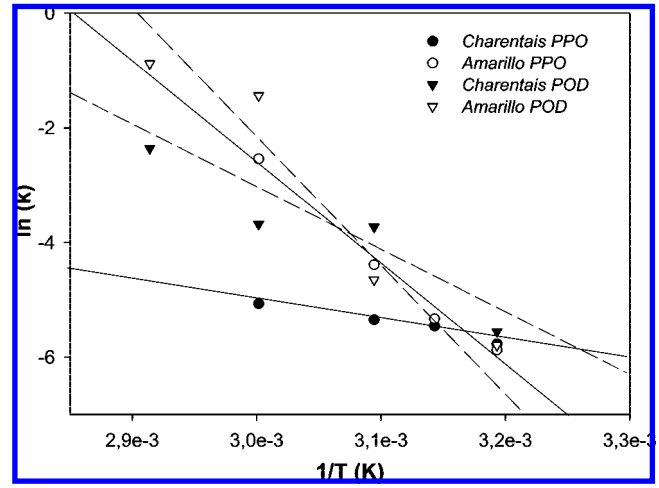

Figure 6. Arrhenius plot for heat inactivation of melon PPO and POD.

$\Delta H^{\#}$ (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 determined from the relationships below as described previously (*32*):

$$\Delta G^{\#} = RT \ln(kT/K_B h) \qquad (2)$$

$$\Delta H^{\#} = \Delta E^{\#} - RT \qquad (3)$$

$$\Delta S^{\#} = (\Delta H^{\#} - \Delta G^{\#})/T \qquad (4)$$

R (8.3145 J mol^{-1} K^{-1}) is the universal gas constant, K_B (1.3806

Table 1. Transition State Parameters for the Heat Inactivation of Crude 'Charentais' and 'Amarillo' PPO and POD (Means ± SD for Triplicate Experiments)[a]

enzymatic extract	$\Delta E^{\#}$ (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (J mol^{-1} K^{-1})
Charentais				
PPO	28.9	26.3 ± 0.6	346.3 ± 8.7	−994.2 ± 4.3
POD	86.3	83.5 ± 0.9	357.5 ± 15.2	−834.2 ± 18.5
Amarillo				
PPO	147.9	145.2 ± 0.6	348.7 ± 11.5	−631.8 ± 21.1
POD	160.6	157.9 ± 0.9	359.3 ± 17.8	−612.8 ± 34.0

[a] $\Delta E^{\#}$, 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.

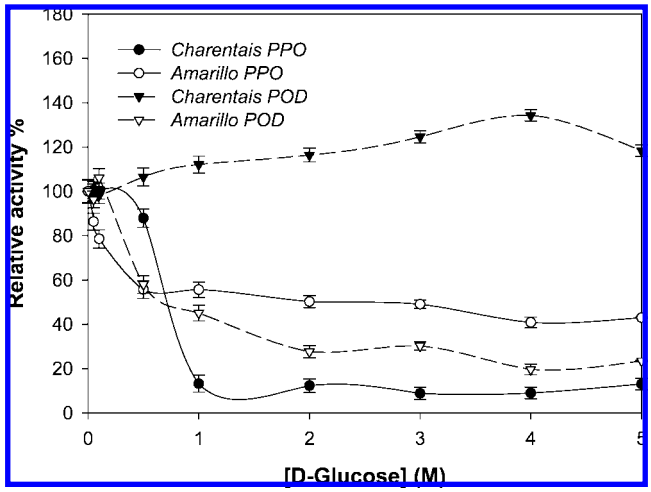

Figure 7. D-Glucose inhibition of crude melon PPO and POD.

× 10^{-23} J K^{-1}) is the Boltzman constant, h (6.6261 × 10^{-34} J s) is the Planck constant, and T is the absolute temperature. Results for these analyses are reported in **Table 1**.

In summary, $\Delta E^{\#}$ was 28.9 kJ mol^{-1} for 'Charentais' PPO heat inactivation, much lower than for that 'Amarillo' PPO heat inactivation (147.9 kJ mol^{-1}). POD activation energy was higher than that of PPO and again with noticeable differences between cultivars (86.3 and 160.6 kJ mol^{-1} for 'Charentais' and 'Amarillo', respectively). The average values of $\Delta H^{\#}$ were 26.3 and 145.2 kJ mol^{-1} for 'Charentais' and 'Amarillo' PPO, respectively, and 83.5 and 157.9 kJ mol^{-1} for 'Charentais' and 'Amarillo' POD, respectively. Then, considering the higher values of $\Delta E^{\#}$ and $\Delta H^{\#}$ for POD in comparison with PPO, it is possible to affirm that melon POD is more thermostable than PPO over the range of temperatures studied. Furthermore, considering the thermal inactivation parameters, differences were also noted between the cultivars, with PPO and POD extracts from 'Amarillo' showing a higher thermostability than those from 'Charentais'.

Sugars Inhibition. The effects of D-glucose as enzymatic inhibitor are shown in **Figure 7**. The percent inhibition was compared with that of the control (100% activity). PPO and POD activities from both cultivars showed a decreasing course as sugar concentration in the assay medium increased, except in POD extract from 'Charentais', keeping its activity in the presence of high D-glucose concentration (up to 5 M). On the other hand, PPO from cultivar 'Charentais' was found to be greatly inhibited even at low sugar concentration in the assay medium (87% loss of activity in presence of 1 M D-glucose). The concentrations of D-glucose required in the assay medium to halve the activity were 0.71 and 2.11 M for 'Charentais' and 'Amarillo' PPO, respectively. The inhibition effects of D-fructose on melon PPO and POD are shown in **Figure 8**. In this case, the increasing concentrations of sugar up to 1 M for 'Amarillo' POD and up to 3 M for 'Amarillo' PPO caused a progressive inactivation of such enzymes, whereas extracts from 'Charentais' were less affected by the presence of sugar. Indeed, PPO from 'Charentais' was shown to be slightly activated by D-fructose, whereas PPO from 'Amarillo' melon retained its original activity up to 0.5 M sugar concentration and then significantly decreased until reaching 59% residual activity at 5 M sugar concentration in the assay medium. It is possible to notice the better resistance to sugars inhibition of both oxidases extracted from the *cantalupensis* melon 'Charentais' in comparison with the *inodorus* type 'Amarillo', except for PPO inhibition. In particular, peroxidase extracts from 'Charentais' were not inhibited by the presence of both sugars, whereas 'Amarillo' extracts were gradually inactivated with increasing sugars concentration.

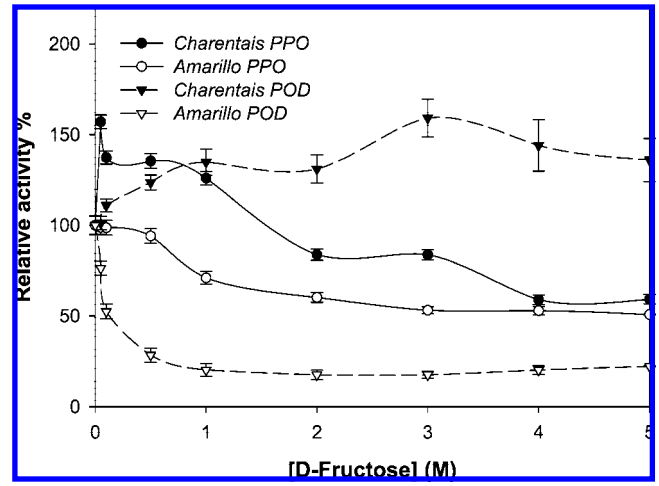

Figure 8. D-Fructose inhibition of crude melon PPO and POD.

Physicochemical Characteristics of Melon Cultivars. Minimally processed 'Charentais' and 'Amarillo' melon cultivars were analyzed during 10 days of storage at 5 °C to evidence possible differences related to the storage suitabilities of the two cultivars. Results are reported in **Table 2**. Both melon cultivars showed similar trends in pH and percentages of titratable acidity (TA) changes during low-temperature fruit storage. Indeed, results showed that the pH significantly increased with storage, with values ranging from 6.68 to 7.03 for 'Charentais' and from 5.85 to 6.27 for 'Amarillo'. On the other hand, TA values significantly decreased throughout storage in both cultivars, between days 0 and 3, showing a decrease of around 30% in cultivar 'Charentais' and 20% in cultivar 'Amarillo'. Neither total soluble solids (TSS) nor dry matter percentages showed significant variations during storage of both cultivars. During storage of melon cubes at 5 °C, total phenolics of cv. 'Charentais' rose by 15% from an initial 0.51 to 0.60 mg/g, whereas cv. 'Amarillo' phenolics showed a slighter increase throughout the storage period and an initial value (0.36 mg/g) about 70% lower than in 'Charentais'. The production of phenolic compounds has been associated with the process of wound healing (*33, 34*) and was previously observed during cold storage of minimally processed cactus pear (*35*) and jicama (*36*).

Melon Browning. Changes in L^*, a^*, b^*, chroma, and hue angle values were chosen to describe the browning development in the samples during storage at 5 °C (**Table 2**). A significant reduction of a^* value was noted in both cultivars at the end of the storage period ($\Delta a^* = 3.1$ and 1.16 between days 0 and 10

Table 2. Physicochemical Properties of Fresh-Cut 'Charentais' and 'Amarillo' Melons during Storage at 5 °C[a]

cultivar	days at 4 °C	pH	TA (g of citric acid/100 g)	TSS (°Brix)	dry matter %	L^*	a^*	b^*	chroma	hue angle	phenolics (mg/g)
Charentais	0	6.68 ± 0.05 c	0.07 ± 0.01 a	13.9 ± 0.6 a	14.4 ± 0.9 a	50.1 ± 3.2 a	20.1 ± 0.9 a	5.7 ± 0.3 a	20.9	3.46	0.51 ± 0.02 b
	3	6.93 ± 0.03 b	0.05 ± 0.005 b	14.8 ± 0.8 a	13.9 ± 0.7 a	46.5 ± 2.8 a	20.0 ± 1.0 a	5.4 ± 0.2 a	20.7	3.60	0.52 ± 0.02 b
	7	7.03 ± 0.03 a	0.03 ± 0.01 c	14.5 ± 0.6 a	14.3 ± 0.4 a	48.7 ± 1.9 a	21.2 ± 1.4 a	5.8 ± 0.1 a	21.9	3.58	0.59 ± 0.03 a
	10	7.01 ± 0.05 ab	0.03 ± 0.002 c	14.5 ± 0.8 a	14.8 ± 0.5 a	48.5 ± 2.0 a	17.0 ± 1.5 b	3.4 ± 0.2 b	17.4	4.93	0.60 ± 0.03 a
Amarillo	0	5.85 ± 0.06 c	0.11 ± 0.01 a	11.2 ± 0.4 a	11.2 ± 0.7 a	77.5 ± 4.1 a	−1.47 ± 0.1 a	12.8 ± 0.7 b	12.9	1.16	0.36 ± 0.02 ab
	3	6.14 ± 0.04 b	0.09 ± 0.005 b	12.2 ± 0.6 a	10.9 ± 0.8 a	76.9 ± 4.2 a	−1.42 ± 0.2 a	12.5 ± 0.2 b	12.6	1.55	0.38 ± 0.02 a
	7	6.27 ± 0.04 a	0.08 ± 0.01 b	11.6 ± 0.5 a	10.9 ± 0.4 a	71.1 ± 4.7 a	−1.35 ± 0.2 a	12.2 ± 0.2 b	12.3	2.45	0.33 ± 0.02 b
	10	6.13 ± 0.02 b	0.09 ± 0.01 b	11.2 ± 0.7 a	11.2 ± 0.3 a	70.6 ± 3.2 a	−2.63 ± 0.2 b	14.0 ± 1.0 a	14.3	0.73	0.39 ± 0.03 a

[a] Within each cultivar, means in a column followed by the same letter are not significantly different at the $P \leq 0.05$ level according to Duncan's multiple-range test.

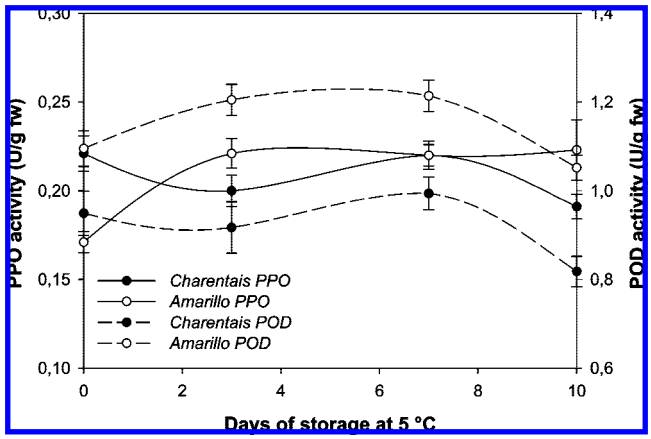

Figure 9. Activity of crude melon PPO and POD during storage at 5 °C.

for cultivars 'Charentais' and 'Amarillo', respectively). A decrease in a^* is associated with loss of typical orange/red color hue during storage of cantaloupe melon, as previosly reported (*37*). Moreover, by calculating ΔE^* [$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$], a widely used parameter for determining color differences perceptible by the human eye, it was possible to note a slight color variation between days 0 and 10 in both cultivars, but this change was more marked in 'Amarillo' ($\Delta E^* = 7.10$ and 4.18 for 'Amarillo' and 'Charentais', respectively).

The activities of PPO and POD during cold storage are shown in **Figure 9**. PPO from cv. 'Charentais' had greater initial activity than that from 'Amarillo' (0.221 and 0.171 units/g of fw, respectively) but did not vary throughout storage, whereas 'Amarillo' PPO increased between days 0 and 3, showing maximum activity (0.223 unit/g of fw). Furthermore, POD was 13.3−23.9% higher in 'Amarillo' during the entire storage period (1.095 and 0.949 units/g of fw initial activity for 'Amarillo' and 'Charentais', respectively), remaining in both cases with small variations until day 10.

To establish the PPO and POD influence on melon browning, relationships between color measurements and enzymatic activities were researched and regression coefficients r^2 for the linear regression fitting of these relations calculated (**Table 3**). It is evident from the results that POD was highly correlated with parameters a^*, b^*, chroma, and hue angle (r^2 from 0.82 to 0.97) in cultivar 'Charentais', whereas it showed less correlation (r^2 from 0.61 to 0.78) in cultivar 'Amarillo'. On the other hand, PPO did not seem to present correlations with browning parameters in both cultivars, except with L^* in cv. 'Charentais' ($r^2 = 0.86$). Furthermore, no correlation was found between browning parameters and the total phenolic content (data not shown). The role of peroxidase in the browning of fruits and vegetables is well-known and reported in several studies about

Table 3. Correlation Coefficients (r^2) between Browning Parameters

		browning parameters				
		L^*	a^*	b^*	chroma	hue angle
Charentais	PPO	0.86	0.69	0.69	0.69	0.63
	POD	0.47	0.97	0.90	0.96	0.82
Amarillo	PPO	0.41	0.09	0.01	0.02	0.05
	POD	0.01	0.61	0.77	0.76	0.78

different products, such as strawberry (*38*) and grape (*39*). Such a role, as previously reported (*40*), could be explained through a synergistic effect of PPO−POD, through the generation of H_2O_2 in PPO-catalyzed reactions, and also the use by POD of semiquinonic intermediates of PPO-catalyzed reactions as oxidizing substrates. However, the relative significance of these two pathways is affected by the nature of the oxidized phenol and therefore by the stability of the corresponding *o*-quinones (*12*). Thus, the results obtained are in agreement with an important role played by POD in enzymatic browning of minimally processed melon, although the presence of PPO activity is fundamental for the involvement of POD.

ABBREVIATIONS USED

PPO, polyphenol oxidase; POD, peroxidase; TA, titratable acidity; TSS, total soluble solids; DOPAC, 3,4-dihydroxyphenylacetic acid; MBTH, 3-methyl-2-benzothiazolinone hydrazone; DMF, dimethylformamide.

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