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Characterization of a Tomato Polyphenol Oxidase and Its Role in Browning and Lycopene Content

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Polyphenol oxidase (PPO) was extracted from five Sicilian varieties of tomato fruit [Pizzutello, Naomi (Hazera), F1 PS212 (Peto seed), Rosa Maletto, and PO228] and assayed with a method using 3-methylbenzothyazolinone hydrazone (MBTH) as chromophore coupling agent. 3,4-Dihydroxyphenylacetic acid was chosen for tomato PPO activity determination. The tomato PPO had maximum activity at pH 4.8. The pH of juice in ripe fruits is between 4.1 and 4.4, a range in which PPO relative activity is between 74 and 87%. The optimum temperature of activity for tomato PPO was 40 °C; the enzyme showed a good relative activity (55% of the maximum) at cold-storage temperature (4 °C). PPO retained 82% relative activity at an NaCl concentration of 0.1 M; at higher concentrations the PPO became gradually inactivated. The commercial variety Naomi is more susceptible to enzymatic browning than the local varieties Pizzutello, Rosa Maletto and PO228, due to higher PPO activity levels. This result confirms the suitability of these local tomato varieties to national markets. Results from storage tests seem to relate PPO activity with color changes associated with browning and lycopene degradation, because lycopene is an antioxidant agent that reconstitutes the polyphenols oxidized by the action of PPO.

KEYWORDS: Tomato fruits; polyphenol oxidase; browning; lycopene content

INTRODUCTION

Browning of damaged tissues of fresh fruits and vegetables mainly occurs from the oxidation of phenolic compounds and contributes significantly to quality loss (*1*). In particular, the primary enzyme responsible for the browning reaction is polyphenol oxidase (PPO; EC 1.14.18.1) (*2*). 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) (*3*). These, in turn, are polymerized to undesirable brown, red, or black pigments (*4*). Other enzymes contribute to these undesirable browning reactions, among which are peroxidase (*5*) and laccase (*6*). 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 (*7*). The prevention of these undesirable reactions has always been a challenge for food scientists. For this reason, PPO has been studied in many fruits and vegetables including apples (*8*), grapes (*9*), pears (*10*), and eggplants (*11*), but no work has investigated the influence of PPO on oxidative browning of tomato fruit (*Lycopersicon esculentum* Mill.) and possible relations with the antagonist action of lycopene, an antioxidant agent naturally present at high levels in tomato fruit and responsible for red color (*12*). In fact, sensory quality involves also the color of the fruit, besides its taste, aroma, and texture (*13*, *14*). In this work, PPO was extracted from tomato fruit and assayed with a method using 3-methylbenzothyazolinone hydrazone (MBTH) as chromophore coupling agent (*8*, *15*). Lycopene content and variation of *L*a*b** color parameters of the samples were evaluated during storage at 40 °C to establish possible relationships between the antagonist actions of PPO and lycopene and sample browning. Polyphenol oxidase extracted from tomato fruit was characterized to determine substrate specificity, kinetic parameters, optimum condition of pH and temperature, thermal stability, and inhibition effects of NaCl.

Enzymatic activity was evaluated on samples from five different Sicilian tomato varieties, of which two are widespread in national markets (Naomi and the hybrid F1 PS212); the other three (Pizzutello, Maletto, and PO228) are novel introductions, chosen with the purpose of valorizing such products and showing their suitability to minimally processed production. Therefore, physicochemical properties (pH, dry matter, total soluble solids, sugar content, titratable acidity, malic and lactic acid contents, ascorbic acid content, lycopene content), PPO activity, and browning during storage were evaluated to

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Table 1. Physicochemical Properties of Different Tomato Varieties[a]

	pH	dry matter (g/100 g of fwt)	soluble solids (°Brix)	total sugars (g/100 g of fwt)	titratable acidity (g/100 g of fwt)	SS/TA	malic acid (mg/100 g of fwt)	lactic acid (mg/100 g of fwt)	ascorbic acid (mg/100 g of fwt)	lycopene (mg/100 g of fwt)
Naomi	4.18 ± 0.03 b	8.05 ± 0.10 a	6.80 ± 0.10 a	3.77 ± 0.15a	1.50 ± 0.11 b	4.53 b	4.31 ± 0.65 d	1.83 ± 0.12 a	27.93 ± 1.13 a	14.83 ± 1.06 b
F1 PS212	4.18 ± 0.03 b	5.65 ± 0.10 e	4.20 ± 0.10 e	2.51 ± 0.12 c	1.15 ± 0.12 c	3.65 c	10.50 ± 0.55 b	0.52 ± 0.08 c	27.93 ± 1.43 a	11.12 ± 1.11 b
Pizzutello	4.10 ± 0.04 c	7.91 ± 0.10 b	6.60 ± 0.10 b	3.76 ± 0.10 a	1.86 ± 0.13 a	3.55 d	24.40 ± 0.60 a	1.78 ± 0.10 a	27.96 ± 1.32 a	15.91 ± 1.32 ab
Rosa Maletto	4.32 ± 0.02 a	7.12 ± 0.10 c	5.10 ± 0.10 d	2.84 ± 0.14 b	1.01 ± 0.11 c	5.05 a	6.76 ± 0.58 c	1.23 ± 0.14 b	27.81 ± 0.98 a	17.73 ± 0.78 a
PO228	4.10 ± 0.03 c	7.51 ± 0.10 d	5.90 ± 0.10 b	2.99 ± 0.12 b	1.80 ± 0.14 a	3.28 e			25.12 ± 1.55 b	17.02 ± 0.66 a

[a] Values represent the mean ± standard error of three replicate samples. Means in the same column followed by the same letter are not significantly different at the $p \leq 0.05$ level according to Duncan's multiple-range test.

determine such suitability and to establish eventual differences among varieties.

MATERIALS AND METHODS

Plant Materials. Samples of five different varieties from the Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA), Catania, Italy, tomato collection were analyzed. The two commercial varieties chosen as reference were the hybrids F1 PS212 (Peto seed) and Naomi (Hazera). The local varieties were Rosa Maletto, Pizzutello, and PO228, widely grown in open fields along the southern coast of Sicily, but apparently also well suited to a protected environment. Pizzutello and Naomi produce cherry tomatoes, F1 PS212 produces round-type tomatoes, and Rosa Maletto and PO228 produce multilocular fruits. All samples were picked and analyzed at red maturity condition (>90% fully red but firm).

Chemicals. MBTH, 3,4-dihydroxyphenylacetic acid (DOPAC), L-DOPA, dopamine, catechol, and 4-methylcatechol were purchased from Sigma (Milan, Italy). All other chemicals were of analytical grade and supplied by Fluka (Milan, Italy). Stock solutions of the phenolic substrates were prepared in 0.15 mM acetic acid to prevent autoxidation. Fifty millimolar sodium acetate (pH 4.2) and citrate phosphate (pH 7.0) buffers were used. The acidic character of MBTH required the use of 50 mM buffer in the assay medium. To dissolve the MBTH−quinone adduct, 2% (v/v), *N*,*N*[1]-dimethylformamide (DMF) was added to the assay medium (*16*, *17*). Eight percent sulfuric acid was used to stop the enzymatic reaction.

Sample Preparation. A 150 g tomato sample was passed through a juice centrifuge, sieved (1.5 mm diameter) to remove seeds, and homogenized using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany) homogenizer set for 60 s.

PPO Extraction and Assay. Forty grams of homogenate was added to 200 mL of cold acetone (−20 °C) and continuously stirred for 10 min. The homogenate was filtered through Whatman 589[2] paper under vacuum on Büchner funnel; the acetone powder, after elimination of the acetone under vacuum, was collected and suspended in 70 mL of 0.1 M citrate−phosphate buffer (pH 7.0) and kept overnight at 4 °C, before being again filtered through Whatman 589[2] paper under vacuum on Büchner funnel. The clear solution was ultrafiltered in a Millipore stirred cell with a 10 kDa membrane (Millipore 8050, Milan, Italy) and utilized as partially purified enzymatic extract.

Enzymatic assay was performed according to a reliable spectrophotometric method, using MBTH to trap the enzyme-generated ortoquinone (*15*, *17*). PPO activity was assayed spectrophotometrically at λ_{max} using five different phenolic substrates (see below) with MBTH. The standard reaction mixture contained 0.9 mL of 40 mM phenolic substrates, 0.1 mL of 2% (w/v) MBTH in methanol, 0.05 mL of DMF, 1.5 mL of 50 mM sodium acetate buffer (pH 4.2), and 0.5 mL of enzymatic extract. Reaction was stopped at different times with 0.5 mL of 8% H_2SO_4. Blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. The protein content was determined according to the Bradford Bio-Rad protein assay using bovine serum albumin as standard (*18*).

Substrate Specificity. PPO activity was measured with five phenolic substrates (catechol, 4-methylcatechol, L-DOPA, DOPAC, dopamine) in order of increasing molarity (0.4 M, 2, 4, 10, 20, 40, 80, and 160 mM). Substrates were dissolved in 0.15 mM acetic acid. Enzyme behavior (at pH 4.2 and 25 °C) was explained by the Michaelis−Menten equation, whereas kinetic parameters (K_m and V_{max}) were calculated by hyperbolic regression analysis (*19*).

Optimum pH and Temperature. PPO activity was determined over a pH range of 2.0−6.8 in 50 mM sodium acetate buffer, using DOPAC (40 mM) as phenolic substrate. Then, tests were carried out at the pH producing maximum activity to find the optimal temperature; PPO activity was assayed at various reaction temperatures as controlled by a circulation water bath. The temperature was varied over the range of 4−70 ± 0.1 °C. DOPAC (40 mM) was used as phenolic substrate.

Thermal Stability. The enzyme solutions in Eppendorf tubes were incubated in a water bath at different temperatures (30, 36, 40, 44, 50 °C) for different times (from 10 to 2880 min). PPO activity was determined at 25 °C and pH 4.2, using DOPAC (40 mM) as phenolic substrate. The percentage residual PPO activity was calculated by comparison with unheated enzyme.

Inhibition Tests. NaCl at different concentrations (50, 100, 200, 300, 400, 500 mM) was dissolved in the assay medium and PPO activity measured at 25 °C and pH 4.2, to determine inhibitor effects of NaCl. Furthermore, to evidence the direct effect of PPO on phenols and lycopene degradation, further storage tests were performed with samples treated with 6% NaCl 6% (w/v) and 0.05% tropolone (w/v) by comparison with an untreated sample.

Physicochemical Properties of Tomato Samples. Titratable acidity and total soluble solids were determined by total free acids neutralization with a 0.1 N NaOH solution (*20*) and by refractometric method, respectively. Total sugars and organic acids (L-malic, L-lactic, L-ascorbic) content was determined spectrophotometrically using Boehringer-Mannheim enzymatic test kits (Monza, Italy). Lycopene from tomato products was extracted with hexane/acetone/ethanol (2:1:1, v/v/v). Absorbance of the hexane extract was measured spectrophotometrically at 472 nm against a hexane blank. Concentration of lycopene was calculated using the extinction coefficient ($E^{1\%}$) of 3450 and expressed as milligrams per 100 g of fresh weight (fwt) (*21*). Total phenolic content was estimated spectrophotometrically with the Folin−Ciocalteu method, using gallic acid as standard.

Color Determination. A Nippon Denshoku NR-3000 colorimeter (Tokyo, Japan) was used to assess tomato pulp color. The measuring aperture diameter was 36 mm, and C/2° was the illuminant/viewing geometry. The color meter was calibrated using the BCR tomato paste reference tile RM 400. Six readings were made on each tomato sample. Analyses were made on a 50 g sample from each variety, having been previously peeled, the pulp homogenized, and bacteriostatic (Thymerosal, Sigma) added. Samples were incubated at 40 °C, and $L^*a^*b^*$ color values were measured every hour (from 0 to 20 h). Chroma and hue values were calculated from a^* and b^* values (*22*).

Statistical Analysis. All determinations were conducted three times at least. 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

Physicochemical Properties of Tomato Varieties. Samples from two commercial tomato varieties (Naomi and F1 PS212) and from three local varieties (Pizzutello, Rosa Maletto, and PO228) were analyzed to evidence eventual differences in physicochemical properties. Results are reported in **Table 1**.

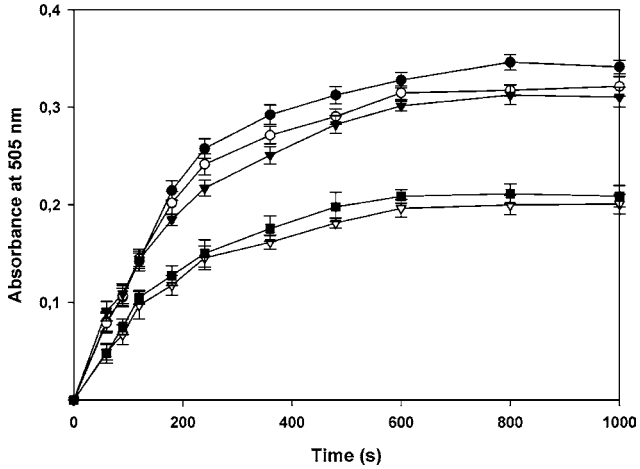

Figure 1. Substrate specificity of tomato PPO: (●) L-DOPA (ϵ = 27220 ± 210 M^{-1} cm^{-1}); (○) dopamine (ϵ = 28050 ± 180 M^{-1} cm^{-1}); (▼) DOPAC (ϵ = 25400 ± 170 M^{-1} cm^{-1}); (△) catechol (ϵ = 18140 ± 200 M^{-1} cm^{-1}); (■) 4-methylcatechol (ϵ = 18120 ± 210 M^{-1} cm^{-1}). All MBTH–substrate adducts showed maximum absorbance at λ = 505 nm.

Major significant differences among cultivars were in dry matter, in which Naomi was found to have the highest value (8.05%), in soluble solids/titratable acidity ratio, and in malic and lactic acid content. Pizzutello showed the highest malic acid content (24.4 mg/100 g of fwt), 2−5 times higher value than that of the other varieties. Among the local varieties, Rosa Maletto had the highest soluble solids/titratable acidity ratio (5.05) and lycopene content (17.7 mg/100 g of fwt). A significant difference in lycopene content was noticed between the commercial and local varieties, whereas ascorbic acid presented no difference among varieties.

Substrate Specificity. Five phenolic substrates (L-DOPA, DOPAC, dopamine, catechol, and 4-methylcatechol) were tested on crude PPO extract to find the one with the best analytical properties. The maximum absorption wavelength of the MBTH adduct was observed at 505 nm for all phenolic substrates tested. In **Figure 1**, absorption at 505 nm versus time plot for each substrate is shown. The five substrates tested have shown a behavior referable to two different groups: substrates L-DOPA, DOPAC, and dopamine are characterized by high extinction coefficient values ($E^{\%}$ from 25000 to 28000 M^{-1} cm^{-1}) and no solubility problems, even at high concentrations; on the other hand, catechol and 4-methylcatechol have shown lower $E^{\%}$ values (18100 M^{-1} cm^{-1}) and worse solubility properties. Relative to substrate group with high extinction coefficient values and good solubility, DOPAC was chosen as the best substrate for PPO activity determination on extracts obtained from tomato samples and for determination of optimum catalysis conditions of the enzyme.

Kinetics Constants. PPO kinetics constants determination was carried out on extracts from Naomi and Pizzutello varieties (**Figure 2**). In both cases the enzyme follows Michaelis−Menten kinetics, showing similar K_m and V_{max} values for DOPAC substrate: K_m = 1.86 (Naomi) and 1.59 (Pizzutello) mM; V_{max} = 0.28 (Naomi) and 0.23 (Pizzutello) units/100 g of fwt.

pH Optimum. The optimum pH for PPO activity differs among fruits. It is around pH 7.0 for PPO in kiwifruit, cherry, and pineapple (*23*, *24*), whereas it is around pH 4.0−5.0 for apple, eggplant, potatoes, and pears (*25*, *26*). The tomato PPO had maximum activity at pH 4.8 (**Figure 3**). The pH of juice in red ripe fruits is between 4.1 and 4.4, over which range PPO relative activity is between 74 and 87%. These results indicate that the pH condition is favored for PPO in red ripe fruit.

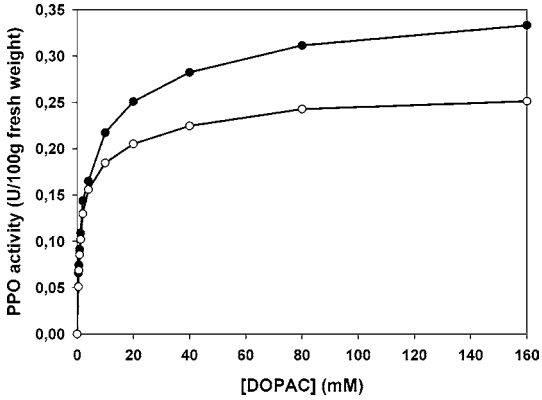

Figure 2. Hyperbolic regression results for tomato PPO (±95% confidence intervals): (●) Naomi (V_{max} = 0.28 ± 0.034; K_m = 1.86 ± 0.48); (○) Pizzutello (V_{max} = 0.23 ± 0.014; K_m = 1.59 ± 0.22).

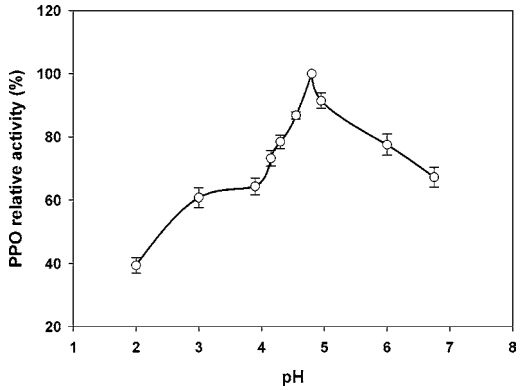

Figure 3. Effect of pH on tomato PPO activity.

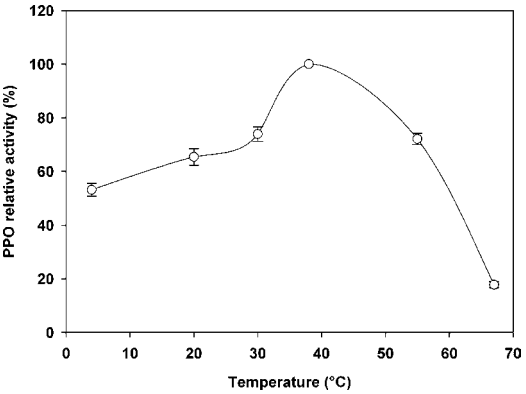

Figure 4. Effect of temperature on tomato PPO activity.

Optimum Temperature. The optimum temperature of activity for tomato PPO was 40 °C (**Figure 4**). The enzyme retained most of its activity (74% of the maximum) over a wide temperature range (30−55 °C). Above 55 °C, the PPO activity declined rapidly as the temperature increased. The enzyme showed a good relative activity (55% of the maximum) at cold-storage temperature (4 °C); this result is important for storage condition applications to fresh produce.

Thermal Stability. The thermostability profiles of PPO extracts from Naomi and Pizzutello are shown in **Figure 5**. The two PPOs are very stable in a temperature range from 30 to 50 °C. The time required to halve the activity was 1528 min at 30 °C, 1121 min at 36 °C, 952 min at 40 °C, 607 min at 44 °C, and 333 min at 50 °C for Naomi; and 1357 min at 30 °C, 1119 min at 36 °C, 928 min at 40 °C, 809 min at 44 °C, and 714 min at 50 °C for Pizzutello. Therefore, although thermal stabilities are similar for the two extracts until 40 °C, Pizzutello

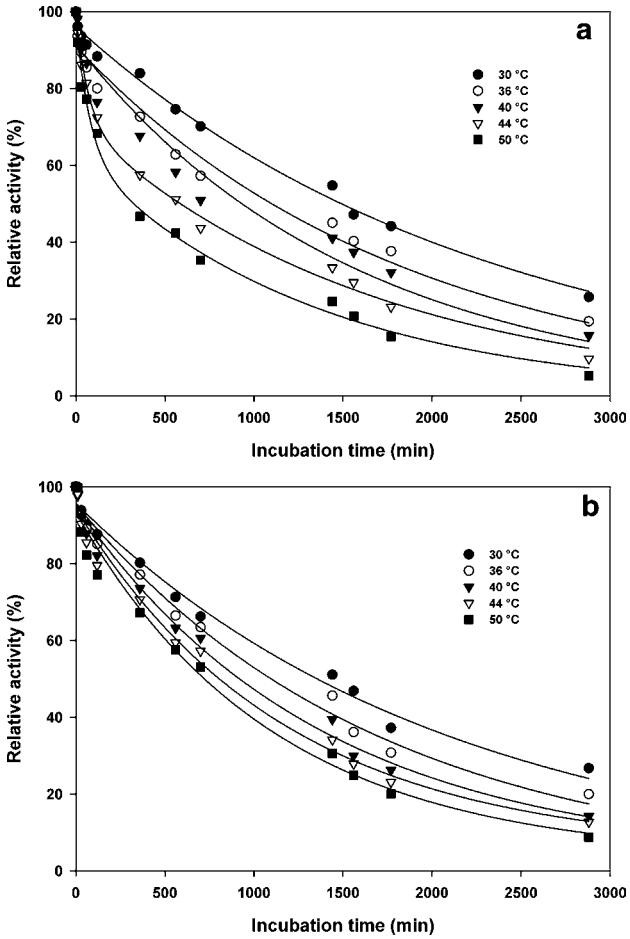

Figure 5. Thermal stability of (**a**) Naomi PPO and (**b**) Pizzutello PPO.

PPO seems to be more stable than Naomi PPO at higher temperatures. PPO is not a very heat-stable enzyme compared to other enzymes responsible for food-quality degradation (*27*), but tomato PPO showed high resistance at temperature (30 °C) even higher than storage temperatures of the fresh produce (from 4 to 25 °C).

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

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

as shown in **Figure 6**. The activation energy ($\Delta E^{\#}$) for crude Naomi and Pizzutello PPO heat inactivation was determined from results in **Figure 6**. Other activation parameters were determined from the relationships below as described previously (28):

$$\Delta G = RT \ln(kT/K_B h) \quad (1a)$$

$$\Delta H^{\#} = \Delta E^{\#} - RT \quad (1b)$$

$$\Delta S^{\#} = (\Delta H^{\#} - \Delta G^{\#})/\mathrm{T} \quad (1c)$$

R (8.3145 J mol^{-1} K^{-1}) is the universal gas constant, K_B (1.3806 $\times$ 10^{-23} J K^{-1}) is the Boltzmann constant, h (6.6261 $\times$ 10^{-34} J s) is the Planck constant, and T is absolute temperature. Results for these analyses are reported in **Table 2**.

To summarize, $\Delta E^{\#}$ was 30.9 kJ mol^{-1} for Naomi PPO heat inactivation as compared to 23.9 kJ mol^{-1} for Pizzutello PPO heat inactivation. At temperatures of 30−50 °C, the average values of $\Delta H^{\#}$ were 28.3 (±0.08) kJ mol^{-1} and 21.3 (±0.08) kJ mol^{-1} for Naomi and Pizzutello PPO, respectively. The value of $\Delta S^{\#}$ was 55.8 (±2.8) J mol^{-1} K^{-1} for Naomi PPO inactivation and 33.6 (±2.4) J mol^{-1} K^{-1} for Pizzutello PPO inactivation.

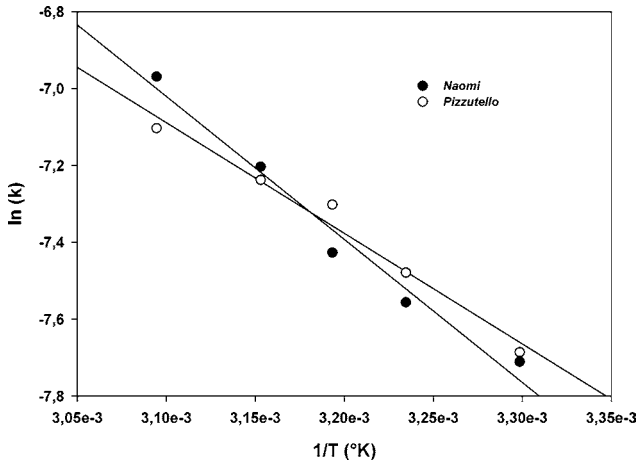

Figure 6. Arrhenius plot for heat inactivation of Naomi and Pizzutello PPO.

Table 2. Transition State Parameters for the Heat Inactivation of Crude Naomi and Pizzutello PPO

temp (°C)	$\Delta E^{\#}$ (J mol^{-1})	$\Delta H^{\#}$ (J mol^{-1})	$\Delta G^{\#}$ (J mol^{-1})	$\Delta S^{\#}$ (J mol^{-1} K^{-1})
		Naomi PPO		
30	30922.5	28403.2	10608.1	58.7
36	30922.5	28353.3	10767.7	56.9
40	30922.5	28320.1	10873.7	55.7
44	30922.5	28286.2	10979.1	54.6
50	30922.5	28236.9	11136.6	52.9
mean[a]	30922.5	28320.1	—[b]	55.8
SD		83.5		2.8
		Pizzutello PPO		
30	23920.1	21400.8	10545.9	35.8
36	23920.1	21350.9	10704.3	34.4
40	23920.1	21317.6	10809.4	33.5
44	23920.1	21284.4	10914.1	32.7
50	23920.1	21234.5	11070.3	31.4
mean[a]	23920.1	21317.7	—[b]	33.6
SD		83.5		2.4

[a] Mean (±SD) for triplicate experiments. [b] Parameter varies with temperature (cf. eq 1a).

Results from **Table 2** suggest that Naomi PPO is slightly more heat-resistant than Pizzutello PPO, apparently as a result of the higher $\Delta H^{\#}$ value for inactivation. In general, $\Delta H^{\#}$ is seen as a measure of the number of non-covalent bonds broken in forming a transition state for enzyme inactivation (*29*).

NaCl Inhibition. The effects of NaCl as PPO inhibitor are shown in **Figure 7**. The percentage inhibition was compared with that of the control (100% activity). PPO activity showed a decreasing course as NaCl concentration in the assay medium increased. The effect of NaCl on crude Naomi PPO activity was similar in pattern to that of crude Pizzutello PPO activity. Such decrease was slight up to a NaCl concentration of 0.1 M (82% PPO relative activity), whereas at higher concentrations PPO became gradually inactivated. It is believed that the action of NaCl is due to its interaction with the copper at the active center of the enzyme (*30*).

PPO Activity. Polyphenoloxidase activity (expressed as units per 100 g of fwt) and PPO activity/dry matter ratio of the five tomato varieties are reported in **Figure 8**. In terms of both PPO activity and PPO/dry matter ratio, the commercial variety Naomi showed highest values (PPO activity = 3.61 units/100 g of fwt,

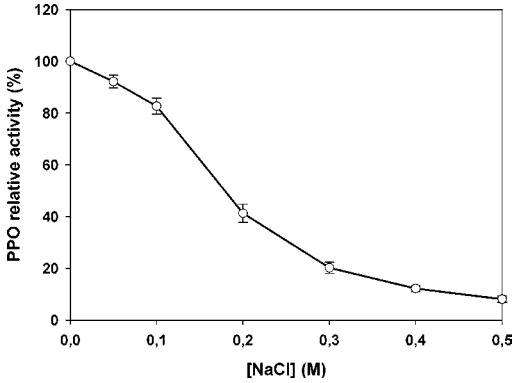

Figure 7. NaCl inhibition of crude tomato PPO.

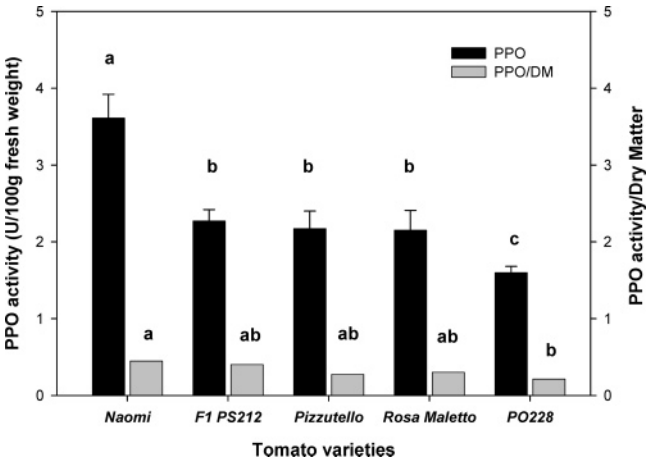

Figure 8. Total PPO activity (units/100 g fwt) and PPO/dry matter ratio of different tomato varieties. Means in columns with the same letter are not significantly different at the $p \leq 0.05$ level according to Duncan's multiple-range test.

PPO activity/dry matter = 0.613), followed by F1 PS212 (PPO activity = 2.27 units/100 g of fwt, PPO activity/dry matter = 0.53), whereas the traditional varieties Pizzutello, Rosa Maletto, and PO228 showed lowest PPO activities (from 1.60 to 2.17 units/100 g of fwt) and PPO/dry matter values (from 0.33 to 0.42). There was no significant difference between F1 PS212 and the local varieties Pizzutello and Rosa Maletto with regard to both PPO activity and PPO/DM ratio. By these results, the commercial variety Naomi is more susceptible to enzymatic browning than the local varieties Pizzutello, Rosa Maletto, and PO228, due to higher PPO activity levels.

Tomato Browning. L^* and a^* values were taken as the browning index of the samples. Such values showed a decreasing course upon storage at 40 °C (**Figure 9**) as b^* and chroma values, whereas hue angle values remained constant (data not shown). Such decrease was consistent for all tomato varieties up to 4 h from the beginning of the test. In particular, lycopene content of the samples decreased upon storage conditions (**Table 3**). To establish the PPO influence on tomato browning and the influence of lycopene as antioxidant agent, the relationships between color measurements and such factors as PPO activity and lycopene content variation were researched. In **Table 3**, all data results from the storage test (20 h at 40 °C) are shown, among which lycopene content and L^*, a^*, b^*, chroma, and hue values vary after 20 h. Initial PPO activity/lycopene content variation, $\Delta(a^*/b^*)$, and $\Delta(a^*/b^*)^2$ ratios were also calculated.

Tomato browning factors such as initial PPO activity, lycopene content variation, and PPO/ΔLyc ratio were put in relation with the variation of principal color parameters [L^*, a^*, b^*, chroma, hue, a^*/b^*, and $(a^*/b^*)^2$] after storage at 40 °C for 20 h. Regression coefficients r^2 for the linear regression fitting of these relationships were calculated (**Table 4**) to establish which relationship was valid for all varieties examined. High r^2 values were obtained for the following relationships: initial PPO activity with Δa^* (0.98) and Δchroma (0.94); ΔLyc with $\Delta(a^*/b^*)$ (0.97), $\Delta(a^*/b^*)^2$ (0.96) and Δhue (0.97); PPO/ΔLyc with Δa^* (0.91) and Δchroma (0.92). The PPO/ΔLyc ratio is better correlated with all color parameter variations (r^2 from 0.86 to 0.92) than PPO activity or ΔLyc alone, except for the relationship with ΔL^*, showing low r^2 values for all relationships studied. To evidence the direct effect of PPO on phenols and lycopene degradation, further storage tests were performed with samples treated with NaCl and tropolone, a strong PPO inhibitor (*31*), by comparison with an untreated sample. Total phenolic and lycopene contents were evaluated during storage. Results are shown in **Figure 10**. The presence of NaCl and tropolone seems to delay the degradation of lycopene and phenols, especially in the first storage hours, compared to the untreated sample. As tropolone has a direct action on the copper active center of the enzyme, NaCl at tested concentrations leads to a decreasing of free water (a_w) available for enzymatic reactions as well as O_2 solubility. Results from storage tests seem to relate the PPO activity with the color changes associated with browning and lycopene degradation, because lycopene is an antioxidant agent that reconstitutes the polyphenols oxidized by the action of PPO.

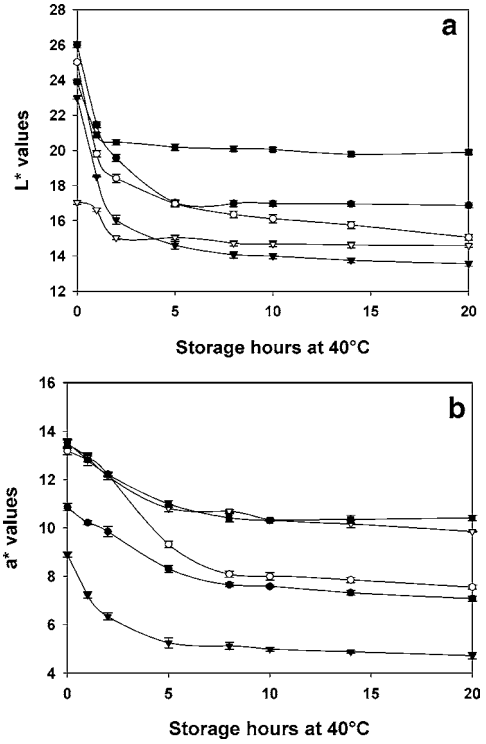

Figure 9. (**a**) L^* and (**b**) a^* values in tomato puree during storage at 40 °C: (○) Naomi; (●) Pizzutello; (▼) F1 PS212; (△) Rosa Maletto; (■) PO228.

Conclusions. Polyphenol oxidase was extracted and assayed from tomato fruits of three Sicilian landraces, Pizzutello, Rosa Maletto, and PO228, and two commercial cultivars widespread in protected cultivation (Naomi and PS212). The substrate specificity was established from values of extinction coefficient as dopamine > L-DOPA > DOPAC > 4-methylcatechol > catechol. Among five substrates tested, DOPAC was the best for tomato PPO activity determination, due to its high extinction

Table 3. Results from Storage Test (20 h at 40 °C) of Tomato Puree[a]

	ΔLyc (mg/100 g of fwt)	PPO*[b]/ΔLyc	Δ*a**	Δ*b**	Δ*L**	Δ(*a**/*b**)	Δ(*a**/*b**)^2	Δchroma	Δhue
Naomi	–4.09 ± 0.58	–0.88	–5.64 ± 0.33	–5.26 ± 0.37	–9.97 ± 0.77	0.123	0.31	–7.69	–0.047
F1 PS212	–6.56 ± 0.71	–0.35	–4.18 ± 0.18	–3.18 ± 0.22	–9.43 ± 0.44	–0.071	–0.17	–5.25	0.030
Pizzutello	–4.75 ± 0.62	–0.46	–3.79 ± 0.21	–3.69 ± 0.41	–9.13 ± 0.57	0.023	0.05	–5.29	–0.011
Rosa Maletto	–6.06 ± 0.35	–0.35	–3.65 ± 0.32	–2.11 ± 0.17	–2.46 ± 0.32	–0.043	–0.14	–4.21	0.012
PO228	–7.52 ± 0.54	–0.21	–3.11 ± 0.28	–1.27 ± 0.18	–4.01 ± 0.33	–0.160	–0.40	–3.23	0.062

[a] Values are reported as mean ± SD for triplicate experiments. [b] Initial PPO activity.

Table 4. Regression Coefficients (r^2) for the Linear Regression Fitting of Browning Parameters

	Δ*L**	Δ*a**	Δ*b**	Δ(*a**/*b**)	Δ(*a**/*b**)^2	Δchroma	Δhue
PPO*[a]	0.370	0.980	0.840	0.819	0.839	0.936	0.790
ΔLyc	0.373	0.594	0.822	0.967	0.959	0.753	0.972
PPO*/ΔLyc	0.374	0.906	0.865	0.895	0.910	0.919	0.872

[a] Initial PPO activity.

coefficient value and excellent solubilization properties. The tomato PPO had maximum activity at pH 4.8. The optimum temperature of activity for tomato PPO was 40 °C; the enzyme halved its relative activity at refrigeration temperature. This result is important for storage condition applications to fresh produce. PPO retained 82% of its relative activity at a NaCl concentration of 0.1 M; at higher concentrations it became gradually inactivated. This result is important for the production process setting of dried tomatoes, typical produce of the Mediterranean diet. The commercial variety Naomi is more susceptible to enzymatic browning than the local varieties Pizzutello, Rosa Maletto, and PO228, due to higher PPO activity levels. This result confirms the suitability of these local tomato varieties to national markets. Results from storage tests seem to relate the PPO activity with the color changes associated with browning and lycopene degradation, because lycopene is an antioxidant agent that reconstitutes the polyphenols oxidized by the action of PPO.

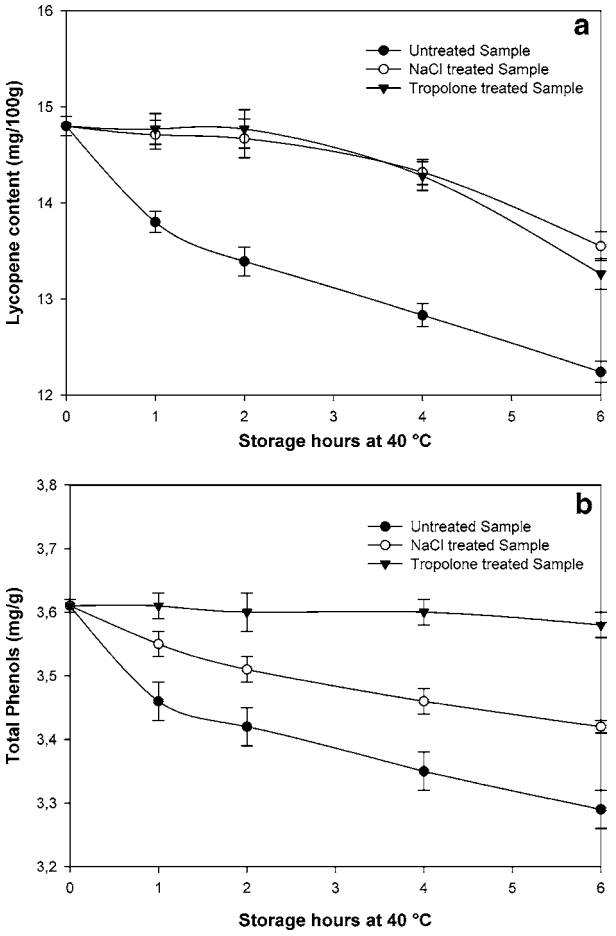

Figure 10. (**a**) Lycopene and (**b**) total phenolic contents in tomato puree during storage at 40 °C

ABBREVIATIONS USED

PPO, polyphenol oxidase; L-DOPA, L-3,4-dihydroxyphenilalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; MBTH, 3-methyl-2-benzothiazolinone hydrazone; DOFATA, Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (Catania, Italy); fwt, fresh weight.

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