Polyphenol Oxidase Activity and Browning in Green Olives

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In five olive (*Olea europaea* L.) varieties, a positive correlation was found between browning rate of the crude homogenate of the fruit and polyphenol oxidase (PPO) activity in the crude enzyme extracted from the acetone powder prepared from each variety. The possibility of the presence of an inhibitor, an inactivator, and/or an activator in the crude enzyme was tested in various ways and ruled out. It was therefore concluded that the differences in the browning rate of the olive varieties studied were directly related to the PPO activities as expressed in the enzymatic extracts.

The browning reaction in fruits and vegetables resulting from mechanical injury during postharvest storage or processing is a widespread phenomenon (Mathews and Parpia, 1975). It is usually commercially undesiderable because of the unpleasant appearance and concomitant development of off-flavor. The browning potential of various fruits, and sometimes in different varieties of the same species, has been shown to be directly related to the phenol level, the polyphenol oxidase (PPO) activity, or a combination of these factors (Vâmos-Vigyàzô et al., 1976; Kahn, 1977; Schaller et al., 1981; Vàmos-Vigyàzô and Nàdudvari-Mârkus, 1982). As concerns green olives, there is little information (Ben-Shalom et al., 1977, 1978). Therefore, this investigation was carried out to test whether there was any correlation between PPO content and browning rate in the fruits of five olive varieties.

MATERIALS AND METHODS

Plant Material. The fruits of five olive (*Olea europaea* L.) varieties "Bella di Spagna", "San Agostino", "San Francesco", "Ascolana Tenera", and "Pasola" were handpicked in September 1983 from trees, 10 years old, grown at the horticultural orchard of Bari University (Italy). Seven samples were harvested for each variety and analyzed immediately.

Enzyme Extractions. These were performed essentially as described by Flurkey and Jen (1978). Fresh olive tissues, 50 g for each sample, were homogenized for 2 min in an Ultra-Turrax homogenizer with 100 mL of cold acetone (-20 °C) and 2.5 g of poly(ethylene glycol) to set the phenolic compounds occurring naturally in the fruit. The green slurry was filtered under suction. The pellet was reextracted with acetone (-20 °C) 3 times, obtaining a white powder that was dried overnight at room temperature to remove residual acetone. Two grams of the acetone powder thus obtained was suspended in 100 mL of 0.05 M phosphate buffer, pH 6.2, containing 1 M KCl. The suspension was stirred for 30 min at 4 °C and then centrifuged at 20000*g* for 20 min at 4 °C. The supernatant (~95 mL) was used as the enzyme extract for the enzyme assay.

Assay Methods. PPO activity was determined spectrophotometrically by using a double-beam recording spectrophotometer (Perkin-Elmer Coleman 124D). Chlorogenic acid, protocatechuic acid, caffeic acid, DL-3,4-dihydroxyphenylalanine, and 4-methylcatechol, purchased from Pfaltz & Bauer, Inc., represented the substrates used to determine the PPO activity of the enzymatic extract from each olive variety. 4-Methylcatechol was recrystallized from *n*-hexane before use. The wavelengths used for determining the activity toward the various substrates, except that for 4-methylcatechol, are described by Interesse et al. (1980); for the latter substrate a wavelength of 410 nm was utilized. The substrate concentration was 0.02 M in sodium citrate buffer, pH 5. Among the substrates tested, 4-methylcatechol was that most rapidly oxidized for all the olive varieties studied. All measurements of PPO activity, therefore, were carried out with 4-methylcatechol as substrate by measuring the change in absorbance at 410 nm at 25 °C. The incubation mixture contained 0.5 mL of enzyme preparation, 1.5 mL of 0.1 M sodium citrate buffer, pH 5, and 1 mL of 0.02 M substrate in the same buffer. The assay mixture without the enzyme solution served as the control. The reaction mixture was oxygenated for 5 min prior to the addition of the enzyme extract. One unit of enzymatic activity was defined as the amount of the enzyme giving, under the above-mentioned conditions, a change in absorbance of 0.05 min^{-1}.

Protein in the enzyme preparation was estimated by following the procedure of Lowry et al. (1951) using crystalline bovine serum albumin (purchased from Sigma Chemical Co.) as a standard.

Preparation of Fruit Homogenate and Measurement of Browning Rate. Fresh fruit pulp of each olive sample was homogenized in a blender for 2 min with 0.05 M sodium phosphate buffer, pH 6.5, in a 1:10 (w/v) ratio. After 5 min at 25 °C the homogenate was quickly filtered through a layer of acid asbestos on No. 41 Whatman filter paper in a Büchner funnel. The rate of browning was expressed as absorbance at 410 nm per gram weight of fruit pulp after a 5-min incubation.

Table I. PPO Activity[a] and Browning Rate in Five Olive Varieties

no.	variety	total units of enzyme act.[b]	rate of browning[b]
1	"Ascolana Tenera"	700.1 ± 0.03 A	60.2 ± 0.02 A
2	"San Agostino"	520.3 ± 0.02 B	45.4 ± 0.03 B
3	"Bella di Spagna"	378.4 ± 0.03 C	31.8 ± 0.06 C
4	"San Francesco"	255.8 ± 0.05 D	21.7 ± 0.05 D
5	"Pascola"	213.5 ± 0.04 E	19.2 ± 0.02 E

[a] The crude enzyme solution was prepared from 2 g of acetone powder of each olive sample. [b] Mean ± SE from Duncan's (1955) multiple range test. Mean values with different capital letters in the same column are significantly different; $p < 0.01$. Seven samples per each variety.

RESULTS AND DISCUSSION

The mean values of PPO content and browning rate are significantly different in the five olive varieties studied (Table I). "Ascolana Tenera" and "San Agostino" show the highest values of PPO activity (700 and 520) and browning rate (60 and 45, respectively), while "San Francesco" and "Pasola" show the lowest values (256 and 214 for PPO content and 22 and 19 for browning rate, respectively). "Bella di Spagna" shows medium values

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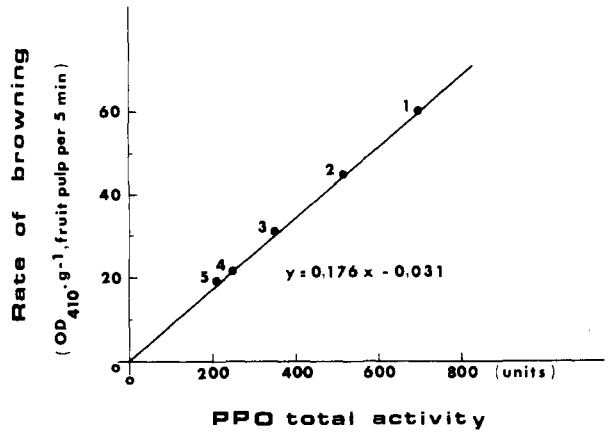

Figure 1. Relationship between rate of browning and PPO total activity in five olive varieties. Variety no. 1–5 are reported in Table I. In the linear regression equation X equals the PPO total activity.

Table II. Effect of Dialysis on PPO Activity of Four Olive Varieties[a]

		enzyme activity[b]	
variety	treatment	units/mL	sp act., units/mg of protein
"Ascolana Tenera"	control	7.37 ± 0.09	2.43 ± 0.05
	dialysis	8.84 ± 0.06	3.43 ± 0.04
"San Agostino"	control	5.59 ± 0.02	2.35 ± 0.04
	dialysis	6.04 ± 0.03	2.84 ± 0.06
"San Francesco"	control	2.75 ± 0.02	1.20 ± 0.07
	dialysis	3.16 ± 0.04	1.44 ± 0.05
"Pasola"	control	2.30 ± 0.03	1.51 ± 0.03
	dialysis	2.74 ± 0.02	2.04 ± 0.05

[a] A portion of the crude enzyme extract of each sample was dialyzed overnight at 4 °C against 0.05 M phosphate buffer, pH 6.2, and centrifuged, and the PPO activity in the supernatant was measured. The nondialyzed enzyme was kept overnight at 4 °C and assayed at the same time as its dialyzed counterpart. [b] Mean ± SE. Seven samples for each treatment. The dialysis mean values are significantly higher than their respective control mean values at the $p < 0.01$ level (t test) (Snedecor, 1956).

compared with those of two above-described groups. A linear relationship has been found between the mean values of the two characteristics studied. This correlation is highly significant ($r = 0.998$) and is reported in the scatter diagram of Figure 1.

The possibilities that the relatively low PPO activity in the enzyme extract of "San Francesco" and "Pasola" was due to the presence of an inhibitor, an inactivator, or factor(s) degrading the enzymes, while the relatively high activity of "Ascolana Tenera" and "San Agostino" was due to an activator, were ruled out on the following bases: (1) The effect of thorough dialysis of the crude enzyme extracts of all samples of the four olive varieties was tested and it was found that dialysis increased significantly in all cases the PPO specific activity from a minimum of 20% in "San Francesco" to a maximum of 41% in "Ascolana Tenera"; the trend of enzymatic activity of dialyzed extracts was the same of that of nondialyzed samples (Table II). (2) The PPO activity extracted from a 1:1 mixture of acetone powder from "Ascolana Tenera" and "San Francesco" was shown to be exactly as expected compared with that obtained when the activities were extracted separately. (3) A mixture of crude enzyme from "Ascolana Tenera" and "San Francesco" was prepared and aliquots of this mixture were immediately assayed: the observed activity was very close to that expected from computation of the activity obtained when the corresponding amounts of each enzyme were assayed separately. Moreover, the possibility that an artifact was formed in the course of preparing the acetone powder from varieties studied was ruled out since the trend of PPO activity in crude enzyme extracts was the same of that observed in filtrates of fruit homogenates obtained according to Ben-Shalom et al. (1977) (data not shown).

It can therefore be concluded that a direct correlation exists between the PPO activity and the rate of browning of the crude homogenate of fruit in the olive varieties studied. Further studies are required to test whether the PPO activity is the sole factor contributing to the differences observed in the browning rate of green olives.

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