Phenolic Compounds Related to the Black Color Formed during the Processing of Ripe Olives

Manuel Brenes-Balbuena, Pedro García-García, and Antonio Garrido-Fernandez*

Instituto de la Grasa, Unidad Estructural de Biotecnología de Alimentos, Apdo 1078, Sevilla, Spain

The composition in phenols of olive, the changes they undergo during the processing of ripe olives, and their relationships with surface color development were studied. The main phenols of these olives are identified as hydroxytyrosol (3,4-dihydroxyphenylacetic acid), verbascoside, tyrosol, vanillic acid, *p*-coumaric acid, and oleuropein. After the sodium hydroxide treatment, they are hydroxytyrosol, tyrosol, vanillic acid, caffeic acid, and *p*-coumaric acid. During the darkening process only the *o*-diphenol compounds hydroxytyrosol and caffeic acid are transformed, and their content diminution in flesh is directly related to the surface color or the surface color development. Thus, they are considered to be the promoters of the dark color obtained during the traditional ripe olive processing.

INTRODUCTION

Processing of ripe olives (by darkening in an alkaline solution) was initiated in California at the beginning of the 20th century. Recently, other countries have also started production. At the moment, the United States and Spain are the main producers and consumers of this kind of commercial table olive.

The procedures everywhere are, in general, very similar and consist of successive treatments of olives with sodium hydroxide solutions (lyes) on 3 consecutive days, penetrating skin, 1 mm into the flesh, and to the pit, respectively. During the intervals between lye treatments, fruits are suspended either in water or diluted brines, through which air is bubbled. Throughout this operation the olives darken progressively both in the flesh and (mainly) on the surface which becomes brown to black. Once the color is obtained, more water is added and aeration continues to pH equilibrium around 7–8 units. Then a ferrous gluconate or lactate solution is added to fix the color, and after 1 day at equilibrium, the product is canned and sterilized, as appropriate for a low-acid food (Cruess, 1958; Fernández et al., 1972). The whole process takes about 1 week (5–9 days).

Darkening can also be achieved just by exposure of the lye-treated olives to the air, washing off the excess of alkali only at the end of the darkening step. However, this operating procedure requires more labor and the fruits often shrivel (McCorkle et al., 1984).

Up to now, there has been only limited research on the olive color change from green-yellow to brown or even black during this processing. However, this darkening has been related to the formation of polymers from the abundant natural phenolic compounds of olives. Oleuropein was the first of them isolated by Panizzi et al. (1960), who also identified its chemical structure as a heterosidic ester of elenolic acid and (dihydroxyphenyl)ethanol. This substance was considered to be responsible for olive darkening (Simpson et al., 1961; Shasha et al., 1963), although the conclusions were not definitive. Recently Amiot et al. (1990) have also studied this compound in relation to the debittering process of green olives.

During the past two decades many other phenolic substances have been isolated and identified from the flesh of fresh olives (Vázquez et al., 1974) as well as from processed fruits (Vázquez and Janer del Valle, 1977), such as rutin, luteolin 7-glucoside, and hydroxycinnamic derivatives. Amiot et al. (1986) have also reported the identification of a new glucoside named verbascoside, in whose structure participate caffeic acid and (dihydroxyphenyl)ethanol. All of these are present in the flesh of olives, and their concentration diminishes throughout ripening (Amiot et al., 1986; Vázquez et al., 1971), a fact that might be in agreement with the observation that olive predisposition to oxidation also decreases with progressive ripening (Hartman, 1958).

Nevertheless, most research on the effects of these phenolics has been in connection with their bactericidal action (Fleming et al., 1973; Ruiz et al., 1990; Nychas et al., 1990) or their enzymatic oxidation, which is a big problem in the processing of green olives. Brown spots that occur after picking are thought to be catalyzed by a diphenol oxidase liberated during fresh olive handling. *o*-Diphenols have been identified as the normal substrate (Sciancalepore, 1985), and oleuropein has been suggested as the main phenol responsible (Goupy et al., 1991). Inhibition of this phenomenon was achieved by introducing the fruits into a diluted NaOH solution immediately after picking (Angel et al., 1979). Thus, it is reasonable to rule out this type of reaction during ripe olive darkening.

In other foods, chemical reactions that produce dark color have already been studied, especially in wine, which is quite similar in its phenolic composition. In this product, it is well established that gallic or caffeic acids begin their oxidation with formation of the corresponding quinones (Tulyathan, 1983) and that oxidation is directly related to oxygen concentration in the solution (Singleton, 1987). The oxidation kinetics of caffeic acid have also been studied in detail recently and depend mainly on solution pH (Cilliers and Singleton, 1989; Cuq and Jaussan, 1991). Once *o*-quinones are formed, these can react with proteins or follow polymerization reactions (Spencer et al., 1988).

In olives, Ralls (1967) suggested that *o*-diphenols in general could be related to these reactions, as Chambionat (1961) had indicated. Since then, nothing else has been done, and very little is known about these transformations. This paper initiates study of the chemical reactions of the phenolic compounds of olive flesh during oxidation in an alkaline medium when fruits are processed as ripe olives and their relationship with the black color development observed during the process.

0021-8561/92/1440-1192$03.00/0

MATERIALS AND METHODS

Olives. Experiments were carried out with fruits of Hojiblanca cultivar (*Olea europaea arolensis*). Olives were picked when they had a green-yellow surface color and were stored under anaerobic conditions in a 6% (w/v) NaCl brine for 7 months before use. An initial addition of 0.4% (w/v) of acetic acid at brining was enough to maintain pH below 4.2 during the whole preservation period. Details of other preservation conditions are given elsewhere (Fernández et al., 1985; UEIBA, 1990).

Experimental Design. Five kilograms of olives was treated with a NaOH solution (2.5% w/v) for 4 h at 34 °C, until the lye reached the pit. Then, 1 kg of these was exposed to air at room temperature (about 20 °C); the fruits were manually moved periodically (every 2–3 h) to avoid white spots. Another 2 kg was put in each of the two experimental devices for the processing of ripe olives; distilled water was added to cover the olives a little in excess (2 L). During the whole time air was bubbled at a rate of 0.3 m^3/h and the pH maintained at approximately 7.0 by addition of 0.1 N HCl regulated by automatic pH meters whose probes were introduced in each container (García et al., 1991). The whole process took around 2 days.

Color of Fruits. Surface color of fruits were measured by reflectance at 700 nm in a Bausch and Lomb spectrophotometer, equipped with a reflectance sphere (Fernández et al., 1985).

Polyphenol Analyses. Approximately 38 g of olive flesh (15 size-graded pitted fruits) was mixed at 4 °C in a blender with enough 80% (v/v) ethanol, containing 1% (w/v) sodium metabisulfite and 6 N HCl, to reduce the pH to 3.0. The following extraction was achieved as described by Amiot et al. (1986).

Polyphenols from the aeration were extracted with ethyl acetate, after pH adjustment to 3.0 with 6 N HCl and addition of 1% sodium metabisulfite.

Both extracts were evaporated under vacuum and the residues dissolved in a 1:1 water/methanol mixture.

Detection and quantification of phenols was carried out by HPLC in a Waters 600E (Millipore, Inc.) apparatus equipped with a diode array detector (Waters 994). A 25 cm × 4 mm i.d. column was used, filled with Spherisorb ODS-2, 5 μm (Supelco, Inc.). A short column (5 cm × 4 mm i.d.) was placed immediately before the main column. The wavelength selected for detection was 280 nm.

Separation was achieved by gradient elution using an initial composition of 95% water, with pH adjusted to 2 with phosphoric acid, and 5% acetonitrile. A flow rate of 1 mL/min was used. Concentration of the latter solvent was increased to 25% in 20 min and up to 50% in another 20 min. To purge the column, the concentration of acetonitrile was raised in 5 min to 75%, maintaining the composition for 10 min. Column re-equilibrium with initial conditions was reached after 15 min at the appropriate gradient.

Phenolic compounds were identified by their retention times and absorption spectra from the 200–380-nm range. The verbascoside spectrum was similar to that obtained by Amiot et al. (1990), and its alkaline hydrolysis originated hydroxytyrosol and caffeic acid. Standards were purchased from Sigma, and hydroxytyrosol was obtained from oleuropein by acid hydrolysis (Vázquez et al., 1974).

RESULTS AND DISCUSSION

The aim of the experimental work was to know the phenolic composition of olives at the beginning of the darkening process, to study the transformations of such substances during the lye treatment [2.5% (w/v) NaOH] and fruit debittering, and to follow the evolution of the compounds formed during the alkaline oxidation that transform the surface color into brown to black.

Figure 1 shows the HPLC chromatograms of the phenolic compounds in the flesh of the oxidized olives. It shows the composition of such fruits after 7 months of preservation in brine and also after the lye treatment. Of the peaks identified, hydroxytyrosol (3,4-dihydroxyphenylacetic acid) and verbascoside were the two most significant. The former has never been reported in olive flesh in such

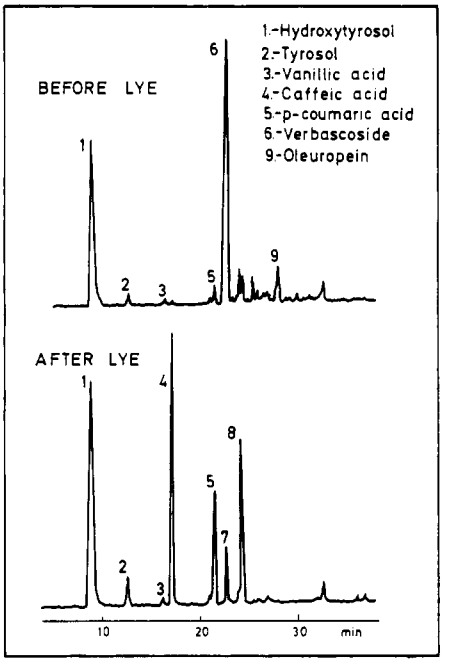

Figure 1. HPLC chromatograms of the phenolic extracts from olive flesh before and after lye treatment.

proportion, so it must come from the acid hydrolysis of oleuropein during the period of preservation in brine when the pH is below 4.2 (which would explain the low oleuropein content in both olives and brine) or (less likely) from the verbascoside partial hydrolysis. In fact, there should have been no, or very little, acid hydrolysis of verbascoside because no caffeic acid was observed in olives before NaOH treatment. Nevertheless, there is always the possibility that the small amount of caffeic acid formed had polymerized; its polymers would not have been extracted by ethyl acetate.

However, after the NaOH solution treatment, caffeic acid was detected in abundance and the hydroxytyrosol presence was increased. This new composition is derived directly from alkaline hydrolysis of the limited amount of oleuropein still present and (mainly) from the verbascoside degradation in such conditions, since diminution of the latter in this process is evident.

Tyrosol (hydroxyphenylacetic acid) was also found both before and after the alkaline action, showing a slight increase. Its presence was in a small quantity and would be due to the hydrolysis of a new glucoside, ligustroside, recently isolated from olive flesh (Leon, 1991), which has a chemical structure similar to that of oleuropein, with tyrosol instead of hydroxytyrosol as the alcohol group. In any case, its diffusion from the olive seed, where it was first identified by Vázquez et al. (1974), is not probable.

Another phenolic substance identified in low proportion and apparently uninfluenced by the lye treatment was vanillic acid, whose presence in olives had already been noted by Vázquez et al. (1974). Although present in stored olives, *p*-coumaric acid is considerably increased by the lye effect and its origin could be certain *p*-coumaric esters which were studied by the same authors.

In addition, there were a few other compounds that could not be identified. In general, they were in small proportion except peaks 7 and 8, which appeared after the lye treatment and whose levels were remarkable when olives underwent the normal process of darkening by both exposure to air or bubbling of air through the water solution.

Changes in polyphenols were closely related to their chemical structures. The concentration of some of them

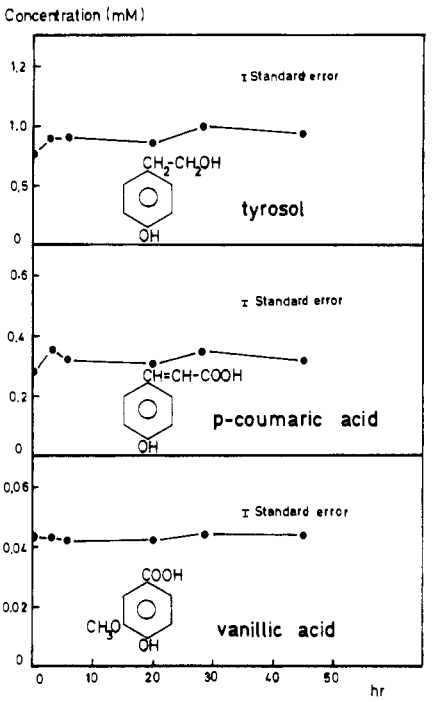

Figure 2. Changes of non *o*-diphenols in olive flesh during oxidation by air exposure.

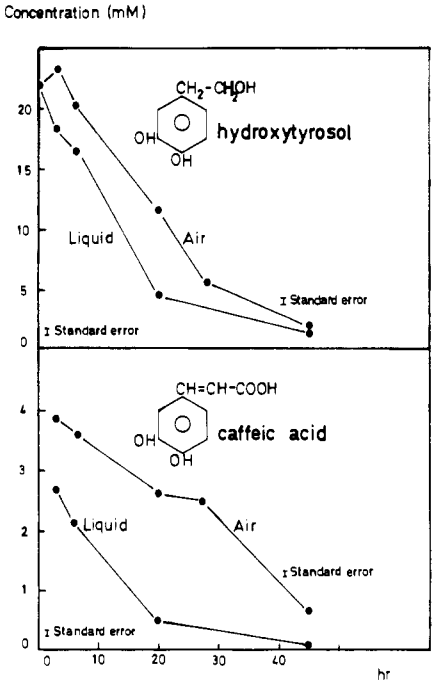

Figure 3. Changes of *o*-diphenols in olive flesh during oxidation by air exposure and aeration of the fruits in a liquid suspension.

remained constant throughout the elaboration process, as Figure 2 shows. These were tyrosol, *p*-coumaric acid, and vanillic acid, and their behavior could be related to the fact that none of them has an *o*-diphenol structure (Tulyathan, 1983).

However, the two most representative phenolic substances of the olive flesh after lye treatment, hydroxytyrosol and caffeic acid, diminished considerably through the oxidation process, as Figure 3 shows. Despite the concentration differences, the oxidation rates were similar for both *o*-diphenols and the flesh content was lower when olives were kept suspended, because of the additional effect

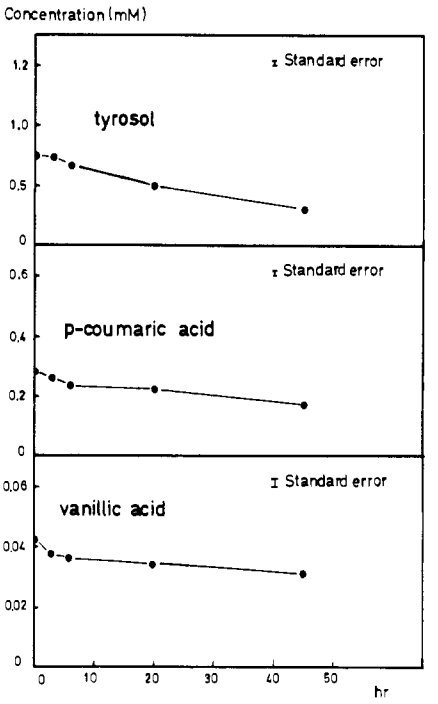

Figure 4. Changes of non *o*-diphenols in olive flesh oxidized by aeration in a liquid suspension.

of dilution into the liquid. This influence was more marked for the caffeic acid, probably due to the higher water solubility.

On the other hand, with simple exposure of fruits to the air the oxidation rate of caffeic acid is constant, although slower than that for hydroxytyrosol, which seems to slow down at the end of the process. In suspension this behavior was observed in both. However, the final concentration was very low in these substances in spite of the fact that the initial hydroxytyrosol content was 5 times that of caffeic acids. This can be explained only on the basis of a severe oxidation of these substances during the air (oxygen) action.

Figure 4 shows the evolution of the non *o*-diphenols in the flesh of olives oxidized in liquid medium. The concentration of tyrosol, *p*-coumaric acid, and vanillic acid diminished very slowly throughout the treatment period, due only to dilution into the surrounding liquid, in which the content of these phenols increased proportionally with time (Figure 5). However, the overall balance was maintained.

o-Diphenol changes in the aerated liquid in which olives were suspended were diverse, as Figure 6 shows. Hydroxytyrosol retained a level around 1.5 mM, very low in relation to the initial concentration in flesh. Caffeic acid decreased with oxidation time, and as with the former compound, its absolute presence in brine was in very low proportion. Such changes indicate that simultaneously to the diffusion process into water there is an oxidation of polyphenols in the flesh itself that could interfere with their transference to the liquid. Some oxidation was also achieved in the solution, whose pH was stabilized between 7 and 8, a slightly alkaline medium that might have favored oxidation.

Finally, Figure 7 shows the relationship between both hydroxytyrosol and caffeic acid and the surface color obtained throughout the darkening process, measured as a percentage of reflectance at 700 nm, which values diminish as the color becomes darker. It can be observed that the transformation of both substances, or diminution of the residual content, was parallel to color development,

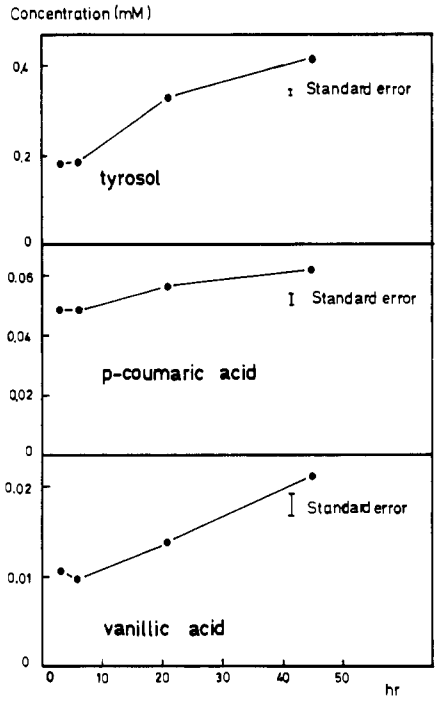

Figure 5. Changes of non *o*-diphenols in the solution of fruits oxidized by aeration in a liquid suspension.

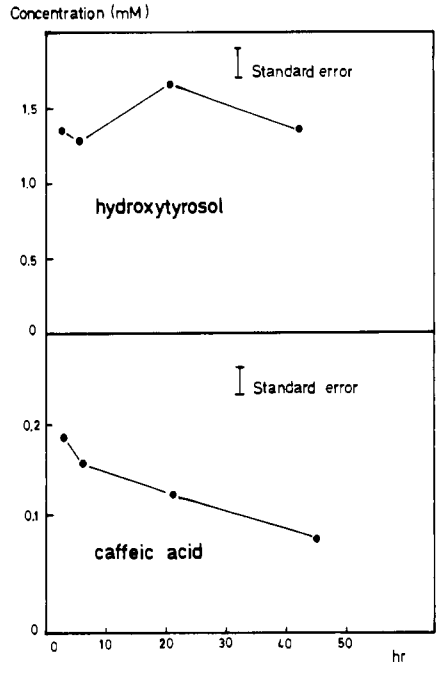

Figure 6. Changes in *o*-diphenols in the solution of fruits oxidized by aeration in a liquid suspension.

with only small differences between the oxidation by air exposure or that by air bubbling in the case of hydroxytyrosol, and a more marked influence of the oxidation conditions in that of caffeic acid. All of these transformations occurred when the olive flesh was alkaline, with a pH about 12.8, which decreased very slowly during the process to reach 11.1 for fruits suspended in water and 12.0 for the others.

Phenols corresponding to peaks 7 and 8, measured as their respective areas, did not change during the oxidation, indicating that, although in moderate abundance, they did not participate in the color formation.

As a conclusion from all of the above assays, it can be stated that brown to black color during the oxidation

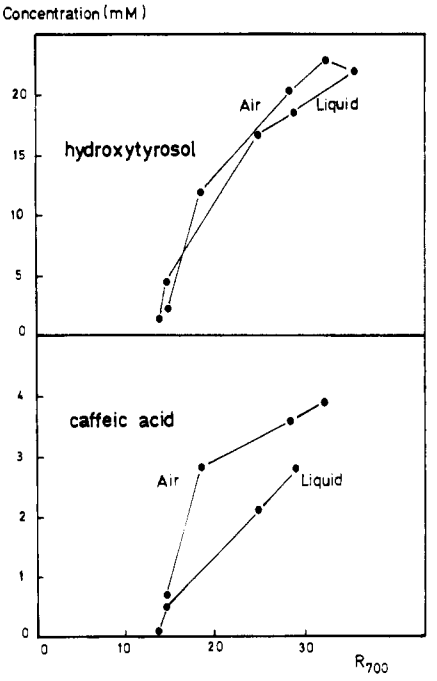

Figure 7. Relationship between *o*-diphenols in olive flesh of both types of oxidation process and surface color development.

process of ripe olive preparation is due mainly to the polymerization of hydroxytyrosol and caffeic acid under the effect of oxygen when fruits are exposed to air or aerated in aqueous suspension. Possible future research indicated includes the individual contribution of each one on color development, as well as their kinetics and color differences attributed to possible *o*-diphenol content in each cultivar.

ACKNOWLEDGMENT

We express our thanks to CICYT (Spanish Government, ALI-88-0115-CO2-01 project) for financial support of this research.

LITERATURE CITED

Amiot, M. J.; Fleuriet, A.; Machiex, J. J. Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food Chem.* **1986**, *34*, 823–826.

Amiot, M. J.; Tachini, M.; Fleuriet, A.; Macheix, J. J. The technological debittering process of olives: characterization before and during alkaline treatment. *Sci. Aliments* **1990**, *10*, 619–631.

Angel, S.; Ben-Shalom, N. Research on olive quality in Israel for processing. I. Prevention of brown spotting in green olives for pickling. *Confructa* **1979**, *24*, 79–84.

Chambionat, A. Contribution to the artificial darkening of green olives. *Cah. Rech. Agron.* **1961**, *No. 11*.

Cilliers, J. J. L.; Singleton, V. L. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. *J. Agric. Food Chem.* **1989**, *37*, 890–896.

Cruess, W. V. Pickles. In *Commercial Fruit and Vegetable Products*; McGraw-Hill: New York, 1958.

Cuq, J. L.; Jaussan, F. Caffeic acid oxidation and antibacterial effects. *Sci. Aliments* **1991**, *11*, 25–36.

Fernández, M. J.; Garrido, A.; González, F.; Durán, M. C.; Cordón, J. L. *Aceitunas tipo negras. El aderezo de aceitunas negras*; Instituto de la Grasa y sus Derivados: Sevilla, Spain, 1972.

Fernández, M. J.; Castro, R.; Garrido, A.; González, F.; González-Cancho, F.; Nosti, M.; Heredia, A.; Minguez, M. I.; Rejano, L.; Durán, M. C.; Sánchez, F.; García, P.; Castro, A. In *Biotecnología de la aceituna de mesa*; Instituto de la Grasa y sus Derivados: Sevilla, Spain, 1985.

Fleming, H. P.; Walter, W. M.; Etchells, J. L. Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Appl. Microbiol.* **1973**, *26*, 777–782.

García, P.; Brenes, M.; Garrido, A. Effect of oxygen and temperature on the oxidation rate during darkening of ripe olives. *J. Food Eng.* **1991**, *13*, 259–271.

Goupy, P.; Fleuriet, A.; Amiot, M. J.; Macheix, J. J. Enzymatic browning, oleuropein content, and diphenoloxidase activity in olive cultivars (Olea europaea, L.). *J. Agric. Food Chem.* **1991**, *39*, 92–95.

Hartman, H. T.; Simone, M.; Vaughn, R. H.; Maxie, E. C. Relation of fruit maturity at harvest to quality of black-ripe processed olives. *J. Am. Soc. Hortic. Sci.* **1958**, *73*, 213–228.

León, R. Studies on the antimicrobial activity of phenolic compounds from olives. Ph.D. Dissertation, University of Seville, Spain, 1991.

McCorkle, K. C.; Wells, H. M.; Scrimshire, H. D.; Beckwith, R. N. Method for producing black ripe olives. U.S. Pat. 4463023, July 31, 1984.

Nychas, G. I. E.; Tasson, S. C.; Board, R. G. Phenolic extract from olives: inhibition of *Staphylococcus aureus*. *Lett. Appl. Microbiol.* **1990**, *10*, 217–220.

Panizzi, L.; Scarpeti, M. L.; Oriente, E. G. Structure of the bitter glucoside oleuropein. Note II. *Gazz. Chim. Ital.* **1960**, *90*, 1449–1485.

Ralls, J. *Basic research on olives*; 46th Annual Technical Report; California Olive Association: Sacramento, 1967; pp 82–88.

Ruiz, J. L.; Rios, R. M.; Fedriani, C.; Olías, J. M.; Rios, J. L.; Jiménez, R. Bactericidal effect of phenolic compounds from green olives on *Lactobacillus plantarum*. *Syst. Appl. Microbiol.* **1990**, *13*, 199–205.

Sciancalepore, V. Enzymatic browning in five olive varieties. *J. Food Sci.* **1985**, *50*, 1194–1195.

Shasha, B.; Leibowitz, J.; Ilany-Feigenbanm, J. On the debittering and darkening of olives. *Isr. J. Chem.* **1963**, *1*, 33–35.

Simpson, F.; Chichester, C. O.; Vaughn, R. H. Compounds responsible for the color of black ripe olives. *J. Food Sci.* **1961**, *26*, 277–283.

Singleton, V. L. Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69–77.

Spencer, C. M.; Cai, Y.; Martin, R.; Gaffuey, S. H.; Goulding, P. N.; Magnolato, D.; Lilley, T. H.; Haslam, E. Polyphenol complexation. Some thoughts and observations. *Phytochemistry* **1988**, *27*, 2397–2409.

Tulyathan, V. Oxidation of phenolics common to Foods and Wine. Ph.D. Dissertation, University of California, Davis, 1983.

UEIBA (Unidad Estructural de Investigación de Biotecnología de Alimentos). Processing of olives darkened by oxidation. In *Table olive processing*; International Olive Council: Madrid, Spain, 1990.

Vázquez, A.; Janer del Valle, M. L. Changes in polyphenols during olive maturation. *Grasas Aceites* **1977**, *28*, 421–426.

Vázquez, A.; Maestro, R.; Graciani, E. Phenolic compounds in olives. I. Poliphenols in flesh. *Grasas Aceites (Seville)* **1971**, *22*, 366–370.

Vázquez, A.; Graciani, E.; Maestro, R. Poliphenol evolution during pickling of green olives. I. Qualitative study. *Grasas Aceites (Seville)* **1974**, *25*, 269–279.

Received for review November 12, 1991. Accepted March 30, 1992.

Registry No. Hydroxytyrosol, 102-32-9; caffeic acid, 331-39-5; tyrosol, 501-94-0; verbascoside, 61276-17-3; vanillic acid, 121-34-6; *p*-coumaric acid, 7400-08-0; oleuropein, 32619-42-4.