# Importance and Evolution of Phenolic Compounds in Olive during Growth and Maturation

Marie-Josèphe Amiot, Annie Fleuriet, and Jean-Jacques Macheix*

The main phenolic compounds (oleuropein, verbascoside, rutin, luteolin 7-glucoside) were separated and determined by HPLC for three varieties (Picholine, Lucques, Salonenque) during the development and maturation of the fruit and for eight other varieties (Bouteillan, Verdale, Cailletier, Zrappola, Tanche, L11, L365, VP7) just during the maturation. Oleuropein content could reach 14% of the dry matter in young fruit and remains very important at harvest for green picked varieties. Furthermore, small-fruit varieties are characterized by high oleuropein and low verbascoside contents, while large-fruit varieties are characterized by low oleuropein and high verbascoside contents.

Several studies concerning phenolic compounds in the olive have already been carried out in relation with the technical problems posed in processing of the fruit (Fernandez Diez, 1971; Vasquez Roncero and Janer del Valle, 1977). In fact these substances are important parameters in the organoleptic qualities of the fruit (Cohen et al., 1967), and *o*-diphenols in particular can play a role in browning (Ben Shalom et al., 1977) or act as antioxidants in the conservation of oil (Vasquez Roncero, 1978). Oleuropein, the main bitter component in the olive (Shasha and Leibowitz, 1961), was revealed in 1908 by Bourquelot and Vintilesco, and in 1960 its structure (Figure 1) was specified as being that of a heterosidic ester of elenolic acid and (dihydroxyphenyl)ethanol (Panizzi et al., 1960). Demethyloleuropein, a demethylated derivative, has also been reported in ripe olives ((Ragazzi et al., 1973). Besides these compounds, there are also flavonol glycosides, in particular rutin and luteolin 7-glucoside (Vasquez Roncero and Janer del Valle, 1974) and derivatives of hydroxycinnamic acids, the main one of which was identified recently as being verbascoside (Fleuriet et al., 1984) and the heterosidic ester of caffeic acid and (dihydroxyphenyl)ethanol (Andary et al., 1982; Figure 1).

The variations of several of these compounds and of total phenols has already been monitored during the ripening of several varieties of olive (Solinas et al., 1975). However, it appeared interesting to obtain more precise quantitative data using separations of the main phenolic compounds in the fruit by HPLC techniques. This investigation was extended to growth and maturation periods to the extent that certain varieties are traditionally picked "green" and some of these, e.g. Lucques and Picholine, are treated by specific techniques (Loussert and Brousse, 1978). Knowledge of the variations in phenolic compounds should make it possible first to obtain better understanding of the relationships that may exist between these substances and the physiology and organoleptic qualities of the fruit and second to provide a more solid basis for processing techniques, thus leading to improved quality.

## MATERIALS AND METHODS

**Plant Material.** The results reported here are for 1984; they confirm and complement data obtained in preceding years. Fruit was picked weekly from olive trees some 20 years old cultivated in the INRA (National Institute of Agronomic Research) experimental orchard in Montpellier. The varieties Picholine, Lucques, and Salonenque were picked during growth and maturation while the five other varieties (Tanche, Bouteillan, Verdale, Cailletier, Zrappola) and three clones in course of selection (L365, L11, VP7) were picked only during maturation.

Each sampling was made of 100 fruits that were first graded and weighed to determine the growth curves. The first analyses of phenolic compounds soon showed that there was high variability from one fruit to another (variation coefficient of 12% in young fruit and nearly 30% in maturing fruit), and the 10 largest fruits in each sample were therefore used to make the batch for analysis. Variability was then considerably lower at less than 10% in every case. The fruits thus selected were immediately frozen in liquid nitrogen and then crushed in a ball grinder (Dangoumau type) after removal of the stone (except for the first four samples in which the endocarp and the seed were crushed and analyzed with the pulp).

**Extraction and Purification of Phenolic Compounds.** The powder obtained (1 g) was immediately homogenized in 80% ethanol in the presence of metabisulfite (2%). After agitation at 4 °C for 20 min followed by filtering, the residue was treated in the same way. Aqueous–Alcohol extracts were collected, and ethanol was evaporated under vacuum. Four successive petroleum ether extractions allowed removal of pigments and most lipids. The phenolic compounds were then extracted by ethyl acetate in the presence of ammonium sulfate (20%), metaphosphoric acid (2%), and methanol (20%) (Fleuriet and Macheix, 1972). After three successive extractions, the ethyl acetate was eliminated and the dry residue dissolved in methanol; the final extract thus obtained was used for UV spectrophometric analysis and HPLC separation.

**Separation and Assaying of Phenolic Compounds by HPLC.** The extracts were analyzed by HPLC (Varian 5000 apparatus) on a Micropak (MCH-5) column [reversed phase, granulometry 5 μm, length 30 cm, internal diameter 4 mm]. An acetonitrile–water gradient (adjusted to pH 2.6 with orthophosphoric acid) was established running from 15 to 40% acetonitrile in water for 20 min. Assaying was carried out by internal calibration with two wavelengths: 280 nm for oleuropein and 340 nm for verbascoside, rutin, and luteolin 7-glucoside, the internal standards selected being coumarin and luteolin respectively, which do not exist in a free state in the fruit. Assaying was carried out in relation with standards supplied by SARSYNTEX, with the exception of verbascoside, which was obtained according to Andary et al. (1982). The results are expressed in milligrams/gram of dry or fresh matter and in milligram per fruit. Several extractions using the same initial plant material resulted in variability of the order of 6%.

## RESULTS AND DISCUSSION

The main phenolic compounds detected by HPLC (Figure 2a,b) were identified as verbascoside (Fleuriet et

Laboratoire de Physiologie Végétale Appliquée, Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France.

0021-8561/86/1434-0823$01.50/0 

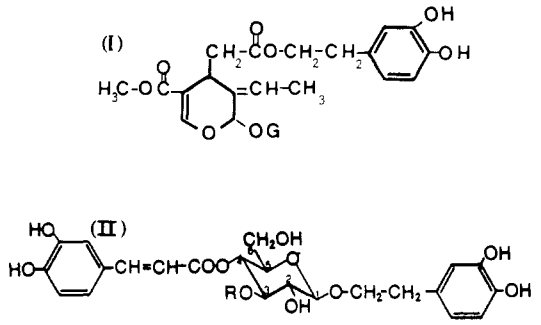

**Figure 1.** Structural formulas of oleuropein (I) and verbascoside (II). G = glucose; R = rhamnose.

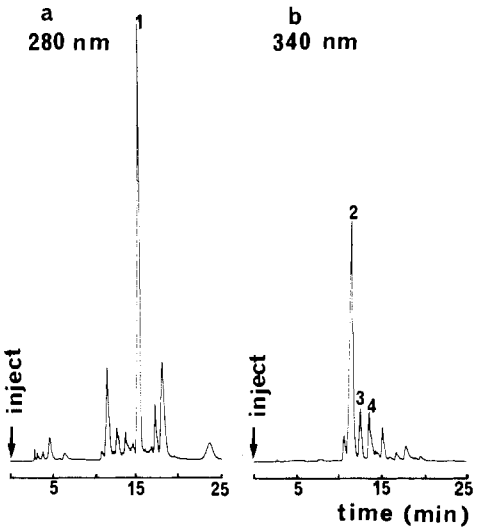


**Figure 2.** Fractionating the phenolic compounds by HPLC at two wavelengths [280 nm (a); 340 nm (b)] from the Lucques variety sampled on Sept 4 1984. Peak identification: (1) oleuropein; (2) verbascoside; (3) rutin; (4) luteolin 7-glucoside.

al., 1984), rutin, and luteolin 7-glucoside. The UV spectra of these extracts showed two peaks at 281 and 330 nm that correspond to the absorption maxima of oleuropein and verbascoside, respectively.

**Variation during Growth and Maturation.** The oleuropein content in the fruit increased rapidly during growth; the maximum obtained depended first on the method of expression of the results—either per fruit or per gram of dry matter (Figure 3a)—or according to the variety. The maximum thus occurred toward 20 Aug for Lucques and Salonenque (Figure 3a,b), with values of 110–120 mg per fruit, and only at the beginning of September for Picholine (Figure 3c) with a value of 70 mg per fruit. Then, during the two successive phases characterizing maturation of the olive (Shulman and Lavee, 1976), very rapid reduction was first observed and then much slower reduction after change in color of the fruit. Verbascoside was not detectable in very young fruits; it accumulated considerably from August onward, reached a maximum distinctly later than oleuropein, and then diminished sharply during ripening and maturation (Figure 3a–c). Flavonoids developed in a similar manner but were present from the first stage of formation of the fruit.

Several interesting remarks can be made about the results above. First, the very high oleuropein content of the olives should be stressed: for example, this compound can form up to 14% of the dry matter in young Picholine. This is exceptional in fruit (Van Buren, 1971; Herrmann, 1978). Even at harvesting of the three varieties picked green before full maturity, oleuropein formed 3–6% of the dry matter, which is still a considerable amount. The ver-

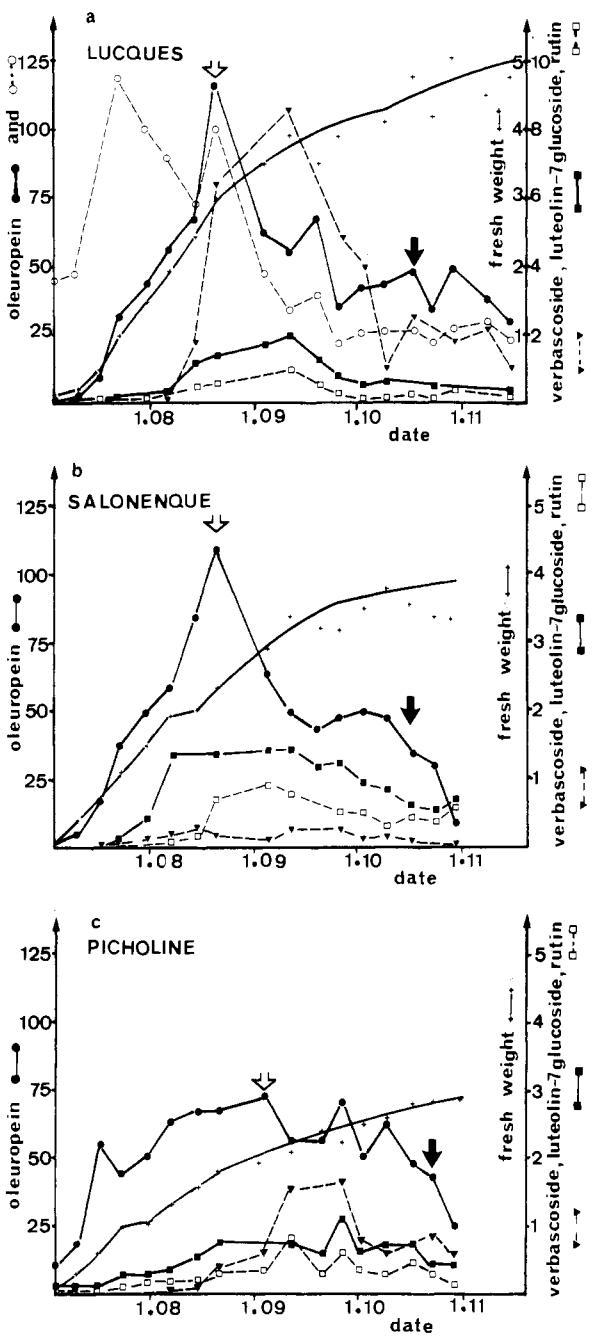


**Figure 3.** Evolution of the main phenolic compounds (mg per fruit) during development and maturation present in fruit Lucques (a), Salonenque, (b), and Picholine (c): ●, oleuropein; ▲, verbascoside; □, rutin; ■, luteolin 7-glucoside; +, fresh weight (g per fruit); O, oleuropein (mg $g^{-1}$ dry weight). Arrows: open, beginning of green maturation; filled, anthocyanins.

bascoside content was much lower (maximum 0.5%) but was still high in relation to glycoside derivatives of hydroxycinnamic acids present in other fruits (Macheix et al., 1977; Herrmann, 1978). In addition, it varies considerably from one variety to another; for example, it is 30 times higher in Lucques than in Salonenque. However, maximum flavonoid contents were very similar in all three varieties; rutin was always the most plentiful since it is probably located in the outermost part of the fruit, as has already been shown in other cases (Tronchet, 1972; Fleuriet, 1976).

Expression of these results in relation to the fresh or dry matter makes it possible to show the maximum oleuropein content better (Figure 3a). The reduction that follows cannot be ascribed to the simple effect of dilution during

growth since the accumulation in the complete organ (per fruit) continues later. This phenomenon has been observed in many fruits (Macheix, 1974; Billot et al., 1978; Fleuriet and Macheix, 1981; Romeyer et al., 1983) and shows the existence of active phenolic synthesis in young fruits. The period of most rapid growth is followed by maturation that takes place in two phases in the olive (Shulman and Lavee, 1976). The first phase, referred to as "green maturation" is characterized by a reduction in chlorophylls and extensive lipid synthesis. The rapid reduction in oleuropein and the peak accumulation of verbascoside and flavonols (Figure 3) is closely associated with this. This comparatively late accumulation of verbascoside that occurs when maturation has commenced should be associated with the previous observations concerning certain glucose derivatives of hydroxycinnamic acids that accumulate in the tomato at the beginning of maturation (Fleuriet and Macheix, 1981) while the quinic derivatives of these acids diminish. The considerable reduction of oleuropein per fruit shows clearly that it is not an inert metabolic compound but is reutilized during the maturation phase. This phenomenon has often been stressed for other phenolic compounds in various plant organs (Strack et al., 1978; Linscheid et al., 1980), and it should certainly be related to the active turnover of certain phenolic metabolites (Molderez et al., 1978; Barz and Köster, 1981). In the case of the olive, the successive evolution of oleuropein and verbascoside and their biochemical relationship may suggest the existence of a metabolic relationship between these two compounds. Nevertheless, no degradation product of oleuropein has been detected during growth of the fruit; (dihydroxyphenyl)ethanol, a constituent compound of oleuropein and of verbascoside and has been revealed in other varieties (Solinas et al., 1975), was not found here in the stages of development examined. It is probable that it is reutilized very rapidly when the oleuropein molecule is broken down. Some varieties (Cailletier, L11) in fact contain demethyloleuropein, another derivative of oleuropein, in increasing amounts during maturation. During this stage, most of the verbascoside disappears without the appearance of the slightest trace of caffeic acid; this is in fact classic in fruits (Macheix et al., 1977). It is perhaps used either directly in the formation of anthocyanins that characterizes maturation or indirectly in possible acylation of these molecules (Kamsteeg et al., 1980); however, it is more probable that the accumulation of anthocyanins is a direct result of a fresh increase in phenylalanine ammonia-lyase activity during maturation as has been shown in cherry (Melin et al., 1977) and grape (Hrazdina and Franzese, 1974).

**Comparison of Phenolic Compound Contents in 11 Varieties.** The study above was extended during maturation to a further eight varieties. The variations obtained first complement those reported above but also further reveal the very considerable quantitative differences in oleuropein contents; extreme values (Picholine, Salonenque) and three other intermediate varieties are shown in Figure 4. Except in the case of three early varieties, Zrappola, Cailletier, and VP7, anthocyanins generally appear when oleuropein contents are low. Comparison of 11 varieties also reveals two interesting relationships. First, there is a fairly close inverse correlation between the size of various varieties of fruit and their oleuropein content (Figure 5); varieties with a high oleuropein content such as Picholine, Cailletier, and Zrappola have small fruit whereas L365 produces large fruit with a very low oleuropein content. This relationship was first observed at the beginning of maturation (Figure 5) and remained valid

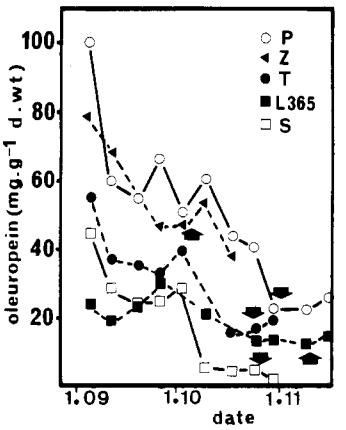


**Figure 4.** Evolution of oleuropein content for five varieties during maturation: P, Picholine; Z, Zrappola; T, Tanche; L365; S, Salonenque. Filled arrows, anthocyanins.

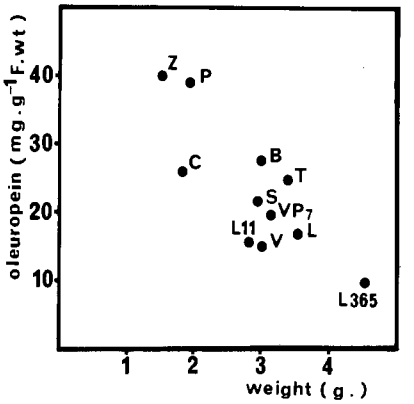


**Figure 5.** Relationship between oleuropein content and weight of fruit for 11 varieties: P, Picholine; L, Lucques; S, Salonenque; B, Bouteillan; V, Verdale; Z, Zrappola; C, Cailletier; T, Tanche; L11; L365; VP7.

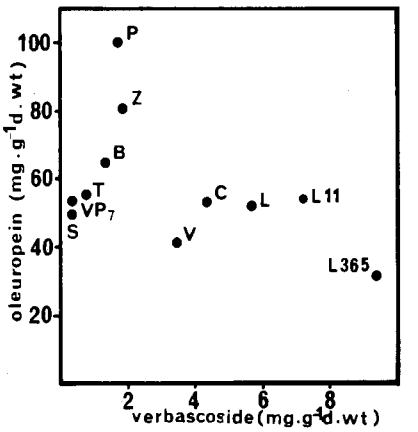


**Figure 6.** Relationship between oleuropein and verbascoside contents for 11 varieties (each value corresponds to the maximum for the two compounds).

until complete maturity of fruit. In addition, it was observed during the same period that the varieties with the highest oleuropein contents were those with the least verbascoside (Figure 6). Picholine and Zrappola, on the one hand, and clones L11 and L365, on the other, are a good illustration of this phenomena whose cause doubtless resides in the metabolic diversity of the various varieties.

These various results were completed by the study of absorbance read from the UV spectra of the extracts at 280 and 330 nm (designated as the ratio 280/330 hereafter). This relationship gives approximate but rapid

indication of the proportion of oleuropein in relation to verbascoside. At the beginning of maturation the various varieties studied were thus divided into several groups: (a) small fruit varieties with high oleuropein and low verbascoside contents characterized by a 280/330 ratio greater than 4 (Picholine, Zrappola); (b) large fruit varieties with a low oleuropein content compared to the other varieties and with a high verbascoside content (these have a 280/330 ratio of less than 2) (L365); (c) other varieties (Bouteillan, Cailletier, Tanche, Lucques, Verdale, Salonenque, L11, VP7) that form a group with practically identical fruit weights and a low verbascoside content, giving a 280/330 ratio of the order of 3. There is no distinct separation between these groups, and it is probable that wider research into varieties would make it possible to find more numerous intermediate situations. In addition, it remains to be shown whether these variations can be generalized to other fruits of the same varieties cultivated under very different conditions; preliminary results obtained on this point for grapes (Boursiquot et al., 1986) would appear to be promising. With these reservations these observations might possibly be the basis for biochemical characterization of varieties of olive.

## CONCLUSIONS

This study makes it possible to stress the quantitative importance of phenolic compounds in the olive. Considerable differences were observed according to the stage of development of the fruit and the varieties studied. However, one of the most original aspects of this work is the revealing of considerable differences between the varieties examined. The causes remain to be determined, together with specification of the metabolic relationships that may exist between oleuropein and verbascoside in the fruit. In addition, the practical consequences of these observations are interesting, particularly with regard to the varieties traditionally picked green before full maturation. Oleuropein contents are still high at picking, but verbascoside is at its most plentiful during this period. The relative importance of these two compounds remains to be determined with regard to the organoleptic qualities of olives for consumption, together with their degradation characteristics during technical processing operations.

**Registry No.** Oleuropein, 32619-42-4; verbascoside, 61276-17-3; rutin, 153-18-4; luteolin 7-glucoside, 5373-11-5.

## LITERATURE CITED


Andary, C.; Wylde, R.; Laffite, C.; Privat, G.; Winternitz, F. *Phytochemistry* **1982**, *21*, 1123–1127.

Barz, W.; Köster, J. In *The Biochemistry of Plants*; Conn, E. E., Ed.; Academic: New York, 1981; Vol. 7, pp 35–84.

Ben Shalom, N.; Kahn, V.; Harel, E.; Mayer, A. M. *J. Sci. Food Agric.* **1977**, *28*, 545–550.

Billot, J.; Hartmann, C.; Macheix, J. J.; Rateau, J. *Physiol. Veg.* **1978**, *16*, 693–714.

Bourquelot, E.; Vintilesco, J. *C.R. Hebd. Seances Acad. Sci.* **1908**, *147*, 533–535.

Boursiquot, J. M.; Sapis, J. C.; Macheix, J. J. *C.R. Hebd. Seances Acad. Sci., Ser. III* **1986**, *302*, 177–180.

Cohen, S.; Lifshitz, A.; Samish, Z. *J. AOAC* **1967**, *50*, 1194–1195.

Fernandez Diez, M. J. In *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic: New York, 1971; pp 265–277.

Fleuriet, A. *Fruits* **1976**, *31*, 117–126.

Fleuriet, A.; Macheix, J. J. *J. Chromatogr.* **1972**, *74*, 339–345.

Fleuriet, A.; Macheix, J. J. *Phytochemistry* **1981**, *20*, 667–672.

Fleuriet, A.; Macheix, J. J.; Andary, C.; Villemur, P. *C.R. Hebd. Seances Acad. Sci., Ser. III* **1984**, *299*, 253–256.

Herrmann, K. In *Fortschritte der Chemie Organischer Naturstoffe*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: Weinheim, 1978; pp 73–132.

Hrazdina, G.; Franzese, A. J. *Phytochemistry* **1974**, *13*, 225–229.

Kamsteeg, J.; Van Brederode, J.; Van Nigtevecht, G. *Phytochemistry* **1980**, *19*, 1459–1462.

Linscheid, M.; Wendisch, D.; Strack, D. *Z. Naturforsch.* **1980**, *35C*, 907–914.

Loussert, R.; Brousse, G. *L'olivier*; G. P. Maisonneuve et Larose: Paris, 1978; pp 389–421.

Macheix, J. J. *Physiol. Veg.* **1974**, *12*, 25–33.

Macheix, J. J.; Rateau, J.; Fleuriet, A.; Bureau, D. *Fruits* **1977**, *32*, 397–405.

Melin, C.; Moulet, A. M.; Dupin, J. F.; Hartmann, C. *Phytochemistry* **1977**, *16*, 75–78.

Molderez, H.; Nagels, A. M.; Parmentier, F. *Phytochemistry* **1978**, *17*, 1747–1749.

Panizzi, L.; Scarpati, M. L.; Oriente, G. *Gazz. Chim. Ital.* **1960**, *90*, 1449–1485.

Ragazzi, E.; Veronese, G.; Guiotto, A. *Ann. Chim.* **1973**, *63*, 13–20.

Romeyer, F. M.; Macheix, J. J.; Goiffon, J. P.; Reminiac, C. C.; Sapis, J. C. *J. Agric. Food Chem.* **1983**, *31*, 346.

Shasha, B.; Leibowitz, J. *J. Org. Chem.* **1961**, *26*, 1948–1954.

Shulman, Y.; Lavee, S. *Plant Physiol.* **1976**, *57*, 490–492.

Solinas, M.; Di Giovacchino, L.; Cucurachi, A. *Ann. Ist. Sper. Eloiot.* **1975**, 5, 3–24.

Strack, D.; Tkotz, N.; Klug, M. *Z. Pflanzenphysiol.* **1978**, *89*, 343–355.

Tronchet, J. *Bull. Soc. Bot.* **1972**, *119*, 25–40.

Van Buren, J. In *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic: London, 1971; Vol. 1, pp 269–304.

Vasquez Roncero, A. *Rev. Fr. Corps Gras* **1978**, *1*, 21–26.

Vasquez Roncero, A.; Janer del Valle, M. L. *Grasas Aceites* **1974**, *25*, 269–279.

Vasquez Roncero, A.; Graciani Constante, E.; Maestro Duran, R. *Grasas Aceites* **1977**, *28*, 421–426.


Received for review November 20, 1985. Accepted May 21, 1986.