

## Phenolic Compounds in Different Olive Varieties

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Phenolic compounds in different olive varieties were determined by HPLC analysis over 2 years. Demethyloleuropein was found in only two (Coratina and Leccino) of the eight varieties studied, so it could be used as varietal marker. Elenolic acid glucoside and hydroxytyrosol can be considered indicators of maturation for olives. In fact, as the olives ripen, their tenor increases whereas oleuropein decreases.

**Keywords:** Phenolic compounds; olive fruit; oleuropein; demethyloleuropein; 3,4-dihydroxyphenylethanol; elenolic acid glucoside

### INTRODUCTION

Phenolic compounds in olive fruits are important factors to consider in order to evaluate the quality of virgin olive oil since they are partly responsible for its autoxidation stability (Vazquez et al., 1975; Gutierrez et al., 1977; Montedoro, 1972; Perrin, 1992) and organoleptic characteristics (Vazquez, 1978). Moreover, they have pharmacological properties (Maestro Duràn et al., 1994), are natural antioxidants (Sheabar and Neeman, 1988; Chimi et al., 1988, 1991; Le Tutour and Guedon, 1992), and inhibit Gram-positive microorganisms involved in the fermentation of olive fruit (Fleming et al., 1973; Brenes et al., 1992, 1995). Oleuropein, the main component that produces bitterness in olives, is a heterosidic ester of elenolic acid and 3,4-dihydroxyphenylethanol (Panizzi et al., 1960). As the fruit ripens, oleuropein progressively decreases (Amiot et al., 1986a,b, 1990) and phenolic compounds, such as demethyloleuropein and 3,4-dihydroxyphenylethanol accumulate (Brenes et al., 1995), as does the nonphenolic compound elenolic acid glucoside, which only corresponds to the secoiridoid part of oleuropein (Amiot et al., 1989).

Other natural phenols that have been identified in olives are the flavonol glycosides, such as quercetin 3-rutinoside (rutin) and luteolin 7-glucoside. They are found in varying amounts, depending on the level of ripeness, and the variety (Ragazzi et al., 1973; Vazquez et al., 1974; Vlahov, 1992).

Since the phenolic content of virgin olive oil is influenced by the variety, location, degree of ripeness and the type of oil extraction procedure used (Solinas et al., 1978; Montedoro and Garofolo, 1984; Amiot et al., 1986; Solinas, 1987; Servili et al., 1996), the aim of this study was to assess the composition and evolution (during the harvest-time) of the phenolic compounds in olives, so as to identify when the olives are at their best maturation stage for harvesting in order to guarantee an optimum phenolic content in the relative oils that are currently under study. In addition, differences in the phenolic compound composition of the varieties typical to the area are being studied.

### MATERIALS AND METHODS

**Plant Material.** In November 1995, approximately 5 kg of olives (*Olea europea* L.) from the Gentile (Larino), Gentile (Colletorto), Coratina, and Leccino varieties were hand-picked in the Molise region at different stages of ripeness.

In November 1996, a further 5 kg were picked in 1 week at peak harvest-time. The varieties consisted of Gentile (Larino), Gentile (Colletorto), Gentile (Santacroce), Coratina, Peranzana, Rosciola, Saligna, and Leccino.

Sampling was limited to the period when olives, in the area considered, are harvested and processed.

**Analysis.** A random selection of 100 g of pulp was immediately frozen in nitrogen and ground. The phenolic compounds were extracted from this powder and purified according to Amiot et al. (1986). Detection and quantification was carried out by HPLC in a Waters 600 apparatus (Milford, MA 01757) with a photodiode array detector (Waters 991) at 20 °C. The 25 cm × 4 mm i.d. column used was filled with Supelcosil ABZ + Plus, 5 μm (Supelco, Inc. Bellefonte, PA), and the flow rate was 1.3 mL/min. The volume of injection was 20 μL. The mobile phase consisted of orthophosphoric acid in water (pH = 2.5) and acetonitrile, with a gradient from 15% to 40% of acetonitrile and the total running time was 20 min.

Oleuropein, quercetin 3-rutinoside, and luteolin 7-glucoside were quantified by using the external standard method at 280 nm for the first compound and at 340 nm for the others. 3,4-Dihydroxyphenylethanol and demethyloleuropein were detected at 280 nm and elenolic acid glucoside was detected at 240 nm and were expressed with the extinction coefficient of oleuropein. 3,4-Dihydroxyphenylethanol, demethyloleuropein and elenolic acid glucoside detections were based on the retention time and the spectrum in a 230–350 nm range.

The 1995 olive fruit samples were also subjected to water and fat analysis.

Determination of water content: sample (100–200 g) was weighed and then dried overnight at 50 °C in a vacuum oven (≤100 mmHg). The sample was cooled for 30 min in a dessiccator and reweighed.

Determination of fat content: a dried sample (2–5 g) was submitted to Soxhlet extraction, using petroleum ether as solvent. After solvent evaporation, the flask containing fat was dried at 100 °C, cooled in a dessiccator, and reweighed.

All the measurements were repeated on triplicate samples; the data reported are the mean of them.

**Color.** A colorimeter (chroma meter type CR-200b, Minolta, Japan) was used to assess the fruits' color and the Hunter colorimetric system was applied ( $L^*$ , lightness;  $a^*$ , redness;  $b^*$ , yellowness) (Hunter, 1979). The measurement was made on 20 fruit samples.

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**Table 1. Composition and Physical Parameters of Different Varieties of Olive Fruit at Harvest Time in 1995<sup>a</sup>**

varieties	harvest-time	fat, % d.b.	water, %	maturation index	puncture force (kg)	<i>L</i> *	<i>a</i> *	<i>b</i> *
Gentile (Larino)	11/01/95	44.7	49.4	2.2	0.47 ± 0.04	44.0 ± 3.2	-3.1 ± 0.2	12.2 ± 0.9
Gentile (Larino)	11/15/95	44.2	54.8	2.6	0.49 ± 0.05	42.7 ± 5.2	-6.5 ± 0.4	17.3 ± 1.5
Gentile (Larino)	11/30/95	46.3	50.6	3.9	0.40 ± 0.05	38.1 ± 3.8	-1.8 ± 0.3	9.8 ± 0.8
Gentile (Colletorto)	11/01/95	36.9	43.1	4.2	0.37 ± 0.03	32.7 ± 4.3	1.0 ± 0.1	-0.1 ± 0.02
Gentile (Colletorto)	11/15/95	42.6	53.5	4.9	0.39 ± 0.05	29.3 ± 4.0	-0.5 ± 0.04	2.7 ± 0.16
Gentile (Colletorto)	11/30/95	43.5	50.1	4.8	0.50 ± 0.06	30.7 ± 2.9	-3.8 ± 0.4	2.3 ± 0.28
Coratina	11/01/95	38.2	50.9	0.4	0.46 ± 0.07	52.0 ± 4.8	-14.4 ± 0.9	24.7 ± 2.6
Coratina	11/15/95	37.0	54.0	0.8	0.60 ± 0.05	53.6 ± 3.5	-19.5 ± 1.2	27.6 ± 2.2
Coratina	11/30/95	38.5	52.2	0.7	0.43 ± 0.04	48.8 ± 4.2	-17.6 ± 1.5	26.9 ± 2.5
Leccino	11/01/95	37.9	47.2	4.8	0.48 ± 0.08	28.4 ± 3.4	-2.1 ± 0.1	1.9 ± 0.12
Leccino	11/15/95	37.5	43.4	4.5	0.48 ± 0.05	31.0 ± 3.7	-1.8 ± 0.1	3.6 ± 0.29

<sup>a</sup> Means ±SD of 20 determinations.

**Table 2. Phenolic Compounds (mg/g of Pulp) in Different Varieties of Olive Fruit during Harvest Time in 1995<sup>a</sup>**

varieties	harvest-time	3,4-dihydroxyphenylethanol	elenolic acid glucoside	demethyloleuropein	quercetin 3-rutinoside	luteolin 7-glucoside	oleuropein
Gentile (Larino)	11/01/95	0.15 ± 0.03	0.32 ± 0.07		0.21 ± 0.03	0.30 ± 0.02	1.12 ± 0.15
Gentile (Larino)	11/15/95	0.48 ± 0.05	1.21 ± 0.19		0.29 ± 0.02	0.30 ± 0.03	1.45 ± 0.19
Gentile (Larino)	11/30/95	0.62 ± 0.08	1.08 ± 0.31		0.40 ± 0.05	0.41 ± 0.05	0.87 ± 0.09
Gentile (Colletorto)	11/01/95	0.40 ± 0.07	0.41 ± 0.05		0.36 ± 0.04	0.15 ± 0.01	2.08 ± 0.29
Gentile (Colletorto)	11/15/95	0.97 ± 0.12	0.95 ± 0.01		0.40 ± 0.05	0.23 ± 0.03	1.91 ± 0.21
Gentile (Colletorto)	11/30/95	1.10 ± 0.15	1.10 ± 0.19		0.54 ± 0.06	0.42 ± 0.05	1.50 ± 0.19
Coratina	11/01/95	0.30 ± 0.06	0.38 ± 0.09	1.32 ± 0.17	0.14 ± 0.02	0.16 ± 0.01	1.44 ± 0.21
Coratina	11/15/95	0.47 ± 0.07	0.32 ± 0.06	1.11 ± 0.12	0.20 ± 0.01	0.23 ± 0.02	1.62 ± 0.18
Coratina	11/30/95	0.52 ± 0.06	0.37 ± 0.04	0.88 ± 0.07	0.29 ± 0.02	0.24 ± 0.02	1.21 ± 0.16
Leccino	11/01/95	0.37 ± 0.05	0.78 ± 0.09	0.41 ± 0.05	0.37 ± 0.04	0.39 ± 0.05	0.97 ± 0.11
Leccino	11/15/95	0.60 ± 0.08	1.41 ± 0.17	1.12 ± 0.15	0.41 ± 0.03	0.41 ± 0.03	0.85 ± 0.09

<sup>a</sup> Means ±SD of three determinations.

**Maturation Index.** The maturation index was determined according to the method proposed by the National Institute of Agronomical Research of Spain, San Jaén Station (Solinas et al., 1987). Briefly the empirical procedure consists in distributing a randomly taken sample of 100 olives in 8 groups according to the skin colors: bright green (group  $N=0$ ), green-yellowish (group  $N=1$ ), green with reddish spots (group  $N=2$ ), reddish-brown (group  $N=3$ ), black with white flesh (group  $N=4$ ), black with < 50% purple flesh (group  $N=5$ ), black with ≥ 50% purple flesh (group  $N=6$ ), black with 100% purple flesh (group  $N=7$ ). The index is given by  $\Sigma(N_i n_i)/100$  where  $N_i$  is the group number and  $n_i$  is the olive number in that group. Maturation index values range from 0 ( $N=0$  bright green olives,  $n=100$ ) to 7 ( $N=100$  purple flesh olives,  $n=100$ ).

**Firmness.** The firmness was determined on 20 fruits by resistance of flesh to penetration, using a puncture test (fruit pressure tester, FT 011, Effegi, Italy). The puncture force was measured with an aluminium probe ( $\varnothing_{16}$ -in. diameter) and the result expressed in kilograms.

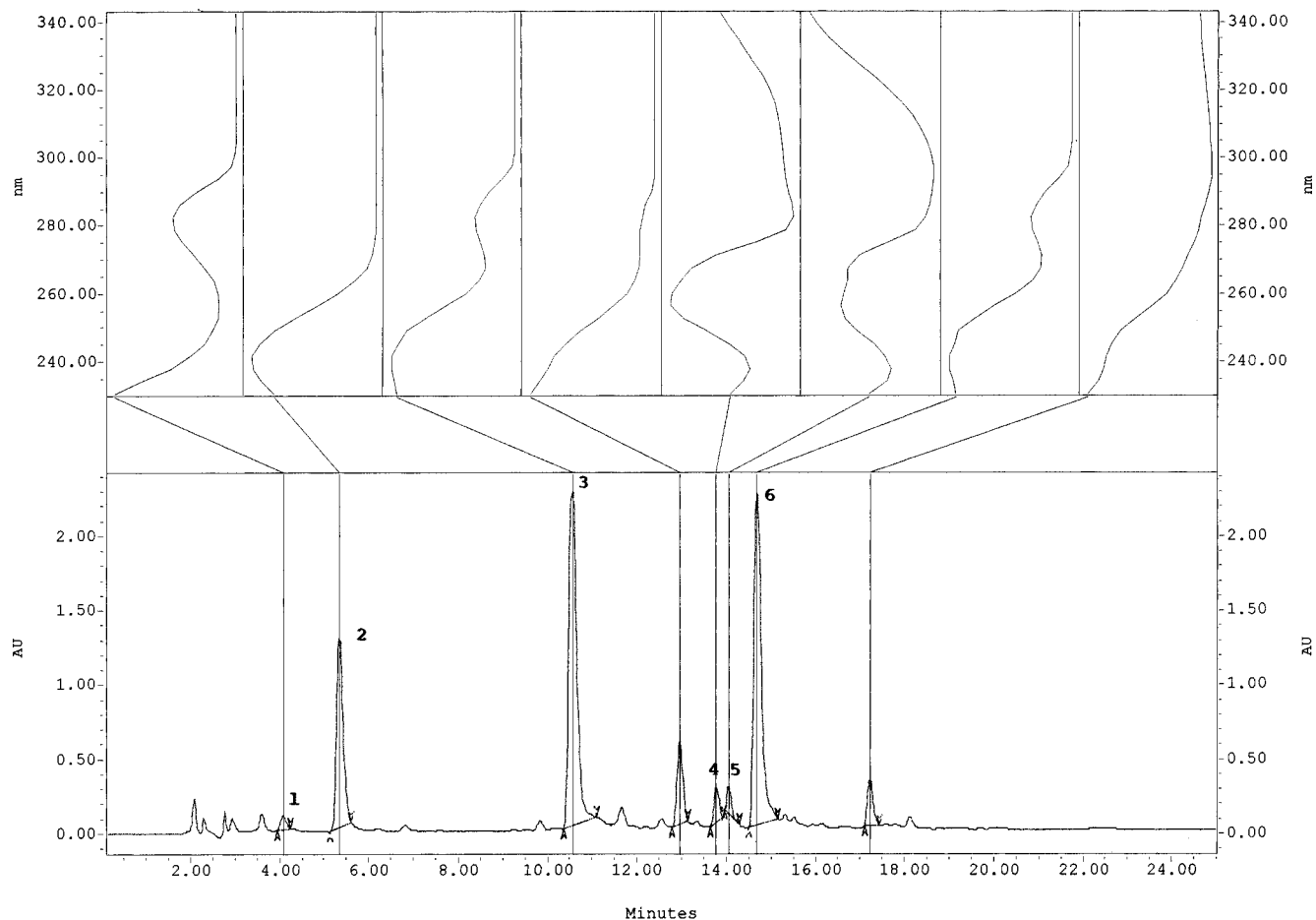
**Statistical Analysis.** The correlations among the chemico-physical parameters of the olive fruits were calculated by using the StatView SE program (Abacus Concepts, Inc., Berkeley, CA, 1984).

**Chemicals and Reference Compounds.** The reagents (Carlo Erba, Milan, Italy) were analytical or HPLC grade, as required. Oleuropein, luteolin-7-glucoside, and rutin were purchased from Extrasynthèse (Z. I. Lyon-Nord, Genay, France).

## RESULTS AND DISCUSSION

The composition and physical parameters of the olive fruits harvested in 1995 are given in Table 1. Water content was affected by seasonal pluviometric behavior. The highest amount was recorded on Nov 15th after 3 days of rain. Differences in fat content, measured on a dry basis, depended on the variety and harvest-time. The correlation coefficients between the chemico-physical parameters of the olive fruits showed that the maturation index (m.i.) was highly correlated to the colorimetric values: m.i. vs.  $L^*$  ( $r = -0.982$ ,  $P \leq 0.0001$ ), m.i. vs.  $a^*$  ( $r = 0.891$ ,  $P \leq 0.0001$ ), m.i. vs.  $b^*$  ( $r = -0.963$ ,  $P \leq 0.0001$ ). Moreover, high correlations between the colo-

rimetric variables were observed:  $L$  vs.  $a^*$  ( $r = -0.873$ ,  $P \leq 0.0001$ );  $L$  vs.  $b^*$  ( $r = 0.965$ ,  $P \leq 0.0001$ );  $a^*$  vs.  $b^*$  ( $r = -0.938$ ,  $P \leq 0.0001$ ). The color values of the 1995 varieties, as they ripened, showed the highest values of lightness and yellowness and lowest values of redness were seen in Coratina olives, since the black maturation phase did not occur in the harvest-time considered. Unlike other fruit, differences in firmness, which depended on the time of harvest and the variety (Bourne, 1979), were not found to be correlated to either the colorimetric values or maturation index. Figure 1 shows the HPLC chromatogram of phenolic extracts from drupes of Coratina at 240 nm with the related UV spectra in the range 230–340 nm. 3,4-Dihydroxyphenylethanol ( $t_R = 4.09$  min) changed during harvest-time in the four varieties studied in 1995 (Table 2), increasing fourfold in Gentile (Larino), from 0.15 to 0.62 mg/g pulp; about three times in Gentile (Colletorto), from 0.40 to 1.10 mg/g of pulp; and nearly twice in Coratina and Leccino. In the interval considered (1 month) oleuropein ( $t_R = 14.69$  min) decreased slightly in the four varieties, while elenolic acid glucoside increased. The data confirm that elenolic acid glucoside and 3,4-dihydroxyphenylethanol are indicators of the maturation of olives, since they increase as the fruit ripens, whereas the amount of oleuropein decreases. This could be correlated to the increased activity of the hydrolytic enzymes (Amiot et al., 1986). In particular glycosidases catalyse the hydrolysis of the oleuropein, with the production of oleuropein aglycon and the dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol (Lo Scalzo et al., 1993; Montedoro et al., 1993; Limiroli et al., 1995). However, due to its hydrophilic nature, 3,4-dihydroxyphenylethanol, the most important antioxidant in virgin olive oil (Chimi et al., 1988), goes into both the water and the oil during the extraction process in amounts that are related to the distribution coefficient and processing conditions. Therefore, in freshly extracted oil, low concentrations of 3,4-dihy-



**Figure 1.** Ultraviolet spectra of phenolic compounds in the Coratina variety. Peak number (240 nm): (1) 3,4-dihydroxyphenylethanol; (2) elenolic acid glucoside; (3) demethyloleuropein; (4) quercetin 3-rutinoside; (5) luteolin 7-glucoside; (6) oleuropein.

**Table 3. Phenolic Compounds (mg/g of Pulp) in Different Varieties of Olive Fruits in 1996<sup>a</sup>**

varieties	harvest-time	3,4-dihydroxyphenylethanol	elenolic acid glucoside	demethyloleuropein	quercetin 3-rutinoside	luteolin 7-glucoside	oleuropein
Leccino	11/14/96	0.54 ± 0.07	0.69 ± 0.08	1.54 ± 0.18	0.41 ± 0.05	0.31 ± 0.02	0.96 ± 0.07
Gentile (Larino)	11/12/96	0.26 ± 0.03	0.99 ± 0.10		0.28 ± 0.02	0.23 ± 0.03	2.37 ± 0.28
Gentile (S. Croce)	11/14/96	0.23 ± 0.02	0.69 ± 0.08		0.19 ± 0.02	0.17 ± 0.02	0.26 ± 0.03
Gentile (Colletorto)	11/15/96		0.87 ± 0.06		0.66 ± 0.07	0.46 ± 0.06	2.06 ± 0.19
Peranzana	11/17/96	0.25 ± 0.03	1.07 ± 0.15		0.44 ± 0.03	0.44 ± 0.03	3.45 ± 0.36
Rosciola	11/15/96	0.48 ± 0.07	0.46 ± 0.06		0.37 ± 0.05	0.60 ± 0.05	0.80 ± 0.09
Saligna	11/14/96	0.05 ± 0.01	0.57 ± 0.07		0.25 ± 0.02	0.29 ± 0.04	0.83 ± 0.07
Coratina	11/17/96	0.31 ± 0.02	0.68 ± 0.05	2.45 ± 0.26	0.32 ± 0.04	0.25 ± 0.03	0.31 ± 0.05

<sup>a</sup> Means ±SD of three determinations.

droxyphenylethanol are generally found (Esti et al., 1996), compared with the high concentration of complex phenols containing 3,4-dihydroxyphenylethanol due to their less hydrophilic nature. Moreover, since oleuropein decreases and 3,4-dihydroxyphenylethanol increases, delaying harvest-time, in the period considered, could be less effective in maintaining the oleuropein derivatives in olive oil. The trend for both quercetin 3-rutinoside and luteolin 7-glucoside content was similar, increasing slightly in the later-picked fruit. The differences found between Coratina and Leccino (an earlier-ripening variety) in demethyloleuropein behavior during ripening (Table 2) were probably due to the varieties being at different stages of maturation on the day of analysis. As previously reported (Amiot et al., 1989), demethyloleuropein can be used as a varietal marker, since it was found in Coratina and Leccino only. This finding was confirmed in the second year of the experiment, when eight varieties were compared (Table

3). Demethyloleuropein content was 1.5 and 2.4 mg/g of olive pulp in Leccino and Coratina respectively, while no trace was found in the other varieties. All the varieties, except for Gentile (Colletorto), contained 3,4-dihydroxyphenylethanol and ranged from 0.05 mg/g in Saligna to 0.53 mg/g in Leccino. The highest oleuropein (3.5 mg/g) content was found in Peranzana, which is a later-ripening variety.

The elenolic acid glucoside ranged from 0.45 mg/g in Rosciola to 1.1 mg/g in Peranzana. There was a slight difference in luteolin 7-glucoside and quercetin 3-rutinoside content between the varieties.

## CONCLUSION

Demethyloleuropein was found in only two (Coratina and Leccino) of the eight varieties studied over the 2 years, so it could be used as varietal marker. Elenolic acid glucoside and 3,4-dihydroxyphenylethanol can be

considered indicators of maturation for olives. In fact, as the olives ripen, their tenor increases, whereas oleuropein decreases. Moreover, since oleuropein decreases and 3,4-dihydroxyphenylethanol increases and due to their different distribution coefficients in both the water and the oil during the extraction process, delaying the harvest-time in the period considered could be less effective to maintaining the oleuropein derivatives in olive oil. Lastly, a highly significant correlation was found between the maturation index and the Hunter color of the olive fruit.

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