# Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 1. Their Extraction, Separation, and Quantitative and Semiquantitative Evaluation by HPLC

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Phenolic compounds have a fundamental importance in the nutritional and sensory characteristics of virgin olive oil. Problems regarding their qualitative and quantitative evaluation have not been completely solved; hence, in this paper the extractive and HPLC methods of analysis are examined and some modifications are presented. By means of these techniques, elenolic acid and four unknown compounds having phenolic behavior were separated from virgin olive oil. Four of them were correlated with total phenols evaluated by means of the Folin–Ciocalteu reagent, and two of them were correlated with olive oil autoxidation stability.

## INTRODUCTION

The importance that phenolic compounds have in oil quality is well-known, especially their correlation with the peroxide number, free fatty acidity, and sensory tests (Vazquez Roncero et al., 1978; Montedoro and Servili, 1989, 1991). Therefore, it is important to evaluate these substances qualitatively and quantitatively (Cantarelli, 1961; Montedoro and Cantarelli, 1969; Montedoro, 1972; Ragazzi and Veronese, 1973a,b; Solinas and Cichelli, 1981, 1982; Cortesi and Fedeli, 1983).

We have found that evaluation of phenolic compounds by the HPLC method (Solinas and Cichelli, 1982) does not agree with the colorimetric determination (Folin–Ciocalteu method) and with the oil autoxidation stability evaluated by the accelerated Swift test, using the Rancimat apparatus (Pannelli and Montedoro, 1989).

These discrepancies lead us to formulate two hypotheses:

(a) The HPLC method we adopted (Servili and Montedoro, 1989), developed by other authors (Cortesi et al., 1981, 1984; Solinas and Cichelli, 1982; Cortesi and Fedeli, 1983), is not sufficiently reliable because the extraction and separation of more complex compounds is inadequate.

(b) The presence in the chromatogram of other, as yet unidentified, phenolic compounds among the different separated and identified compounds (simple phenols) may contribute to the explanation of the observed differences.

Hence, we revised the procedure starting with sample preparation since this phase is particularly delicate because of the presence of compounds which are easily degradable by peroxidation (Montedoro and Cantarelli, 1969; Montedoro, 1972; Servili and Montedoro, 1989).

Acid hydrolysis of the extract and subsequent use of chromatographic techniques and spectrophotometric evaluation of the derivatives were also used in an attempt to identify the compounds. In addition, we evaluated their concentrations in different oils to see if there was a relationship with autoxidation stability and with the Folin–Ciocalteu values.

This first paper describes the modified analytical method and gives some data on an initial attempt to characterize the hydrolyzable fraction.

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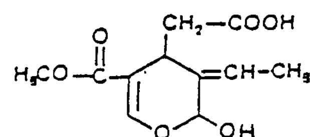


**Figure 1.** Elenolic acid.

## MATERIALS AND METHODS

**Materials.** *Oils.* Two different models were used:

(a) An olive oil was washed repeatedly with absolute methanol until the phenolic compounds were completely eliminated. The extractions were checked directly with the Folin–Ciocalteu reagent (Montedoro, 1972) and indirectly with TLC (Ragazzi and Veronese, 1973a,b). Standard phenolic compounds were added to the oil.

(b) Virgin olive oils from Umbria, Apulia, and Liguria, with different total phenolic contents, were examined. Free fatty acidity, peroxide number, and autoxidation stability of these oils ($n = 10$) grouped by phenolic concentrations are reported in Table I.

*Standard Compounds.* (3,4-Dihydroxyphenyl)ethanol was synthesized in the laboratory according to the procedure of Schoepf et al. (1949). Protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, and cinnamic acid were very pure products obtained from Fluka Co. (Buchs, Switzerland). Oleuropeine glycoside was obtained from Extrasynthèse Co. (Z. I. Lyon-Nord, Genay, France); (*p*-hydroxyphenyl)ethanol was obtained from Janssen Chemical Co. (Beerse, Belgium).

Elenolic acid (Figure 1) was obtained by hydrolysis of the pure oleuropeine glycoside following the procedure of Walter et al. (1973).

The compound was collected and purified by TLC, using as a stationary phase a 0.25-mm silica gel layer from Whatman (London) and benzene/methanol/acetic acid (45:8:1 v/v) as a mobile phase.

**Methods.** *Free acidity and peroxide number* were determined according to the Italian *Official Methods of Analysis of Oils and Fats* (Istituto Poligrafico dello Stato, 1964).

*Colorimetric Evaluation of Total Phenols.* The total phenols were determined calorimetrically at 765 nm using the Folin–Ciocalteu reagent and are expressed as gallic acid on a methanolic extract of virgin olive oil obtained as follows: 10 mL of a solution of methanol/water (80:20 v/v) plus Tween 20 (2% v/w) was added to 10 g of olive oil and mixed with an Ultra-Turrax T 25 at 15000*g* for 1 min and centrifuged at 5000*g* for 10 min; the extraction was repeated two times. To eliminate the oil

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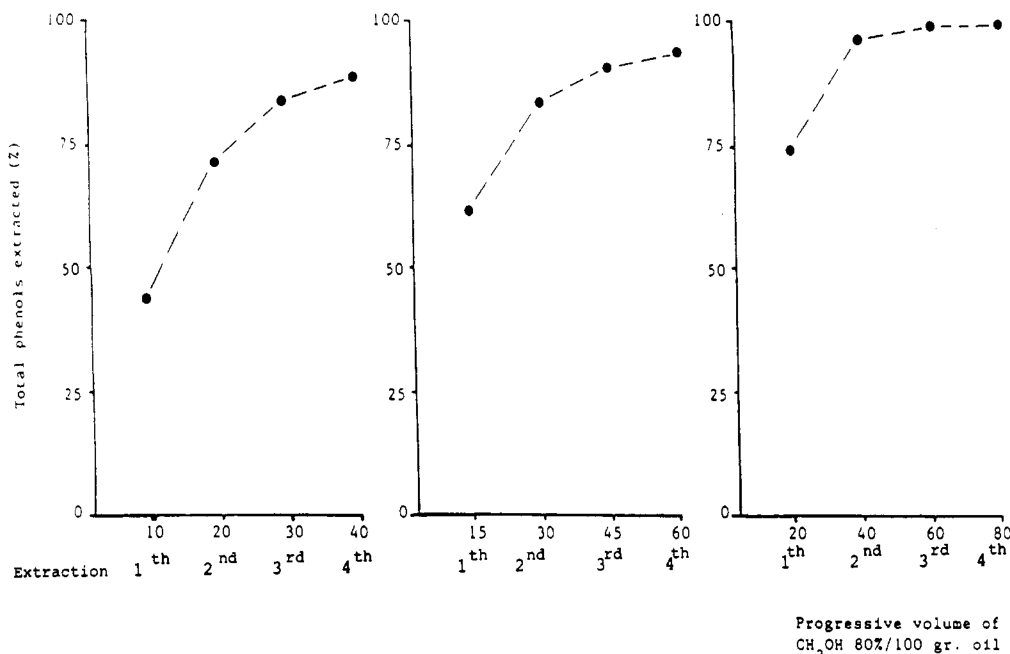


**Figure 2.** Extraction number and volume of 80% methanol in the phenolic extraction of the virgin olive oil.

**Table I. Free Acidity, Peroxide Number, and Autoxidation Stability of Virgin Olive Oils Grouped by Total Phenols**

| | low (total phenols 50–200 mg/kg) | | medium (total phenols 200–500 mg/kg) | | high (total phenols 500–1000 mg/kg) | |
|---|---|---|---|---|---|---|
| | av[a] | range | av[a] | range | av[a] | range |
| free acidity, % w/w of oleic acid | 0.48 | 0.28, 1.00 | 0.26 | 0.19, 0.37 | 0.26 | 0.19, 0.31 |
| peroxide no., mequiv of $O_2$/kg | 12.9 | 4.3, 23.0 | 9.9 | 4.0, 19.8 | 5.8 | 2.0, 9.8 |
| autoxidation stability, h | 4.9 | 2.75, 9.50 | 12.9 | 7.3, 16.0 | 20.1 | 16.4, 25.3 |

[a] $n = 10$.

**Table II. Influence of Solvent Mixtures on the Extraction of Phenolic Compounds from Olive Oil**

| | | total phenols extracted, mg/kg | | |
|---|---|---|---|---|
| oil/solvent | w/v | methanol 60% | methanol 80% | methanol 100% |
| control oil | | 89.7 | 108.8 | 89.1 |
| oil/hexane | 1:1 | 94.4 | 108.8 | 47.9 |
| oil/petroleum ether | 1:1 | 94.4 | 108.1 | 45.8 |
| oil/chloroform | 10:1 | 83.5 | 101.7 | 83.2 |
| oil/[hexane/petroleum ether (9:1)] | 1:1 | 94.4 | 108.8 | 45.8 |
| oil/[hexane/petroleum ether/chloroform (9:1:5)] | 1:1 | a | 101.7 | a |

[a] The separation of this mixture was impossible.

droplets, the methanolic extract was kept for 24 h at −20 °C (Montedoro and Cantarelli, 1969; Montedoro, 1972; Montedoro et al., 1978).

*Autoxidation Stability.* Two different procedures were used. (a) The accelerated automatic Swift test was carried out using the Rancimat apparatus (Methrohm Co., Basel, Switzerland) at 120 °C with an air flow of 20 L/h; the results are expressed as induction time (hours) (Laubli and Bruttel, 1986; Servili and Montedoro, 1989).

(b) For the spontaneous autoxidation test, two virgin olive oil samples of 400 g each with two different concentrations of total phenols (82 and 498 mg/kg) were put into open white glass bottles (1 L) and were exposed to indirect sunlight at room temperature. The autoxidation was followed by peroxide number evaluation. At 50 mequiv of $O_2$/kg peroxide number and 100 mequiv of $O_2$/kg peroxide number, the samples were drawn for the HPLC analysis of phenols.

*Phenolic Extractions for HPLC Analysis.* To extract phenolic compounds from the virgin olive oil, the following solvents were compared: absolute methanol (Solinas et al., 1975, 1978; Solinas and Cichelli, 1981); methanol/water (80:20 v/v) (Montedoro and Cantarelli, 1969; Montedoro, 1972); methanol/water (60:40 v/v).

**Table III. Purification of Phenolic Extracts with Different Solvents**

| solvent | v/v | vol, mL | total phenols after two washings, mg/kg of oil |
|---|---|---|---|
| control extract | | | 232 |
| hexane | | 40 | 220 |
| petroleum ether 40–70 | | 40 | 220 |
| chloroform | | 40 | a |
| hexane/petroleum ether | 9:1 | 40 | 220 |
| hexane/petroleum ether/chloroform | 9:1:5 | 40 | 103 |
| acetonitrile + hexane | 1:4 | 50 | 230 |

[a] The separation with this solvent was impossible.

These solvents were used directly in the virgin olive oil or in mixtures of oil/hexane (1:1 w/v), oil/petroleum ether (1:1 w/v), oil/chloroform (10:1 w/v), oil/[hexane/petroleum ether (9:1 v/v)] (1:1 w/v), or oil/[hexane/petroleum ether/chloroform (9:1:5 v/v)] (1:1 w/v) (Vazquez Roncero et al., 1973, 1978; Ragazzi and Veronese, 1973a,b.

The mixtures above reported were mixed with a vortex at 5000*g* for 2 min and centrifuged at 5000*g* for 10 min.

Three different volumes of methanol/water (80:20 v/v) were used: 10, 15, and 20 mL/100 g of virgin olive oil; for each volume, four successive extractions were tested.

*Extract Purification for HPLC Analysis.* Different solvents were used in this phase: hexane, petroleum ether 40–70, chloroform (Vazsquez Roncero et al., 1978), hexane/petroleum ether (9:1 v/v), hexane/petroleum ether/chloroform (9:1:5 v/v).

The procedure was as follows: The methanolic extract was concentrated in vacuum under a stream of nitrogen at <35 °C until it reached a syrupy consistency; it was then subjected to two consecutive washings with 20 mL of the same solvents previously reported. In addition, the acetonitrile plus hexane mixture was evaluated. In this case, the extract, brought to a syrupy consistency, was added to 10 mL of acetonitrile and was washed twice with 20 mL of hexane.

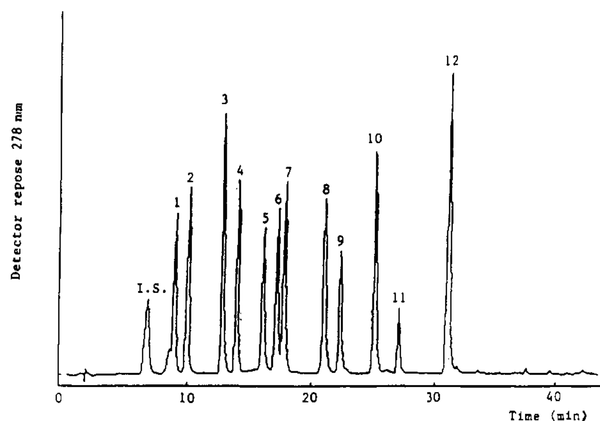


**Figure 3.** Fractionation of the standard phenolic compounds extracted from washed olive oil by HPLC at 278 nm. Peak numbers: (I.S.) gallic acid; (1) (3,4-dihydroxyphenyl)ethanol; (2) protocatechuic acid; (3) (p-hydroxyphenyl)ethanol; (4) p-hydroxybenzoic acid; (5) vanillic acid; (6) caffeic acid; (7) syringic acid; (8) p-coumaric acid; (9) ferulic acid; (10) o-coumaric acid; (11) oleuropeine glycoside; (12) cinnamic acid.

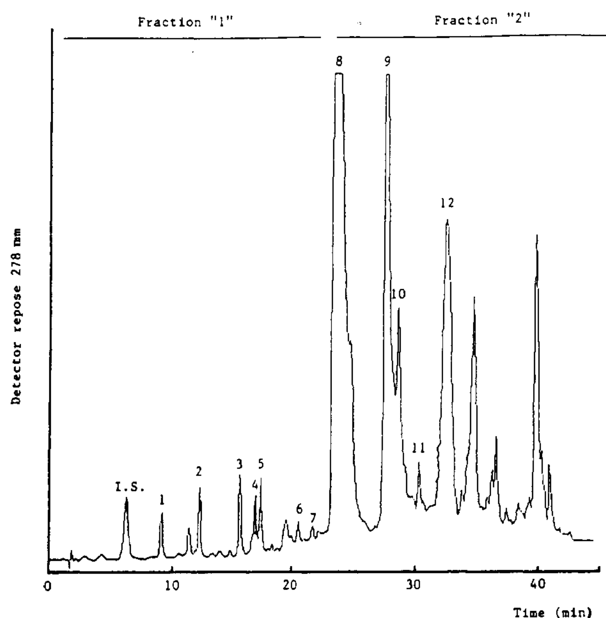


**Figure 4.** Fractionation of the phenolic extract of virgin olive oil by HPLC at 278 nm. Peak numbers: (I.S.) gallic acid; (1) (3,4-dihydroxyphenyl)ethanol; (2) (p-hydroxyphenyl)ethanol; (3) vanillic acid; (4) caffeic acid; (5) syringic acid; (6) p-coumaric acid; (7) ferulic acid; (8) RT 23.50; (9) RT 27.70; (10) RT 28.40; (11) cinnamic acid; (12) RT 32.50.

*High-Pressure Liquid Chromatography (HPLC).* By modification of previous methods (Cortesi et al., 1981; Solinas and Cichelli, 1981, 1982), the following procedure was adopted: The HPLC system was composed of a Varian 5000 chromatograph with a 150 mm × 4.6 mm $C_{18}$ Erbasil column, coupled with a Varian Polychrom 9060 UV photodiode spectrophotometer; the eluates were detected at 278 and 239 nm at 25 °C. The flow rate was 1 mL/min; the mobile phase used was 2% acetic acid (pH 3.1) in water (A) vs methanol (B) for a total running time of 45 min, and the gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 10 min, and 0% A/100% B in 10 min until the end of running. Samples were dissolved in methanol, and 10 μL of this solution was injected in the column.

The spectrophotometric evaluation of the individual compounds was obtained during the HPLC analysis by recording the UV spectra (in the range from 190 to 367 nm) by means of the photodiode array detector.

*Thin-Layer Chromatography (TLC).* The stationary phase used was a 0.25-mm silica gel layer from Whatman. The mobile phases were as follows: A, toluene/ethyl formate/formic acid (50:40:10 v/v); B, benzene/methanol/acetic acid (45:8:1 v/v); C, ethyl acetate/methanol/water (100:16.5:13.5 v/v); D, chloroform/ethyl acetate/formic acid (50:40:5 v/v). One hundred microliters of the phenolic extract dissolved in methanol (containing 10 mg of total phenols/mL) was applied on the layer.

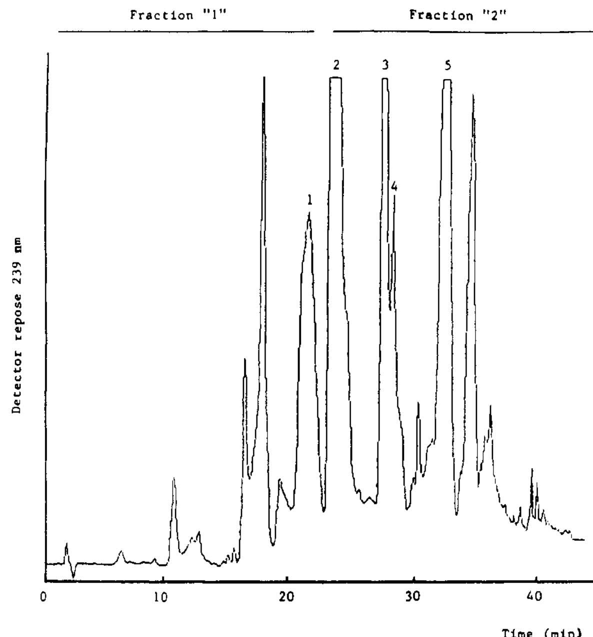


**Figure 5.** Fractionation of the phenolic extract of virgin olive oil by HPLC at 239 nm. Peak numbers: (1) elenolic acid; (2) RT 23.50; (3) RT 27.70; (4) RT 28.40; (5) RT 32.50.

**Table IV. Relative Response Factor (RRF) and Retention Time (RT) of the Identified Phenols in Olive Oil**

| | concn, ppb | RRF[a] av | RRF[a] sp[b] | RT, min |
|---|---|---|---|---|
| (3,4-dihydroxyphenyl)-ethanol | 1389–5555 | 2.158662 | 0.02083 | 9.27 |
| protocatechuic acid | 811–3244 | 1.031627 | 0.73256 | 9.90 |
| (p-hydroxyphenyl)-ethanol | 2819–11276 | 2.500605 | 0.20318 | 12.50 |
| p-hydroxybenzoic acid | 872–3488 | 0.978595 | 0.09929 | 13.47 |
| vanillic acid | 564–2256 | 0.662263 | 0.07224 | 15.60 |
| caffeic acid | 416–1664 | 0.793771 | 0.02446 | 16.37 |
| syringic acid | 414–1656 | 0.546135 | 0.04581 | 16.93 |
| p-coumaric acid | 303–1212 | 0.239840 | 0.02166 | 20.38 |
| ferulic acid | 305–1220 | 0.357532 | 0.03472 | 21.63 |
| o-coumaric acid | 297–1188 | 0.260573 | 0.01936 | 24.36 |
| oleuropein glycoside | 2280–9120 | 8.435775 | 0.61582 | 26.46 |
| cinnamic acid | 283–1132 | 0.181422 | 0.01408 | 30.62 |

[a] Relative to gallic acid (internal standard). [b] $n = 4$.

*Detection of TLC spots* was done by (a) spraying with Folin–Ciocalteu reagent followed by ammonia vapors, (b) spraying with p-toluenesulfonic acid plus vanillin and heating at 105 °C for 10 min (Montedoro and Cantarelli, 1969; Ragazzi and Veronese, 1973a,b; Walter et al., 1973), and (c) leaving the layers in the air and evaluating the browning of the spots after 24 h of exposure at room temperature.

*Multiple linear regression analysis* was employed using the STATGRAPHICS program (STSC Inc., 1987) considering HPLC peak area numbers 8–10 and 12 in relation to total phenols and autoxidation stability for 30 virgin olive oil samples.

## RESULTS AND DISCUSSION

**Phenolic Extraction for HPLC Analysis.** The best results were obtained using methanol/water (80:20 v/v), in agreement with the data in the literature (Montedoro and Cantarelli, 1969; Montedoro et al., 1978; Vazquez Roncero et al., 1978), as summarized in Table II.

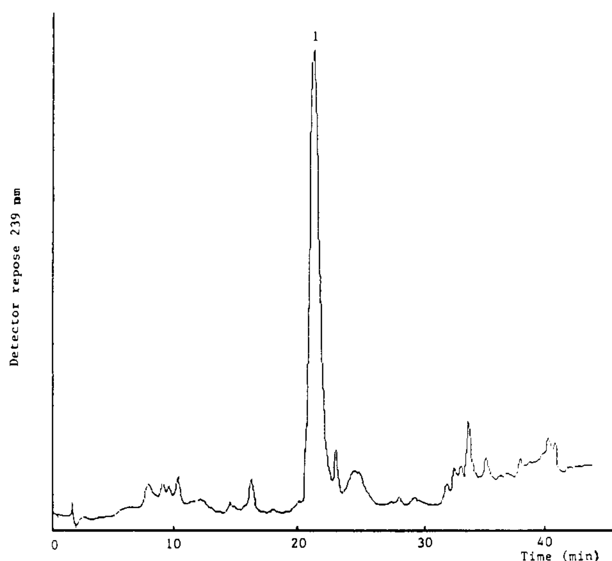


Figure 6. HPLC chromatogram of elenolic acid.

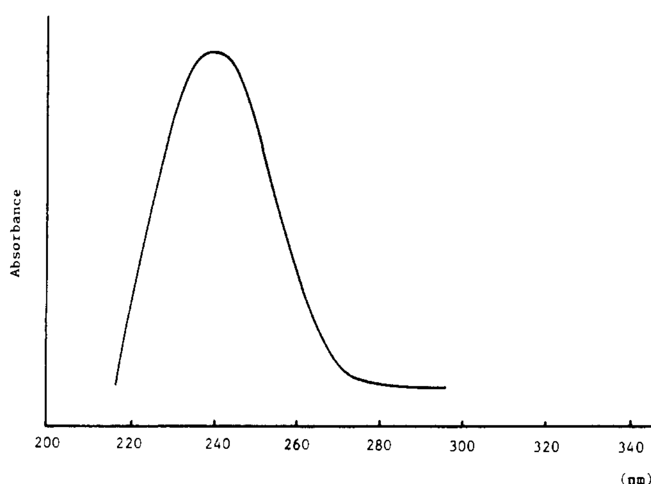


Figure 7. Ultraviolet spectra (UV) of elenolic acid (RT 21.20) obtained in 2% acetic acid in water and methanol during the HPLC analysis.

The addition of various specific lipid solvents (hexane, petroleum ether, chloroform) to the oils did not increase the phenolic concentration of the extracts.

The optimum extraction was achieved by extracting 100 g of oil with 2 volumes of 20 mL of solvent (Figure 2).

**Extract Purification for HPLC Analysis.** The data in Table III show that hexane is the most selective solvent, especially when used in addition to acetonitrile. The latter solvent, being immiscible with hexane, solubilizes the phenolic fraction only, while both polar and neutral lipids remain in the other phase.

**Chromatographic Separation by HPLC.** The chromatograms of a "washed" oil with the addition of known amounts of phenols and of a virgin olive oil are given, respectively, in Figures 3, 4, and 5. The first chromatogram (Figure 3) shows a satisfactory resolution for all standard components. The values of their concentrations (parts per billion), the relative response factors (RRF), and the retention times, calculated from four repetitions, are given in Table IV. The data show the reproducibility of the method, as shown by the low standard deviation values.

Examination of the chromatograms of the virgin olive oil obtained at two different wavelengths (278 and 239 nm) (Figures 4 and 5) provides some new information. The retention times and the respective absorptions at

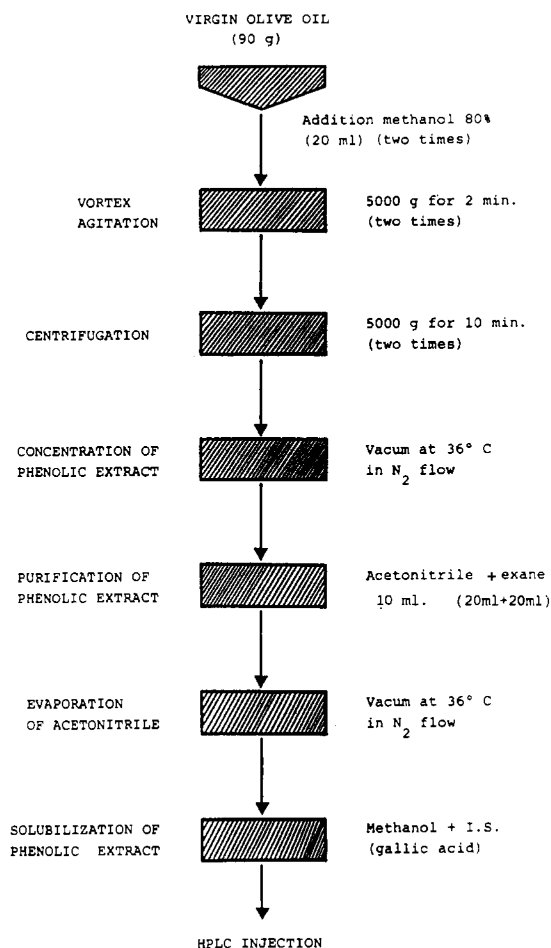


Figure 8. Multiphase process used in phenolic HPLC analysis of virgin olive oil.

Table V. Physical and Chemical Properties of Elenolic Acid

| | peak RT 21.20 | elenolic acid standard | refs |
|---|---|---|---|
| HPLC RT | 21.20 | | 21.20 |
| TLC $R_f$ | | | |
| A[a] | 0.42 | 0.42 | 0.43[d] |
| B[a] | 0.78 | 0.78 | 0.88[e] |
| TLC specific reaction | | | |
| air oxidation | neg[c] | neg | |
| Folin–Ciocalteau | neg | neg | |
| PTSA + V[b] | gray | gray | gray[e] |
| spectrometry exam | | | |
| max, $\mu$m | 239 | 239 | 239[d,e] |

[a] A, benzene/methanol/acetic acid (45:8:1). B, chloroform/ethyl acetate/formic acid (50:40:15). [b] PTSA + V = p-toluenesulfonic acid + vanillin. [c] Negative reaction. [d] Walter et al. (1973). [e] Amiot (1986).

278 nm in the UV spectra in the range from 200 to 340 nm of the components corresponding to peaks 1–7 and 11 are identical with those provided by the pure reference compounds, previously reported (Figure 3) (Montedoro and Cantarelli, 1969; Montedoro, 1972; Solinas et al., 1975; Vazquez Roncero et al., 1978; Solinas and Cichelli, 1981), while the compounds corresponding to peaks 8–10 and 12 (with retention times of 23.50, 27.70, 28.40, and 32.50 min, respectively) are unknown.

For the first time in a virgin olive oil, the occurrence of elenolic acid has been identified by comparison of its $R_f$, RT, and UV spectra with those of an authentic standard (Figures 6 and 7 and Table V).

The chromatographic method described above was used to examine the phenolic composition of different virgin

**Table VI. Phenolic Composition in Virgin Olive Oils with Different Concentrations of Total Phenols**

| | low (total phenols 50–200 mg/kg) | | medium (total phenols 200–500 mg/kg) | | high (total phenols 500–1000 mg/kg) | |
|---|---|---|---|---|---|---|
| | av[a] | range | av[a] | range | av[a] | range |
| fraction 1 | | | | | | |
| (3,4-dihydroxyphenyl)ethanol | 1.0[b] | 0.0, 3.0 | 23.5[b] | 3.2, 74.4 | 10.7[b] | 5.2, 15.3 |
| (*p*-hydroxyphenyl)ethanol | 33.5 | 0.7, 94.0 | 61.6 | 0.5, 267.2 | 8.7 | 3.4, 13.1 |
| vanillic acid | 3.2 | 1.0, 9.3 | 5.3 | 1.2, 12.4 | 4.4 | 1.1, 10.2 |
| caffeic acid | 1.5 | 0.5, 7.2 | 3.5 | 0.0, 8.3 | 14.7 | 8.0, 22.3 |
| syringic acid | 5.0 | 0.6, 13.5 | 3.2 | 0.0, 8.2 | 5.1 | 0.0, 10.5 |
| *p*-coumaric acid | 5.8 | 1.6, 14.4 | 13.4 | 8.3, 20.0 | 22.0 | 13.6, 30.7 |
| ferulic acid | 1.9 | 0.5, 3.7 | 13.3 | 2.8, 37.2 | 13.7 | 10.1, 19.4 |
| cinnamic acid | 2.8 | 0.0, 9.1 | 29.1 | 7.6, 61.0 | 34.8 | 22.6, 53.5 |
| elenolic acid | 376.4 | 167.6, 661.3 | 919.8 | 583.3, 1633.6 | 1020.4 | 680.5, 2358.0 |
| fraction 2 | | | | | | |
| peak 8[c] | 50.0[b] | 1.0, 169.3 | 390.0[b] | 55.2, 442.4 | 1146.0[b] | 852.3, 2059.4 |
| peak 9 | 32.0 | 10.5, 84.4 | 129.0 | 24.0, 328.3 | 255.6 | 98.5, 355.6 |
| peak 10 | 196.0 | 133.4, 359.2 | 207.0 | 61.5, 432.4 | 115.0 | 86.3, 146.6 |
| peak 12 | 25.8 | 4.0, 56.3 | 159.2 | 92.4, 278.6 | 328.6 | 158.5, 605.3 |

[a] $n = 10$. [b] Peak area: $n \times 10^4$. [c] See Figure 4.

**Table VII. Final Model of Multiple Regression Analysis between Total Phenols, Determined with the Folin–Ciocalteu Reagent, and Some Phenolic Compounds: Model Fitting Results for Total Phenols[a]**

| independent variable | coeff | SE | *F* ratio | *t* value | sig level |
|---|---|---|---|---|---|
| constant | 77.304 | 29.412 | 80.665 | 2.628 | 0.017 |
| peak 8[b] | 0.170 | 0.052 | 214.234 | 3.269 | 0.004 |
| peak 9 | 0.976 | 0.212 | 22.256 | 4.600 | 0.000 |
| peak 12 | 0.450 | 0.192 | 5.506 | 2.345 | 0.031 |

[a] $R^2$ adjusted 0.922; SE 79.963; $F(3,26,0.95)$ 2.99; total degrees of freedom 29; confidence level 95%. [b] See Figure 4.

**Table VIII. Phenolic Composition of Virgin Olive Oil with Low Total Phenols Measured with HPLC during the Oxidation Process**

| | starting oxidation peroxide no. 18, mequiv of $O_2$/kg | after 75 days peroxide no. 50, mequiv of $O_2$/kg | after 324 days peroxide no. 100, mequiv of $O_2$/kg |
|---|---|---|---|
| fraction 1 | 131 ± 7[a] | 150 ± 9 | 148 ± 9 |
| (*p*-hydroxyphenyl)ethanol | 107 ± 5 | 131 ± 6 | 129 ± 7 |
| fraction 2 | 152 ± 9 | 126 ± 7 | 126 ± 7 |
| peak 9[b] | 7.1 ± 0.4 | 3.0 ± 0.2 | |
| peak 10 | 111 ± 5 | 103 ± 5 | 101 ± 4 |

[a] Peak area ($n \times 10^4$) averaged from three data ± standard deviation. [b] See Figure 4.

olive oils coming from different growing areas and varieties grouped by total phenolic concentrations. The data given in Table VI denote some fundamental differences regarding both known (fraction 1) and unknown substances (fraction 2), as found by other authors (Cortesi and Fedeli, 1983).

In fraction 1, the presence of (*p*-hydroxyphenyl)ethanol was found in all samples, regardless of their phenolic concentration, while the important concentration of (3,4-dihydroxyphenyl)ethanol was found in oils having phenol content greater 200 ppm.

In fraction 2, the substances corresponding to peaks 8–10 and 12 represent about 70–80% of the total HPLC peak area.

Peaks 8, 9, and 12 are significantly correlated with the total phenols determined colorimetrically (Table VII).

The compounds corresponding to peaks 8, 9, and 12 tend to progressively disappear together with (3,4-dihydroxyphenyl)ethanol in the oils subjected to spontaneous autoxidation tests (Tables VIII and IX).

The antioxidant power of (3,4-dihydroxyphenyl)ethanol has been well ascertained (Chimi et al., 1988; Servili and Montedoro, 1989).

**Table IX. Phenolic Composition of Virgin Olive Oil with High Total Phenols Measured with HPLC during the Oxidation Process**

| | starting oxidation peroxide no. 8, mequiv of $O_2$/kg | after 235 days peroxide no. 50, mequiv of $O_2$/kg | after 460 days peroxide no. 100, mequiv of $O_2$/kg |
|---|---|---|---|
| fraction 1 | 288 ± 15[a] | 248 ± 14 | 201 ± 7 |
| (3,4-dihydroxyphenyl)ethanol | 77.2 ± 4.6 | 23.4 ± 1.5 | |
| (*p*-hydroxyphenyl)ethanol | 176 ± 12 | 208 ± 11 | 191 ± 12 |
| fraction 2 | 1494 ± 72 | 679 ± 37 | 240 ± 11 |
| peak 8[b] | 249 ± 10 | 37.2 ± 1.7 | |
| peak 9 | 325 ± 18 | 90.4 ± 4.4 | |
| peak 10 | 448 ± 20 | 303 ± 16 | 215 ± 9 |
| peak 12 | 285 ± 14 | 97.0 ± 4.7 | |

[a] Peak area ($n \times 10^4$) averaged from three data ± standard deviation. [b] See Figure 4.

**Table X. Final Model of Multiple Regression Analysis between Unknown Peaks and Oil Antoxidation Stability: Model Fitting Result for Autoxidation Stability[a]**

| independent variable | coeff | SE | *F* ratio | *t* value | sig level |
|---|---|---|---|---|---|
| constant | 7.104 | 0.841 | | 8.451 | 0.000 |
| peak 8[b] | 0.010 | 0.001 | 135.241 | 11.629 | 0.000 |

[a] Adjusted $R^2$ 0.827; SE 3.209; $F(1,28,0.95)$ 4.20; total degrees of freedom 29; confidence level 95%. [b] See Figure 4.

Only peak 8 turned out to be correlated to the oil autoxidation stability (Table X).

**Conclusions.** A reproducible analytical method has been developed which separates and quantifies 17 compounds, 12 of which are identified (Figure 8). Elenolic acid, derived from the hydrolysis of oleuropeine, was identified for the first time in virgin olive oil.

An important concentration of (3,4-dihydroxyphenyl)ethanol is found in oils with a total phenol content greater than 200 ppm, while (*p*-hydroxyphenyl)ethanol is present in all olive oils. Four unknown compounds appear to be important because their concentrations are significantly correlated both with total phenols and with oil autoxidation stability.

ACKNOWLEDGMENT

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**Registry No.** (3,4-Dihydroxyphenyl)ethanol, 10597-60-1; (*p*-hydroxyphenyl)ethanol, 2380-91-8; vanillic acid, 121-34-6; caffeic acid, 331-39-5; syringic acid, 530-57-4; *p*-coumaric acid, 7400-08-0; ferulic acid, 1135-24-6; cinnamic acid, 621-82-9; elenolic acid, 34422-12-3.