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Phenolic compounds and stability of virgin olive oil—Part I

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The stability of 24 samples of Greek virgin olive oil was examined in relation to total polyphenol content, and individual phenols were determined by reversed-phase HPLC. Tyrosol, the major olive-oil phenol, is not correlated with the shelf-life of the oil. Total polyphenol content and hydroxytyrosol-to-tyrosol ratio have a significant linear correlation with the resistance of the oil to autoxidation.

INTRODUCTION

It has been reported (Vazquez Roncero, 1978; Gutfinger, 1981) that virgin olive oils with a high 'polyphenol' content are more resistant to autoxidation. Polyphenols are part of the so-called 'polar fraction' of virgin olive oil, which is usually obtained by extraction with methanol : water systems. Vazquez Roncero (1978) and Gutfinger (1981) found a good correlation between the polyphenol content, as determined by the Folin-Ciocalteau colorimetric method, and the stability of the oil.

Although the presence of phenolic compounds has been related to the shelf-life of the oil, it is not clear which particular compounds are mainly responsible for the antioxidant activity. The polar fraction is very complex and many components remain unidentified (Solinas & Cichelli, 1981; Cortesi & Fedeli, 1983; Solinas, 1987). Papadopoulos and Boskou (1991) tried to estimate the protection factors of major phenols present in virgin olive oil and to assess their contribution to its stability. If the more effective phenolic antioxidants of this oil were known, high-performance liquid chromatography (HPLC) methods, which are currently applied to the analysis of the phenols, could be modified to determine only these important constituents. As a result, the information needed could be obtained by shorter-time chromatographic procedures, which are much easier to carry out.

This work was undertaken to determine phenols in samples of virgin olive oil by HPLC, to correlate their content to the oil stability and to compare correlation coefficients with those obtained using the conventional colorimetric method for the determination of the 'total polyphenol content'.

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MATERIALS AND METHODS

Samples

Samples of Greek virgin olive oil were provided by a plant located in the area of Athens or collected by the authors from various regions of Greece.

Reagents and standards

Methanol for oil extraction (pro-analysis), acetic acid (pro-analysis) and hexane (extra pure) were purchased from Merck (Darmstadt, Germany); methanol (HPLC Chromasolv) was Riedel de Haen (Seelze, Germany); Folin-Ciocalteau reagent was obtained from Sigma Co. (St. Louis, MO, USA). Protocatechuic, vanillic, *p*-coumaric, *o*-coumaric and 3,4-dihydroxyphenylacetic acids were purchased from Sigma Co.; caffeic and *p*-hydroxybenzoic acids from Fluka (Buchs, Switzerland); and 4-hydroxyphenyl-ethanol (tyrosol) from Aldrich Chem. Co. (Milwaukee, WI, USA). 3,4-Dihydroxyphenylethanol (hydroxytyrosol) was prepared by acidic hydrolysis of oleuropein, according to the method described by Graciani Constante and Vazquez Roncero (1980). Oleuropein was kindly offered by Dr R. Maestro Duran (Instituto de le Grasa y sus Derivados, Seville, Spain).

Apparatus

All analytical separations were performed with a Spectra Physics Liquid Chromatograph (Model 8800) equipped with a variable-wavelength UV detector (Spectra Chrom 100) and an electronic integrator (Spectra Physics, Model 4290); injection was by means of a Rheodyne injection valve (Model 7125) with a 10-μl fixed loop (Rheodyne, CA, USA). A visible spectrometer (Model Spectronic 20, Bausch and Lomb) was used for the determination of the total phenol content, according to the colorimetric method proposed by Swain & Hillis (1959).

Sample preparation

The polar fraction of virgin olive oil was obtained using the method described by Vazquez Roncero *et al.* (1976): 50 g of oil dissolved in hexane (50 ml) was extracted with methanol : water (60 : 40, v/v, 3 × 30 ml). Each extract was treated once with hexane (50 ml). The three extracts were combined and the solvent was evaporated to dryness in a flash evaporator (40°C); the residue in methanol was transferred into a volumetric flask (5 ml) and aliquots of this solution were used for both the colorimetric and reversed-phase HPLC analysis.

Colorimetric determination of the total polyphenol content

The polar fraction solution (2 ml) was transferred into a 25-ml volumetric flask and Folin-Ciocalteau reagent (0·5 ml) was added; after 3 min, 1 ml of a saturated sodium-carbonate solution was added and the flask was made up to volume with water and stored in the dark for 1 h; the absorbance of the solution was measured at 725 nm. The total polyphenol content was expressed as ppm of caffeic acid. A calibration curve was prepared using concentrations of the standard ranging from 1 to 120 μg per 25 ml of solution.

Reversed-phase HPLC determination of phenolic compounds

The chromatographic separation was achieved on a Spherisorb ODS-2 5μ column (250 × 4·6 i.d.; Anachem, Luton, Bedfordshire, UK). A linear gradient elution at a flow rate of 1 ml min^{-1} was used. Elution system : solvent A—acetic-acid : water (3 : 97, v/v); solvent B—methanol. Gradient range: 0–60 min, 6–37% B in A; 60–70 min, 37–100% B in A; 70–90 min, 100% B; 90–105 min, 100–6% B in A; 105–130 min, 6% B. UV detector and integrator settings were 280 nm, 0·002 Aufs and attenuation × 32, chart speed × 25 cm min^{-1}, peak threshold × 235, respectively. Identification of peaks was based on relative retention times and spiking. Calibration plots were established using solutions of the standards, according to the quantification procedure proposed by Tsimidou *et al.* (1992) using four standards.

Stability test

Samples of oil (5 g each) were transferred into a series of opened, transparent glass bottles of 8 cm volume and 3 cm cross-section, and the filled bottles were stored at 63°C in the dark. Peroxide values were determined periodically according to the AOCS (1969) method).

RESULTS AND DISCUSSION

Twenty-four samples of virgin olive oil were chromatographed. Hydroxytyrosol and tyrosol were the major

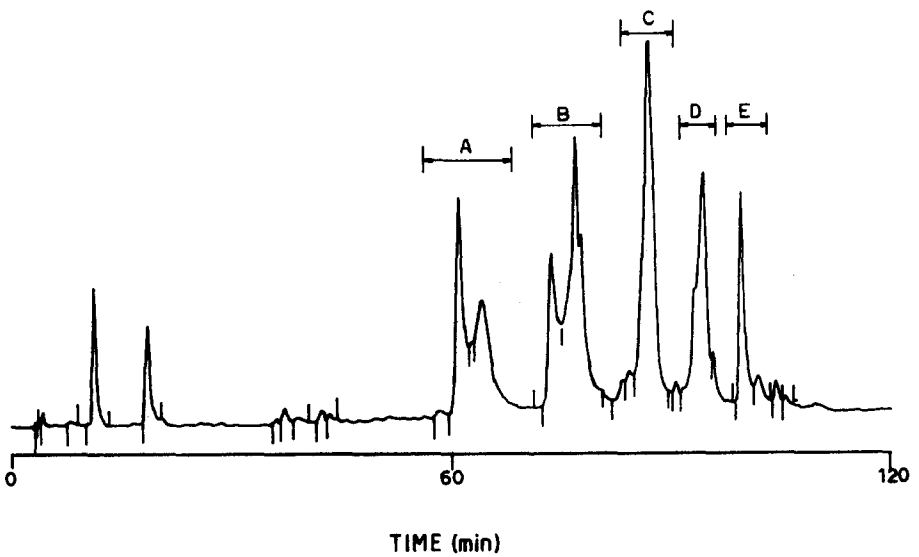

Fig. 1. Reversed-phase HPLC of the polar fraction of a sample of virgin olive oil. (Chromatographic conditions were as described by Tsimidou *et al.* (1992). The elution system was extended using only methanol. Fractions A, B, C, D and E were collected for further study.)

Table 1. Phenolic compounds and stability of virgin olive oil

Oils	HPLC data (ppm)				Colorimetric data (ppm)	Stability data[a]
	Hydroxytyrosol	Tyrosol	Total phenols	Hydroxytyrosol: tyrosol	Total phenols	
1	4·6	4·6	9·4	1·0	175·0	92
2	4·9	8·4	13·7	0·6	82·4	56·5
3	2·4	5·1	7·7	0·5	47·0	18
4	1·7	3·9	5·7	0·4	75·0	42
5	1·4	1·7	3·2	0·8	62·5	37
6	39·4	29·8	69·6	1·3	153·7	57·5
7	12·5	7·1	20·1	1·8	242·5	119
8	11·9	4·7	16·9	2·5	125·0	62
9	9·4	7·1	16·8	1·3	207·5	57·5
10	0·7	13·3	14·0	0·05	34·3	33
11	4·4	3·7	8·2	1·2	125·0	100
12	1·4	6·3	7·8	0·2	82·5	45·5
13	1·3	1·6	3·3	0·8	53·0	27·5
14	2·4	3·3	6·8	0·8	53·7	47
15	3·3	6·6	10·6	0·5	68·7	67
16	5·6	6·2	12·7	0·9	187·5	58
17	1·5	6·4	8·0	0·2	30·0	30
18	1·9	2·0	3·9	1·0	112·5	61
19	3·4	2·5	5·9	1·4	112·5	58
20	0·9	0·8	2·0	1·1	18·7	29
21	16·7	6·2	23·7	2·7	207·5	115
22	1·2	4·6	6·0	0·3	28·0	26
23	5·8	2·6	8·5	2·2	180·0	113
24	12·8	7·7	20·6	1·7	110·0	70

[a] Days needed for peroxide values to attain 70 meq of active oxygen per kilogram of oil.

phenols in all the samples. Protocatechuic, vanillic, *p*-coumaric, *o*-coumaric, *p*-hydroxybenzoic and syringic acids were detected in trace amounts (less than 1 ppm) in most of the samples examined.

The tyrosol, hydroxytyrosol and total phenol content as determined by HPLC, the total phenol content based on the Folin-Ciocalteau colorimetric determination and the stability data are presented in Table 1. Regression analysis is presented in Table 2.

As shown in Table 2, tyrosol, the main olive-oil phenol, is not correlated with the stability of the oil. These results are in accordance with the work by Papadopoulos and Boskou (1991), who found a low antioxidant activity for this phenol compared to butylated hydroxytoluene (BHT). Hydroxytyrosol content seems to be of some importance, but the total phenol content, as determined by the Folin-Ciocalteau reagent, is better correlated with the stability of the oil. On the other hand, the sum of the chromatographically identified phenols does not correlate at all. This suggests that other compounds present in the polar fraction, which are not eluted under the chromatographic conditions usually applied, may contribute to the antioxidant effect. The nature of these compounds has not been studied, although some efforts were made in the past to correlate their presence with quality characteristics or the addition of processed olive oil (Cortesi & Fedeli, 1983; Cortesi *et al.*, 1985; Solinas, 1987). In order to investigate further the polar fraction, the reversed-phase HPLC system applied in this study was extended and the whole profile of the methanol: water extract of the oil was observed (Fig. 1). This extension revealed the presence of a considerable number of unidentified components that absorb at 280 nm. Five separate fractions (A, B, C, D and E), corresponding to the five major groupings of unidentified peaks, were collected after 12 repeated injections. All of these fractions were positive to the Folin-Ciocalteau reagent.

Table 2. Correlation of virgin olive oil stability with phenol content

Phenol	Coefficient of correlation (r)
Hydroxytyrosol	0·344
Tyrosol	−0·021
Total phenol content (reversed-phase HPLC)	0·092
Total phenol content (Colorimetric method)	0·816
Hydroxytyrosol: tyrosol	0·713

It must also be stressed that the hydroxytyrosol-to-tyrosol ratio has a significant linear correlation with the stability of the oil (r = 0·713). This seems to be very promising for predicting the stability of this valuable natural product, because these two phenols can be eluted, even isocratically, within 10 min. Thus, the whole reversed-phase HPLC procedure is dramatically

simplified. However, this finding has to be confirmed by the analysis of more olive-oil samples.

CONCLUSION

It is clear that, besides the phenols, the presence of which has been repeatedly reported, other phenolic compounds are also present in olive oil. The contribution of these unknown compounds to the stability of the oil may be significant. The concentration of hydroxytyrosol and the ratio of hydroxytyrosol-to-tyrosol ratio may prove to be of practical importance if the role of other antioxidant factors is to be fully elucidated.

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