

### Research Note

# Determination of Phenolic Acids in Virgin Olive Oil

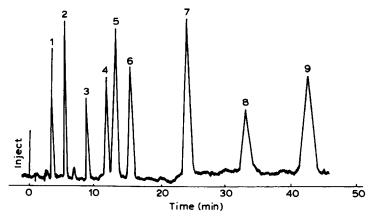
#### ABSTRACT

Phenolic compounds, which are present in significant amounts in virgin olive oil, are almost completely destroyed during the refining process of the oils. Numerous phenolic acids were found in oil samples and caffeic, p-coumaric, syringic, vanillic, ferulic, protocatechuic and p-hydroxybenzoic acids have been identified. Among these, p-coumaric acid was predominant in all virgin olive oil samples. They can play important roles as antioxidants and influence the flavour of the oil.

#### INTRODUCTION

Phenolic compounds of olives are normally found as constituents of vegetation water. During the oil extraction from the olive fruits they are distributed between the organic and aqueous phases. An important part of the phenolic fraction is retained in the oil after the oil extraction process (Fedeli, 1977).

An HPLC technique was developed for the separation and quantitation of the phenolic acids by Wulf and Nagel (1976). Phenolic compounds of virgin olive oil have been investigated by using several chromatographic techniques in recent years. Numerous phenolic acids have been identified in virgin olive oil which have a strong influence on flavour of the oil and they can also play a biochemical role as antioxidants (Solinas & Cichelli, 1982; Cortesi & Fedeli, 1983). The antioxidant properties vary with the type and concentration of phenolic acid (Dziedzic & Hudson, 1984). The levels of phenolic compounds in olives differ widely among varieties and locations



**Fig. 1.** Separation of phenolic acid standard mixtures by HPLC. 1, Gallic; 2, protocatechuic; 3, p-hydroxybenzoic; 4, vanillic; 5, caffeic; 6, syringic; 7, p-coumaric; 8, ferulic; 9, O-coumaric.

(Gutfinger, 1981). The purpose of this study was to investigate the phenolic acids in virgin olive oil by using an HPLC technique.

#### MATERIALS AND METHODS

Samples of virgin olive oil were obtained from different locations in Turkey. The oils were commercially produced by different extraction processes. The phenolic compounds were extracted from the oils according to the method described by Graciani *et al.* (1980). The extracts were analysed by HPLC (Waters Associates) on a Bondopak  $C_{18}$  column,  $30 \, \text{cm} \times 4 \, \text{mm}$  i.d. Phenolic acid standards were purchased from Sigma and Merck. The standard solutions of the phenolic acids were prepared in acetonitrile—water (4:1 v/v

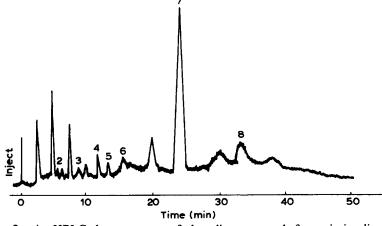


Fig. 2. An HPLC chromatogram of phenolic compounds from virgin olive oil.

Phenolic acid	Sample No.										
	1	2	3	4	5	6	7	8	9	10	11
Caffeic —		_		_				0.34		_	_
p-Coumaric	2.76	1.39	1.73	1.08	3.58	3.86	0.73	2.45	10.37	4.14	0.5
Syringic	1.46	0.99	0.49		1.10	0.49		_	0.79	0.83	_
Vanillic	0.65		0.33		0.85	0.41		_	0.83		
Ferulic	0.81		_		_	0.11	_	_	1.25	_	
Protocatechuic		_	_			Trace	_		Trace		
p-hydroxybenzoic	Trace	_	Trace			0.20			0.25	_	

**TABLE 1** Phenolic Acids in Virgin Olive Oil Samples  $(\mu g/g)^a$ 

at a concentration of 25 mg per 50 ml). A water-methanol-acetic acid mixture (95:15:5) (v/v) as mobile phase and an absorbance detector at 254 nm were used. The flow rate for the mobile phase was 1·4 ml/min and a sensitivity of 0·02 (AUSF). Recorder chart speed was 0·25 cm/min. Phenolic acids were identified by comparing their retention times with the values of the standards and the concentrations of phenolic acids were calculated from calibration curves of peak heights versus amounts of injected standards.

#### **RESULTS AND DISCUSSION**

The main phenolic acids (caffeic, syringic, p-hydroxy benzoic, ferulic, vanillic and protocatechuic) were separated and determined (Figs 1 and 2). The amounts of each phenolic acid in the oil samples are given in Table 1, which showed that p-coumaric acid was the predominant acid in all olive oil samples. In this study, a major part of the phenolic acids was comprised of p-coumaric, syringic and vanillic while p-hydroxy benzoic, syringic and vanillic were reported qualitatively previously by Graciani et al. (1980). o-Coumaric and gallic acids were detected in any olive oil samples. Solinas and Cichelli (1982), in Italy, determined five phenolic acids quantitatively and their findings are in accordance with the results found by us.

#### REFERENCES

Cortesi, N. & Fedeli, E. (1983). Polar components of virgin olive oil. *Nota. I Riv. Ital.* Sost. Grasse., LX, 341-52.

<sup>&</sup>quot; Mean values for three replications.

<sup>-,</sup> Not detected.

- Dziedzic, S. Z. & Hudson, B. J. F. (1984). Phenolic acids and related compounds as antioxidants for edible oils. *Food Chemistry.*, 14, 45-51.
- Fedeli, E. (1977). Lipids of olives. *Prog. Chem. Fats Other Lipids*, 15, Pergamon Press, Oxford, pp. 57-74.
- Graciani, C. E., Colchero Yela, C. & Vazquez, R. A. (1980). Study of polar compound of the olive oil by HPLC. Grasas y Aceites., 31(2), 85-9.
- Gutfinger, T. (1981). Polyphenols in olive oils. J. Amer. Oil Chem. Soc., 58, 966-8. Solinas, M., Cichelli, A. (1982). Il dosagio per GLC & HPLC delle sostanze fenoliche dell'olio di oliva. La Riv. della Soc. Ital. di Scienza dell'alim Anns., 11(4) 223-30.
- Wulf, L. W & Nagel, C. W. (1976). Analysis of phenolic acids and flavonoids by HPLC. *Journal of Chromatography*, 116, 271-9.

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(Received 9 October 1989; revised version received and accepted 28 February 1990)