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DEVELOPMENT OF AN HPLC/DAD METHOD FOR DETERMINATION OF PHENOLIC PROFILE IN PORTUGUESE OLIVE FRUITS

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INTRODUCTION

Polyphenolic compounds influence the sensorial properties of olive fruits and virgin olive oils and are important markers for studying fruit characteristics of different cultivars and for controlling oil production processes (Romani *et al.*, 1999).

A few chromatographic methods have been used to study the phenolic compounds of olive fruit (Soler-Rivas *et al.*, 2000; Capasso *et al.*, 1992; Ficarra *et al.*, 1991; de Laurentis *et al.*, 1997; Baracco *et al.*, 1995). This communication reports the development of a new HPLC/DAD methodology to separate, identify and quantify the phenolic compounds from Portuguese olive fruit cultivars (*Cobrançosa*, *Madural* and *Verdeal*).

EXPERIMENTAL

Extraction of Phenolic Compounds from Olive Fruits. Each olive fruit sample (ca. 1.5 g) was thoroughly mixed with methanol until complete extraction of the phenolic compounds (negative reaction to NaOH 20%) The methanolic extract was filtered, concentrated to dryness under reduced pressure (40ºC) and redissolved in acid water (pH 2 with HCl) (≈ 50 mL). The aqueous solution was then passed through an ISOLUTE C18 (NEC) column, previously conditioned with 60 mL of methanol and 140 mL of acid water (pH 2 with HCl). The loaded cartridge was washed with n-hexane and phenolic compounds were eluted with methanol. The methanolic extract was evaporated to dryness under reduced pressure (40ºC), redissolved in methanol (4 mL) and 20 µL were analysed by HPLC.

HPLC Analysis of Phenolic Compounds. Separation of phenolics was achieved with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 x 0.46 cm; 5µm, particle size) column. The solvent system used was a gradient of water-formic acid (19:1) (A) and methanol (B): 0' - 5% B, 3' - 15% B, 13' - 25% B, 25' - 30% B, 35' - 35% B, 39' - 40% B, 42' - 45% B, 45' - 45% B, 50' - 47% B, 60' - 48% B, 64' - 50% B, 66' – 100% B. The solvent flow rate used was 0.9 mL/min. Detection was achieved with a Gilson diode array detector (DAD), and chromatograms were recorded at 280 and 320 nm. Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

RESULTS AND DISCUTION

The results demonstrate that the *Cobrançosa* cultivar has the same qualitative composition as *Madural*, being characterised by the presence of nine identified compounds: hydroxytyrosol, 5-*O*-caffeoylquinic acid, verbascoside, luteolin 7-*O*-glucoside, oleuropein, rutin, apigenin 7-*O*-glucoside, quercetin 3-*O*-rhamnoside and luteolin (Figure 1). *Verdeal* cultivar exhibited a similar phenolic composition, but verbascoside was not present.

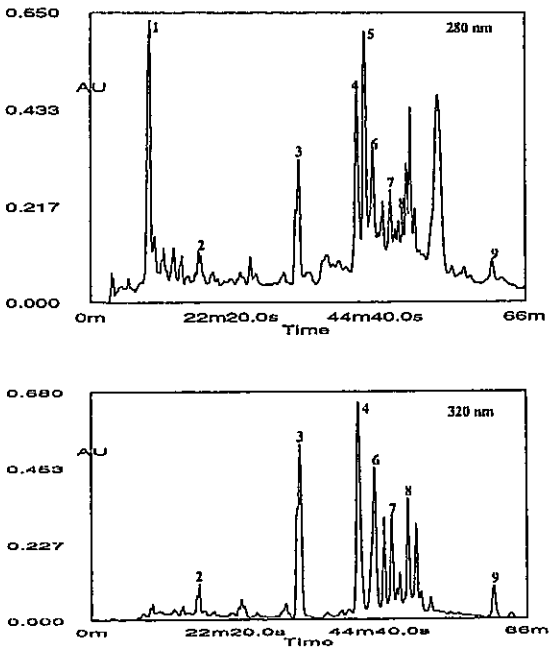

Figure 1. HPLC phenolic profile of a olive fruit sample. (1) hydroxytyrosol; (2) 5-*O*-caffeoylquinic; (3) verbascoside; (4) luteolin 7-*O*-glucoside; (5) oleuropein; (6) rutin; (7) apigenin 7-*O*-glucoside; (8) quercetin 3-*O*-rhamnoside; (9) luteolin.

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