Biologically-active Phytochemicals in Food

Analysis, Metabolism, Bioavailability and Function

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PHENOLIC COMPOUNDS IN THREE PORTUGUESE OLIVE FRUITS VARIETIES

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1 INTRODUCTION

Phenolic compounds constitute a large and heterogeneous class of compounds with a very wide distribution in *taxa* of higher plants. In spite of this almost ubiquity, experimental evidence has demonstrated that each plant species is characterised by the presence of a limited number of compounds.

Within each species the nature of these compounds can vary from organ to organ[1] but is constant enough towards several other factors. These facts have been used, in recent years, in the characterisation of several food products of plant origin, by their phenolic profile. Factors that can induce some variability in the qualitative composition of the phenolic fraction of a given species are strong environmental stress and the existence of races or varieties[1]. With this fact in mind, we are initiating a study on the possible utilisation of phenolic profiles in the discrimination of three different olive fruit cultivars (*Cobrançosa*, *Madural* and *Verdeal*) from Trás-os-Montes, one of the most important regions of olive tree cultivation in Portugal.

2 EXPERIMENTAL

2.1 Extraction and Purification of Phenolic Compounds from Portuguese Olive Fruits

The lyophilised olive fruit samples (*Cobrançosa*, *Madural* and *Verdeal* cultivars) were extracted with methanol, until complete extraction of the phenolic compounds (negative reaction to NaOH 20%). After evaporation of the solvent, the residue obtained was dissolved in acid water (pH 2 with HCl) and passed through a preconditioned C18 SEP-PAK (NEC) cartridge. Each column was preconditioned 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 then concentrated to dryness, redissolved in methanol and analysed by HPLC.

2.2 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.

3 RESULTS AND DISCUSSION

The methodology used for the sample preparation originated extracts free from interferences and the gradient used for HPLC gave well resolved chromatograms (Figures 1 and 2) useful for future quantification (ongoing work).

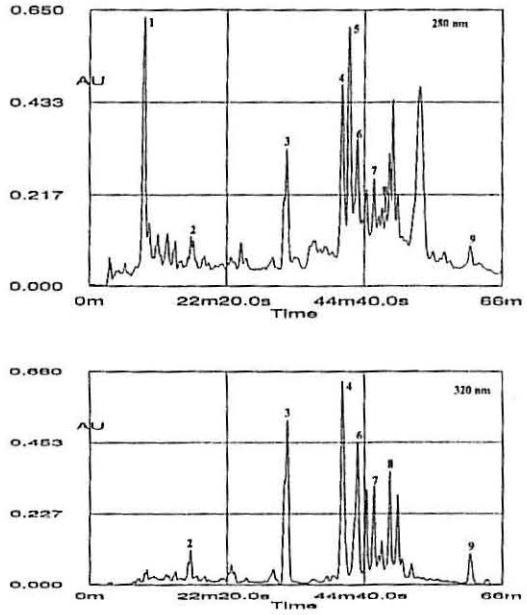

Figure 1 *HPLC profile of Madural olive fruit. (1) hydroxytyrosol,; (2) 5-O-caffeoylquinic; (3) verbascoside; (4) luteolin 7-O-glucoside; (5) oleuropein; (6) rutin; (7) apigenin 7-O-glucoside; (8) quercetin 3-O-rhamnoside; (9) luteolin.*

The extracts obtained from *Cobrançosa* and *Madural* cultivars exhibited a similar qualitative composition; both of them showed the presence of hydroxytyrosol, oleuropein, 5-*O*-caffeoylquinic, verbascoside, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside, quercetin 3-*O*-rhamnoside and luteolin (Figure 1). The extract obtained from *Verdeal* cultivar showed a very similar composition, exception made for verbascoside that was not detected (Figure 2).

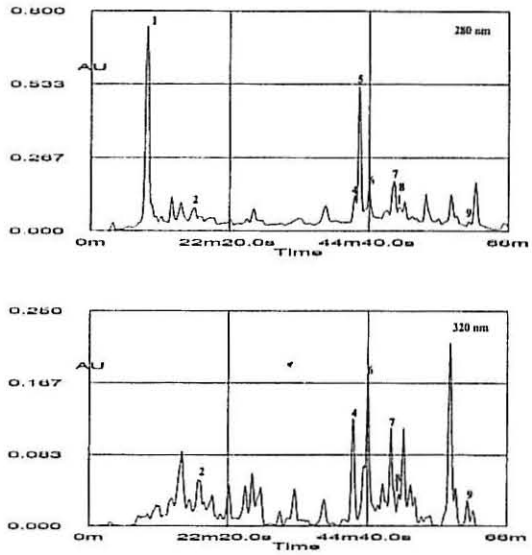

Figure 2 *HPLC profile of Verdeal olive fruit. (1) hydroxytyrosol,; (2) 5-O-caffeoylquinic; (4) luteolin 7-O-glucoside; (5) oleuropein; (6) rutin; (7) apigenin 7-O-glucoside; (8) quercetin 3-O-rhamnoside; (9) luteolin*

4 REFERENCES

1. D. Ryan and K. Robards, *Analyst*, 1998, **123**, 31.