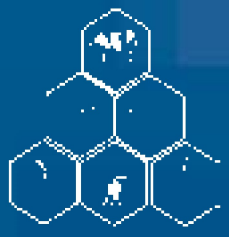

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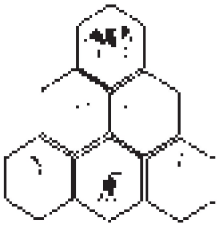

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Development of an HPLC/DAD method for determination of phenolic profile in portuguese olive fruits

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Introduction

Phenolics are characteristic constituents of green plants occurring in virtually all parts of the plant but with quantitative distributions that vary between different organs of the plant and within different populations of the same plant species. Phenolics in olive pulp and oil constitute a complex mixture and the complete chemical nature has not, as yet, been elucidated[1].

Phenolic compounds may contribute to fruit quality in a number of ways; for example, by contributing to sensory attributes, such as colour and flavour, and through the contribution of some specific phenolics, in particular oleuropein[2], to the intense bitterness of the olive fruit.

Factors contributing to the variability in phenolic distribution include cultivar and genetics, geographical origin, maturity, climate, position on tree, rootstock and agricultural practices. In the case of processed products, technological processes to which olive fruits are exposed may also impact significantly on the phenolic content[1].

Here we studied the following aspects of the determination of polyphenols in Portuguese olive fruits: extraction, chromatographic separation, and quantification.

Experimental

Extraction of Phenolic Compounds from Portuguese Olive Fruits

The lyophilised olive fruit samples 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.

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; 5mm, 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.

Results and Discussion

Results of quantification of phenolic compounds applied to three Portuguese olive fruit cultivars – *Cobrançosa, Madural and Verdeal* – are shown in Table 1. The extract obtained from *Cobrançosa* olive fruit has the same qualitative composition as that obtained from *Madural* olive fruit, being characterised by the presence of, at least, ten identified compounds: hydroxytyrosol, oleuropein, 5-*O*-caffeoylquinic, verbascoside, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside, quercetin 3-*O*-rhamnoside and luteolin. *Verdeal* olive fruit exhibited a similar phenolic composition, but verbascoside was not present. In all cases, several unidentified anthocyanins were detected.

Table 1
Phenolic compounds of three Portuguese olive fruit cultivars (g/kg)[a]

Phenolic compounds	Cultivars		
	Cobrançosa	Madural	Verdeal
Hydroxytyrosol	1.440 (0.0284)	4.468 (0.1445)	0.558 (0.0028)
5-O-Caffeoylquinic	0.002 (0.0001)	0.004 (0.0001)	0.001 (0.00002)
Verbascoside	0.044 (0.0008)	0.047 (0.0009)	-
luteolin 7-O-glucoside	0.218 (0.0040)	0.841 (0.0074)	0.037 (0.00005)
Oleuropein	1.976 (0.0327)	5.374 (0.5946)	5.706 (0.1132)
Rutin	0.505 (0.0041)	0.959 (0.0155)	0.158 (0.0019)
Apigenin 7-O-glucoside	0.038 (0.0009)	0.135 (0.0034)	0.015 (0.0001)
Quercetin 3-O-rhamnoside	0.060 (0.0013)	0.114 (0.0060)	0.019 (0.0002)
Luteolin	0.026 (0.0005)	0.054 (0.0014)	0.002 (0.0005)

a Values are expressed as mean (standard deviation) of three determinations

In conclusion, the proposed procedure is simple, rapid, sensitive, reproducible and accurate, suitable for routine analysis of phenolics in olive fruits. This procedure also allows the discrimination of different cultivars of Portuguese olive fruits.

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