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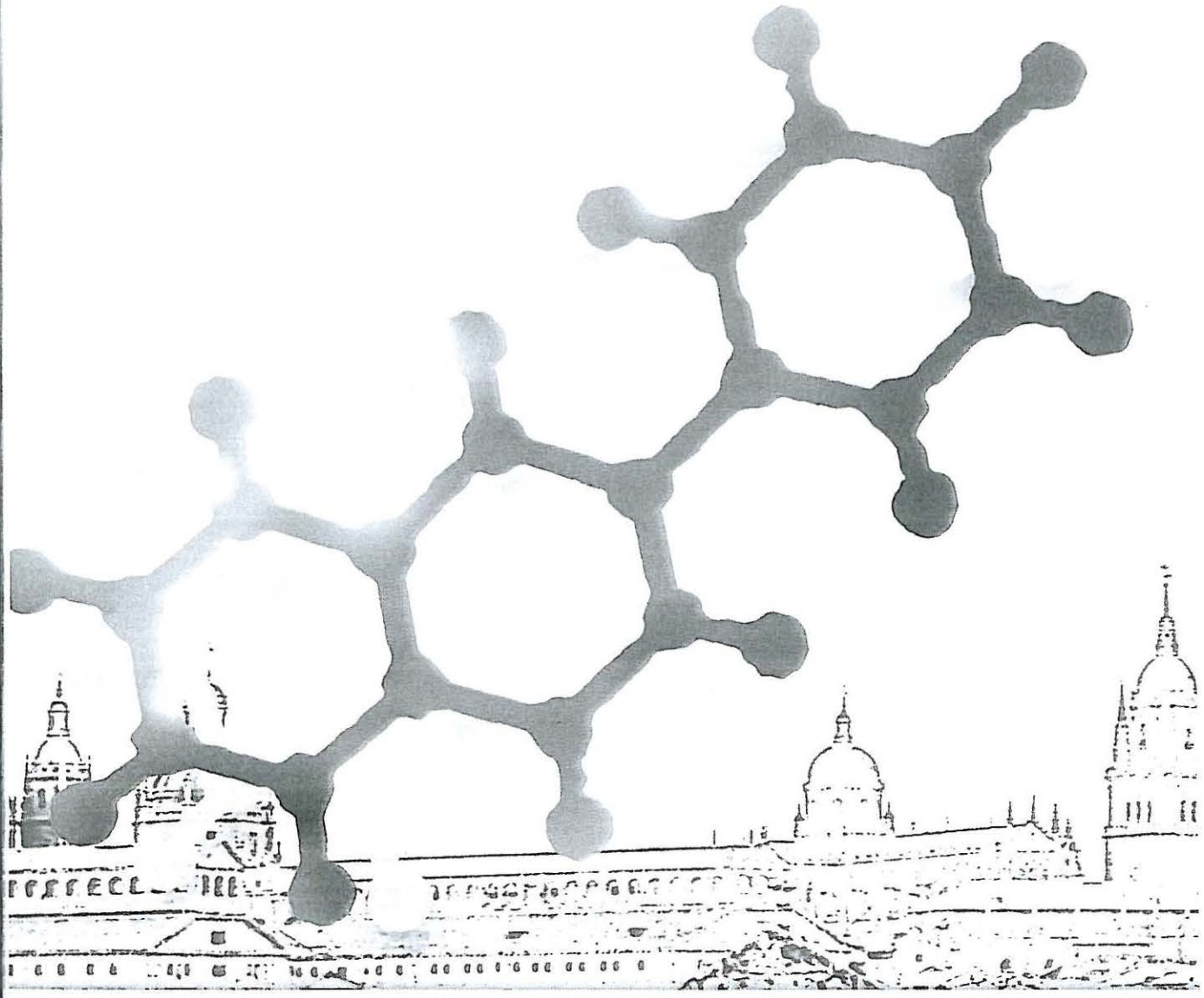

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Determination of phenolic compounds in Portuguese wild mushrooms

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Abstract. We report the determination of phenolic compounds in five Portuguese wild mushrooms (*Agaricus arvensis*, *Agaricus silvicola*, *Lactarius deliciosus*, *Lepista nuda* and *Ramaria botrytis*) by reversed phase HPLC-DAD-ESI/MS. Three phenolic compounds were detected, identified and quantified: protocatechuic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid. *p*-Hydroxybenzoic acid was found in all the mushroom samples, being the main phenolic compound with the exception of *R. botrytis*. This species presented the highest content in phenolic compounds mainly due to the contribution of protocatechuic acid.

Introduction. In recent years oxidative stress, induced by reactive oxygen species (ROS) that are generated by normal metabolic activity as well as lifestyle factors such as smoking, exercise and diet, have been implicated in the causation and progression of several chronic diseases. Antioxidants that can mitigate the damaging effects of ROS have been the focus of recent research [1]. Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of certain forms of cancer and cardiovascular diseases [2]. Although the protective effects have been primarily attributed to the well-known antioxidants, such as Vitamin C, Vitamin E and β-carotene, plant phenolics may also play a significant role [3]. Phenolic compounds exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions [3]; many of these biological functions have been attributed to their free radical scavenging and antioxidant activity. The use of mushrooms extracts as antioxidants is becoming increasingly popular [4,5] generating a great interest in the identification of individual antioxidant components such as phenolic compounds. Studies concerning the analysis of the phenolic components of Portuguese wild mushrooms can be found in the literature, particularly for *Cantharellus cibarius* [6], *Suillus bellini*, *Tricholomopsis rutilans*, *Hygrophorus agathosmus*, *Amanita rubescens*, *Russula cyanoxantha*, *Boletus edulis*, *Tricholoma equestre*, *Suillus luteus*, *Suillus granulatus* [7], *Fistulina hepatica* [8]. Nevertheless, being the Northeast of Portugal one of the European regions with higher wild edible mushroom diversity, it is important to characterize the phenolic composition of other species also important and with gastronomic relevance. In this study, individual phenolic compounds profile of five Portuguese wild mushrooms (*Agaricus arvensis*, *Agaricus silvicola*, *Lactarius deliciosus*, *Lepista nuda* and *Ramaria botrytis*) was obtained by high-performance liquid chromatography coupled to photodiode array detector and mass spectrometer (HPLC-DAD-ESI/MS).

Materials and Methods. *Samples.* Wild mushrooms were collected under live pine or oak trees in Bragança (Northeast of Portugal), in autumn 2006, and were identified according to several authors.

Phenolics Extraction. Each mushroom sample (3 g) was extracted with acetone:water (80:20; 50 mL) mixture at -20°C for 6h. The extract was put in an ultrasonic bath for 15 min, centrifuged at 4000 *g* for 10 min, and filtered through Whatman nº 4 paper. The residue was then extracted with three additional 50 mL portions of the acetone:water mixture. The combined extracts were evaporated at 30 ºC to remove acetone. The aqueous phase was washed with hexane, and then submitted to a liquid-liquid extraction with ethyl ether (3 x 50 mL) and ethyl acetate (3 x 50 mL). The organic phases were evaporated at 30 ºC to dryness, redissolved in water:methanol (80:20), and filtered through a 0.22 µm disposable LC filter disk for HPLC analysis.

HPLC analysis. The extracts were analyzed on an analytical HPLC unit, using a Spherisorb ODS2 3 µm (4.6 x 150 mm) column. The solvent system used was a gradient of acetic acid (2.5%) (A), acetic acid/acetonitrile (90:10) (B), and acetonitrile (C), at a solvent flow rate of 0.5 mL/min. Detection was carried out in a diode array detector (DAD), using 280 nm as preferred wavelength and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet. The compounds in each sample were identified by

comparing their retention times and UV-vis spectra with standards, and from their mass spectra. Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

Results and Discussion. It was possible to identify and quantify three phenolic compounds (protocatechuic, *p*-hydroxybenzoic and *p*-coumaric acids). No flavonoids or other relevant polyphenols could be detected in the samples analysed. *p*-Hydroxybenzoic acid was the main phenolic compound found in all the mushroom samples, with exception of *R. botrytis* which presented protocatechuic acid as main compound (Table 1). Other authors had already reported the presence of *p*-hydroxybenzoic acid in other mushroom species, such as *A. rubescens*, *T. equestre* and *R. cyanoxantha* [7], and *p*-coumaric acid in *C. cibarius* [6] and *F. hepatica* [8]. *R. botrytis* showed the highest phenolic compounds concentration (356.7 mg/Kg, dry matter).

Table 1. *Phenolic compounds composition in Portuguese wild mushrooms.*

	Phenolic compounds (mg/kg, dry matter)			
	protocatechuic acid (15.1 min)	*p*-hydroxybenzoic acid (22.9 min)	*p*-coumaric acid (41.7 min)	Total phenolic compounds
A. arvensis	n.d	70.13 ± 1.20	48.67 ± 3.40	118.8 ± 4.6
A. silvivola	n.d	238. 7 ± 12.4	45.72 ± 1.19	284.4 ± 11.2
L. deliciosus	n.d	12.37 ± 1.64	n.d	12.37 ± 1.64
L. nuda	33.5 ± 0.50	29.31 ± 1.54	3.75 ± 0.56	66.53 ± 2.62
R. botrytis	342.7 ± 10.2	14.00 ± 0.77	n.d	356.7 ± 9.4

n.d – not detected

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Celestino Santos Buelga
President of the Scientific Commitee ICP2008