Phenolic profile of seventeen Portuguese wild mushrooms

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ABSTRACT

Analysis of phenolic compounds in seventeen Portuguese wild mushroom species was carried out by high-performance liquid chromatography coupled to photodiode array detection (HPLC-DAD). Protocatechuic, *p*-hydroxybenzoic, *p*-coumaric and cinnamic acid were found and quantified. *Fistulina hepatica* showed the highest phenolic acids concentration (111.72 mg/Kg, dw) due to the significant contribution of protocatechuic (67.62 mg/Kg) and *p*-hydroxybenzoic (41.92 mg/kg) acids. The edible mushrooms analyzed could be directly used in the human diet to combat oxidative stress, while inedible species could represent a source of extractable phenolic compounds to be used as additives in the food industry or as components in pharmaceutical and cosmetic formulations, due to their well-known antioxidant properties.

***Keywords*:** Wild mushrooms; edibility; phenolic compounds, HPLC-DAD.

1. Introduction

The implication of oxidative and nitrosative stress in the etiology and progression of several acute and chronic clinical disorders such as cancer, cardiovascular and neurodegenerative diseases, has led to the suggestion that natural antioxidants may have health benefits as prophylactic agents. These antioxidants may help the endogenous defence system, assuming a major importance as possible protector agents reducing oxidative damage (Ferreira, Barros, & Abreu, 2009). Against this background, the possibility of including mushrooms, which contain significant amounts of bioactive phytochemicals, in our diets may provide desirable health benefits, beyond that of basic nutrition.

The antioxidants found in mushrooms are mainly phenolic compounds, having been quantified in many different species mainly from Finland (Mattila et al., 2001), India (Puttaraju, Venkateshaiah, Dharmesh, & Somasundaram, 2006; Jayakumar, Thomas, & Geraldine, 2009), Korea (Kim et al., 2008), Portugal (Ribeiro et al., 2006; Ribeiro, Valentão, Baptista, Seabra, & Andrade, 2007; Ribeiro et al., 2008; Barros, Dueñas, Ferreira, Baptista, & Santos-Bulega, 2009) and Turkey (Yaltirak, Aslim, Ozturk, & Alli, 2009). Phenolic compounds have specific health effects even though they are non-nutritive compounds. In our diet they might provide health benefits associated with reduced risk of chronic diseases which may relate to their ability to reduce agents by donating hydrogen and quenching singlet oxygen. Antioxidant properties of phenolic compounds also play a vital role in the stability of food products, as well as in the antioxidative defence mechanisms of biological systems (Wright, Johnson, & DiLabio, 2001).

It is our interest to characterize the phenolic composition of mushroom species and to understand if differences exist between the phenolic profile of edible and non edible species.

2. Materials and Methods

2.1. Samples

Seventeen mushroom species were collected in different ecosystems of the Northeast of Portugal (**Table 1)**. The morphological identification of the wild macrofungi was made according to macro and microscopic characteristics (Marchand, 1971-1986; Bon, 1988; Courtecuisse & Duhem, 2005). Representative voucher specimens were deposited at the herbarium of *Escola Superior Agrária de Bragança*. After taxonomic identification, the mushrooms were immediately lyophilized (Ly-8-FM-ULE, Snijders, Holland).

2.2. Standards and reagents

Acetonitrile 99.9% was of HPLC grade from Lab-Scan (Lisbon, Portugal). Other solvents were of analytical grade purity and were also supplied by Lab-Scan. Phenolic standards were from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA) before use.

2.3. Sample preparation

Each mushroom sample (~3 g) was extracted using an acetone:water (80:20; 30 ml) mixture at -20°C for 6h. After 15 min in an ultrasonic bath, the extract was centrifuged at 4000*g* for 10 min and filtered through Whatman nº 4 paper. The residue was then extracted with two additional 30 ml portions of the acetone:water mixture. The combined extracts were evaporated at 40 ºC under reduced pressure to remove acetone (rotary evaporator Büchi R-210). The aqueous phase was washed with *n*-hexane and then submitted to a liquid-liquid extraction with diethyl ether (3 × 30 ml) and ethyl acetate (3 × 30 ml). The

88 organic phases were evaporated at 40 ºC to dryness, redissolved in water:methanol (80:20)
89 and filtered through a 0.22 µm disposable LC filter disk for HPLC analysis.
90
91 *2.4. HPLC analysis*
92 The phenolic extracts were analysed using HPLC equipment consisting of an integrated
93 system with a Varian 9010 pump, a Varian Pro star diode array detector (DAD) and a
94 Jones Chromatography oven column heater (model 7981). Data were analysed using Star
95 chromatography workstation version 6.41 software (Varian). The chromatographic
96 separation was achieved with an Aqua (Phenomenex, Torrance, CA) reverse phase C_{18}
97 column (3 µm, 150mm x 4.6mm i.d.) thermostatted at 30 ºC. The mobile phase and the
98 gradient employed was described previously (Barros et al., 2009). Injection volume was 20
99 µl. Detection was carried out in a diode DAD, using 280 nm as the preferred wavelength.
100
101 **3. Results and Discussion**
102 Protocatechuic, *p*-hydroxybenzoic, *p*-coumaric and cinnamic acids were identified and
103 quantified in some of the analysed samples (**Table 2**) by comparing their chromatographic
104 characteristics and absorption spectra with that of the standard compounds.
105 *Fistulina hepatica* showed the highest concentration of phenolic acids mostly due to the
106 contribution of protocatechuic and *p*-hydroxybenzoic acids (**Table 2**). Mushrooms have
107 developed chemical defence mechanisms (against insects and microorganisms) analogous
108 to those in plants, such as the production of phenolic compounds. In fact, phenolic
109 compounds have been shown to protect the plant cell wall during UV, salt, or pathogenic
110 stress (Signore, Romeo, & Giaccio, 1997). Indeed, other authors (Ribeiro et al., 2007)
111 reported the presence of caffeic, *p*-coumaric and ellagic acids in *Fistulina hepatica*

112 collected in the same region (Bragança, Portugal) in 2004. However, we did not find any of
113 those phenolic compounds in our sample.
114 Instability of phenol content over time after collection is often observed, probably due to
115 enzymatic and oxidative decomposition. This fact, together with the different stress
116 conditions at which mushrooms were submitted, as well as the different extraction
117 methodology applied (in addition to possible genetic variability) could explain the
118 differences observed between the present study and other studies in what concerns the
119 phenolic profile of *Fistulina hepatica* (Ribeiro et al., 2007) and *Hydnum repandum*
120 (Puttaraju et al., 2006). In addition, in our study it was possible to detect and quantify
121 cinnamic acid in a Portuguese sample of the latter mushroom, while other authors also
122 quantified tannic, gallic and protochatecuic acids in a sample from India (Puttaraju et al.,
123 2006).
124 No phenolic acids were detected in *Laccaria amethystina*, *Lepista inversa* and *Russula*
125 *delica*. Nevertheless, Yaltirak et al. (2009) found gallic acid, catechin, caffeic acid and
126 rutin in a sample of *Russula delica* from Turkey. These authors used an extraction
127 methodology with ethanol in a soxhlet apparatus at 60 ºC. We avoided heat due to the fact
128 that phenolic compounds are unstable and readily become non-antioxidative under heating
129 and in the presence of antioxidants (Yen & Hung, 2000; Barros, Baptista, Correia, Morais,
130 & Ferreira, 2007). However, the results obtained by Yaltirak et al. (2009) support the view
131 that heat may increase phenolics concentration. Also, Choi, Lee, Chun, Lee, & Lee (2006)
132 described that heat treatment of Shiitake increased the overall content of free polyphenolic
133 and flavonoid compounds. The authors explained that heat treatment might produce
134 changes in their extractability due to the disruption of the plant cell wall - thus bound
135 polyphenolic and flavonoid compounds may be released more easily relative to those of
136 raw materials.

Figure 1 compares the total phenolic compounds according to the edibility of each wild mushroom sample. *Fistulina hepatica*, *Hygrophorus agathosmus*, *Tricholoma atrosquamosum* and *Suillus collinitus* are edible mushrooms and showed the highest content of phenolics. Although the sample number of inedible mushrooms and mushrooms with questionable edibility was lower than that of the edible species, it can be concluded that *Tricholoma sulphureum* was the only inedible mushroom that yielded a reasonable phenolic content. Therefore, despite being inedible, mushrooms are an important source of phenolic compounds that could be extracted and included in formulations to prevent oxidative stress. This state in humans is originated by continuous exposure to chemicals and contaminants that lead to an increase in the amount of free radicals in the body, causing irreversible oxidative damage to biomolecules (e.g. lipids, proteins, DNA). Therefore, antioxidants and particularly phenolic compounds may decrease the risks of several chronic diseases such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans (Halliwell, 1996).

Overall, *Fistulina hepatica* revealed the highest concentration of phenolic compounds. The phenolic profile of this sample and of *Hydnum repandum* and *Russula delica* has already been described. However, we pointed out some differences in the results obtained. Furthermore, this is the first time that phenolic compounds in the other fourteen species are described. The edible mushrooms could be directly used in the human diet to combat oxidative stress, taking advantage on the synergistic and/or additive effects of all the compounds present therein (Liu, 2004), while inedible species could represent a source of extractable phenolic compounds to be used as additives in the food industry or as components in pharmaceutical and cosmetic formulations, due to their well-known antioxidant properties.

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232 **Table 1**. Information on the wild mushroom samples collected.

Species	Common name	Edibility	Ecossystem
Fistulina hepatica (Schaeff.:Fr.)	Beefsteak Fungus	Edible	*Quercus pyrenaica*
Hydnum repandum (L.: Fr.)	Hedgehog	Edible	Mixed stands
Hygrophoropsis aurantiaca (Wulf.: Fr.) Mre.	False Chanterelle	Questionable	Mixed stands
Hygrophorus agathosmus	Gray almond waxy cap	Edible	Mixed stands
Hygrophorus olivaceo-albus	None	Edible	Mixed stands
Laccaria amethystina (Bolt. ex Fr.) R.Maire	Amethyst Deceiver	Edible	*Quercus pyrenaica*
Lactarius aurantiacus (Fr.)	Orange milkCap	Edible	Mixed stands
Lactarius salmonicolor (Heim y Leclair)	None	Edible	*Quercus pyrenaica*
Lepista inversa (Scop.: Fr.)	Tawny funnel cap	Edible	Mixed stands
Mycena haematopus (Pers) P. Kumm.	Bleeding mycena	Questionable	Mixed stands
Russula caerulea (Pers) Fr.	Humpback brittlegill	Edible	*Pinus pinaster*
Russula delica (Fr.)	Milk-white brittlegill	Edible	Mixed stands
Russula sardonia Fr.	Primrose brittlegill	Inedible	*Pinus pinaster*
Suillus collinitus (Fr.) Kuntz	None	Edible	*Quercus pyrenaica*
Suillus mediterraneensis (Jacquetant & Blum) Redeuilh	None	Inedible	*Quercus pyrenaica*
Tricholoma atrosquamosum (Cheval) sacc.	None	Edible	Mixed stands
Tricholoma sulphureum (Bull.: Fr.) Kumm.	Sulphur knight	Inedible	*Quercus pyrenaica*

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Table 2. Phenolic acids (mg/Kg dw) found in the mushroom samples (mean ± standard deviation; n=3).

	protocatechuic acid (22.3 min)	*p*-hydroxybenzoic acid (33.4 min)	*p*-coumaric acid (52.3 min)	cinnamic acid (63.7 min)	Total phenolic acids
Fistulina hepatica	67.62 ± 1.66	41.92 ± 8.95	nd	2.16 ± 0.10	111.72 ± 7.19
Hydnum repandum	nd	nd	nd	4.51 ± 1.25	4.51 ± 1.25
Hygrophoropsis aurantiaca	nd	nd	nd	3.52 ± 0.88	3.52 ± 0.88
Hygrophorus agathosmus	17.92 ± 0.20	nd	8.65 ± 0.20	46.04 ± 0.23	72.61 ± 0.62
Hygrophorus olivaceo-albus	nd	7.38 ± 0.19	nd	0.85 ± 0.15	8.23 ± 0.44
Laccaria amethystina	nd	nd	nd	nd	nd
Lactarius aurantiacus	nd	nd	nd	9.18 ± 0.55	9.18 ± 0.55
Lactarius salmonicolor	nd	3.40 ± 0.42	nd	2.64 ± 0.14	6.04 ± 0.29
Lepista inversa	nd	nd	nd	nd	nd
Mycena haematopus	1.02 ± 0.06	nd	nd	2.96 ± 0.03	3.98 ± 0.03
Russula caerulea	nd	nd	nd	2.58 ± 0.04	2.58 ± 0.04
Russula delica	nd	nd	nd	nd	nd
Russula sardonia	nd	nd	nd	0.43 ± 0.01	0.43 ± 0.01
Suillus collinitus	5.18 ± 0.21	14.14 ± 1.15	nd	1.34 ± 0.17	20.66 ± 1.12
Suillus mediterraneensis	1.38 ± 0.09	2.04 ± 0.08	nd	0.98 ± 0.09	4.40 ± 0.26
Tricholoma atrosquamosum	nd	nd	79.34 ± 4.51	7.43 ± 0.42	86.67 ± 4.10
Tricholoma sulphureum	nd	2.55 ± 0.17	nd	23.18 ± 0.85	25.73 ± 1.03

nd- not detected

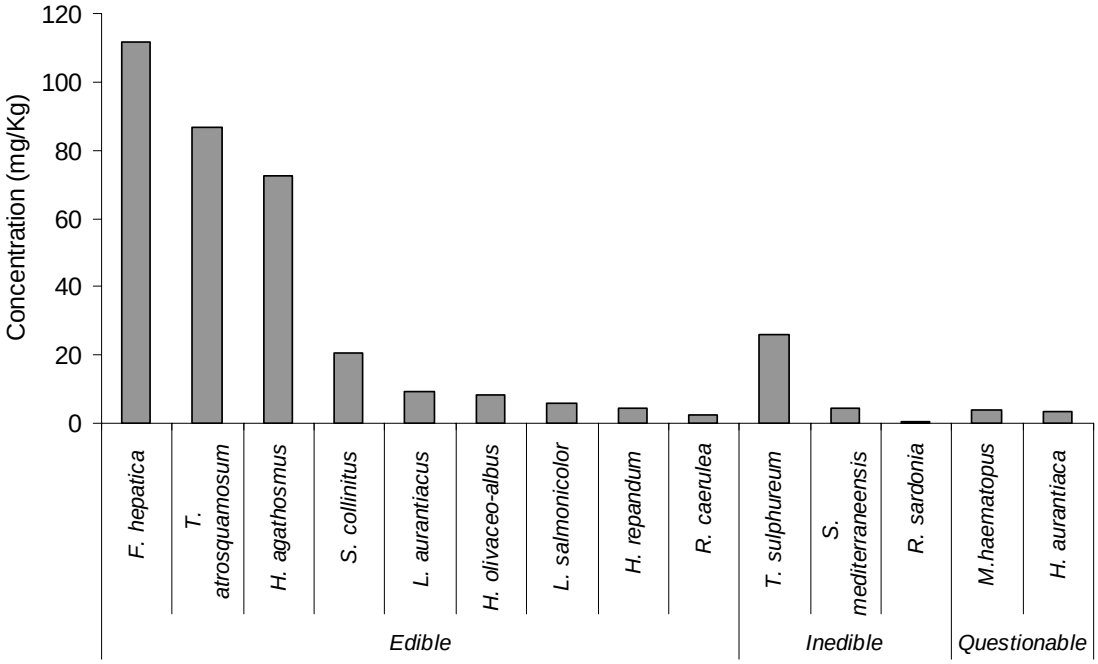

Figure 1. Comparison of phenolic acids content according to edibility of the wild mushrooms.