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# Effects of trophism on nutritional and nutraceutical potential of wild edible mushrooms

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ABSTRACT

Consumption of wild growing mushrooms has been preferred to eating of cultivated fungi in many countries of central and Eastern Europe. Nevertheless, the knowledge of the nutritional value of wild growing mushrooms is limited. The present study reports the effects of trophism on mushrooms nutritional and nutraceutical potential. *In vitro* antioxidant properties of five saprotrophic (*Calvatia utriformis, Clitopilus prunulus, Lycoperdon echinatum, Lyophyllum decastes,* and *Macrolepiota excoriata*) and five mycorrhizal (*Boletus erythropus, Boletus fragrans, Hygrophorus pustulatus, Russula cyanoxantha,* and *Russula olivacea*) wild edible mushrooms were accessed and compared to individual compounds identified by chromatographic techniques. Mycorrhizal species revealed higher sugars concentration (16–42 g/100 g dw) than the saprotrophic mushrooms (0.4–15 g/100 g). Furthermore, fructose was found only in mycorrhizal species (0.2–2 g/100 g). The saprotrophic *L. decastes*, and the mycorrhizal species *B. erythropus* and *B. fragrans* gave the highest antioxidant potential, mainly due to the contribution of polar antioxidants such as phenolics and sugars. The bioactive compounds found in wild mushrooms give scientific evidence to traditional edible and medicinal uses of these species.



## 1. Introduction

Mushrooms are appreciated all over the world not only by their texture and flavour, but also by their chemical, nutritional (Kalač, 2009) and functional properties (Leskosek-Cukalovic et al., 2010). Wild mushrooms are rich in minerals and have high levels of water, proteins, fibbers and carbohydrates. Mushrooms also have low fat levels being excellent to include in low caloric diets (Agahar-Murugkar & Subbulakshmi, 2005; Díez & Alvarez, 2001; Heleno, Barros, Sousa, Martins, & Ferreira, 2009). Therefore, edible species are highly nutritive and have been compared to meat, eggs and milk, since they reveal a composition in amino acids similar to animal proteins (Longvah & Deosthale, 1998).

Consumption of wild growing mushrooms has been preferred to eating of cultivated fungi in many countries of central and Eastern Europe (Kalač, 2009). Wild edible fungi are collected for food and to earn money in more than 80 countries. Collection and consumption within countries varies from the extensive and intensive patterns of China to more restricted use by indigenous people in South America. Substantial quantities are eaten through personal collections that may go unrecorded. The nutritional value of wild edible fungi should not be underestimated: they are of comparable value with many vegetables and in notable cases have a higher food value (Boa, 2004).

Mushrooms are consumed as a delicacy, and particularly for their specific aroma and texture. Both fresh and preserved fruiting bodies of tens of species can be culinary-processed in different manners. However, the knowledge of the nutritional value of wild growing mushrooms is limited when compared with vegetables (Kalač, 2009).

Saprotrophic fungi derive their nutrients from dead organic material, *e.g.*, agricultural crop residues, wood of dead trees, animal dung, *etc.* (Chang & Miles, 2004). The saprotrophic wild edible fungi, though less important in terms of volumes collected and money earned from local sales, are important in nutrient recycling. The saprotrophic species are the basis for the hugely valuable global business in cultivated mushrooms, currently valued at around US $23 billion each year. This is an increasing source of income for small-scale enterprises in developing countries (Boa, 2004).

Otherwise, fungi that live in the soil in symbiotic association with roots of vascular plants in woodlands and in forest ecosystems are very important ecologically and economically. These associations are referred to as mycorrhizae (fungus root association). There are some mycorrhizal mushrooms but it is difficult to bring these wild mushrooms into cultivation because they are the products of a fungus root association. These mushrooms have a mutualistic symbiotic relationship with trees. In these partnerships, the fungi obtain their carbohydrates from the plant roots. The root hosts, in turn, are supplied with inorganic mineral nutrients absorbed from the soil by fungal mycelia. Mycorrhizal

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fungi in plant roots have, indeed, been demonstrated to strongly stimulate the growth of their hosts (Chang & Miles, 2004; Martins, 2008).

Wild edible fungi play an important ecological role. Many of the leading species live symbiotically with trees and this mycorrhizal association sustains the growth of native forests and commercial plantations in temperate and tropical zones (Boa, 2004).

In the present work, we intend to evaluate the effects of trophism in mushrooms nutritional and nutraceutical potential. *In vitro* antioxidant properties of five saprotrophic (*Calvatia utriformis, Clitopilus prunulus, Lycoperdon echinatum, Lyophyllum decastes,* and *Macrolepiota excoriata*) and five mycorrhizal (*Boletus erythropus, Boletus fragrans, Hygrophorus pustulatus, Russula cyanoxantha,* and *Russula olivacea*) wild edible mushrooms were accessed and compared to individual compounds identified by chromatographic techniques.

## 2. Material and methods

### 2.1. Mushroom species

Five wild edible saprotrophic mushroom species and five wild edible mycorrhizal mushroom species were collected in Bragança (Northeast Portugal). Information about the collected species is provided in Table 1. Taxonomic identification of sporocarps was made according to several authors (Bon, 1988; Courtecuisse & Duhem, 2005; Frade & Alfonso, 2005), and representative voucher specimens were deposited at the herbarium of School of Agriculture of Polytechnic Institute of Bragança. All the species were lyophilised (Ly-8-FM-ULE, Snijders, Holland), reduced to a fine dried powder (20 mesh) and kept at −20 °C until further analysis.

### 2.2. Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, gallic acid, ascorbic acid, tocopherols (α, β, δ and γ isoforms), sugars (D(−)fructose, D(+)mannitol and D(+)trehalose) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

### 2.3. Macronutrients

Samples were analysed for chemical composition (moisture, protein, fat, carbohydrates and ash) using the AOAC procedures (1995). Protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Carbohydrates were calculated by difference: Carbohydrates = 100 − (g protein + g fat + g ash). Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

### 2.4. Fatty acids

Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID) as described previously by the authors (Heleno et al., 2009), and after a trans-esterification procedure with methanol:sulphuric acid 95% toluene 2:1:1 (v/v/v). The equipment was a DANI model GC 1000 instrument with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 μm $d_f$). The FID temperature was 260 °C. The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 30 °C/min ramp to 125 °C, 5 °C/min ramp to 160 °C, 20 °C/min ramp to 180 °C, 3 °C/min ramp to 200 °C, 20 °C/min ramp to 220 °C and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed as a relative percentage of each fatty acid.

### 2.5. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), after extraction with 80% aqueous ethanol at 80 °C, as previously described by the authors (Heleno et al., 2009). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). The chromatographic separation was achieved with a Eurospher 100–5 $NH_2$ column (4.6 × 250 mm, 5 μm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase was acetonitrile:deionized water, 7:3 (v/v) at a flow rate of 1 ml/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Data were analysed using Clarity 2.4 Software (DataApex). Quantification was made using the internal standard method (raffinose, 5 mg/ml) and the results were expressed in g per 100 g of dry weight.

### 2.6. Vitamins

Tocopherols content was determined following a procedure previously optimized and described by the authors (Heleno, Barros,

**Table 1**
Information about the wild edible species analysed.

| Scientific name | English name | Habitat | Date of collection | Trophism |
|---|---|---|---|---|
| *Calvatia utriformis* (Bull.) Jaap. | Mosaic puffball | Mixed stands | October 2009 | Saprotrophic |
| *Clitopilus prunulus* (Scop. ex Fr.) P. Kumm | Sweetbread | Mixed stands | November 2009 | Saprotrophic |
| *Lycoperdon echinatum* Pers. | Spring puffball | Pinus sp. | November 2009 | Saprotrophic |
| *Lyophyllum decastes* (Fries: Fries) Singer | Fried chicken | Mixed stands | November 2009 | Saprotrophic |
| *Macrolepiota excoriata* (Schaeff.) M.M. Moser | Unknown | Mixed stands | October 2009 | Saprotrophic |
| *Boletus erythropus* (Pers.) | Dotted stem bolete | *Castanea sativa* | October 2010 | Mycorrhizal |
| *Boletus fragrans* (Vittadini) | Unknown | *Castanea sativa* | October 2010 | Mycorrhizal |
| *Hygrophorus pustulatus* (Persoon : Fries) Fries | Spotted-stalk | Pinus sp. | November 2009 | Mycorrhizal |
| *Russula cyanoxantha* (Schaeff.) Fr. | Charcoal burner | Mixed stands | October 2010 | Mycorrhizal |
| *Russula olivacea* (Schaeff.) Fr. | Unknown | Quercus sp. | October 2010 | Mycorrhizal |