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, Macrolepiota excoriata*) and five mycorrhizal (*Boletus erythropus, Boletus fragrans, Hygrophorus pustulatus, Russula cyanoxantha, 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 *Lyophyllum decastes*, and the mycorrhizal species *Boletus erythropus* and *Boletus 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.

Keywords**:** Edible Mushrooms; Saprotrophic; Mycorrhizal; Nutrients; Nutraceuticals

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 (Díez & Alvarez, 2001; Agahar-Murugkar & Subbulakshmi, 2005; 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 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, Macrolepiota excoriata*) and five mycorrhizal (*Boletus erythropus, Boletus fragrans, Hygrophorus pustulatus, Russula cyanoxantha, 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 mychorrizal 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 ºC 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 ºC. 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 ºC. The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30ºC/min ramp to 125 ºC, 5ºC/min ramp to 160 ºC, 20ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. 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 ºC, 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 30ºC (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, Sousa, Martins, & Ferreira, 2010), after subsequent extraction with methanol and hexane. Analyses was performed by the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 × 4.6 mm) normal-phase column from YMC Waters operating at 30ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on

the fluorescence signal response, using the internal standard method (tocol in hexane, 50 μg/ml), and the results were expressed in μg per 100 g of dry weight.

Ascorbic acid was determined after extraction of a fine dried powder (20 mesh; 150 mg) with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtration through a Whatman Nº 4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichloroindophenol (9 ml) and the absorbance was measured after 30 min at 515 nm against a blank (AnalytikJena 200 spectrophotometer). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (6×10^{-3} - 0.1 mg/ml), and the results were expressed as mg per 100 g of dry weight.

2.7. Total phenolics

Samples (~1.5 g) were extracted stirring with 40 ml of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 20 ml portion of methanol. The combined methanolic extracts were evaporated at 35 ºC under reduced pressure, re-dissolved in methanol at 20 mg/ml, and stored at 4 ºC for further use. Phenolics were determined by *Folin-Ciocalteu* assay. The extract solution (1 ml) was mixed with *Folin-Ciocalteu* reagent (5 ml, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/l, 4 ml). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (9.4×10^{-3} – 0.15 mg/ml), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

2.8. Bioactivity

In vitro assays already described by the authors (Barros, Carvalho, & Ferreira, 2010; Heleno et al., 2010), were used to evaluate the antioxidant activity of the samples. Different concentrations of the extracts were employed to find EC_{50} values: extract concentration providing 50% of antioxidant activity (or 0.5 of absorbance in reducing power assay) calculated from the graphs of antioxidant potential against extract concentration. Trolox was used as standard.

2.8.1. DPPH radical-scavenging activity. This assay was performed in 96-well microtiter plates using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each of the 96-wells of the plate consisted of one of the different concentrations of the extracts (30 μl) and aqueous methanolic solution (80:20, v/v, 270 μl) containing DPPH radicals ($6x10^{-5}$ mol/l). The mixture was left standing for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\% \text{ RSA} = [(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

2.8.2. Reducing power. This assay was also performed using microtiter plates and the Microplate Reader described above. Different concentrations of the extracts (0.5 ml) were mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The mixture (0.8 ml) was poured into

the wells of a 48-well microplate, as also deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance was measured at 690 nm.

2.8.3. Inhibition of β-carotene bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 ºC under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into test tubes containing different concentrations of the extracts (0.2 ml). The tubes were shaken and incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100.

2.9. Statistical analysis

For each mushroom species three samples were assayed and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

The results of the proximate chemical composition and energetic value obtained for the saprotrophic and mycorrhizal wild edible mushrooms are shown in **Table 2**.

The moisture ranged from 78.00 g/100 g fw in *B. fragrans* to 93.03 g/100 g fw in *H. pustulatus*. Protein was found in high levels and varied between 16.80 g/100 g dw in *R. cyanoxantha* and 25.52 g/100 g dw in *L. decastes*. Fat ranged from 0.75 g/100 g dw in *B. erythropus* to 3.06 g/100g dw in *H. pustulatus*, making wild mushrooms ideal to be included in low caloric diets. Carbohydrates, calculated by difference, were also an abundant macronutrient and ranged from 43.38 g/100 g dw in *R. olivacea* to 76.29 g/100 g dw in *B. fragrans*. Ash content varied between 4.74 g/100 g dw in *B. fragrans* and 37.78 g/100 g dw in R. *olivacea*. On the basis of the proximate analysis, it was observed that 100 g dw of these mushrooms assure, on average, 335 kcal. The highest values were found in *B. fragrans*, while *R. olivacea* gave the lowest energy contribution (**Table 2**). It was not possible to distinguish saprotrophic and mycorrhizal mushrooms based on their macronutrients composition. The quantities found are in agreement with other studies on different mushroom species (Heleno et al., 2009; Kalač, 2009).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of the studied saprotrophic and mycorrhizal wild edible mushrooms are shown in **Table 3**. Up to twenty-six fatty acids were detected in most of the samples. The major fatty acid found was linoleic acid (C18:2n6), with the exceptions of *Lyophyllum decastes* and *Hygrophorus pustalatus* where oleic acid (C18:1n9) predominated, contributing to the prevalence of MUFA in the last two species. The studied species also revealed palmitic acid (C16:0) as a major fatty acid. The UFA oleic and linoleic acids were also reported as main fatty acids in *Boletus erythropus* from Canada (Pedneault, Angers, Gosselin, & Tweddell, 2006) and in *Russula cyanoxantha* from Portugal (Ribeiro, Pinho, Andrade,

Baptista, & Valentão, 2009). Nevertheless, the sample of *Russula cyanoxantha* herein studied revealed C18:2>C18:1 with prevalence of PUFA, while a sample collected in 2006 gave C18:1>C18:2 with prevalence of MUFA (Ribeiro et al., 2009). No relevant differences were observed between the fatty acids profiles of saprotrophic and mycorrhizal wild edible mushrooms.

Mannitol and trehalose were the main sugars in the studied mushrooms (**Table 4**). The presence of fructose (0.15 to 2.26 g/100g dw) was detected only in mycorrhizal species. Mycorrhizal fungi do not have the capacity to hydrolyze sucrose to glucose and fructose because they lack the hydrolytic enzyme. The only way these fungi have to use sucrose is in symbiosis, where the disaccharide is hydrolysed by an apoplastic or cell-wall bound invertase derived from the host plant. Sucrose produced by the plant is transported to roots, where it effluxes to the apoplast and is hydrolysed by plant extracellular invertase into glucose and fructose. In the acidified apoplast, glucose is absorbed by high affinity hexose transporters. Fructose, also released by invertase, exerts feedback inhibition on invertase activity, whereas glucose inhibits fructose uptake by the fungus. As glucose concentration in the apoplast declines, the fungus switches to absorb fructose, which then releases the feedback inhibition on invertase, allowing renewed sucrose hydrolysis. Glucose and fructose are not diffused back to the apoplast from the fungal mycelia because they are converted into fungal-specific carbohydrates (Smith & Read, 1997; Orcutt & Nilsen, 2000, Nehls, Mikolajewski, Magel, & Hampp, 2001). It is known that fungi convert plant carbon sources, such as glucose and fructose to trehalose, arabitol and mannitol (Hughes & Mitchell, 1995; Nehls, Mikolajewski, Magel, & Hampp, 2001). Sucrose is hydrolyzed by plant glycosyl hydrolase family 32 enzymes (GH32) into its constituent monosaccharides to meet plant cellular demands. Plant biotrophic fungi exhibit a wide range of ability to access plant-

synthesized sucrose, but most genomes of mycorrhizal taxa lack GH32 genes. Reliance on plant GH32 enzyme activity for carbon acquisition in these symbionts supports earlier predictions of possible plant control over carbon allocation in the mycorrhizal symbiosis (Parrent, James, Vasaitis, & Taylor, 2009). Furthermore, mycorrhizal species revealed mannitol as the most abundant sugar (15.25 to 36.97 g/100 g dw; **Table 4**). Due to its sweetener characteristic, mannitol becomes very attractive from the point of view of food industry, because it is a dietary carbohydrate that has reduced caloric value, since it is only partially absorbed in the small intestine. In fact, this alcohol-sugar derivative finds important applications in pharmaceutical, cosmetic and food industries (Oliveira, Ferreira, & Souza, 2009).

Mycorrhizal mushrooms revealed higher levels of mannitol and trehalose, and therefore total sugars than saprotrophic species. *B. fragrans* and *B. erythropus* revealed the highest levels of total sugars (42.37 and 34.46 g/100 g dw, respectively) including trehalose (3.14 and 4.84 mg/100 g dw, respectively). This disaccharide (composed of two glucose molecules bound by an alpha, alpha-1,1 linkage, with no reducing power) is known to be one of the sources of energy in most living organisms and can be found in bacteria, fungi, insects, plants, and invertebrates. Furthermore, trehalose protects organisms against various stresses, such as dryness, freezing, and osmopressure. Trehalose has good stabilizing functions, namely, preventing starch retrogradation, protein denaturation, and lipid degradation. This saccharide shows good sweetness like sucrose, and in the food industry it is used as a sweetener (Higashiyama, 2002).

α-Tocopherol was found in all the species, but γ- and δ-tocopherols were the major vitamers (**Table 5**). β-tocopherol was found only in two saprotrophic species: *L. echinatum* and *L. decastes*. The latter species presented the highest content of tocopherols (170.79 μg/g dw) while the mycorrhizal mushrooms *B. erythropus* and *B.*

fragrans revealed the lowest content (19.16 and 22.23 µg/g dw, respectively). Ascorbic acid was found in high levels. The highest ascorbic acid concentration was found in the saprotrophic mushroom *C. prunulus* (400.36 mg/100 g dw), while the lowest value was found in the mycorrhizal mushroom *B. fragrans* (81.32 mg/100 g dw). Nevertheless, this behavior was not generalized, which indicates that vitamins contents are not dependent of mushrooms trophism.

Vitamin E (tocopherols and tocotrienols) is a potent antioxidant with anti-inflammatory properties. Several lines of evidence suggest that it has potential beneficial effects with regard to cardiovascular disease. Tocopherols supplementation in human subjects and animal models has been shown to decrease lipid peroxidation, superoxide production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase as well as by decreasing the expression of scavenger receptors, particularly important in the formation of foam cells (Singh, Devaraj, & Jialal, 2005). Vitamin C (ascorbic acid) is also involved in chemical reactions of cellular metabolism. Epidemiological studies indicate that deficiencies in ascorbic acid can generate long-dated oxidative stress-associated pathologies (Chepda, Perier, Chamson, & Frey, 1999).

The antioxidant properties of saprotrophic and mycorrhizal mushrooms were evaluated through scavenging activity on DPPH radicals (examining the capacity to decrease the absorbance of DPPH solution at 517 nm), reducing power (measuring the conversion of Fe^{3+}/ferricyanide complex to the ferrous form) and lipid peroxidation inhibition by β-carotene-linoleate system (measuring the inhibition of β-carotene bleaching, after neutralization of the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models). The results, including EC_{50} values, are given in **Figures 1-3**, that show an increase in antioxidant properties with the increase of extract concentration.

All the samples revealed antioxidant properties independently of their trophism. The saprotrophic *Lyophyllum decastes*, and the mycorrhizal species *Boletus erythropus* and *Boletus fragrans* revealed the highest radical scavenging effects (EC_{50} values ~0.3 mg/ml; **Figure 1**) which is in agreement with their highest concentration in polar antioxidants such as phenolics (48, 39 and 34 mg GAE/g extract, respectively; **Figure 4**) and sugars (34 and 42 g/100 g dw, respectively for the mycorrhizal species; **Table 4**). The same behaviour was observed for reducing power: The saprotrophic *Lyophyllum decastes*, and the mycorrhizal species *Boletus erythropus* and *Boletus fragrans* gave the highest reducing power (lowest EC_{50} values 0.97, 1.42 and 1.52 mg/ml, respectively; **Figure 2**). *Boletus erythropus* revealed the highest percentage of lipid peroxidation inhibition (EC_{50} value 0.58 mg/ml; **Figure 3**), followed by *Boletus fragrans* (0.96 mg/ml) and *Lyophyllum decastes* (0.98 mg/ml; **Figure 3**).
Russula cyanoxantha (mycorrhizal species) was already studied by Ribeiro et al. (2008) that report DPPH scavenging activities of 0.18, 0.11 and 0.08 mg/ml for entire mushroom, stipe and cap, respectively. The immunomodulator and antitumor activities in pre-clinical animal models of the saprotrophic *Lyophyllum decastes* was reported and attributed to linear (1→3) and (1→6)-β-D-glucans (Ferreira, Vaz, Vasconcelos, & Martins, 2010). Nonetheless, as far as we know, there are no reports on antioxidant properties of the other mushrooms.

Overall, saprotrophic and mycorrhizal mushrooms revealed the same profile in macronutrients, fatty acids and vitamins. Nevertheless, mycorrhizal species revealed a higher concentration in sugars than the saprotrophic mushrooms, and fructose (ketohexose) was detected only in the first species. The saprotrophic *Lyophyllum decastes*, and the mycorrhizal species *Boletus erythropus* and *Boletus fragrans* gave the

highest antioxidant potential, mainly due to the contribution of polar antioxidants such as phenolics and sugars.

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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

Table 2. Proximate chemical composition and energetic value of saprotrophic and mycorrhizal wild edible mushrooms. In each column different letters mean significant differences ($p<0.05$).

Species	Moisture (g/100g fw)	Total fat (g/100g dw)	Crude protein (g/100g dw)	Ash (g/100g dw)	Carbohydrates (g/100g dw)	Energy (kcal/100g dw)
Calvatia utriformis	78.00 ± 1.36 e	1.90 ± 0.01 cb	20.37 ± 0.49 bcd	17.81 ± 0.22 d	59.91 ± 0.40 c	338.26 ± 0.61 d
Clitopilus prunulus	89.78 ± 1.46 b	1.01 ± 0.06 ed	18.13 ± 0.37 cd	30.19 ± 2.50 b	50.66 ± 2.21 d	284.30 ± 5.80 f
Lycoperdon echinatum	85.24 ± 0.48 cd	1.22 ± 0.20 ed	23.52 ± 2.20 ba	9.43 ± 0.23 e	65.83 ± 2.09 b	368.34 ± 0.66 bc
Lyophyllum decastes	87.38 ± 1.40 cb	2.10 ± 0.12 b	25.52 ± 3.49 a	7.38 ± 0.64 fe	64.99 ± 2.96 cb	380.98 ± 1.82 ba
Macrolepiota excoriata	88.92 ± 1.57 b	1.55 ± 0.10 cd	25.28 ± 2.64 a	28.98 ± 1.11 cb	44.19 ± 2.14 e	291.84 ± 3.51 fe
Boletus erythropus	88.36 ± 1.49 b	0.75 ± 0.02 e	20.92 ± 0.05 bc	25.90 ± 0.28 c	52.44 ± 0.20 d	300.15 ± 0.88 e
Boletus fragrans	77.99 ± 0.07 e	1.83 ± 0.17 cb	17.15 ± 0.04 d	4.74 ± 0.19 f	76.29 ± 0.27 a	390.19 ± 0.06 a
Hygrophorus pustulatus	93.03 ± 0.79 a	3.06 ± 0.51 a	18.64 ± 0.40 cd	14.04 ± 0.14 d	64.26 ± 0.72 cb	359.16 ± 1.40 c
Russula cyanoxantha	85.44 ± 0.99 cd	1.52 ± 0.52 cd	16.80 ± 0.06 d	7.03 ± 0.87 fe	74.65 ± 1.01 a	379.46 ± 0.61 ba
Russula olivacea	84.58 ± 1.01 d	1.99 ± 0.44 cb	16.84 ± 0.05 d	37.78 ± 5.20 a	43.38 ± 3.71 e	258.84 ± 14.71 g

Table 3. Fatty acids composition (percent) of saprotrophic and mycorrhizal wild edible mushrooms. In each row different letters mean significant differences (p<0.05).

	Calvatia utriformis	*Clitopilus prunulus*	*Lycoperdon echinatum*	*Lyophyllum decastes*	*Macrolepiota excoriata*	*Boletus erythropus*	*Boletus fragrans*	*Hygrophorus pustulatus*	*Russula cyanoxantha*	*Russula olivacea*
C6:0	0.36 ± 0.04	0.03 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	0.10 ± 0.01	0.16 ± 0.02	0.02 ± 0.00	0.04 ± 0.01	0.09 ± 0.03	0.13 ± 0.01
C8:0	0.04 ± 0.00	0.07 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.09 ± 0.03	0.03 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C10:0	0.05 ± 0.00	0.12 ± 0.04	0.03 ± 0.01	0.03 ± 0.00	0.06 ± 0.01	0.09 ± 0.02	0.03 ± 0.00	0.04 ± 0.01	0.11 ± 0.02	0.20 ± 0.02
C12:0	0.20 ± 0.01	0.23 ± 0.04	0.20 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.55 ± 0.02	0.27 ± 0.01	0.05 ± 0.00	0.37 ± 0.00	0.37 ± 0.06
C13:0	0.05 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.09 ± 0.00	0.01 ± 0.00	nd	0.04 ± 0.01	nd
C14:0	0.48 ± 0.02	0.23 ± 0.05	0.56 ± 0.05	0.21 ± 0.00	0.22 ± 0.00	1.38 ± 0.12	0.45 ± 0.08	0.15 ± 0.04	0.39 ± 0.04	0.29 ± 0.02
C15:0	2.20 ± 0.03	0.82 ± 0.05	2.68 ± 0.42	0.62 ± 0.00	0.20 ± 0.00	1.87 ± 0.03	0.74 ± 0.07	0.43 ± 0.03	0.47 ± 0.01	0.17 ± 0.02
C16:0	13.54 ± 0.14	10.11 ± 0.30	10.82 ± 0.35	10.95 ± 0.04	17.83 ± 0.05	21.33 ± 1.46	14.91 ± 0.12	6.96 ± 0.34	12.95 ± 0.22	16.13 ± 0.39
C16:1	0.22 ± 0.03	0.78 ± 0.04	0.39 ± 0.07	0.37 ± 0.00	0.79 ± 0.01	1.23 ± 0.17	1.70 ± 0.15	0.15 ± 0.02	0.56 ± 0.04	1.31 ± 0.04
C17:0	1.12 ± 0.01	0.22 ± 0.04	0.77 ± 0.07	0.37 ± 0.00	0.17 ± 0.00	0.82 ± 0.10	0.27 ± 0.01	0.12 ± 0.02	0.15 ± 0.01	0.10 ± 0.00
C18:0	2.43 ± 0.07	2.47 ± 0.11	2.08 ± 0.14	4.88 ± 0.02	1.94 ± 0.01	4.15 ± 0.02	2.39 ± 0.10	3.96 ± 0.16	11.10 ± 0.15	2.78 ± 0.03
C18:1n9	6.00 ± 0.13	20.42 ± 0.70	9.16 ± 0.66	47.43 ± 1.43	10.21 ± 0.36	14.74 ± 1.19	19.80 ± 0.81	51.53 ± 0.05	28.39 ± 1.11	25.99 ± 0.12
C18:2n6	70.29 ± 0.32	59.92 ± 1.97	69.92 ± 0.71	32.03 ± 1.39	66.19 ± 0.05	48.76 ± 0.86	56.89 ± 0.62	34.74 ± 0.39	43.65 ± 1.45	50.20 ± 0.28
C18:3n6	nd	0.04 ± 0.01	0.06 ± 0.01	0.08 ± 0.00	nd	nd	nd	nd	nd	nd
C18:3n3	0.57 ± 0.00	1.25 ± 0.00	0.12 ± 0.02	0.19 ± 0.00	0.07 ± 0.01	1.09 ± 0.04	0.19 ± 0.01	0.09 ± 0.01	0.08 ± 0.03	0.06 ± 0.02
C20:0	0.34 ± 0.01	0.37 ± 0.07	0.42 ± 0.07	0.35 ± 0.00	0.14 ± 0.00	0.44 ± 0.02	0.19 ± 0.01	0.21 ± 0.04	0.13 ± 0.00	0.39 ± 0.02
C20:1	0.06 ± 0.01	0.25 ± 0.02	0.06 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	nd	0.13 ± 0.03	0.16 ± 0.03	0.08 ± 0.00	0.05 ± 0.01
C20:2	0.07 ± 0.02	0.31 ± 0.03	0.06 ± 0.01	0.03 ± 0.01	0.07 ± 0.00	0.09 ± 0.01	0.12 ± 0.02	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
C20:3n6	nd	0.06 ± 0.03	0.08 ± 0.01	0.08 ± 0.02	nd	nd	0.06 ± 0.00	nd	nd	0.04 ± 0.01
C20:4n6	nd	0.18 ± 0.01	0.29 ± 0.04	0.18 ± 0.02	0.02 ± 0.00	nd	0.01 ± 0.00	0.01 ± 0.00	nd	nd

C20:3n3+C21:0	0.04 ± 0.00	0.08 ± 0.02	0.08 ± 0.01	0.06 ± 0.00	0.01 ± 0.00	0.07 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	nd
C20:5n3	0.20 ± 0.02	0.19 ± 0.01	0.20 ± 0.02	0.11 ± 0.00	0.04 ± 0.00	0.10 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.12 ± 0.00	0.53 ± 0.02
C22:0	0.78 ± 0.08	0.66 ± 0.04	0.74 ± 0.07	0.62 ± 0.01	0.28 ± 0.04	0.74 ± 0.17	0.42 ± 0.01	0.54 ± 0.06	0.30 ± 0.03	0.48 ± 0.02
C23:0	0.15 ± 0.04	0.39 ± 0.13	0.27 ± 0.04	0.40 ± 0.04	0.13 ± 0.05	0.42 ± 0.08	0.15 ± 0.03	0.06 ± 0.01	0.12 ± 0.03	0.09 ± 0.02
C24:0	0.65 ± 0.04	0.72 ± 0.09	0.79 ± 0.04	0.71 ± 0.00	1.26 ± 0.22	1.54 ± 0.13	0.89 ± 0.03	0.61 ± 0.11	0.67 ± 0.05	0.59 ± 0.03
C24:1	0.19 ± 0.03	0.07 ± 0.01	0.14 ± 0.03	0.06 ± 0.01	0.05 ± 0.00	0.09 ± 0.03	0.24 ± 0.03	nd	0.03 ± 0.00	0.04 ± 0.01
SFA	**22.37 ± 0.22 c**	**16.45 ± 0.84 f**	**19.45 ± 0.11 e**	**19.31 ± 0.03 e**	**22.49 ± 0.29 c**	**33.62 ± 1.78 a**	**20.75 ± 0.09 d**	**13.18 ± 0.39 g**	**26.90 ± 0.36 b**	**21.75 ± 0.39 dc**
MUFA	**6.47 ± 0.11 i**	**21.51 ± 0.83 e**	**9.75 ± 0.56 h**	**47.94 ± 1.44 b**	**11.10 ± 0.37 g**	**16.06 ± 0.99 f**	**21.86 ± 0.71e**	**51.85 ± 0.01a**	**29.06 ± 1.06 c**	**27.40 ± 0.07 d**
PUFA	**71.16 ± 0.33 a**	**62.04 ± 1.67 c**	**70.80 ± 0.67 a**	**32.75 ± 1.41 h**	**66.41 ± 0.08 b**	**50.32 ± 0.80 e**	**57.38 ± 0.62 d**	**34.98 ± 0.39 g**	**44.04 ± 1.43 f**	**50.85 ± 0.32 e**

nd- not detected; C- Carbon atoms; SFA- saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA- polyunsaturated fatty acids.

Table 4. Sugars composition of saprotrophic and mycorrhizal wild edible mushrooms. In each column different letters mean significant differences (*p*<0.05).

	Fructose	Mannitol	Trehalose	Total sugars (g/100g dw)
Calvatia utriformis	nd	nd	0.40 ± 0.01 f	0.40 ± 0.01 e
Clitopilus prunulus	nd	0.95 ± 0.10 e	0.95 ± 0.16 edf	1.90 ± 0.27 ed
Lycoperdon echinatum	nd	0.85 ± 0.03 e	1.38 ± 0.09 ed	2.23 ± 0.06 ed
Lyophyllum decastes	nd	11.03 ± 0.74 d	3.98 ± 0.54 b	15.01 ± 1.28 c
Macrolepiota excoriata	nd	4.10 ± 0.04 e	2.75 ± 0.04 c	6.85 ± 0.09 d
Boletus erythropus	1.72 ± 0.03 b	27.90 ± 0.30 b	4.84 ± 0.51 a	34.46 ± 0.24 b
Boletus fragrans	2.26 ± 0.11 a	36.97 ± 0.33 a	3.14 ± 0.26 c	42.37 ± 0.60 a
Hygrophorus pustulatus	0.15 ± 0.05 d	35.37 ± 5.24 a	3.00 ± 0.00 c	38.52 ± 5.29 ba
Russula cyanoxantha	0.34 ± 0.04 c	16.18 ± 0.61 c	1.64 ± 0.32 d	18.16 ± 0.26 c
Russula olivacea	0.23 ± 0.03 dc	15.25 ± 0.24 dc	0.71 ± 0.07 ef	16.19 ± 0.34 c

Table 5. Vitamins composition of saprotrophic and mycorrhizal wild edible mushrooms. In each column different letters mean significant differences (p<0.05).

Sample	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols (µg/100 g dw)	Ascorbic acid (mg/100 g dw)
Calvatia utriformis	8.74 ± 0.30 cd	nd	56.43 ± 0.37 d	nd	65.17 ± 0.68 d	152.89 ± 4.60 c
Clitopilus prunulus	10.02 ± 0.31 cd	nd	nd	18.98 ± 1.27 c	29.00 ± 1.59 fg	400.36 ± 18.46 a
Lycoperdon echinatum	9.42 ± 0.25 cd	48.21 ± 5.54 a	73.92 ± 1.57 c	nd	131.55 ± 3.72 c	166.13 ± 8.89 c
Lyophyllum decastes	13.73 ± 0.30 b	8.30 ± 1.47 b	135.23 ± 0.26 a	13.53 ± 0.66 dc	170.79 ± 0.77 a	385.55 ± 11.68 a
Macrolepiota excoriata	18.73 ± 3.06 a	nd	94.34 ± 8.69 b	44.36 ± 5.82 a	157.43 ± 0.19 b	176.85 ± 28.81 c
Boletus erythropus	1.52 ± 0.32 e	nd	17.64 ± 1.95 e	nd	19.16 ± 2.27 g	169.20 ±23.01 c
Boletus fragrans	1.93 ± 0.04 e	nd	10.30 ± 1.63 fe	10.00 ± 1.32 de	22.23 ± 2.91 g	81.32 ± 4.75 d
Hygrophorus pustulatus	8.09 ± 0.56 d	nd	103.34 ± 12.22 b	15.72 ± 0.36 dc	127.15 ± 11.30 c	387.80 ± 7.01 a
Russula cyanoxantha	10.46 ± 0.07 cd	nd	21.92 ± 2.34 e	6.48 ± 0.98 e	38.86 ± 1.28 fe	189.61 ± 53.93 c
Russula olivacea	11.73 ± 0.85 cb	nd	11.84 ± 1.37 fe	26.18 ± 0.72 b	49.75 ± 1.23 e	247.38 ± 65.03 b

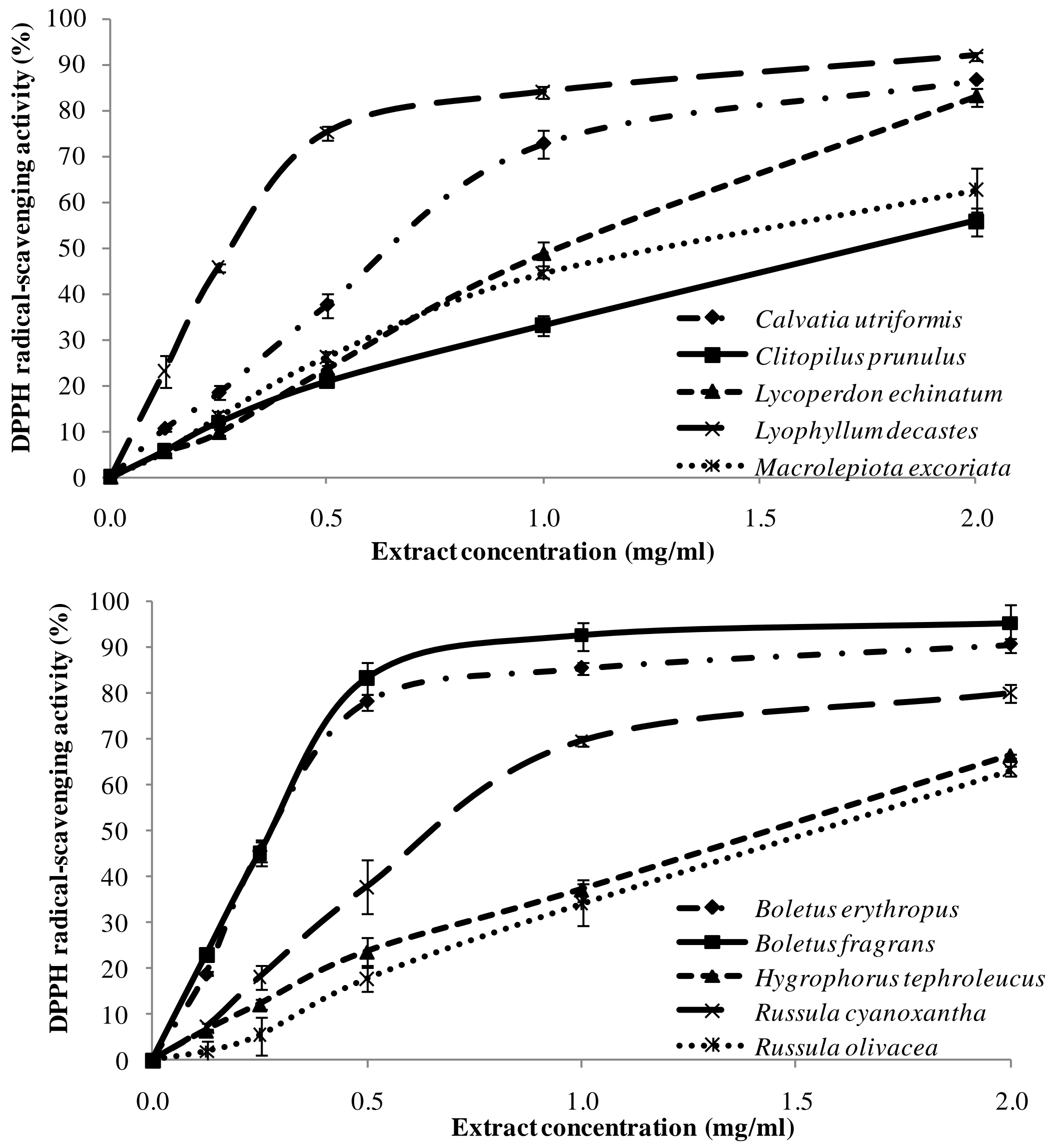


Figure 1. DPPH radical scavenging activity of the wild edible mushrooms.

EC_{50} values of saprotrophic species (mg/ml): *Calvatia utriformis* 0.68 ± 0.04; *Clitopilus prunulus* 1.75 ± 0.13; *Lycoperdon echinatum* 1.04 ± 0.08; *Lyophyllum decastes* 0.29 ± 0.01; *Macrolepiota excoriata* 1.32 ± 0.09.

EC_{50} values of mycorrhizal species (mg/ml): *Boletus erythropus* 0.28 ± 0.02; *Boletus fragrans* 0.28 ± 0.01; *Hygrophorus tephroleucus* 0.74 ± 0.02; *Russula cyanoxantha* 0.69 ± 0.03; *Russula olivacea* 1.47 ± 0.08.

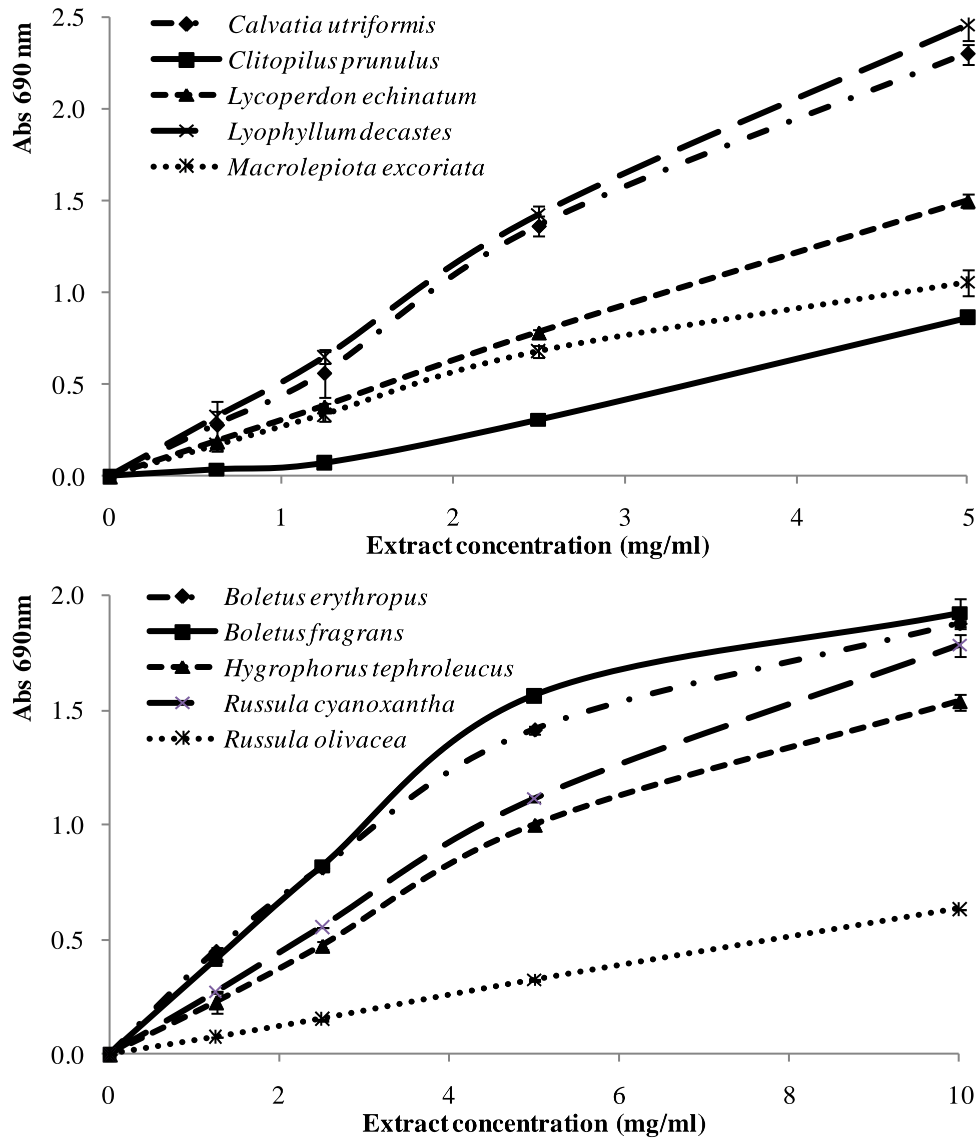
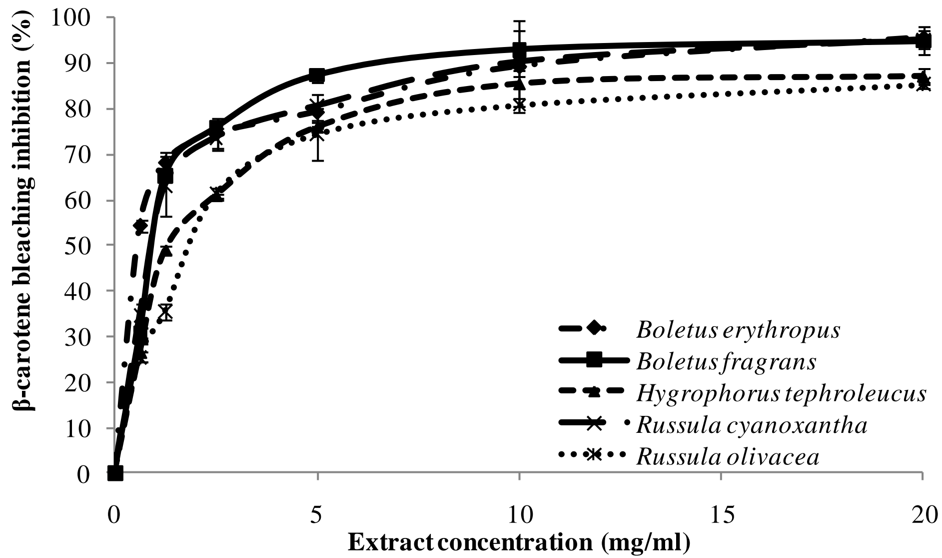


Figure 2. Reducing power of the wild edible mushrooms.

EC_{50} values of saprotrophic species (mg/ml): *Calvatia utriformis* 1.16 ± 0.27; *Clitopilus prunulus* 3.36 ± 0.03; *Lycoperdon echinatum* 1.61 ± 0.05; *Lyophyllum decastes* 0.97 ± 0.04; *Macrolepiota excoriata* 1.84 ± 0.03.

EC_{50} values of mycorrhizal species (mg/ml): *Boletus erythropus* 1.42 ± 0.05; *Boletus fragrans* 1.52 ± 0.02; *Hygrophorus tephroleucus* 2.61 ± 0.08; *Russula cyanoxantha* 2.26 ± 0.00; *Russula olivacea* 7.85 ± 0.01.



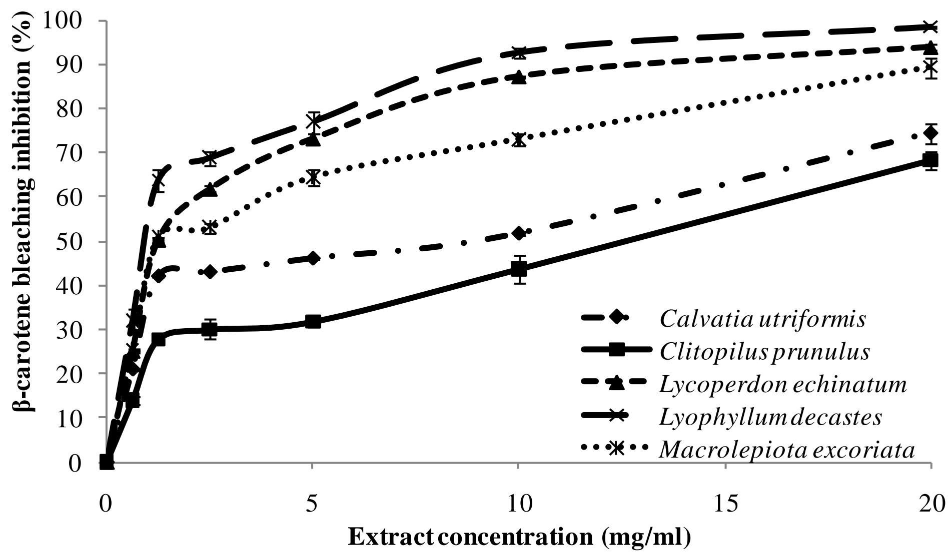


Figure 3. β-carotene bleaching inhibition of the wild edible mushrooms.

EC_{50} values of saprotrophic species (mg/ml): *Calvatia utriformis* 8.40 ± 0.20; *Clitopilus prunulus* 12.06 ± 0.30; *Lycoperdon echinatum* 1.24 ± 0.01; *Lyophyllum decastes* 0.98 ± 0.04; *Macrolepiota excoriata* 1.23 ± 0.03.

EC_{50} values of mycorrhizal species (mg/ml): *Boletus erythropus* 0.58 ± 0.02; *Boletus fragrans* 0.96 ± 0.02; *Hygrophorus tephroleucus* 1.28 ± 0.04; *Russula cyanoxantha* 0.98 ± 0.07; *Russula olivacea* 1.95 ± 0.04.

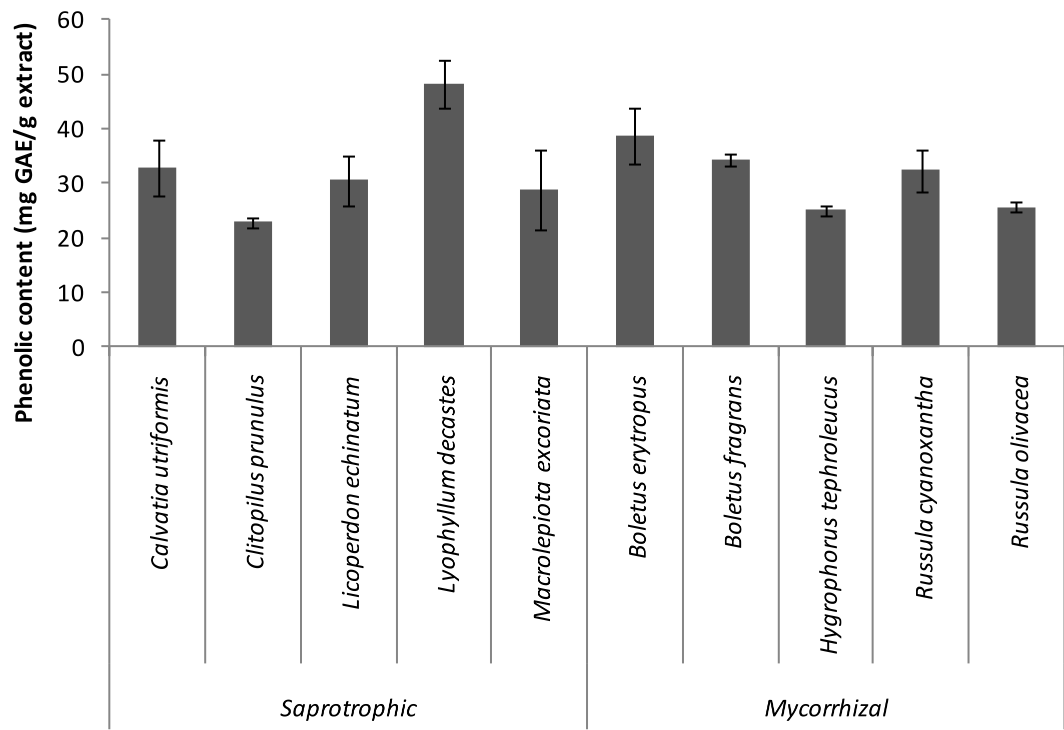


Figure 4. Phenolic content in the saprotrophic and mycorrhizal wild edible mushrooms.