JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Composition and Biological Properties of Portuguese Wild Mushrooms: A Comprehensive Study

LILLIAN BARROS, BRUNA A. VENTURINI, PAULA BAPTISTA, LETÍCIA M. ESTEVINHO, AND ISABEL C. F. R. FERREIRA*

CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

The chemical composition and biological properties of Portuguese wild mushrooms (*Cantharellus cibarius*, *Hypholoma fasciculare*, *Lepista nuda*, *Lycoperdon molle*, *Lycoperdon perlatum*, *Ramaria botrytis*, *Tricholoma acerbum*) were evaluated in order to assess these products as sources of nutrients and nutraceuticals. The analyzed mushrooms contain very useful phytochemicals such as phenolics, tocopherols, ascorbic acid, and carotenoids. All of the species proved to have antioxidant activity (measured by four different methods), being more significant for *R. botrytis* (EC_{50} values < 1 mg/mL). *Lycoperdon* species were resistant to all of the tested microorganisms, and the other samples revealed antimicrobial activity selectively against Gram-positive bacteria, with very low minimal inhibitory concentration, in some cases, even lower than the standard. The combination of bioactive compounds and rich nutritional composition (high contents in protein and carbohydrates, low content in fat with the precious contribution of unsaturated fatty acids and the absence of *trans* fatty acids) in the mushroom makes it a very special food.

KEYWORDS: Mushrooms; chemical compounds; antioxidant activity; antimicrobial activity

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*). There is convincing scientific evidence in support of the association between diet and chronic diseases such as cancer, cardiovascular disease, diabetes, and osteoporosis. Epidemiological studies have consistently shown an inverse association between the 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. Flavonoids are the most common and widely distributed group of plant phenolics and have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumor promotion (*4*). The health benefits of tocopherol as a bioactive compound are well documented. α-Tocopherol, the principal form of vitamin E, is a lipid-soluble antioxidant, and it functions as a chain-breaking antioxidant for lipid peroxidation (LP) in cell membranes and also as a scavenger of ROS such as singlet oxygen. It is considered to serve as the first line of defense against LP, and it protects polyunsaturated fatty acids (PUFAs) in cell membranes from free radical attack through its scavenging activity in biomembranes at early stages of LP (*5*). The antioxidant properties of carotenoids have been suggested as being mainly responsible for their beneficial effects (*6*). Particularly, β-carotene has been found to be inversely associated with cancer risk in epidemiologic studies and showed promising results in laboratory assays. Also, the role of lycopene in the prevention of chronic diseases has been evaluated in epidemiological studies as well as in tissue culture experiments using human cancer cell lines, animal studies, and also human clinical trials (*6*).

The biological properties of mushrooms, namely, antioxidant (*7–9*) and antimicrobial properties (*10–13*), have been described and attributed to their high content in antioxidants (*14–16*) and antibiotics, respectively. Besides their pharmacological characteristics, wild mushrooms are becoming more and more important in our diet for their nutritional value, including high protein and low fat/energy contents (*17–20*). The fatty acid

* Author to whom correspondence should be addressed (e-mail iferreira@ipb.pt; telephone +351-273-303219; fax +351-273-325405).

10.1021/jf8003114 CCC: $40.75
Published on Web 04/25/2008

composition may also have beneficial effects on blood lipid profiles. Substitution of saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) leads to increased high-density lipoprotein (HDL) cholesterol and decreased low-density lipoprotein (LDL) cholesterol, triacylglycerol, lipid oxidation, and LDL susceptibility to oxidation (*5*).

Northeastern Portugal, due to climatic conditions and flora diversity, is one European region with higher wild edible mushroom diversity, some of them with great gastronomic relevance. Despite the immense popularity of this food in the region and its increasing exportation to foreign countries (particularly Spain, France, and Italy), data regarding the nutritive value of the wild mushroom varieties available in the region are very meager. The high nutritional quality and unique flavors of these mushrooms are likely to be lost if not documented. Therefore, it is now imperative that a nutritional database of these mushrooms is set up to retain the information on these unique species and for a better management and conservation of these natural resources and the habitats related to them.

Herein, we present a study of the chemical composition and biological properties of Portuguese wild mushrooms (*Cantharellus cibarius*, *Hypholoma fasciculare*, *Lepista nuda*, *Lycoperdon molle*, *Lycoperdon perlatum*, *Ramaria botrytis*, *Tricholoma acerbum*) in order to assess these products as sources of nutrients and nutraceuticals. Chemical analysis included determination of proteins, fats, ash, and carbohydrates and individual profiles in sugars, fatty acids, and tocopherols by chromatographic techniques. Other compounds such as phenolics, flavonoids, carotenoids, and ascorbic acid were also determined. Biological characterization was focused on the evaluation of mushrooms' bioactive properties such as antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Samples. Samples of *Cantharellus cibarius* L:Fries, *Lepista nuda* (Bull ex Fr) Cook, *Lycoperdon molle* (Pers.:Fr) Ricken, *Lycoperdon perlatum* Pers.:Pers., *Hypholoma fasciculare* (Huds) Quél, *Tricholoma acerbum* (Bull.:Fr) Quél, and *Ramaria botrytis* (Pers.:Fr.) Ricken were collected under live pine trees (*Pinus* sp.) for the first two species and under oak trees (*Quercus pyrenaica* Willd.) for the last species, in Bragança (northeastern Portugal), in autumn 2006. Taxonomic identification was made according to several authors (*21, 22*), and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. All of the samples were lyophilized (Ly-8-FM-ULE, Snijders), reduced to a fine dried powder (20 mesh), and submitted to chemical composition and antioxidant activity analysis.

Standards and Reagents. Acetonitrile 99.9%, *n*-hexane 95%, and ethyl acetate 99.8% were of HPLC grade from Laboratory-Scan (Lisbon, Portugal). All other solvents were of analytical grade purity: methanol and diethyl ether were supplied by Laboratory-Scan, whereas toluene and sulfuric acid were supplied by Sigma Chemical Co. (St. Louis, MO). The fatty acid methyl ester (FAME) reference standard mixture 37 [fatty acids C4−C24; (standard 47885-U)] was purchased from Sigma, as were also other individual fatty acid isomers, tocopherol standards (α, β, γ, and δ), and the standards used in the antioxidant activity assays: 2-*tert*-butyl-4-methoxyphenol (BHA), *tert*-butylhydroquinone (TBHQ), L-ascorbic acid, α-tocopherol, gallic acid, and (+)-catechin. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). The standards used in the antimicrobial activity assays, ampicillin and cycloheximide, as also butylated hydroxytoluene (BHT), were purchased from Merck (Darmstadt, Germany). All other chemicals were obtained from Sigma Chemical Co. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems).

Chemical Composition. *Chemical parameters.* Samples of mushrooms were analyzed for chemical composition (moisture, protein, fat, carbohydrates, and ash) using the AOAC procedures (*23*). The crude protein content (N × 4.38) of the samples was estimated by the macroKjeldahl method; the crude fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C; reducing sugars were determined by dinitrosalicylic acid (DNS) method. Total carbohydrates were calculated by difference: total carbohydrates = 100 − (g of moisture + g of protein + g of fat + g of ash). Total energy was calculated according to the following equation: energy (kcal) = 4 × (g of protein + g of carbohydrate) + 9 × (g of lipid).

Fatty Acids. Fatty acids were determined by gas−liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the following transesterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol/sulfuric acid/toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 °C and 160 rpm; then 5 mL of deionized water was added, to obtain phase separation; the FAMEs were recovered with 5 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a microcolumn of sodium sulfate anhydrous to eliminate the water; the sample was recovered in a vial with Teflon, and before injection, the sample was filtered with a 0.2 μm nylon filter from Millipore. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID, and a Macherey-Nagel column (30 m × 0.32 mm i.d. × 0.25 μm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/min ramp to 240 °C, and held for 11 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. For each analysis 1 μL of the sample was injected in GC. 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 in relative percentage of each fatty acid.

Sugars. Free sugars were determined by high-performance liquid chromatography coupled to a refraction index detector (HPLC-RI) based on the method used by Harada et al. (*24*) with minor modifications. Dried powder (1.0 g) was extracted with 40 mL of 80% aqueous ethanol at 80 °C for 30 min. The resulting suspension was centrifuged at 15000*g* for 10 min. The supernatant was concentrated at 60 °C under reduced pressure and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 mL. Soluble sugars were determined by using HPLC (Knauer, Smartline system) at 35 °C. The HPLC system was equipped with a Knauer Smartline 2300 RI detector and with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer). The mobile phase was acetonitrile/deionized water, 7:3 (v/v), at a flow rate of 1.25 mL/min. The results are expressed in grams per 100 g of fresh weight, calculated by internal normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards. The sugar standards used for identification were purchased from Sigma Chemical Co.: L(+)-arabinose, D(−)-fructose, D(+)-galactose, D(+)-glucose anhydrous, lactose 1-hydrate, maltose 1-hydrate, D(+)-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose monohydrate, L(+)-rhamnose monohydrate, D(+)-sucrose, D(+)-trehalose, and D(+)-xylose.

Tocopherols. BHT solution in hexane (10 mg/mL; 100 μL) and IS solution in hexane (δ-tocopherol; 1.6 μg/mL; 250 μL) were added to the sample prior to the extraction procedure. The samples (∼500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min) and centrifuged (5 min, 4000*g*), and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 1 mL of *n*-hexane, dehydrated with anhydrous sodium sulfate, filtered through a 0.22 μm disposable LC filter disk, transferred into a dark injection vial, and analyzed by HPLC. The HPLC equipment consisted of an integrated

Table 1. Moisture, Proximate Chemical Composition (Grams per 100 g of Fresh Weight), and Energy Value (Kilocalories per 100 g of Fresh Weight) of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

sample	moisture	total fat	crude protein	ash	carbohydrates	reducing sugars	energy
Cantharellus cibarius	92.38 ± 0.31 ba	0.22 ± 0.04 a	4.09 ± 0.09 a	0.88 ± 0.05 c	2.44 ± 0.33 bc	0.26 ± 0.00 c	28.18 ± 1.39 ba
Lepista nuda	93.77 ± 0.98 a	0.11 ± 0.01 b	3.70 ± 0.61 b	1.15 ± 0.19 c	1.55 ± 0.98 c	0.10 ± 0.00 d	20.85 ± 4.68 b
Lycoperdon perlatum	88.65 ± 2.08 c	0.05 ± 0.01 d	1.94 ± 0.18 c	3.62 ± 0.37 a	5.74 ± 1.87 ba	0.44 ± 0.01 a	31.18 ± 6.90 ba
Lycoperdon molle	89.09 ± 1.27 bc	0.08 ± 0.02 cd	1.83 ± 0.04 c	2.20 ± 0.25 b	6.80 ± 1.57 a	0.42 ± 0.03 b	35.25 ± 6.01 a
Ramaria botrytis	89.77 ± 1.15 bc	0.14 ± 0.01 b	4.08 ± 0.18 a	0.90 ± 0.02 c	5.12 ± 1.02 ba	0.45 ± 0.00 a	38.03 ± 4.69 a

[a] In each column different letters indicate significant differences ($p < 0.05$).

Table 2. Fatty Acid Composition (Percent) of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	Cantharellus cibarius	*Lepista nuda*	*Lycoperdon molle*	*Lycoperdon perlatum*	*Ramaria botrytis*
C6:0	0.06 ± 0.01	0.09 ± 0.00	1.05 ± 0.06	0.56 ± 0.05	0.17 ± 0.05
C8:0	0.10 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.02 ± 0.00
C10:0	0.05 ± 0.00	0.10 ± 0.01	0.30 ± 0.02	0.15 ± 0.03	0.05 ± 0.00
C12:0	0.02 ± 0.00	0.11 ± 0.02	0.14 ± 0.01	0.15 ± 0.04	0.02 ± 0.00
C14:0	0.09 ± 0.00	0.33 ± 0.01	0.46 ± 0.01	0.40 ± 0.01	0.11 ± 0.01
C15:0	0.14 ± 0.00	0.79 ± 0.00	2.53 ± 0.06	1.86 ± 0.26	1.00 ± 0.01
C16:0	7.19 ± 0.14	11.77 ± 0.07	13.74 ± 0.17	12.90 ± 0.06	9.91 ± 0.03
C16:1	0.20 ± 0.00	0.51 ± 0.11	0.24 ± 0.00	0.32 ± 0.08	0.22 ± 0.03
C17:0	0.09 ± 0.00	nd	0.90 ± 0.01	0.48 ± 0.08	0.70 ± 0.00
C18:0	3.34 ± 0.01	2.39 ± 0.00	2.35 ± 0.03	2.95 ± 0.01	2.36 ± 0.05
C18:1n9c	8.13 ± 0.01	29.53 ± 0.04	8.58 ± 0.07	4.59 ± 0.05	43.93 ± 0.14
C18:2n6c	50.01 ± 0.09	51.48 ± 0.11	64.15 ± 0.42	70.69 ± 0.87	38.32 ± 0.07
C18:3n3	0.10 ± 0.00	0.21 ± 0.00	0.06 ± 0.00	0.22 ± 0.05	0.02 ± 0.00
C20:0	0.18 ± 0.00	0.27 ± 0.01	0.36 ± 0.02	0.63 ± 0.02	0.13 ± 0.01
C20:1c	27.98 ± 0.20	0.05 ± 0.00	nd	nd	0.44 ± 0.04
C20:2c	0.13 ± 0.01	0.09 ± 0.00	0.35 ± 0.01	nd	0.28 ± 0.00
C20:3n6	nd	0.10 ± 0.01	nd	nd	0.08 ± 0.00
C20:3n3 + C21:0	0.12 ± 0.01	0.07 ± 0.00	0.53 ± 0.09	0.27 ± 0.06	0.04 ± 0.00
C20:5n3	0.09 ± 0.00	0.15 ± 0.00	0.82 ± 0.02	0.33 ± 0.02	0.16 ± 0.01
C22:0	0.23 ± 0.01	0.55 ± 0.02	1.22 ± 0.02	1.32 ± 0.27	0.86 ± 0.04
C23:0	0.06 ± 0.00	0.29 ± 0.01	0.50 ± 0.03	0.48 ± 0.03	0.17 ± 0.01
C24:0	0.51 ± 0.02	0.78 ± 0.08	1.43 ± 0.01	1.64 ± 0.06	0.88 ± 0.06
C24:1	1.23 ± 0.04	0.21 ± 0.02	0.23 ± 0.05	nd	0.10 ± 0.01
total SFA	12.04 ± 0.52 e	17.58 ± 0.08 c	25.04 ± 0.27 a	23.57 ± 0.52 b	16.38 ± 0.06 d
total MUFA	37.54 ± 0.14 b	30.32 ± 0.02 c	9.04 ± 0.01 d	4.91 ± 0.14 e	44.69 ± 0.07 a
total PUFA	50.42 ± 0.65 d	52.10 ± 0.10 c	65.92 ± 0.25 b	71.52 ± 0.65 a	38.91 ± 0.02 e

[a] Different letters indicate significant differences ($p < 0.05$). nd, not detected.

system with a Smartline pump 1000 (Knauer), a degasser system Smartline manager 5000, an AS-2057 autosampler, and a 2500 UV detector at 295 nm (Knauer) connected in series with an FP-2020 fluorescence detector (Jasco) programmed for excitation at 290 nm and emission at 330 nm. Data were analyzed using Clarity 2.4 software (DataApex). The chromatographic separation was achieved with a Polyamide II (250 × 4.6 mm) normal-phase column from YMC Waters operating at 30 °C (7971 R Grace oven). 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, and the injection volume was 20 μL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in mushroom samples are expressed in nanograms per gram of fresh mushroom.

Other Bioactive Compounds. Phenols, flavonoids, ascorbic acid, and carotenoids were determined according to procedures previously described by us (*12*). For phenolic compounds determination, gallic acid was used to calculate the standard curve (0.01−0.4 mM; $Y = 2.8557X - 0.0021$; $R^2 = 0.9999$), and the results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract. For flavonoid contents determination, (+)-catechin was used to calculate the standard curve (0.022−0.34 mM; $Y = 0.9629X - 0.0002$; $R^2 = 0.9999$), and the results were expressed as milligrams of (+)-catechin equivalents (CEs) per gram of extract. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020−0.12 mg/mL; $Y = 3.4127X - 0.0072$; $R^2 = 0.9905$), and the results were expressed as milligrams of ascorbic acid per gram of extract. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) $= -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$; β-carotene (mg/100 mL) $= 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$. The results were expressed as micrograms of carotenoid per gram of extract.

Biological properties. *Sample Preparation for Antioxidant and Antimicrobial Activities Assays.* The samples (∼3 g) were extracted by stirring with 100 mL of methanol at 25 °C at 150 rpm for 24 h and filtered through Whatman no. 4 paper. The residue was then extracted with two additional 100 mL portions of methanol, as described earlier. The combined methanolic extracts were evaporated at 40 °C to dryness and redissolved in methanol for antioxidant activity assays or in DMSO for antimicrobial activity assays, at a concentration of 50 mg/mL, and stored at 4 °C for further use.

Antioxidant Activity. (a) DPPH Radical-Scavenging Activity (RSA). Various concentrations of mushroom extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The RSA was calculated as a percentage of DPPH discoloration using the equation % 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. The extract concentration providing 50% of RSA (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHA and α-tocopherol were used as standards.

(b) Reducing Power. Various concentrations of mushroom methanolic extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10%

Table 3. Sugar Composition (Grams per 100 g of Fresh Weight) of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	mannitol	trehalose	maltose	melezitose	total sugars
Cantharellus cibarius	1.06 ± 0.02 b	0.85 ± 0.01a	nd	nd	1.91 ± 0.03 a
Lepista nuda	0.05 ± 0.00 c	0.75 ± 0.03 b	0.10 ± 0.00 a	nd	0.85 ± 0.03 c
Lycoperdon perlatum	0.02 ± 0.00 d	0.29 ± 0.01 c	nd	nd	0.31 ± 0.01 d
Lycoperdon molle	nd	0.17 ± 0.05 d	nd	nd	0.17 ± 0.05 e
Ramaria botrytis	1.20 ± 0.01 a	0.20 ± 0.01 d	nd	0.02 ± 0.00 a	1.42 ± 0.00 b

[a] In each column different letters indicate significant differences ($p < 0.05$). nd, not detected.

Table 4. Tocopherol Composition (Nanograms per Gram of Fresh Weight) of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	α-tocopherol	β-tocopherol	γ-tocopherol	total
Cantharellus cibarius	13.40 ± 0.76 dc	2.87 ± 0.06 e	nd	16.27 ± 0.76 e
Hypholoma fasciculare	16.06 ± 0.08 dc	40.46 ± 0.40 b	25.69 ± 0.45 a	82.20 ± 0.63 b
Lepista nuda	7.95 ± 0.49 d	12.13 ± 0.37 c	14.64 ± 0.18 b	34.72 ± 0.43 c
Lycoperdon molle	27.13 ± 0.42 a	nd	nd	27.13 ± 0.42 dc
Lycoperdon perlatum	25.23 ± 0.76 ba	nd	nd	25.23 ± 0.76 de
Ramaria botrytis	20.54 ± 0.88 bc	229.81 ± 0.99 a	nd	250.35 ± 1.35 a
Tricholoma acerbum	13.73 ± 0.29 dc	6.53 ± 0.49 d	2.79 ± 0.17 c	23.05 ± 0.92 de

[a] In each column different letters indicate significant differences ($p < 0.05$). nd, not detected.

trichloroacetic acid (TCA) (w/v) had been added, the mixture was centrifuged at 1000 rpm for 8 min (Centorion K24OR-2003 refrigerated centrifuge). The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. BHA and α-tocopherol were used as standards.

(c) Inhibition of β-Carotene Bleaching. The antioxidant activity of mushroom extracts was evaluated by the β-carotene linoleate model system. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of this solution was pipetted into a 100 mL round-bottom flask. After the chloroform had been removed at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of the mycelium extracts. The tubes were shaken and incubated at 50 °C 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. Absorbance readings were then recorded at 20 min intervals until the control sample had changed color. A blank, devoid of β-carotene, was prepared for background subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equation: LPO inhibition = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. TBHQ was used as standard.

(d) Inhibition of Lipid Peroxidation Using Thiobarbituric Acid Reactive Substances (TBARS). Brains were obtained from pig (*Sus scrofa*) of body weight ~150 kg, dissected, and homogenized with a Polytron in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate, which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the mushroom extracts (0.2 mL) in the presence of $FeSO_4$ (10 μm; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of TCA (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)−TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (percent) was calculated using the following formula: inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHA was used as standard.

Antimicrobial Activity. (a) Microorganisms and Culture Conditions. Microorganisms labeled CECT were obtained from the Spanish type culture collection (CECT) of Valencia University, whereas microorganisms labeled ESA were clinically isolated strains from different biological fluids, identified by the Microbiology Laboratory of Escola Superior Agrária de Bragança. Gram-positive (*Bacillus cereus* CECT 148, *Bacillus subtilis* CECT 498, *Staphylococcus aureus* ESA 7 isolated from pus) and Gram-negative (*Escherichia coli* CECT 101, *Pseudomonas aeruginosa* CECT 108, *Klebsiella pneumoniae* ESA 8 isolated from urine) bacteria and fungi (*Candida albicans* CECT 1394, *Cryptococcus neoformans* ESA 3 isolated from vaginal fluid) were used to screen sample antimicrobial activity. Microorganisms were cultured aerobically at 37 °C (Scientific 222 oven model, 2003) in nutrient agar medium for bacteria and at 30 °C (Scientific 222 oven model, 2003) in sabouraud dextrose agar medium for fungi.

(b) Test Assays for Antimicrobial Activity. A screening of antibacterial activities against the Gram-negative and Gram-positive bacteria and fungi was performed, and the minimal inhibitory concentration (MIC) was determined by an adaptation of the agar streak dilution method based on radial diffusion (*12*). Suspensions of the microorganisms were prepared to contain approximately 10^8 cfu/mL, and the plates containing agar medium were inoculated (100 μL). A 50 μL volume of each sample was placed in a hole (depth = 3 mm, diameter = 4 mm) made in the center of the agar. Under the same conditions, different DMSO solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. DMSO was chosen as the best solvent after comparative toxicity assays, which proved its nontoxicity. The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria or fungi, after 24 h. The inhibition halos corresponding to the MICs were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition halo was measured three times (three different plates), and the average was considered. A control using only inoculation was also carried out.

Statistical Analysis. For each of the mushroom species three samples were analyzed, and also all of 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 the SAS v. 9.1.3 program.

RESULTS AND DISCUSSION

Chemical Composition. The results of the chemical composition and estimated energetic value (expressed on fresh weight basis) obtained for the edible mushroom species are shown in **Table 1**. The moisture ranged from 88.65 g/100 g in *L. perlatum* to 93.77 g/100 g in *L. nuda*. Protein was found in high levels and varied between 1.83 g/100 g in *L. molle* and

Table 5. Total Bioactive Compounds of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	phenols (mg/g)	flavonoids (mg/g)	ascorbic acid (mg/g)	β-carotene (μg/g)	lycopene (μg/g)
Cantharellus cibarius	1.75 ± 0.50 e	0.47 ± 0.05 f	0.40 ± 0.02 a	5.77 ± 0.41 e	1.95 ± 0.28 d
Hypholoma fasciculare	17.67 ± 0.27 b	5.09 ± 0.48 b	0.09 ± 0.00 e	24.62 ± 0.76 b	11.90 ± 0.51 b
Lepista nuda	6.31 ± 0.13 d	3.36 ± 0.50 c	0.23 ± 0.03dc	2.52 ± 0.25 g	0.98 ± 0.13 e
Lycoperdon molle	11.48 ± 0.52 c	2.45 ± 0.08 d	0.34 ± 0.08 b	4.48 ± 0.23 f	2.19 ± 0.15 d
Lycoperdon perlatum	10.57 ± 0.17 c	2.10 ± 0.14 ed	0.21 ± 0.02 d	12.50 ± 0.53 c	6.39 ± 0.34 c
Ramaria botrytis	20.32 ± 1.87 a	16.56 ± 0.30 a	0.27 ± 0.04 c	10.41 ± 0.48 d	1.51 ± 0.22 ed
Tricholoma acerbum	5.53 ± 0.63 d	1.87 ± 0.71 e	0.22 ± 0.04 dc	75.48 ± 2.01 a	39.65 ± 1.33 a

[a] In each column different letters indicate significant differences ($p < 0.05$).

Table 6. EC_{50} Values (Milligrams per Milliliter) Obtained for the Antioxidant Activity of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	DPPH scavenging activity	reducing power	β-carotene bleaching inhibition	lipid peroxidation inhibition
Cantharellus cibarius	19.65 ± 0.28 a.	8.72 ± 0.03 a.	8.40 ± 0.87 a	8.59 ± 0.73 a
Hypholoma fasciculare	1.13 ± 0.03 f	0.95 ± 0.01 f	0.86 ± 0.02 f	1.55 ± 0.53 e
Lepista nuda	4.41 ± 0.01 b	3.53 ± 0.09 b	4.21 ± 0.09 c	5.80 ± 0.07 b
Lycoperdon molle	3.23 ± 0.09 e	2.27 ± 0.00 e	1.92 ± 0.05 e	3.31 ± 0.23 d
Lycoperdon perlatum	3.95 ± 0.04 c	2.96 ± 0.01 d	2.49 ± 0.06 d	4.64 ± 1.40 c
Ramaria botrytis	0.66 ± 0.00 g	0.68 ± 0.00 g	0.67 ± 0.01 f	1.01 ± 0.02 e
Tricholoma acerbum	3.60 ± 0.08 d	3.27 ± 0.02 c	5.89 ± 0.28 b	6.20 ± 0.67 b

[a] In each column different letters mean significant differences ($p < 0.05$).

Table 7. Antimicrobial Activity of the Wild Mushrooms (Mean ± SD; $n = 3$)[a]

	MIC (μg/mL)							
sample	*B. cereus*	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. peumoniae*	*C. albicans*	*C. neoformans*
Cantharellus cibarius	5 (++++)	5 (++++)	5 (++++)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Hypholoma fasciculare	500 (+++)	5 (++++)	500 (++++)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Lepista nuda	5 (++++)	5 (++++)	5 (++++)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Lycoperdon molle	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Lycoperdon perlatum	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Ramaria botrytis	50 (++++)	50 (++++)	5 (++++)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
Tricholoma acerbum	500 (−)	500 (++++)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)	500 (−)
ampicillin	3.13 (++++)	12.5 (++++)	6.25 (++++)	6.25 (++++)	6.25 (++++)	6.25 (++++)	NT	NT
cycloheximide	NT	NT	NT	NT	NT	NT	12.5 (++)	6.25 (++++)

[a] No antimicrobial activity at the higher tested concentration (−), inhibition zone < 1 mm; slight antimicrobial activity (+), inhibition zone 2−3 mm; moderate antimicrobial activity (++), inhibition zone 4−5 mm; high antimicrobial activity (+++), inhibition zone 6−9 mm; strong antimicrobial activity (++++), inhibition zone > 9 mm. Standard deviation ± 0.5 mm. NT, not tested.

4.09 g/100 g in *C. cibarius*. Fat ranged from 0.05 g/100 g in *L. perlatum* to 0.22 g/100 g in *C. cibarius*. The wild mushrooms were rich sources of protein and had low amounts of fat, making them an ideal snack material, which is in agreement with other studies of different mushroom species (*17–20*). Carbohydrates, calculated by difference, were also an abundant macronutrient and ranged from 1.55 g/100 g in *L. nuda* and 6.80 g/100 g in *L. molle*. Reducing sugars are only a small part of carbohydrates content because polysaccharides such as chitin and starch are the most abundant mushroom carbohydrates (*25*). Ash content varied between 0.88 g/100 g in *C. cibarius* and 3.62 g/100 g in *L. perlatum*. Being a good source of protein and carbohydrate, mushrooms fall between most legumes and meat (*26*) and prove to be excellent foods that can be used in low-calorie diets for their low contents of fat and energy.

Among all of the studied species, the chemical composition of only *C. cibarius* had already been described (*19*), but from a different country. Despite some similarities in the composition of Portuguese and Indian *C. cibarius* samples, it is known that the chemical compositions of mushrooms are affected by a number of factors, namely, mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions, and preparation of the substrates (*25*).

On the basis of the proximate analysis, it can be calculated that an edible portion of 100 g of these mushrooms assures, on average, 31 kcal. The highest values are guaranteed by *R. botrytis*, whereas *L. nuda* gives the lowest energy contribution (**Table 1**).

The results for fatty acid composition, total SFA, MUFA, and PUFA of the studied mushrooms are shown in **Table 2**. In general, the major fatty acid found in the studied samples was linoleic acid (C18:2), followed by oleic acid (C18:1) and palmitic acid (C16:0). In fact, it is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushrooms flavor (*27*). Besides the 3 main fatty acids already described, 20 more were identified and quantified. PUFA were the main group of fatty acids in all species with the exception for *R. botrytis*, in which MUFA were the main group. Nevertheless, UFAs predominate over SFA for all of the studied mushroom species, ranging from 75 to 88%. This is consistent with the observations that, in mushrooms, unsaturated fatty acids predominate over the saturated in the total fatty acid content (*17, 18*). Considering total MUFA content, *L. perlatum* had the lowest value but contained the highest PUFA content, due to the higher contribution of linoleic acid. *L. molle* had the highest SFA value, due to the presence of higher amounts of palmitic acid. Trans isomers of unsaturated fatty acids were not detected in the studied mushrooms; some of these compounds have been related to increased risk of

cardiovascular disease, being negatively correlated with plasma HDL-cholesterol concentration and positively correlated with plasma LDL-cholesterol level (*5*).

The sugar compositions (**Table 3**) of the edible mushrooms presented mannitol and trehalose as the main sugars. For *C. cibarius* (1.06 g/100 g) and *R. botrytis* (1.20 g/100 g) mannitol was the most abundant sugar, whereas trehalose predominates in *L. nuda*, *L. perlatum*, and *L. molle*, ranging from 0.17 to 0.75 g/100 g. The accumulation of these sugars in the fruit bodies of other species was already reported (*24, 28*). Nevertheless, the present study describes for the first time the presence of maltose (disaccharide) and melezitose (nonreducing trisaccharide).

Overall, *R. botrytis* revealed the highest energy value, but with the highest protein content. It also presented the highest MUFA and the lowest SFA levels, which may be relevant because the substitution of SFA with MUFA leads to an increase in HDL cholesterol and decreases in LDL cholesterol, triacylglycerol, lipid oxidation, and LDL susceptibility to oxidation (*5*). The sugar composition presents the highest percentage of mannitol, which functions to provide support and expansion of the fruit body as other sugar alcohols. *R. botrytis* is one of the largest of the coral fungi and is considered to be an excellent edible by some mycophagists.

The tocopherol content was determined in seven Portuguese mushroom species (**Table 4**), including five edible samples (nutritional composition above-described) and two nonedible species (*Hypholoma fasciculare* and *Tricholoma acerbum*). The results obtained in the analysis of mushroom samples point to the existence of differences in tocopherol composition among different species. α-Tocopherol was found in all of the species, being the major compound for *C. cibarius*, *L. molle*, *L. perlatum*, and *T. acerbum*; β-tocopherol was the major compound for *R. botrytis* and *H. fasciculare*, whereas γ-tocopherol was the major compound in *L. nuda*. *R. botrytis* presented the higher content of tocopherols (250.35 ng/g of fresh weight), whereas *C. cibarius* revealed the lowest content (16.27 ng/g). Some authors published tocopherol determination in other mushrooms, but using a different methodology (*14, 15*). In the present work we did not perform a saponification step in the sample preparation, because saponification is more time-consuming and laborious, eventually leading to tocopherols degradation due to their high sensivity to light, heat, and oxygen. We also introduced an antioxidant protector to minimize tocopherol loss. α-Tocopherol was already determined by Elmaster et al. (*14*) in *L. nuda* from Turkey, but the results were expressed per milligram of extracts.

Table 5 presents phenol, flavonoid, ascorbic acid, and carotenoid concentrations obtained in the mushroom extracts. Phenols were the major antioxidant components found in the extracts (1.75−20.32 mg/g), followed by flavonoids (0.47−16.56 mg/g). Ascorbic acid was found in small amounts (0.09−0.40 mg/g), and β-carotene and lycopene were found in only vestigial amounts (<0.08 mg/g). *R. botrytis* revealed a higher content in phenol and flavonoid compounds, which significantly ($p < 0.05$) decreased in the other species.

Biological Properties. The bioactive properties (antioxidant and antimicrobial) were evaluated using the whole extract, which is a complex mixture of phytochemicals with additive and synergistic effects. To screen the antioxidant properties, several chemical and biochemical assays using animal cells were performed: reducing power (measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form), scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers), inhibition of β-carotene bleaching (by neutralizing the linoleate free radical and other free radicals formed in the system that attack the highly unsaturated β-carotene models), and inhibition of lipid peroxidation in brain tissue (measured by the color intensity of MDA−TBA complex).

All of the species proved to have antioxidant activity (**Table 6**), being more significant for *R. botrytis* (lower EC_{50} values). *C. cibarius* presented the lowest antioxidant properties (higher EC_{50} values), which are compatible with its lower phenol and tocopherol contents. These properties seem to be related to total phenolic and tocopherol contents. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (*28*). Tocopherol functions as a chain-breaking antioxidant for lipid peroxidation in cell membranes and also as a scavenger of ROS such as singlet oxygen (*5*).

Table 7 shows the antimicrobial screening of mushroom extracts against *B. cereus*, *B. subtilis*, *S. aureus* (Gram-positive), *E. coli*, *P. aeruginosa*, *K. peumoniae* (Gram-negative) bacteria, and *C. albicans* and *C. neoformans* (fungi). *Lycoperdon* species were resistant to all of the tested microorganisms, and the other samples revealed antimicrobial activity selectively against Gram -positive bacteria, with very low MICs. Particularly, *C. cibarius* and *L. nuda* presented MIC values against *B. subtilis* and *S. aureus*, even lower than that of the standard ampicillin. Despite the existence of some studies demonstrating that phenolic compounds possess antimicrobial activity (*29*), herein a relationship between phenol content and antimicrobial properties seems not to exist. In fact, there are other compounds, such as steroids, oxalic acid, sesquiterpenoids, and epipolythiopiperazine-2,5-diones isolated from mushrooms, that proved to have antimicrobial activity (*30*).

The edibility seems not to be related to bioactive properties because one of the nonedible mushrooms (*H. fasciculare*) revealed high antioxidant and antimicrobial activities and high phenol and tocopherol contents, whereas the other one (*T. acerbum*) presented lower bioactive properties.

In conclusion, the analyzed mushrooms contain very useful phytochemicals such as phenolics, tocopherols, ascorbic acid, and carotenoids and revealed interesting antioxidant and antimicrobial properties. The combination of their bioactive compounds and rich nutritional composition (high contents in protein and carbohydrates, low contents in fat with the precious contribution of unsaturated fatty acids and the absence of *trans* fatty acids) makes them very special. This study contributes not only to a better knowledge of the product but also to its valorization.

LITERATURE CITED

(1) Halliwell, B. Antioxidants in human health and disease. *Annu. Rev. Nutr.* **1996**, *16*, 33–50.

(2) Bazzano, L. A.; He, J.; Ogden, L. G. Legume consumption and risk of coronary heart disease in US men and women: NHANES I epidemiologic follow-up study. *Arch. Intern. Med.* **2001**, *161*, 2573–2578.

(3) Soobrattee, M. A.; Neergheen, V. S.; Luximon-Ramma, A.; Aruoma, O. I.; Bahorun, T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat. Res.* **2005**, *579*, 200–213.

(4) Marchand, L. L. Cancer preventive effects of flavonoids—a review. *Biomed. Pharmacother.* **2002**, *56*, 296–301.

(5) Kanu, P. J.; Zhu, K.; Kanu, J. B.; Zhou, H.; Qian, H.; Zhu, K. Biologically active components and nutraceuticals in sesame and related products: a review and prospect. *Trends Food Sci. Technol.* **2007**, *18*, 599–608.

(6) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216.
(7) Barros, L.; Ferreira, M.-J.; Queirós, B.; Ferreira, I. C. F. R.; Baptista, P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem.* **2007**, *103*, 413–419.
(8) Lo, K. M.; Cheung, P. C. K. Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. *Food Chem.* **2005**, *89*, 533–539.
(9) Mau, J.-L.; Lin, H.-C.; Chen, C.-C. Antioxidant Properties of several medicinal mushrooms. *J. Agric. Food Chem.* **2002**, *50*, 6072–6077.
(10) Turkoglu, A.; Duru, M. E.; Mercan, N.; Kivrak, I.; Gezer, K. Antioxidant and antimicrobial activities of *Laetiporus sulphurous* (Bull.) Murrill. *Food Chem.* **2007**, *101*, 267–273.
(11) Hirasawa, M.; Shouji, N.; Neta, T.; Fukushima, K.; Takada, K. Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (shiitake, an edible mushroom). *Int. J. Antimicrob. Agents* **1999**, *11*, 151–157.
(12) Barros, L.; Calhelha, R. C.; Vaz, J. A.; Ferreira, I. C. F. R.; Baptista, P.; Estevinho, L. M. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms. *Eur. Food Res. Technol.* **2007**, *225*, 151–156.
(13) Hatvani, N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. *Int. J. Antimicrob. Agents* **2001**, *17*, 71–74.
(14) Elmastas, M.; Isildak, O.; Turkekul, I.; Temur, N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J. Food Compos. Anal.* **2007**, *20*, 337–345.
(15) Tsai, S.-Y.; Tsai, H.-L.; Mau, J.-L. Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea*, and *Boletus edulis*. *LWT* **2007**, *40*, 1392–1402.
(16) Valentão, P.; Andrade, P. B.; Rangel, J.; Ribeiro, B.; Silva, B. M.; Baptista, P.; Seabra, R. M. Effect of the conservation procedure on the contents of phenolic compounds and organic acids in chanterelle (*Cantharellus cibarius*) mushroom. *J. Agric. Food Chem.* **2005**, *53*, 4925–4931.
(17) Longvah, T.; Deosthale, Y. G. Compositional and nutritional studies on edible wild mushroom from northeast India. *Food Chem.* **1998**, *63*, 331–334.
(18) Diéz, V. A.; Alvarez, A. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chem.* **2001**, *75*, 417–422.
(19) Agahar-Murugkar, D.; Subbulakshmi, G. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chem.* **2005**, *89*, 599–603.
(20) Barros, L.; Baptista, P.; Correia, D. M.; Casal, S.; Oliveira, B.; Ferreira, I. C. F. R. Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from northeast Portugal. *Food Chem.* **2007**, *105*, 140–145.
(21) Moser, M. In *Keys to Agarics and Boleti (Polyporales, Boletales, Agaricales, Russulales)*; Roger Phillips: London, U.K., 1983.
(22) Courtecuisse, R.; Duhem, B. In *Mushrooms and Toadstools of Britain and Europe*; HarperCollins Publishers: London, U.K., 1995.
(23) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Arlington VA, 1995.
(24) Harada, A.; Gisusi, S.; Yoneyama, S.; Aoyama, M. Effects of strain and cultivation medium on the chemical composition of the taste components in fruit-body of *Hypsizygus marmoreus*. *Food Chem.* **2004**, *84*, 265–270.
(25) Manzi, P.; Aguzzi, A.; Pizzoferrato, L. Nutritional value of mushrooms widely consumed in Italy. *Food Chem.* **2001**, *73*, 321–325.
(26) FAO/WHO Protein quality evaluation. Report of the joint FAO/WHO expert consultation. *Food and Nutrition Paper 51*; Food and Agriculture Organizations and the World Health Organization: Rome, Italy, 1989.
(27) Maga, J. A. Mushroom flavor. *J. Agric. Food Chem.* **1981**, *29*, 1–4.
(28) Decker, E. A. Phenolics: prooxidants or antioxidants. *Nutr. Rev.* **1997**, *55*, 396–407.
(29) Puupponen-Pimiä, R.; Nohynek, L.; Meier, C.; Kähkönen, M.; Heinonen, M.; Hopia, A.; Oksman-Caldentey, K.-M. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Mic.* **2001**, *90*, 494–507.
(30) Lindequist, U.; Niedermeyer, T. H. J.; Jülich, W.-D. The pharmacological potential of mushrooms. *eCam* **2005**, *2*, 285–299.

Received for review January 30, 2008. Revised manuscript received March 6, 2008. Accepted March 9, 2008. We are grateful to the Foundation for Science and Technology (PPCDT/AGR/56661/2004) for financial support of this work.

JF8003114