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Effects of Conservation Treatment and Cooking on the Chemical Composition and Antioxidant Activity of Portuguese Wild Edible Mushrooms

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The effects of processing and cooking practices on the chemical composition and antioxidant activity of Portuguese wild edible mushroom species (*Lactarius deliciosus*, *Macrolepiota mastoidea*, *Macrolepiota procera*, and *Sarcodon imbricatus*) were investigated. Dried, frozen, and cooked samples were analyzed for proximate constituents (moisture, fat, crude protein, ash, and carbohydrates) and nutritional value. Fatty acid and sugar profiles were also obtained by gas−liquid chromatography/flame ionization detection and high-performance liquid chromatography/refraction index, respectively. The antioxidant properties were evaluated by several biochemical assays: 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, reducing power, inhibition of β-carotene bleaching, and inhibition of lipid peroxidation in brain tissue using thiobarbituric acid reactive substances. Results of this study show that mushroom species and processing and cooking practices are all effective determinants for either chemical composition or antioxidant properties. Cooked samples proved to have lower nutrient concentrations and lower antioxidant activities than either dried or frozen samples. In what concerns fatty acids and sugar individual profiles, only cooking proved to be relevant: The cooked samples presented higher monounsaturated fatty acid and lower polyunsaturated fatty acid and sugars contents.

KEYWORDS: Mushrooms; conservation treatment; cooking; chemical composition; antioxidant activity

INTRODUCTION

Wild mushrooms are becoming more and more important in our diet for their nutritional (*1*−*3*) and pharmacological (*4*−*6*) characteristics. The high protein and low fat/energy contents of wild edible mushrooms, reported by many workers (*7*−*12*) including our research group (*13*), make them excellent foods for use in low caloric diets. Concerning the pharmacological potential such as antimicrobial (*14*), antiviral, antitumor, anti-allergic, immunomodulating, anti-inflammatory, antiatherogenic, hypoglycemic, and hepatoprotective properties (*15*), mushrooms have also become attractive as a functional food and as a source for the development of drugs and nutraceuticals. Among them, phenolic compounds exhibit potent antioxidant activities (*16*−*22*). Antioxidants can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation (LPO), and the consumption of antioxidant-rich foods could bring diverse physiological benefits to the consumer, such as protection against human diseases associated with oxidative stress, like coronary heart disease and cancer (*23*).

Nevertheless, edible mushrooms are characterized by a short shelf life (1−3 days at room temperature), linked to the occurrence of postharvest changes. These changes are due to the high moisture content of the carpophores and to the high activity of enzymes such as protease or polyphenol oxidase, responsible for protein and sugar decrease and for a browning reaction during storage (*24*). The drying and deep-freezing processes have been used to increase storage stability and facilitate mushroom consumption without seasonal constraints (*25*). Chemical and nutritional characteristics of mushrooms are closely linked not only to species but also on processing (*3*, *10*, *11*) and cooking (*25*−*27*). Despite this evidence, there are no detailed studies on the influence in fatty acid and sugars profiles, particularly on mushrooms from Northeast Portugal, one of the European regions with a higher wild edible mushrooms diversity. There are also reports about the changes in the contents of health-promoting compounds and antioxidant activities of several vegetables after cooking, such as broccoli (*28*), cabbages (*29*), amaranth, cowpea, peanut, pumpkin, and sweet potato leaves (*30*). Nevertheless, there are no reports for mushrooms concerning this aspect.

The objective of this study was to evaluate the modifications induced by different conservation treatments (drying and freezing) or cooking in the chemical compositions and antioxidant properties of four Portuguese wild edible mushroom species

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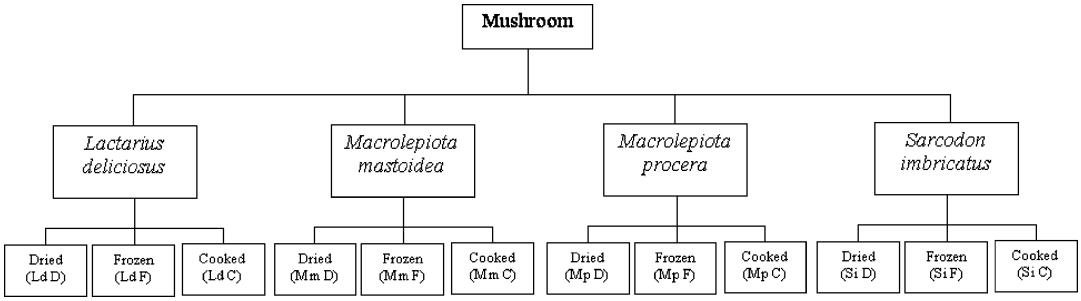
Chart 1



(*Lactarius deliciosus*, *Macrolepiota mastoidea*, *Macrolepiota procera*, and *Sarcodon imbricatus*). The entire extracts were used to achieve health benefits due to the additive and synergistic effects of all of the phenolics present in the extracts, to stimulate the use of mushrooms not only for their nutritional properties but also as a source for the development of drugs and nutraceuticals.

MATERIALS AND METHODS

Samples. Samples of *L. deliciosus* (L.) Gray, *S. imbricatus* (L.) P. Karst, *M. mastoidea* (Fr.) Singer, and *M. procera* (Scop.) Singer were collected under live pine trees (*Pinus* sp.) for the first two species and under oak trees (*Quercus pyrenaica* Willd.) for the lasts species, in Bragança (Northeast of Portugal), in autumn 2006. Taxonomic identification was made according to several authors (*31*, *32*), and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. After collection and taxonomic identification, all of the mushrooms were immediately submitted to different treatments, according to **Chart 1**.

Drying was performed in an oven at 40 °C; freezing was carried out at −20 °C. Mushrooms were cooked with olive oil (extra virgin), salt, and onions; this procedure corresponded to the cooking practice used for these Portuguese mushroom species. After cooking, the onions and excess olive oil were removed. All of the samples were lyophilized (Ly-8-FM-ULE, Snijders) and submitted to chemical composition and antioxidant activity analyses.

Standards and Reagents. All reagents were of analytical grade purity: Methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal); toluene was from Riedel-de-Haen; and sulfuric acid was from Fluka. The fatty acid methyl ester (FAME) reference standard mixture 37 [fatty acids C4−C24; standard 47885-U was from Supelco (Bellefonte, PA)] was purchased from Sigma (St. Louis, MO), as also were other individual fatty acid isomers and the standards used in the antioxidant activity assays: BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (*tert*-butylhydroquinone), L-ascorbic acid, α-tocopherol, gallic acid, and (+)-chatequin. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals were obtained from Sigma Chemical Co. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, United States).

Chemical Composition. Samples of mushrooms were analyzed for chemical composition (moisture, protein, fat, carbohydrates, and ash) using the AOAC procedures (*33*). The crude protein content ($N \times 4.38$) 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 method. Total carbohydrates were calculated by the difference: total carbohydrates = 100 − (g moisture + g protein + g fat + g ash). The total energy was calculated according to the following equations: energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid).

Fatty Acid Composition. Fatty acids were determined by gas−liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the following trans-esterification procedure: Fatty acids were methylated with 5 mL of methanol:sulfuric acid:toluene 2:1:1 (v:v), for 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 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.

Sugar Composition. 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. (*34*) 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 mm × 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 g/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.

Antioxidant Activity. *Sample Preparation.* 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 #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, redissolved in methanol at a concentration of 50 mg/mL, and stored at 4 °C for further use.

Determination of Antioxidant Components. For phenolic contents determination (*35*), 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytikijena 200−2004 spectrophotometer). 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 mg of gallic acid equivalents per g of extract.

Table 1. Moisture (g/100 g of Fresh Weight), Proximate Chemical Composition (g/100 g of Dry Weight), and Energetic Value (kcal/100 g of Dry Weight) of the Wild Edible Mushrooms (Mean ± SD; $n = 3$)[a]

samples	moisture	total fat	crude protein	ash	carbohydrates	reducing sugars	energy
L. deliciosus D	90.05 ± 1.84 a	6.47 ± 0.70 b	17.87 ± 1.62 c	14.28 ± 0.22 a	60.30 ± 2.73 a	0.48 ± 0.11 b	370.90 ± 3.97 c
L. deliciosus F	88.25 ± 3.56 a	8.45 ± 3.49 b	24.33 ± 1.81 b	9.53 ± 1.60 b	57.68 ± 2.97 a	0.54 ± 0.01 ba	404.17 ± 17.94 b
L. deliciosus C	65.74 ± 0.77 b	35.95 ± 2.74 a	29.64 ± 1.96 a	4.53 ± 0.08 c	28.77 ± 3.91 b	0.66 ± 0.02 a	561.63 ± 13.43 a
M. mastoidea D	88.69 ± 3.69 a	2.55 ± 0.13 c	21.89 ± 2.31 b	7.96 ± 0.30 b	67.60 ± 1.43 a	0.30 ± 0.02 c	380.89 ± 1.77 b
M. mastoidea F	89.80 ± 0.8 a	3.05 ± 0.17 b	24.51 ± 3.97 a	11.76 ± 0.49 a	60.68 ± 1.26 b	0.46 ± 0.04 b	368.22 ± 3.98 c
M. mastoidea C	70.30 ± 1.87 b	63.03 ± 0.02 a	10.10 ± 0.46 c	3.54 ± 0.58 c	23.38 ± 0.44 c	0.67 ± 0.04 a	700.96 ± 2.21 a
M. procera D	90.01 ± 1.73 a	1.45 ± 0.13 b	7.62 ± 0.08 c	9.86 ± 0.72 b	80.38 ± 0.19 a	0.32 ± 0.01 c	365.01 ± 0.59 b
M. procera F	89.85 ± 1.85 a	2.18 ± 0.07 b	9.36 ± 0.91 b	9.16 ± 0.59 c	79.28 ± 1.41 a	0.56 ± 0.04 b	379.87 ± 6.05 b
M. procera C	64.36 ± 7.88 b	57.43 ± 2.93 a	11.85 ± 0.13 a	5.73 ± 0.09 a	16.40 ± 2.68 b	0.79 ± 0.02 a	623.67 ± 11.14 a
S. imbricatus D	93.89 ± 5.20 a	3.45 ± 0.18 c	29.98 ± 0.30 a	12.14 ± 0.32 a	54.43 ± 0.76 b	0.49 ± 0.01 a	368.69 ± 1.04 c
S. imbricatus F	92.95 ± 1.23 a	8.94 ± 2.99 b	25.71 ± 4.23 a	8.31 ± 0.31 b	55.98 ± 3.45 b	0.46 ± 0.02 a	404.01 ± 6.48 b
S. imbricatus C	68.54 ± 1.78 b	16.21 ± 1.15 a	15.02 ± 0.33 b	4.82 ± 0.32 c	63.95 ± 1.50 a	0.48 ± 0.04 a	461.74 ± 6.05 a

[a] In each row and for each species, different letters mean significant differences ($p < 0.05$). D, dried; F, frozen; and C, cooked.

For flavonoid contents determination (*36*), the mycelium extract (250 μL) was mixed with 1.25 mL of distilled water and 75 μL of a 5% $NaNO_2$ solution. After 5 min, 150 μL of a 10% $AlCl_3 \cdot H_2O$ solution was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the mixture. The solution was mixed well, and the intensity of pink color was measured at 510 nm. (+)-Chatequin 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 mg of (+)-chatequin equivalents per g of extract.

DPPH Radical-Scavenging Activity. 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 radical scavenging activity (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 radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHA and α-tocopherol were used as standards.

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% tricloroacetic acid (w/v) was 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.

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 was 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. 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 a standard.

Inhibition of LPO 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 mushrooms 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 trichloroacetic acid (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 (%) 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% LPO inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHA was used as a standard.

Statistical Analysis. For each one 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 error (SE) or standard deviation (SD). The effects of conservation treatments (drying, freezing) and cooking on the mushrooms chemical composition and antioxidant activity were analyzed using one-way analysis of variance 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

Effects on the Chemical Composition. The results of the chemical composition and estimated energetic value obtained for the wild mushroom species are shown in **Table 1**. The moisture ranged from 64.36 g/100 g of fresh weight in cooked *M. procera* and 93.89 g/100 g in dried *S. imbricatus*. The dry matter content of raw mushrooms was higher as compared to cooked mushrooms, but no significant differences were obtained between dried and frozen samples. Carbohydrates were the predominant macronutrients and ranged from 16.40 g/100 g in cooked *M. procera* and 80.38 g/100 g in the corresponding dried sample. Protein was the second component most abundant and varied between 7.62 g/100 g in dried *M. procera* and 29.98 g/100 g in *S. imbricatus* dried sample. Fat ranged from 1.45 g/100 g in dried *M. procera* and 63.03 g/100 g in cooked *M. mastoidea*. The high protein and carbohydrate and low fat characteristics of the edible wild mushrooms have been previously reported by other workers (*7*, *10*, *11*). The ash content

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

	Ld D	Ld F	Ld C	Mm D	Mm F	Mm C	Mp D	Mp F	Mp C	Si D	Si F	Si C
C10:0	1.99 ± 0.22	1.82 ± 0.02	0.18 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	ND	0.15 ± 0.03	0.01 ± 0.00	ND	0.02 ± 0.00	0.04 ± 0.02	ND
C12:0	0.24 ± 0.01	0.35 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	ND	0.06 ± 0.01	0.04 ± 0.01	ND	0.02 ± 0.00	0.02 ± 0.00	ND
C14:0	0.17 ± 0.00	0.14 ± 0.01	0.02 ± 0.00	0.26 ± 0.00	0.21 ± 0.00	0.02 ± 0.00	0.32 ± 0.03	0.20 ± 0.01	0.01 ± 0.00	0.18 ± 0.00	0.14 ± 0.01	0.02 ± 0.00
C15:0	0.28 ± 0.01	0.30 ± 0.01	0.04 ± 0.00	0.18 ± 0.00	0.11 ± 0.00	0.01 ± 0.00	0.35 ± 0.03	0.33 ± 0.02	0.01 ± 0.00	0.87 ± 0.00	0.91 ± 0.00	0.03 ± 0.00
C16:0	6.60 ± 0.11	6.87 ± 0.16	10.19 ± 0.25	16.97 ± 0.12	16.83 ± 0.07	10.49 ± 0.09	19.80 ± 0.26	18.48 ± 0.14	10.30 ± 0.12	13.61 ± 0.08	14.46 ± 0.11	10.60 ± 0.01
C16:1	0.19 ± 0.01	0.26 ± 0.01	0.48 ± 0.01	2.33 ± 0.04	2.82 ± 0.02	0.60 ± 0.01	1.06 ± 0.01	0.72 ± 0.02	0.49 ± 0.00	0.35 ± 0.00	0.42 ± 0.01	0.52 ± 0.00
C17:0	0.15 ± 0.00	0.13 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.06 ± 0.00	0.12 ± 0.00	0.21 ± 0.02	0.14 ± 0.01	0.12 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.12 ± 0.00
C18:0	44.38 ± 1.34	43.13 ± 0.63	9.32 ± 1.36	1.26 ± 0.04	0.55 ± 0.01	2.83 ± 0.03	2.38 ± 0.06	2.09 ± 0.03	2.81 ± 0.19	3.57 ± 0.13	3.82 ± 0.48	3.27 ± 0.01
C18:1n9c	21.63 ± 0.40	24.41 ± 1.76	64.80 ± 0.42	16.61 ± 0.32	17.36 ± 0.28	71.88 ± 0.11	9.04 ± 0.29	14.46 ± 0.19	73.70 ± 0.34	50.04 ± 0.28	52.40 ± 0.74	72.32 ± 0.34
C18:2n6c	23.19 ± 1.23	21.13 ± 1.33	12.90 ± 1.13	60.59 ± 0.45	60.69 ± 0.34	12.16 ± 0.02	64.55 ± 0.34	62.14 ± 0.26	10.67 ± 0.35	28.59 ± 0.30	25.24 ± 1.16	11.04 ± 0.38
C18:3n3	0.41 ± 0.13	0.50 ± 0.04	0.91 ± 0.02	0.08 ± 0.01	0.11 ± 0.01	0.98 ± 0.00	0.06 ± 0.00	0.11 ± 0.02	0.99 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	1.02 ± 0.01
C20:0	0.18 ± 0.01	0.19 ± 0.00	0.40 ± 0.00	0.12 ± 0.01	0.06 ± 0.00	0.41 ± 0.00	0.16 ± 0.01	0.11 ± 0.01	0.42 ± 0.01	0.92 ± 0.01	1.02 ± 0.02	0.45 ± 0.01
C20:1c	0.03 ± 0.00	0.01 ± 0.00	0.23 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.25 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.25 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.26 ± 0.00
C20:2c	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.08 ± 0.01	0.09 ± 0.00	ND	0.11 ± 0.01	0.06 ± 0.01	0.01 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	ND
C22:0	0.16 ± 0.00	0.13 ± 0.04	0.12 ± 0.00	0.12 ± 0.03	0.07 ± 0.01	0.12 ± 0.00	0.24 ± 0.01	0.17 ± 0.00	0.12 ± 0.01	0.59 ± 0.03	0.69 ± 0.02	0.12 ± 0.01
C23:0	0.02 ±0.01	0.32 ± 0.16	0.02 ± 0.00	0.09 ± 0.06	0.04 ± 0.02	0.01 ± 0.00	0.09 ± 0.02	0.10 ± 0.02	0.02 ± 0.01	0.25 ± 0.05	0.07 ± 0.01	0.04 ± 0.01
C24:0	0.20 ± 0.01	0.10 ± 0.09	0.06 ± 0.02	0.75 ± 0.06	0.61 ± 0.01	0.07 ± 0.00	0.89 ± 0.06	0.63 ± 0.02	0.06 ± 0.00	0.58 ± 0.02	0.35 ± 0.03	0.17 ± 0.01
total SFA	54.36 ± 1.72	53.48 ± 1.14	20.51 ± 1.65	19.88 ± 0.33	18.57 ± 0.13	14.08 ± 0.13	24.63 ± 0.55	22.31 ± 0.29	13.88 ± 0.34	20.69 ± 0.34	21.57 ± 0.71	14.82 ± 0.20
total MUFA	21.85 ± 0.40	24.69 ± 1.78	65.51 ± 0.43	19.04 ± 0.37	20.27 ± 0.30	72.73 ± 0.12	10.17 ± 0.31	15.23 ± 0.21	74.44 ± 0.35	50.46 ± 0.29	52.88 ± 0.75	73.09 ± 0.34
total PUFA	23.65 ± 1.36	21.68 ± 1.38	13.84 ± 1.19	60.76 ± 0.47	60.89 ± 0.35	13.14 ± 0.02	64.72 ± 0.35	62.31 ± 0.28	11.67 ± 0.37	28.69 ± 0.30	25.37 ± 1.18	12.06 ± 0.39

[a] ND, not detected.

varied between 3.54 g/100 g in cooked *M. mastoidea* and 14.28 g/100 g in dried *L. deliciosus*. Reducing sugars are only a small part of the carbohydrate contents since polysaccharides such as chitin and starch are the most abundant mushroom carbohydrates. The heat inherent to the cooking process could lead to polysaccharides hydrolysis and subsequent sugars release; accordingly, cooked samples present higher amounts of reducing sugars.

Regarding results on a fresh basis, cooking procedures significantly increased nutrient concentration by decreasing the water content; nevertheless, if values are calculated on a dried basis (**Table 1**), significant carbohydrate and protein losses can be observed. These results are in agreement with other studies on different mushroom species. Manzi et al. (*25*, *26*) reported that cooking processes result in a loss of moisture and a subsequent concentration of nutrients but also that cooking may promote a loss of nutrients due to interactions among constituents, chemical reactions, solubility in cooking medium, and (or) thermal degradation. The same authors (*25*) described a significant decrease for dry matter, fat, protein, and carbohydrates after cooking (70−72%) in dried samples and a concentration increase (90−94%) in fresh samples of *Agaricus bisporus*, *Pleurotus ostreatus*, and *Boletus* sp. Dikeman et al. (*27*) also reported cooking losses and, therefore, a concentration of dry matter constituents in *A. bisporus*, *Grifola frondosa*, and *Lentinus edodes*, especially for carbohydrates (starch and total dietary fiber).

The significantly higher fat amount found in the cooked mushrooms might be due to the presence of some olive oil used in the cooking procedure, as it can be observed in the fatty acids profile presented below. On the basis of the proximate analysis, it can be calculated that 100 g of the cooked mushrooms assures significantly higher energy values. The highest values are guaranteed by cooked *M. mastoidea* (700.96 kcal), while dried *M. procera* (365.01 kcal) gives the lowest energy contribution (**Table 1**). An apparent greater stability of nutrients and subsequent higher energetic contribution was observed in frozen mushrooms, when compared with the more severe drying treatment. Despite all of these conclusions, variation in composition among mushrooms may be due to several other factors including mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions, and preparation of the substrates (*25*, *27*).

Effects on the Fatty Acid Composition. The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied mushrooms are shown in **Table 2**. In general, for noncooking samples, the major fatty acids found were linoleic acid (C18:2; ∼60% for *Macrolepiota* species and ∼25% for *L. deliciosus* and *S. imbricatus*) and oleic acid (C18:1; ∼50% for *S. imbricatus*, ∼20% for *L. deliciosus*, and ∼15% for *Macrolepiota* species), followed by palmitic acid (C16:0; ∼ 10% for *L. deliciosus* and ∼15% for the other species). For *L. deliciosus* mushrooms, stearic acid (C18:0) was also found in significant amounts, being the most abundant fatty acid (∼44%). This fact was already observed by us in a previous work (*13*). The prevalence of linoleic (∼65%), oleic (∼10%), and palmitic (∼20%) acids was also observed by Longvah and Deosthale (*10*) in the analysis of Indian mushrooms, *Schizophyllum commune* and *L. edodes*. In another study with *Tricholoma* species, oleic (∼57%) and linoleic (∼28%) acids were also the main fatty acid constituents; other fatty acids detected were found only in small amounts (*11*). Besides the four main fatty acids already described, 13 more were identified and quantified. There were no differences in the fatty acid profiles of dried and frozen samples **(Table 2)**, which is in agreement with the results obtained for total fat presented in **Table 1**. Unsaturated fatty acids (UFAs) predominate over SFAs in all of the mushroom samples (dried, frozen, and cooked), with the exception of *L. deliciosus* due to the abundance of C18:0 in this case. This is consistent with the observations that in fresh mushrooms, UFAs predominate over the saturated, in the total fatty acid content (*10*, *11*). Particularly for cooked samples, the PUFA contents decreased while MUFA percentages increased. SFAs slightly decreased in all of the samples except in *L. deliciosus*. Considering the total MUFA contents, cooked *M. procera* had the highest value (74.44%) but contained the lowest PUFA contents (11.67%). Otherwise, dried *M. procera* presented the lowest MUFA contents (10.17%) and the highest PUFA values (64.72%). In fact, for the cooked samples, a considerable decrease in linoleic acid contents was observed, and for *L. deliciosus*, a decrease was also observed in the stearic acid content probably due to some heating degradation. Another reason could be the heating promoting transformation of linoleic acid into 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and which might

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

	mannitol	trehalose	total sugars
L. deliciosus D	15.41 ± 1.90 a	0.88 ± 0.17 c	16.29 ± 1.75 a
L. deliciosus F	13.90 ± 0.41 a	3.50 ± 0.25 a	17.40 ± 0.66 a
L. deliciosus C	10.19 ± 0.13 b	2.24 ± 0.01 b	12.43 ± 0.13 b
M. mastoidea D	7.84 ± 0.14 a	4.11 ± 0.24 a	11.95 ± 0.20 a
M. mastoidea F	4.60 ± 0.97 b	5.11 ± 1.17 a	9.71 ± 2.11 ba
M. mastoidea C	7.54[b] ± 0.11 a	ND	7.54 ± 0.11 b
M. procera D	4.73 ± 0.26 b	2.92 ± 0.13 b	7.66 ± 0.40 b
M. procera F	6.51 ± 0.11 a	7.63 ± 0.58 a	14.13 ± 0.69 a
M. procera C	2.27[a] ± 0.45 c	1.19 ± 0.02 c	3.46 ± 0.44 c
S. imbricatus D	19.57 ± 1.62 a	6.03 ± 0.26 a	25.61 ± 1.87 a
S. imbricatus F	25.31 ± 1.48 a	4.99 ± 0.41 b	30.30 ± 1.77 a
S. imbricatus C	11.78 ± 1.72 b	3.44 ± 0.49 c	15.22 ± 2.21 b

[a] In each row and for each species, different letters mean significant differences ($p < 0.05$). [b] Values corresponding to mannitol + glucose; D, dried; F, frozen; C, cooked; and ND, not detected.

contribute to cooked mushrooms flavor (*37*). Because oleic acid is the most abundant fatty acid in olive oil (*38*), the increase in oleic acid percentage observed in all of the cooked samples is probably due to the presence of some olive oil used in mushrooms preparation.

Effects on the Sugar Composition. In what concerns sugar composition (**Table 3**), mushrooms showed some homogeneity. All of them presented mannitol and trehalose as the main sugars, while other sugars were present either only in small amounts (*M. mastoidea* and *M. procera*) or were not detected (*L. deliciosus* and *S. imbricatus*). The accumulation of these sugars in the fruit bodies of other mushrooms such as *Hypsizygus marmoreus*, *A. bisporus*, *Flammulina velutipes*, and *L. edodes* has been already reported (*34*). Mannitol was the most abundant sugar in all of the studied species ranging from 2.27 g/100 g of dry weight for cooked *M. procera* and 25.31 g/100 g for frozen *S. imbricatus*. Other authors (*39*) also reported this sugar concentration up to 50% dry weight in *A. bisporus* mushrooms. Sugar alcohols, particularly mannitol, function to provide support and expansion of the fruit body; this sugar alcohol has half the calories of sugar and is half as sweet, and because it is poorly absorbed by the body, it does not raise insulin levels as much as sugar and also does not promote tooth decay (*27*).

The values obtained for sugars concentrations were higher than those obtained for reducing sugars (**Table 1**) since trehalose, an abundant sugar in mushrooms fruiting bodies, is an α-linked disaccharide and therefore a nonreducing sugar. No significant differences between total sugars of dried and frozen samples were observed. Cooked samples presented significantly lower sugar concentrations, expressed as dry weight, which is in agreement with the previously discussed carbohydrates results. A significant decrease in trehalose concentration (glucose disaccharide), which probably was converted into glucose, due to the heating process, was also observed. The chromatograms obtained for cooked *Macrolepiota* species presented broad peaks, probably including glucose and mannitol, which have similar retention times. Nevertheless, the increase in glucose was not enough to balance the decrease in mannitol + glucose concentration caused by heated sugars degradation.

Effects on the Antioxidant Compounds. Table 4 shows EC_{50} values obtained in the antioxidant activity assays of wild edible mushrooms submitted to different conservation treatments and cooking and also their phenol and flavonoid concentrations. Dried samples revealed a higher content in phenol (ranging from 3.06 mg/g in *S. imbricatus* and 3.40 mg/g in *L. deliciosus*) and flavonoid (ranging from 0.99 mg/g in *M. procera* and 2.71 mg/g in *L. deliciosus*) compounds. The amounts found in the cooked samples significantly decreased when compared with the contents found in the other samples. Phenolic compounds are unstable and easily become nonantioxidative under heating and in the presence of antioxidants; thus, heat used in the cooking procedure could destroy the structures of polyphenols and cause a decrease in their antioxidant activity (*16*, *40*). Nevertheless, at low heating temperatures, an increase in phenolics concentration may occur. This can be observed in the dried mushrooms that present higher phenols than the frozen samples (**Table 4**). Choi et al. (*41*) described that heat treatment of Shiitake increased the overall content of free polyphenolic and flavonoid compounds. The authors explained that heat treatment might produce changes in their extractability due to the disruption of the plant cell wall; thus, bound polyphenolic and flavonoid compounds may be released more easily relative to those of raw materials. Thermal processing is generally applied to extend the shelf life of food products. However, it is well-known that natural nutrients could be significantly lost during the thermal processing due to the fact that most of the bioactive compounds are relatively instable to heat. Therefore, heat-processed foods are considered to have a lower health-promoting capacity than the corresponding fresh ones. However, recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment (*42*, *43*). In the present study, the results showed that low heating temperatures (40 °C) increased the bioactive compounds contents (dried samples) while higher heating temperatures destroyed those compounds (cooked samples).

The enhanced antioxidant activity in noncooked samples could be explained by the increased amount of antioxidant compounds, particularly phenolic compounds (*21*, *22*). Also, other authors (*29*) described the loss of total antioxidant activity in blanched cruciferous vegetables. Yen and Hung (*16*) suggested that alkali was an effective factor that caused a decrease in the antioxidative activity of the herb of *Mesona procumbens* Hemsl. extracts and that heating enhanced this effect. The authors attribute the decrease in antioxidant activity to the decrease in the contents of the phenolic components. Other authors reported that heat treatment could also deactivate endogenous oxidative enzymes, explaining the increased antioxidant contents by preventing enzymatic oxidation causing loss of the antioxidant compound in the raw plant materials (*43*). This could explain the higher antioxidant activity (lower EC_{50} values) obtained for dried samples in comparison to frozen samples in the present study. Many antioxidant compounds are mainly present as a covalently bound form with insoluble polymers. Therefore, it is suggested that heat treatment might disrupt the cell wall and liberate antioxidant compounds from an insoluble portion of mushroom, which, in turn, increases the pool of bioaccessible antioxidant compounds. Another reason for the improved antioxidant activity could be due to the formation of novel compounds having antioxidant activities during heat treatment or thermal processing (*41*).

Effects on the Antioxidant Properties. All of the species proved to have antioxidant activities, namely, RSA and LPO inhibition capacities. Nevertheless, different species revealed different properties according to each tested method; dried samples were more efficient (lower EC_{50} values), as was already described. *M. mastoidea* cooked samples proved to have lower antioxidant properties (higher EC_{50} values), which is compatible with its lower phenol contents. As an example, **Figure 1** shows

Table 4. EC_{50} Values Obtained in the Antioxidant Activity Assays and Bioactive Compounds Contents in the Wild Edible Mushrooms (Mean ± SD; $n = 3$)[a]

	antioxidant properties (EC_{50} values; mg/mL)				bioactive compounds (mg/g)	
	DPPH scavenging activity	reducing power	β-carotene bleaching inhibition	lipid peroxidation inhibition	phenols	flavonoids
L. deliciosus D	16.31 ± 0.24 c	4.98 ± 0.02 b	3.76 ± 0.24 b	26.40 ± 0.03 c	3.40 ± 0.18 a	2.71 ± 0.12 a
L. deliciosus F	20.54 ± 0.59 b	4.65 ± 0.02 b	3.74 ± 0.04 b	29.63 ± 0.08 b	2.95 ± 0.24 b	1.91 ± 0.07 b
L. deliciosus C	33.70 ± 1.69 a	17.66 ± 0.08 a	17.86 ± 3.80 a	35.85 ± 0.03 a	1.23 ± 0.14 c	0.89 ± 0.06 c
M. mastoidea D	8.18 ± 0.07 c	4.35 ± 0.01 c	6.48 ± 1.10 b	24.20 ± 0.03 c	3.08 ± 0.17 a	2.10 ± 0.15 a
M. mastoidea F	8.49 ± 0.10 b	4.44 ± 0.04 b	8.92 ± 0.72 a	34.42 ± 0.05 b	2.69 ± 0.37 b	1.56 ± 0.11 b
M. mastoidea C	25.60 ± 0.13 a	4.79 ± 0.03 a	8.10 ± 0.93 a	>50 a	1.13 ± 0.09 c	1.08 ± 0.11 c
M. procera D	5.38 ± 0.50 c	4.18 ± 0.02 b	5.19 ± 0.16 b	>50 a	3.17 ± 0.92 a	0.99 ± 0.05 a
M. procera F	6.95 ± 1.24 b	4.49 ± 0.02 a	6.23 ± 1.23 ba	>50 a	2.59 ± 0.17 a	0.90 ± 0.11 a
M. procera C	8.82 ± 0.36 a	3.96 ± 0.04 c	6.51 ± 1.08 a	>50 a	2.45 ± 0.21 a	0.20 ± 0.11 b
S. imbricatus D	5.82 ± 0.06 c	4.41 ± 0.03 c	3.53 ± 0.20 c	38.17 ± 0.03 c	3.06 ± 0.10 a	1.52 ± 0.03 a
S. imbricatus F	10.98 ± 0.17 b	5.94 ± 0.05 b	4.45 ± 0.25 b	41.14 ± 0.03 b	2.22 ± 0.31 b	1.12 ± 0.03 b
S. imbricatus C	11.82 ± 0.20 a	8.74 ± 0.16 a	16.64 ± 1.08 a	44.42 ± 0.13 a	1.69 ± 0.38 c	1.02 ± 0.04 c

[a] In each row and for each species, different letters mean significant differences ($p < 0.05$). D, dried; F, frozen; and C, cooked.

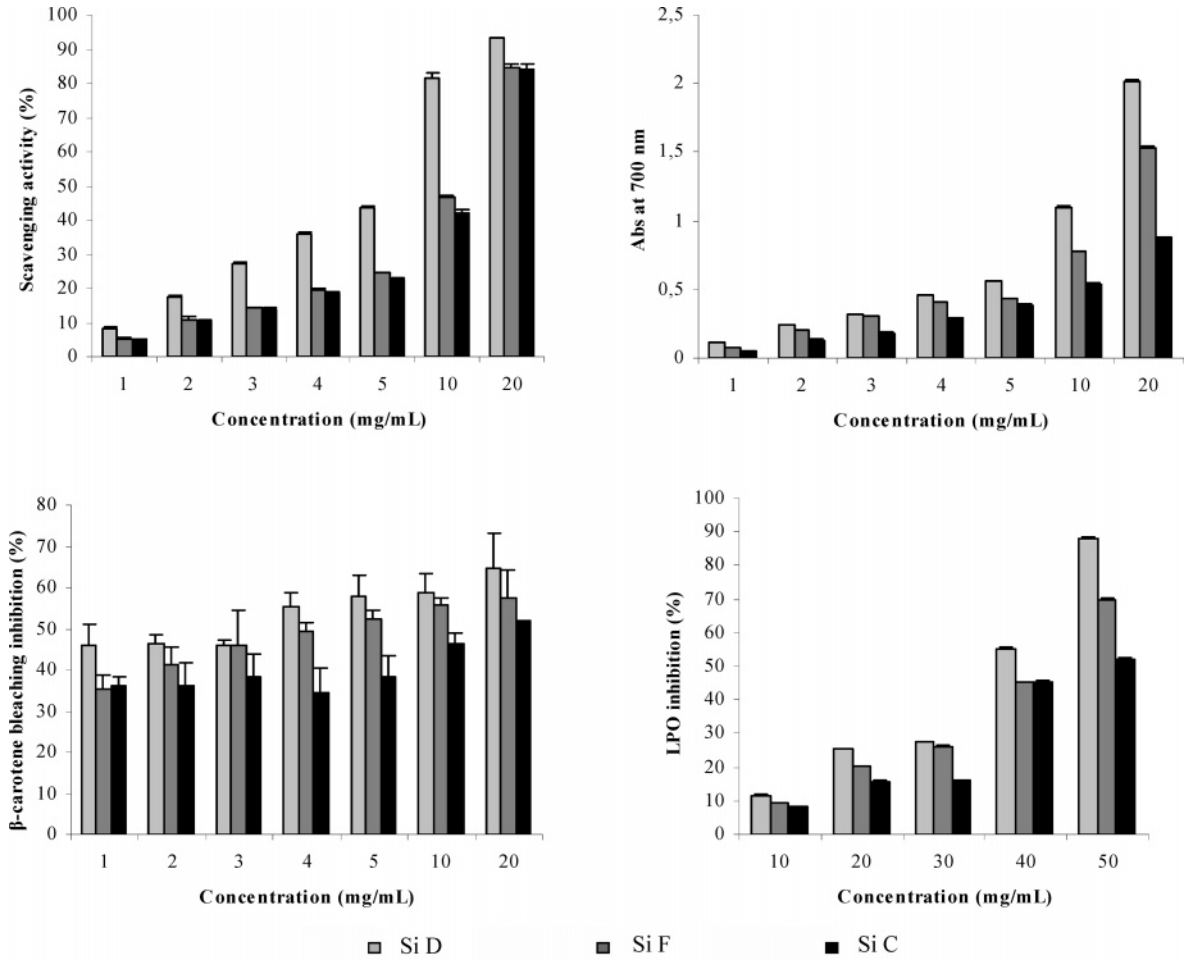


Figure 1. Antioxidant activity of *S. imbricatus* extracts: Scavenging activity on DPPH radicals (%), reducing power, hemolysis inhibition (%), and LPO inhibition (%). Each value is expressed as a mean ± SE ($n = 3$).

the antioxidant activity of *S. imbricatus* after different treatment procedures examined as a function of their concentration. Several biochemical assays were used to screen the antioxidant properties: 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, which attack the highly unsaturated β-carotene models), and inhibition of LPO in brain tissue (measured by the color intensity of the MDA−TBA complex). The assays were performed in the whole extract, since it could be more beneficial than isolated constituents; a bioactive individual component can change its properties in the presence of other compounds present in the extracts (*44*). According to Liu (*45*), additive and synergistic effects of

phytochemicals in fruits and vegetables are responsible for their potent bioactive properties, and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This explains why no single antioxidant can replace the combination of natural phytochemicals to achieve the health benefits. Analysis of **Figure 1** revealed that the antioxidant activity increased with a concentration increase; very good results were obtained at higher extracts concentrations. The antioxidant activity was better (lower EC_{50} values) in chemical assays than in the biochemical assay using animal cells (LPO inhibition) (**Table 4** and **Figure 1**). The decrease in the antioxidant activity of cooked samples might have resulted in the destruction of phenolic hydroxyl groups due to high heat temperature.

In conclusion, both conservation treatment and cooking proved to have influence in mushroom nutritional values and their antioxidant properties. Cooked samples proved to have lower nutrient concentrations and lower antioxidant activities than either dried or frozen samples. Nevertheless, for fatty acid and sugar individual profiles, only the cooking procedure seems to be relevant; the cooked samples presented higher MUFA and lower PUFA and sugars contents.

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