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Journal of Nutrition and Food Science Research

Agaricus brasiliensis (Sun mushroom): antioxidant capacity and HMG-CoA reductase inhibition potential of dried powder formulations in vitro and hepatic alterations in hypercholesterolemic rats

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Received Date: April 19, 2022
Accepted Date: April 20, 2022
Published Date: May 19, 2022

Abstract

This study investigues the chemical composition of the mushroom Agaricus brasiliens is prepared in different commercially available forms: dehydrated whole mushrooms, mushroom powder, and capsules. The most active form was subsequently examined in vivo to investigate its possible effects on liver tissue. For the in vitro assay, the antioxidant potential via DPPH radical and hypocholesterolemic through the activity of HMG-CoA reductase were analyzed. Regarding the in vivo test, 24 female albino Fischer rats (aged 80–95 days; average weight, 205 g) were fed either the AIN-93M modified standard diet (C) or a hypercholesterolemic dietcontaining 25 % soybean oil and 1 % cholesterol supplemented with mushroom powder (HAb) or simvastatin (HS). Animals received the diets for 8 weeks. After this period, the liver was separated for histopathological analysis. Their nutritional components were compared, as well as the antioxidant activity (DPPH free radical assay) and 2-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitory effect. All samples showed high contents of fiber and protein, plus low levels of lipid (1–2%) except for the capsule form (10.3%). They all inhibited 2,2-diphenyl-1-picrylhydrazyl free radicals to a large extent (70–83%), thus demonstrating high antioxidant activity. Chemical characterization using HPLC revealed that tocopherol in dehydrated whole A. brasiliensis is a potent antioxidant and quenches the reactive oxygen species. The ethanolic extracts of samples were found to efficiently inhibit HMG-CoA reductase expression. Significant changes were observed in the liver tissue of animals that had *Agaricus brasiliensis* and Simvastatin added to their diet. Our results, taken together, confirm the significant presence of bioactive compounds in Agaricus brasiliensis, reflecting its ability to act as a food with functional properties. These results reinforce the importance of complementary studies to evaluate the conditions of cultivation and obtainment of the bioactive compounds and their possible interference in the composition and, consequently, in the biological activity of *Agaricus brasiliensis*.

Key words

*Agaricus brasiliensis;*bioactive compounds;antioxidant activity; hypocholesterolemic activity; rats; hepatic steatosis.

Abbreviations

Agaricus brasiliensis, A. brasiliensis; 2-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, HMG-CoA reductase; 2,2-diphenyl-1-picrylhydrazyl, DPPH; (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox; Association of Official Analytical Chemists, AOAC; Reactive oxygen species, ROS; low-density lipoprotein, LDL; neutral detergent fiber, NDF.

1.Introduction

Mushrooms have been used for thousands of years around the world as food and both preventative and curative medicine. Nowadays, various mushrooms are finding new applications as functional foods by combining their medicinal and culinary properties. However, data about the nutritional composition of mushrooms mostly come from studies performed in Korea, China, and Japan, where mushrooms are widely consumed and affordable to most of the population. However, since the composition of mushrooms depends greatly on their growth environment and preparation method, there is a need to study mushrooms that are grown and used outside East Asia (Matila et al. 2000; Sales-Campos et al., 2009).

The *Agaricus brasiliensis* mushroom is native to Brazil and considered a useful food supplement. For example, studies have shown that it displays anti-inflammatory and antioxidant effects (Oliveira et al., 2007; Savoie et al., 2008). It has been used in alternative medicine to treat cancer, diabetes mellitus, hyperlipidemia, atherosclerosis, chronic viral hepatitis, and bacterial infections (Kimura et al., 2004). However, little information is available on the specific compounds that provide its antioxidant effect.

Hypercholesterolemia is known to accelerate the development of cardiovascular diseases and the progression of atherosclerotic lesions. Reactive oxygen species (ROS) play a central role in the pathogenesis of hypercholesterolemia (Anandhi et al., 2013). Plant and fungi rich in phenolic compounds can reduce hypercholesterolemia due to their antioxidant effect. In these organisms, the phenolic compounds are generated as secondary metabolites. Besides inhibiting free radicals, they have been shown to chelate metals and inhibit lipoxygenase (Decker, 1997). The most common, naturally occurring phenolic antioxidants include phenolic acids, tannins, flavonoids, and tocopherols (Angelo and Jorge, 2007). Flavonoids are especially important due to their antioxidant, antiplatelet, anti-inflammatory, and antiallegenic effects.

They may also inhibit certain enzymes related to tumorigenesis such as lipoxygenase and cyclooxygenase. Flavonoids also have significant effects on macrophage-mediated low-density lipoprotein (LDL) oxidation. It is known that the capacity of macrophages to oxidize LDL depends on the oxidative state of the cell, which is determined in turn by the balance between pro-oxidants and antioxidants. Flavonoids exert their effect by inhibiting cellular oxygenases and activating cellular antioxidants (Kim et al., 2004).

The therapeutic potential of a food on cholesterol metabolism could be evaluated by its effect on 2-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Studies have shown that mushrooms of the genus Pleurotus can reduce blood cholesterol as they contain lovastatin, a pharmacological agent widely used to decrease serum cholesterol via inhibiting HMG-CoA reductase (Gunde-Cimerman et al., 1995). Therefore, the effect of A. brasiliensis on cholesterol metabolism was examined here by evaluating its effect on HMG-CoA reductase. Mushrooms may be commercially available in raw or processed forms (canned or dried). Research has shown that the processing and storage conditions affect their composition and therefore the functional properties (De Pauli, 2010). Initially, we tested the potential of *Agaricus brasiliensis*, given its characteristics, to act as a therapeutic target in the control of hyperlipedemia. However, the chemical composition of the different commercial forms found of this particular mushroom species, and even the way in which this composition could influence the biological activities, remain unclear and often with conflicting results.

In this study, the chemical composition and therapeutic potential of A. brasiliensis were evaluated in three commonly available preparations (namely dehydrated whole mushrooms, powder, and capsule), in order to evaluate the bioactive compounds with possible antioxidant and cholesterol-reducing activities. Additionally, the effects of the product that showed the best results in relation to its chemical characterization was examined with respect to its potential as a functional food, specifically in relation to the effects on liver tissue.

2. Materials and Methods

2.1 In vitro test

2.1.1. Sample collection

Whole dried mushrooms (Sample A) were obtained from the southern region of Minas Gerais, Brazil. According to the supplier, the cultivation method was adapted from that of Agaricus bisporus (Champignon de Paris). The cultivation consisted of several phases: composting (phase I), pasteurization and conditioning of the substrate (phase II), inoculation and incubation (phase III), coverage of the colonized substrate with a cover layer (phase IV), and mushroom production and harvesting (phase V). During the growing period, the average temperature ranged between 16 and 28 °C. After harvesting, the mushrooms were cut in half lengthwise and dehydrated at a constant temperature (45 °C to 55 °C) for 6 h (Pascholati, 1998).

The dehydrated mushrooms were then packaged and sold (Dias, 2010). Sample B was purchased as a powder in central Minas Gerais, and no information about the composition was provided. Sample C in capsule form was purchased from a natural food store in central Minas Gerais. According to the label, it was composed of dehydrated mushroom powder (A. brasiliensis Murril), gelatin capsule (gelatin hydrolysate 90.1% and purified water 9.9%), and silicon dioxide as antihumectant. The following nutritional information for one serving (1.8 g) was provided: 5 kcal/21 kJ, 1 g carbohydrates, 0 g protein, 0 g total fat, 0 g saturated fat, 0 g trans-fat, 0 g dietary fiber, and 0 g sodium. The gelatin capsule was discarded, and only the contents were used in this study.

2.1.2 Sample preparation

The samples were dried in an oven at 60 °C, ground, packed in glass containers, and stored at room temperature until analyses. All analytical procedures were performed in triplicate.

2.1.3. Chemicals

The following chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA): 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and Folin-Ciocalteu reagent. All chemicals and solvents were of analytical grade.

2.1.4. Determination of nutritional composition of samples

Analyses were performed in the Laboratory of Bromatology, Department of Foods, Nutrition School, Federal University of Ouro Preto. The levels of moisture, ash, total lipids, protein, and fiber were measured in triplicate. The moisture, protein, and ash contents were determined according to the method published by the Association of Official Analytical Chemists (AOAC, 1984). Specifically, the moisture content was obtained by drying in an oven at 105 ºC for about 5 h to a constant weight, the protein content (N × 4.38) was estimated using the Kjeldahl method, and the ash content was determined in an oven at 550 °C. The lipid content was determined by extraction with petroleum ether in a Soxhlet apparatus, as described in the Analytical Standards of the Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008). The fiber content (cellulose, hemicelluloses, and lignin) was determined using neutral detergent fiber (NDF) in a fiber digestion apparatus (Tecnal TE-149, TECNAL, Piracicaba, Brazil) according to the manufacturer's instructions. The carbohydrate content was calculated by difference. The energy content was calculated according to the equation: energy (kcal) = 4 × [mass of protein (g) + mass of carbohydrates (g)] + [9 × mass of lipids (g)].

2.1.5. Chemical characterization of A. brasiliensis by HPLC-DAD-ESI-MS

The analyses were performed using diode array high precision liquid chromatography (Waters, Milford, MA, USA) with coupled diode detector (DAD), and a mass spectrometer equipped with electrospray ionization (ESI) operated under the following conditions: capillary voltage 3500 V; positive and negative ion mode; capillary temperature 320 °C; voltage source 5 kV; vaporizer temperature 320 °C; corona needle current 5 mA, and gas and nitrogen sheath of 27 psi. The full scan mode (100–2000u) was used. ESI-mass spectrometry (MS/MS) data were collected using an Acquity UPLC (Waters) system, with argon as the collision gas and a collision energy of 30 eV. Chromatographic separation was performed on Acquity BEH UPLC ™ RP-18 column (1.7 µM, 50 mm × 2 mm ID; Waters). The two eluent phases were (1) 0.1% formic acid in water (solvent A) and (2) 0.1% formic acid in acetonitrile (solvent B). The elution time was 0–11 min, with a linear gradient of 5% to 95% of solvent B. The flow rate was 0.3 mL/min-1, and the sample injection volume was 4.0 mL. Ultraviolet (UV) absorption spectra were recorded from 190 to 450 nm. MS analysis was performed on the quadrupole instrument equipped with a negative mode electrospray source (ion spray voltage: -4 kV; orifice voltage: -60 V).

2.1.6. Sample preparation for antioxidant capacity assays

Samples were extracted according to a previously reported procedure with some modification. Briefly, 80% methanol (10 mL) was added to the sample, and the mixture was stirred in a vortex apparatus for 2 min and then stood for 30 min. The supernatant was collected after centrifugation at 9279.4 × g for 8 min (Ramirez-Anguiano et al., 2007).

2.1.6.1. DPPH radical-scavenging ability

The antioxidant capacity was determined using the free radical DPPH according to the method described by Brand-Williams, Cuvelier, and Berset (Brand-Willians, 1995). Trolox (6-hydroxy-2-5-7-8-tetrametilcromo-2-carboxylate acid; 1 mL in 80 mL of methanol) was used as a standard antioxidant. The antioxidant activity was measured in terms of light absorbance (A) by the radical at 515 nm, and the inhibition percentage was determined according to the equation below: % Inhibition of DPPH radical = (Acontrol – Asample/Acontrol) × 100

2.1.7. Determination of HMG-CoA reductase inhibition

Mushroom powders (50 mg/mL) were mixed with water, ethanol/water (1:1, v/v), ethanol, or methanol. The obtained suspensions were shaken in a vortexer for 1 min and centrifuged at 13362 × g for 2 min.

HMG-CoA reductase activity was measured using the supernatant and commercial HMG-CoA Reductase Assay kit (Sigma, Madrid, Spain) according to the user's manual. The assay is based on measuring the decrease in optical absorbance at 340 nm, which represents the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the catalytic subunit of HMG-CoA reductase in the presence of the substrate HMG-CoA. The mushroom supernatant (20 µL) was added to a 96-well plate, and the change in absorbance was monitored at 37 °C using a microplate reader (EL808, Elisa BioTek Instruments). Pravastatin was used as a positive control. This analysis was only carried out for Sample A.

2.2.In vivo tests

2.2.1. Animals

Twenty-four female albino Fischer rats were obtained (aged 80–95 days; average weight, 205 g) from the Experimental Nutrition Laboratory, Department of Food, School of Nutrition, Federal University of Ouro Preto. The rats were kept in individual cages in a room under controlled conditions (24 °C, 55 % humidity, 12-h light/dark cycle). During the experiment, food and water were available ad libitum. The experimental protocol for the use of animals was approved by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (No. 2011/69).

2.2.2 Diet and experimental design

Rats were fed either the AIN-93M modified standard diet (Reeves, Nielsen, Fahey, 1993) or a hypercholesterolemic diet containing 25 % soybean oil and 1 % cholesterol (Matos et al., 2005) supplemented with mushroom powder or simvastatin. The oil and cholesterol levels of the hypercholesterolemic diet were identified based on the work of Matos et al. (2005) and adapted for the AIN-93M diet. We divided the animals into four groups of 6 animals each. The control group (C) received the standard diet; the hypercholesterolemic group (H) received the hypercholesterolemic diet; the hypercholesterolemic plus simvastatin group (HS) received the hypercholesterolemic diet plus simvastatin (in the form of macerated tablets); and the hypercholesterolemic plus A. brasiliensis group (HAb) received the hypercholesterolemic diet and A. brasiliensis. The C group received the standard diet, and the H, HS, and HAb groups received the hypercholesterolemic diet in metabolic cages for 2 weeks prior to the 6-week experimental period when each group received their respective treatments.

2.2.3. Sample preparation

After 6 weeks of treatment, the rats were fasted for 12 h, anesthetized with isoflurane (Cristália, Itapira, SP, Brazil), and euthanized by exsanguination. To determine the sérum component levels, blood samples were collected in 5-mL test tubes and centrifuged at 1500×g for 15 min. The livers of the animals were also removed, rinsed in saline solution, weighed, immersed in liquid nitrogen, and stored immediately at −80 °C for subsequent analysis.

2.2.4. Histological analysis

Liver tissue fragments were fxed in 10% bufered formalin for 72 h, dehydrated, cleared, and embedded in parafn. Tissue sections (4 μm) were cut using a microtome (Leica, Wetzlar, Germany), mounted on microscope slides, stained with Masson's trichrome, and photographed at 400× magnifcation (Leica Application Suite) using a Leica DM5000 microscope coupled to a digital camera. Fibrosis and infammation quantifcation were assessed by evaluation of the total tissue area (1.5×106 μm2) using 15 images of randomly-selected felds of tissue sections, per animal, obtained using Leica QWin software.

2.3. Statistical analysis

All data were analyzed using the Kolmogorov–Smirnov normality test. Data that were consistent with a normal distribution were evaluated by univariate analysis of variance (one-way ANOVA, followed by Dunnett's or Bonferroni's post-test) using GraphPad PRISM® software version 5 for Windows (San Diego, CA, USA). Differences were considered significant when $p < 0.05$. Data were expressed as the mean ± SD. For data that did not follow a normal distribution, the nonparametric Kruskal–Wallis and Dunn's post-tests were used. In this case, the data were presented as the median plus range.

3. Results

3.1. Sample composition

Table 1 summarizes the wt.% composition of mushroom samples. All samples showed high contents of fiber (30–50%) and protein (18–25%), while the ash and moisture contents were 6–7% and ~9%, respectively. We observed that all samples had low lipid levels (1 to 2%), except for Sample C (the capsule form, 10.3%).

Table 1: Percentage composition of different formulations of A. brasiliensis.

NUTRIENTS	A	B	C
Moisture and Volatile	9.37 ± 0.06a	9.03 ± 0.03b	9.03 ± 0.04b
Ash	6.50 ± 0.01c	6.32 ± 0.04c	7.47 ± 0.01a
Proteins†	20.72 ± 1.24b	18.80 ±0.46b	20.39 ±1.20b
Lipids	1.91 ± 0.02b	1.78 ± 0.15b	10.34 ±0.16a
Fiber (NDF)	39.93 ± 1.44b	50.98 ±0.52a	30.19 ±1.51d
Carbohydrates**	21.48 ± 1.12a	13.09 ±0.70b	22.58 ±2.54a
Caloric value (kcal/100 g)	185.00±2.52c	143.60±1.76d	264.90 ± 6.72a

A: Whole dried mushroom; **B:** Powdered mushroom; **C:** Capsule form.

* Analyses were performed in triplicate. Data are shown as the mean ± standard deviation

NDF = neutral detergent fiber.

† Conversion factor from nitrogen to protein: 4.38.

** Carbohydrates = 100- (% moisture + % protein + % lipids + % ash + % fiber.

3.2. Chemical characteristics

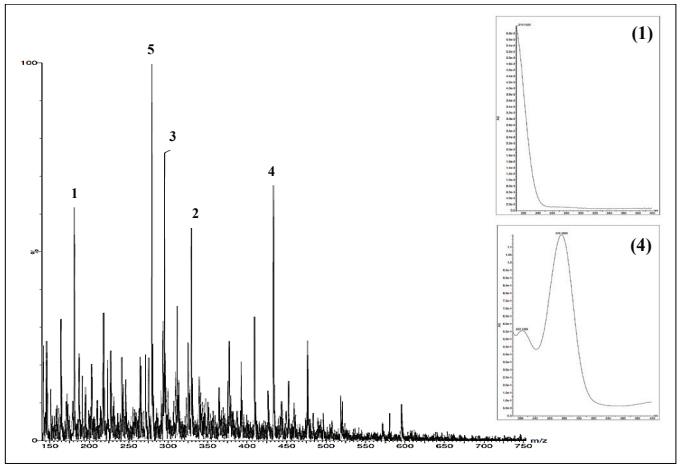
Three fatty acids were identified from the mushrooms and represented by peaks 2, 3, and 5 (Table 2 and Figure 1). They are proposed to be unsaturated fatty acids. Mannitol and a tocopherol derivative were also tentatively identified (peaks 1 and 4 respectively, Table 2). Their UV absorption spectra are shown as insets in Figure 1. The mass spectra obtained were compared with data from the literature. Searches were performed in the available databases by the mass/charge ratio (m/z) obtained in the spectra.

Table 2: Compounds identified in whole dried A. brasiliensis mushroom by HPLC-DAD-ESI-MS.

Peak	Compound	Formula	TR (min)	UV (nm)	LC-MS [M-H]- (m/z)
1	Mannitol	C6H13O6	0.68	-	181.1462
2	Fatty acid	C17H33O5	4.73	-	329.5222
3	Fatty acid	C18H31O3	6.88	-	295.5479
4	5.7-Bis(hydroxymethyl)-□-tocopherol	C27H45O4	7.18	222.1; 276.1	433.4049
5	Fatty acid	C18H31O2	8.61	-	279.3448

*** TR:** retention time in minutes, UV: ultraviolet absorption peak wavelength in nanometers, LC-MS [M-H]- (m/z):liquid chromatography coupled to negative mode mass spectrometry, mass/charge ratio.

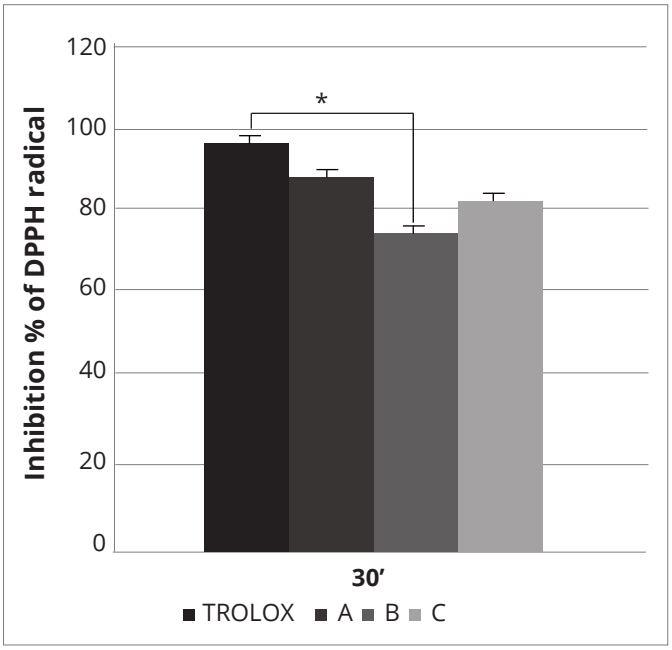
Figure 1: HPLC-DAD-ESI-MS profile of whole dried A. brasiliensis mushroom. Insets: UV absorption spectra (190 to 450 nm) of 1 (mannitol) and 4 (5,7-bis(hydroxymethyl)-α-tocopherol).



3.3. Antioxidant capacity

All samples of A. brasiliensis were found to significantly neutralize the free radical DPPH, with the ability ranging from 70% in Sample C to 83% in Sample A (Figure 2).

Figure 2: Antioxidant activity of the different formulations of A. brasiliensis measured by DPPH scavenging activity assay.

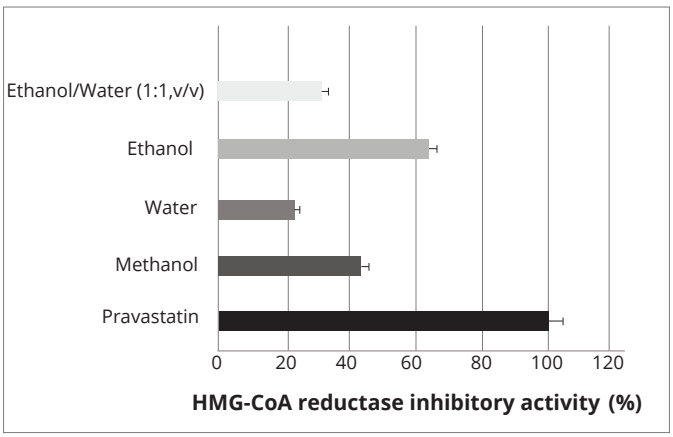


*****Data are shown as the mean ± standard deviation. Analyses were performed in triplicate using 0.1 g/mL A. brasiliensis and 1000 μM Trolox. **A:** Whole dried mushroom; **B:** Powdered mushroom; **C:** Capsule form; DPPH: 2,2-diphenyl-1-picrylhydrazyl. * p<0.05 vs. Trolox.

3.4. A. brasiliensis as a source of HMG-CoA reductase inhibitors

Only the extracts of Sample A were evaluated for HMG-CoA reductase inhibition, since this sample was found to have the most promising nutritional composition and antioxidant activity. The inhibition of HMG-CoA reductase varied significantly (~22 to ~73%) depending on the extraction solvent (Figure 3), being the highest in the ethanol extract and lowest in the aqueous extract.

Figure 3: HMG-CoA reductase inhibitory activities of A. brasiliensis extracts obtained using whole dried mushroom in ethanol/water (1:1, v/v), ethanol, water, and methanol.

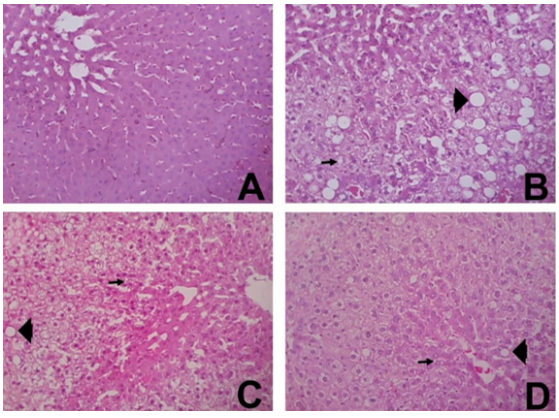


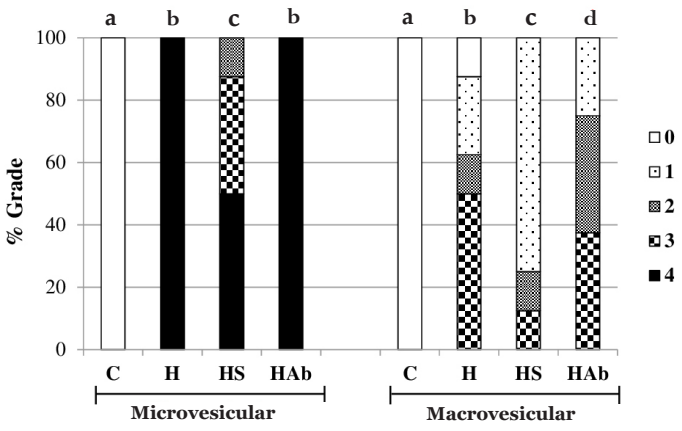
* (▫)Hydroalcoholic extract; (▪) Ethanolic extract; (■) Aqueous extract; (■) Methanolic Extract; (■): Pravastatin. Analyses were done in triplicate.

3.5. Effects of *Agaricus brasiliensis* on liver tissue

Histological sections of the liver stained by H&E showed that the C group presented a histological aspect compatible with the normality of the organ, as can be seen in Figure 4. The hypercholesterolemic (Fig.4) and Hypercholesterolemic + A. brasiliensis (Fig.4) groups showed moderate presence of hepatocytes presenting macrovesicular steatosis and intense presence of microvesicular steatosis. On the other hand, the hypercholesterolemic + Simvastatin group (Fig.4) showed a moderate to severe presence of microvesicular steatosis, followed by a slight presence of hepatocytes in macrovesicular steatosis.

Figure 4: Photomicrographs of liver histological sections stained with Hematoxylin and Eosin. Arrowhead: Macrovesicular steatosis; Arrow: Microvesivular steatosis. C: Control group (A); H: Hypercholesterolemic Group (B); HAb: Hypercholesterolemic group + 1% of *Agaricus brasiliensis* (C); HS: Hypercholesterolemic group + 0.008% Simvastatin (D). Presence of steatosis: 0, none; 1, 10%; 2, 10 to 33%; 3, 33 to 66% and 4, >66%. Different superscript letters indicate a statistically significant difference between columns ($p < 0.0001$). Data analyzed using the chi-square test. Bar = 50 micrometers. ANOVA followed by the Bonferroni's test.





4. Discussion

Foods containing functional nutrients are in high demand, as they combine nutrition with health benefits (George et al.,2005). Edible mushrooms typically contain significant amounts of protein and carbohydrate and low levels of fat, making them nutritious especially compared to animal products (Toor and Savage, 2006; Brito and Volp, 2010). However, as previously mentioned, there are only limited data regarding the composition of mushrooms that are cultivated in Brazil.

Other researchers have examined the nutritional contents in A. brasiliensis, which are affected by numerous factors. The growing conditions, soil, climate, and the choice of substrate appear to be the most important ones according to Furlani and Godoy (2005). Additionally, there are choices in the correction factor used to calculate the protein content from the organic nitrogen content. A value of 6.25 is used for most foods, i.e., assuming that proteins are fully digestible and contain 16% nitrogen. Nitrogen could exist in forms other than proteins, however that is usually negligible in most foods. Since mushrooms have a significant amount of chitin in their cell walls, a different correction factor should be used (Furlani and Godoy, 2005). Here, we used a value of 4.38, which assumes that only 70% of nitrogenous compounds in the mushroom are digestible by the human body. Using the correction actor of 6.25, Oliveira, Oliveira, Lima, and Villas Boas (1999) measured the protein percentage of dried A. brasiliensis to be 30.13%. Chemical analysis of the same mushroom by Celso showed similar values (28.94–35.88%), although the correction factor was not specified there (Eira, 2003). Their data showed that A. brasiliensis has a higher protein content than Pleurotus ostreatus (oyster mushroom) and Lentinula edodes (shiitake mushroom). These values also varied according to the strain used and whether the basidiomata stadium was open or closed. Pedroso and Tamai (2001) found that dehydrated A. brasiliensis basidiocarp contains 40 to 45% protein. Here, our value of 18–25% contradicts the findings of Pedroso and Tamai (2001) but is similar to that found for L. edodes (19.49%) (Eira, 2003).

Edible mushrooms generally have a low lipid content (less than 5% dry weight). The values found in our study (0.9 to 1.9%) were similar to those reported by other authors for both the same species (Oliveira et al., 1999) and Pleurotus spp (Sturion and Oetterer, 1995). The exception was Sample C, which contained about 10% lipid. This value was high compared to those reported in a recent study for A. brasiliensis and L. edodes capsules (1.82% and 1.01%, respectively) (Carneiro et al., 2013).

Besides water, carbohydrates are the most abundant component in mushrooms, and their total content varies according to the species (typically 35–70% dry weight) (Diez and Alvarez, 2001; Longvah and Deosthale, 1998; Mau et al., 2001). In most of the literature, the carbohydrate content in mushrooms is calculated by difference (Manzi et al., 2001; Yang

et al., 2001). The results in our study were 13–22%, which do not agree with the results of other studies (34.78% and 38.3%) (Oliveira et al., 1999; Eira, 2003). Our values are also lower compared to those of A. bisporus (54.12%), L. edodes (69.58%), and Pleurotus spp. (65.82%) (Furlani and Godoy, 2005). Further, our phytochemical analysis using HPLC identified mannitol in A. brasiliensis. This finding corroborates the literature reports that polyols such as glucose, trehalose, mannitol, and arabinitol are the main sugars in the fruiting bodies of edible mushrooms (Barros et al., 2008; Cai et al., 2013; Martins, Reis, Ferreira, 2021).

Reported fiber contents in literature vary a great deal due to the use of several different methodologies. However, many studies do not mention the method used, and so a comparison is difficult. According to Mizuno (2002), A. brasiliensis contains on average of 20–28% dietary fiber that includes β-glucans, chitin, hemicelluloses, and pectin. Using the method of Weende (which only measured the cellulose and insoluble lignin fractions), Oliveira and collaborators (1999) found a fiber content of 14.57% for the same species. In comparison, this study employed the neutral detergent method that accounts for cellulose, hemicellulose, and lignin. Given the importance of fiber in the diet, the choice of fiber-testing method is important for minimize possible errors. Recently, we measured the amount of total dietary fiber in whole dehydrated mushrooms by the gravimetric enzymatic method, and the results revealed a fiber content of about 24% (3.64% soluble fraction and 20.4% insoluble) (De Miranda et al, 2015). These data highlight the need for a careful description of the chosen measurement method, which should be appropriate for the specific food matrix.

From the above discussions, there is a great deal of variation in the reported chemical composition of A. brasiliensis, and so further work is needed to determine it more definitively. In addition, variations resulting from the processing should also be considered, as temperature and humidity can interfere directly with the composition and quality of the product.

Rufino and collaborators (2007) discussed numerous possible methods to measure the antioxidant capacity, such as peroxyl radical capture [oxygen radical absorbance capacity assay (ORAC) and total peroxy radical-trapping antioxidant potential assay (TRAP)], metal reduction [ferric reducing ability of plasma assay (FRAP) and cupric reducing antioxidant capacity assay (CUPRAC)], hydroxyl radical capture (deoxyribose method), organic radical capture [2,2'- azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay and DPPH assay], and quantification of the products formed during lipid peroxidation [thiobarbituric acid reactive substances (TBARS) oxidation of LDL, oxidation of the co-carotene]. In this study, the DPPH assay was used. In this method, the analyte is added to a methanolic solution of DPPH radicals, whose subsequent reaction can be monitored by the decrease in absorbance at 515 nm.(Brand- Willians, 1995). The results are expressed as the percentage reduction of DPPH radical concentration. Our data showed that A. brasiliensis was effective in reducing the DPPH radicals, and that the efficacy depends on the commercial form. These results are consistent with those found by Savoie and collaborators (2008), who reported that A. brasiliensis had higher antioxidant potential than the other mushroom species tested.

We found that the the more processed mushroom samples (i.e. powder and capsule) had lower concentrations of phenolic compounds and flavonoids than the whole ones (data not shown). The content of phenolic compounds can vary greatly due to their rapid degradation by oxidation (Carneiro et al., 2013). Therefore, a lower phenolic content would be expected in the more processed foods. Furthermore, phenolic compounds are known to be secondary metabolites, and their production is influenced by the fungal growth conditions (Di Pauli, 2010). This may also partly explain the variation between the different samples. Palacios and collaborators (2011) evaluated the antioxidant activity of eight species of edible mushrooms (A. bisporus, Boletus edulis, Calocybe gambosa, Cantharellus cibarius, Craterellus cornucopioides, Hygrophorus marzuolus, Lactarius delicious, and Pleurotus ostreatus) and found their phenolic contents to vary from 1 to 6 mgGAE/g, being the highest in B. edulis. Although our work did not determine the exact structure of the phenolic compounds, studies have suggested that the antioxidant property of A. brasiliensis is associated with a high concentration of tocopherols (Tsai et al., 2007).

A positive correlation had been reported between antioxidant activity and phenolic content in mushrooms (Tsai et al., 2007; Yen et al., 1993; Cheung and Cheung, 2005). This correlation was also observed in our study for the three kinds of mushroom samples. However, phytochemical analysis of A. brasiliensis by HPLC performed in this study revealed a different profile from previous descriptions for this and other mushroom species. Specifically, we identified the tocopherol derivative (5,7-bis(hydroxymethyl)-δ-tocopherol), while Tsai and collaborators (Tsai et al., 2007) found a significant amount in tocopherol in aqueous extracts of A. brasiliensis. For these authors, the observed antioxidant activity was directly related to the phenolic content, especially the tocopherol content. Regarding the flavonoid content, no UV or MS peaks associated with these compounds were observed. Flavonoids have been identified in some fungal species such as Aspergillus candidus, Phallus impudicus, and Fistulina hepatica (Ribeiro et al., 2007), however animals and fungi are not capable of synthesizing these compounds themselves (Iwashina et al., 2000). Some authors suggested that only plants have the biosynthetic pathway for producing flavonoids. For example, Barros et al. (2009) analyzed 16 edible mushroom species and found significant amounts of phenolic compounds, mostly phenolic acids, by HPLC-DAD-ESI/MS. However, the presence of flavonoids was not identified by this method (Barros et al. 2009).

An increasing number of studies have demonstrated the cholesterol-lowering effects of various mushrooms (Bobek et al., 1993; Cheung, 1996; Cheung, 1998; Bobek et al., 1998; Fukushima et al., 2001; Berger et al., 2004). In general, the presence of substances such as lovastatin and eritadenina makes these fungi important in searching for treatments of cardiovascular diseases (Guillamón et al., 2010). In A. brasiliensis, several phenolic compounds along with the β-glucans and chitin are considered responsible for the cholesterol- lowering effect. These phenolic compounds may reduce the risk of cardiovascular diseases by regulating lipid metabolism and preventing hypercholesterolemia. Kim and collaborators (2004) reported the antioxidant effects of lovastatin and the flavonoid naringin. According to their study, naringin was able to increase the activity of some antioxidant enzymes (superoxide dismutase, catalase, and glutathione reductase) and at the same time reduce HMG-CoA reductase activity by about 30%. Mushrooms of the genus Pleurotus are considered potential producers of lovastatin—a potent inhibitor of HMG-CoA reductase. Kim and collaborators (2004) had measured the inhibitory effect of the methanol extracts. The amount of lovastatin varies depending on the age and the specific part of the mushroom. In the early growth stage, it is uniformly distributed in small sporocarps, and thus there is a substantial difference in concentration between the pileus and the stipe. However, as the mushroom grows, this difference can change (Pascholati, 1998). In our study, only the HMG-CoA reductase inhibition by Sample A was measured, since the whole dried mushrooms showed the highest antioxidant effect. This enzyme inhibition was generally between 22% (aqueous extract) to 70% (ethanol extract). Therefore, the solvent choice has a large effect on the amount of active compound extracted. This result differs from a previous study, which reported that methanol could effectively extract the compounds required for strong HMG-CoA reductase inhibition (Gil- Ramirez et al., 2011). Additionally, many studies demonstrate the therapeutic potential of A. brasiliensis aqueous extracts, and lovastatin is expected to be less soluble in water than in methanol. Therefore, it is especially surprising that our aqueous and methanol extracts were ineffective.

Regarding to tests to verify its biological potential, the addition of *Agaricus brasiliensis* to the diet promoted significant changes in the liver tissue, especially with regard to macrovesicular steatosis. Nakamura et al., when evaluating the supplementation of mice submitted to a diet high in fat and cholesterol for 21 weeks with *Agaricus brasiliensis* KA21 (AGA) found an attenuation in the development of hepatic fibrosis, which is a characteristic feature of late-stage NASH. The authors associated this change with the reduction in hepatic oxidative stress induced by the administration of AGA. Yamanaka et al. (2014) found that the way the mushroom is cultivated (indoor or outdoor) directly interferes not only in its composition, but also in its ability to act in the protection of liver tissue. In this study, outdoor cultivation significantly enhanced the antioxidative activity of A. brasiliensis fruiting bodies, being more effective at protecting against CCl4-induced liver injury than mushrooms grown in a greenhouse. The administration of A. brasiliensis hydroalcoholic extract to rats represents not only the ingestion of metabolic precursors, but also the ingestion of substances that, even at low concentrations, can exert important signaling functions in the liver as well as in the organism as a whole. Although the study by de Oliveira et al. (2010) was carried out in part on animals on a standard diet, their research helps us to understand our results. The dose used, the form of administration of the mushroom and even the duration of the experiment are directly associated with the data found in our study. Although previous studies carried out by our group together with this group have suggested *Agaricus brasiliensis* as a possible therapeutic target for hyperlipidemias, studies are needed to establish the best way to use it so that it can exert its full biological potential.

5. Conclusions

We have shown that the chemical composition of A. brasiliensis varies greatly between differently processed samples and between different studies. In our work, the largest difference from the literature was the much higher lipid content in the capsule form. However, our results confirmed that A. brasiliensis has significant nutritional value, being high in protein and fiber and low in fat. Furthermore, this mushroom exhibited significant hypocholesterolemic and antioxidant potential, as well as proved to be an important source of phenolic compounds (notably tocopherol). These characteristics are dependent of the muchroom preparation process. Dehydrated whole mushrooms were the most effective and had a higher antioxidant content than the powdered and capsule forms. This work supports the use of A. brasiliensis as a functional food, and highlights the effects of different preparation methods on the medicinal effectiveness of mushrooms.

CRediT authorship contribution statement

Aline Mayrink de Miranda: conceptualization; data curation; formal analysis; investigation; writing - original draft methodology; writing - review and editing.

Lorena Souza e Silva: data curation; formal analysis; investigation; methodology.

Jéssica Ariela Teixeira Costa: helped in preparation of mushrooms and execution of research.

Wanderson Geraldo de Lima: helped in execution of histopathological analysis.

Geraldo Célio Brandão and Gustavo Henrique Bianco de Souza: helped in execution of physico-chemical methods.

Marcelo Eustáquio Silva: helped in the experimental design and in the supply and handling of the animals and execution of research.

Maria Lúcia Pedrosa: conceptualization and supervision.

Acknowledgments

The authors thank the Federal University of Ouro Preto, and acknowledge the financial support provided by the following Brazilian government agencies: Fundação de Amparo à

Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Capacitação de Pessoal de Nível Superior (CAPES/Brazil).

Conflict of Interests

The authors declare no conflict of interest.

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