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Original Nutritional value of *Agaricus sylvaticus*; mushroom grown in Brazil

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Abstract

The bromatological characterization of the Agaricus sylvaticus species (A. sylvaticus), known as the Sun Mushroom and cultivated in Brazil, is necessary to determine substances with pharmacological and nutritional potential, in view its safe use in food and in human medicine. The purpose of the present study was to determine the chemical composition of the A. sylvaticus mushroom grown in Brazil. Mushrooms were obtained in dehydrated form from a producer in Minas Gerais State. Through this study it was able to observe the fungus' rich chemical composition, highlighting the variety and quantity of minerals as well as its high protein content. There are many components of this mushroom that have medicinal properties, which are recognized as excellent antioxidants. Results also proved that the composition of A. sylvaticus presented differences when compared to the chemical composition of other Agaricaceae fungi.

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Key words: Therapeutic fungi. Chemical composition. Protein. Mushroom. Cancer.

Abbreviations

A. brasiliensis: Agaricus brasiliensis.
A. sylvaticus: Agaricus sylvaticus.
AOAC: Association of Official Analytical Chemists.
DCFI: 2, 6-dichlorophenol indophenol sodium.
FAO: Food and Agriculture Organization.
Gla: Gamma carboxyglutamic acid.
HPLC: High performance liquid chromatography.
MAPA: Ministério da Agricultura, Pecuária e Abas-

tecimento.

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EL VALOR NUTRITIVO DE AGARICUS SYLVATICUS; SETAS CULTIVADAS EN BRASIL

Resumen

En la caracterización bromatológica del género Agaricus sylvaticus (A. sylvaticus), conocido como la seta del sol y cultivado en Brasil, es necesario determinar las sustancias con potencial farmacológico y nutritivo con el objetivo de un uso seguro en la alimentación y la medicina humana. El objetivo de este estudio fue determinar la composición química de la seta A. sylvaticus cultivada en Brasil. Se obtuvieron las setas en su forma deshidratada de un cultivador del estado de Minas Gerais. A través de este estudio pudimos observar la rica composición química del hongo, destacando la variedad y cantidad de minerales así como su alto contenido en proteínas. Esta seta contiene muchos componentes con propiedades medicinales, que se sabe que son excelentes antioxidantes. Los resultados también muestran que la composición de A. sylvaticus mostraba diferencias al compararla con la composición química de otros hongos de la familia Agaricaceae.

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Palabras clave: Hongos terapéuticos. Composición química. Proteínas. Setas. Cáncer.

UFG: Universidade Federal de Goiás. WHO: World Health Organization.

Introduction

Due to their high nutritional value, mushrooms have been widely consumed by people seeking a healthier and more nutritional diet. Some mushrooms are considered nutraceuticals, that is, functional foods, being that in addition to their high protein content, low concentration of total fats, added to a significant concentration of vitamins and minerals, they contain antioxidants that are extremely important in the cure, treatment, and prevention of various diseases, including cancer.¹

In Brazil, the consumption of mushrooms by the population is still considered low, but mushrooms of the *Agaricus* genus are becoming very popular owing to their attributed medicinal properties, often associ-

ated to the presence of bioactive compounds with medicinal value, such as phenolic compounds, polyketides, terpenes and steroids, which are recognized as excellent antioxidants.²

Several investigations related to dietary supplementation with *A. sylvaticus* mushroom have shown positive results in patients with colorectal cancer in postoperative phase reducing the deleterious effects caused by the disease itself and by conventional treatment,³ also in the improvement of gastrointestinal changes of these patients.^{4,5}

According to Furlani & Godoy,⁶ the concentration of macro and micronutrients in food is directly related to the benefits they play in humans and animals.

The aim of this study was to evaluate the chemical composition of the *A. sylvaticus* fungus (Sun Mushroom) with respect to protein, lipids, carbohydrates, dietary fiber, minerals, fat soluble vitamins and Vitamin C.

Materials and methods

Obtainment of sample of A. sylvaticus *mushroom* (*Sun Mushroom*)

A sample of dehydrated *A. sylvaticus* mushroom (Sun mushroom), was obtained from a producer in Minas Gerais State. To allow greater extraction of its components, the mushroom was mashed up in a Willey type (Model ET-648, Tecnal Brand mill). The physical and chemical analysis were performed at the Physical Chemistry Laboratory of the Food Research Center, School of Veterinary Medicine (accredited by MAPA - Ministério da Agricultura, Pecuária e Abastecimento) and the Laboratory of Food Biochemistry, Pharmacy School, both from Universidade Federal de Goiás - UFG, from March to June 2010.

Chemical characterization

The whole analysis, in duplicate, has followed the official methods established by MAPA, by the Association of Official Analytical Chemists (AOAC).7-10 Moisture analysis were performed using a kiln at 105 $^{\circ}$ C ± 3 $^{\circ}$ C for 24 hours and total ash by means of sample calcination in a muffle furnace at 550 °C for 12 hours. The Kjedahl method was utilized for protein determination, using a 6.25 correction factor. Sample fat content was detected by continuous "Soxhlet" device type extraction. Determination of total dietary fiber was based on sequential enzymatic digestion of the dried mushroom sample with alphaamylase thermo-stable; protease and amyloglucosidase. The determination of carbohydrates was calculated by the difference, using rates obtained by moisture analysis, fixed mineral residue, proteins and lipids.

Evaluation of minerals

The determination of minerals was performed by means of atomic absorption spectrometry (spectrometer GBC Brand, Model 932AA), in duplicate. The search for iron, zinc, manganese, sodium, potassium, cobalt, copper, calcium and magnesium made was possible, as the laboratory where these tests were performed only contained specific cathode lamps for each of these minerals.

Evaluation of fat-soluble vitamins

Fat-soluble vitamins were determined by high performance liquid chromatography (HPLC), in duplicate. This analysis was used to determine the oil extracted lipids, stored at 10 °C for conservation. Gilson brand liquid chromatography was used with a stationary phase column E-18, column 10 cm/4.6 mm and 5 micras particles. Methanol was used for the mobile phase, utilizing an isocratic working system with 100% methanol and 1 mL/min flow. Variable wavelength was used for each vitamin studied.

Evaluation of Vitamin C

The determination of Vitamin C was performed in triplicate, following the Tillmans Method with titration of standard solution of ascorbic acid and oxalic acid solution with DCFI solution (2, 6-dichlorophenol indophenol sodium), and the solutions used were prepared as described by Instituto Adolfo Lutz¹¹ for Tillmans Method. To determine Vitamin C it was obtained an aqueous, non fractioned extract of *A. sylvaticus* mushroom from diluted dehydrated mushrooms ground in water, kept under agitation at room temperature for one hour.

Results and discussion

Chemical composition of Agaricus sylvaticus

The nutritional value of food is commonly expressed according to the chemical composition or percentage of homogeneous groups of substances in one hundred grams of food, which are: moisture, lipids, proteins, carbohydrates, fiber and ash¹¹ table I shows the results found by analyzing the chemical composition of dehydrated *A. sylvaticus* mushroom.

As they have high nutritional value, mushrooms have been identified as alternatives for a healthier diet rich in proteins. They are highly recommended in countries with high rates of malnutrition,¹³ or for people who need a high protein diet with low lipid content.¹⁴ Observation noted that the *A. sylvaticus* mushroom grown in Brazil contains high protein content (41.16%). However,

Table I Bromatological composition (% per 100 g) of dehydrated A. sylvaticus mushroom cultivated in Brazil in 2010								
A. sylvaticus	6.31	7.38	41.16	6.60	36.21	2.34		

*Results are shown in % in 100 g sample.

*The chemical analysis of this study was performed in duplicate.

*The methodology of the chemical analysis used with dehydrated A. sylvaticus mushroom is described by AOAC: Moisture (kiln 105 °C), ash (muffle furnace at 550 °C), proteins (Kjedahl), lipids (Soxhlet), Carbohydrate (difference from the other constituents of 100%), and dietary fiber (by enzymatic digestion of the sample).

although some authors compare the nutritional value of mushrooms to that of beef (approximately 14.8%),¹⁵ it should be taken into account the biological utilization of protein, since the *Agaricus brasiliensis* mushroom presented, in some studies,¹⁶ low concentrations of essential amino acids necessary for animal growth in experiments, as well as other native cultivated mushrooms in the far east.¹⁷

In 2005 a survey was conducted on the chemical composition of *A. sylvaticus* grown in Brazil by the Japan Food Research Laboratories.¹⁸ For the dehydrated mushroom, were found values of 4.4 g/100 g of moisture, 39.4 g/100 g of protein, 3.0 g/100 g of lipid, 45.6 g/100 g of carbohydrate and 7.6 g/100 g of minerals. The *A. sylvaticus* mushroom grown in Brazil in 2010 showed higher values of moisture content (6.31%), lipids (6.60%) and protein (41.16%), which can be explained taking into account the differences in growing region, climate, genetic mutations,¹⁸ conditions which are probably better in the areas cultivated today.

According to Minhoni et al.,²⁰ the qualitative characteristics of mushrooms are also influenced by species, strain, post-harvest processing, the basidiomata development stage, part of basidiomata and substrate. Braga et al.,²¹ highlight age, environment and locality, as factors influencing the variations in protein content of mushrooms. According to these authors, young mushrooms are richer in protein than the more mature and open ones. In works performed by Shibata & Demiate,²² the authors observed that smaller mushrooms have higher protein content, mainly at the pileus.

In addition to high-protein content, the A. sylvaticus mushroom contains high biological value, since it presents all the essential amino acids,²³ as shown by research conducted by the Japan Food Research Laboratories¹⁸ on the A. sylvaticus grown in Brazil. Such research detected 1.71 g/100 g levels of arginine, 1.55 g/100g levels of lysine, 0.62 g/100 g levels of histidine, 1.11 g/100 g levels of phenylalanine, 0.83 g/100 g levels of tyrosine, 1.72 g/100 g levels of leucine, 1.01 g/100 g levels of isoleucine, 0.39 g/100 g levels of methionine, 1.28 g/100 g levels of valine, 1.75 g/100 g levels of alanine, 1.25 g/100 g levels of glycine, 1.26 g/100 g levels of proline, 5.73 g/100 g levels of glutamic acid, 1.20 g/100 g levels of serine, 1.2 g/100 g levels of threonine, 2.35 g/100 g levels of aspartic acid, 0.43 g/100 g levels of tryptophan and 0.36 g/100 g levels of cystine.

According to Henriques et al.,¹⁶ it is important to check the standards set by FAO/WHO (Food and Agriculture Organization/World Health Organization) for essential amino acid contents such as lysine and leucine, so that the mushroom protein will not be considered as low-quality protein and digestibility. In such case, this mushroom should not be indicated as the only source of protein to ensure satisfactory growth levels.

The wealth of nutrients from the *A. sylvaticus* mushroom is of great importance in terms of public health, since the Brazilian population has a high number of obese people.¹⁴ According to results related to amounts of protein and lipids in the present study, *A. sylvaticus* mushroom can be presented as an important alternative for healthy food, assisting those who seek better quality of life. The *A. sylvaticus* mushroom could be used as food in a mixed diet with other protein sources, or be added to other foods in the hope of enriching the product, as suggested by Monteiro,²⁴ in adding the *A. brasiliensis* mushroom to tomato sauce.

With respect to the lipid content in this study, 6.60% of this nutrient was detected in the *A. sylvaticus* mushroom. According to Borchers et al.,²⁵ although mushrooms contain small quantities of total fat, they have a high percentage of polyunsaturated fatty acids (PUFA) and low content of saturated fatty acids and cholesterol. According to Novaes & Novaes,¹⁶ crude fat of mushrooms consists of several classes of lipids, including free fatty acids, mono-di and triglycerides, sterols, terpenoids and phospholipids, especially lecithin.

The amount of carbohydrates found in the *A. sylvaticus* mushroom was 36.21%. According to Shibata & Demiate,²² carbohydrate content increases when the strain of mushrooms has increased size, and upon analyzing the carbohydrate content of the pileus, a lower concentration of this nutrient is presented when compared to the strain.

In a study by Copercom,²⁶ the chemical composition of other mushrooms of the *Agaricus* genus, *A. brasiliensis* in dried state showed the following results: water (7.5%), protein (36.6%), lipids (3.4%), fiber (6.8%), ash (7.3%), and carbohydrates (38.3%). Comparing these results with those of the present work, we see that only the ash content of the fungi studied was similar.

On aiming to analyze the chemical composition of two strains of *Agaricus Blazei Murrill*, Shibata & Demiate,²² protein values of 34.80% to 39.80%, fiber

values of 7.35% to 9.65%, ash values of 6.99% to 7.89%, lipid values of 0.80% to 3.68% and carbohydrate values of 46.22% to 41.41% were found, which also differ from those results presented in this paper.

A study on *A. sylvaticus* mushroom detected an amount of 2.34% of dietary fiber. According to Novaes & Novaes,¹⁶ the dietary fiber contained in mushrooms has adverse physical action on the absorption of toxic, harmful and carcinogenic substances. Numerous studies show that the fibers are associated to a lower incidence of colorectal cancer, since it accelerates faecal excretion by laxative action, reducing time spent in the intestines. By studying the chemical composition of edible mushrooms, Andrade et al.²⁷ observed that crude fiber content varies depending on the part of the mushroom like the stalk, pileus or the whole basidiomata.

Characterization of minerals present in the Agaricus sylvaticus mushroom

Table II presents the mineral composition of nine minerals researched in *A. sylvaticus* fungus according to the conditions and limitations of the laboratory used in this study.

Among micronutrients, substances required by the body in small quantities for normal operation are zinc, copper, selenium, manganese, chromium, molybdenum and iron.²⁸

Significant amounts of iron were found (726.90 mg/100 g) in the *A. sylvaticus*, which makes the mushroom a rich source of this mineral. According to Crichton et al.,²⁹ iron works in oxygen transport, DNA synthesis, redox reactions in the electron transport chain, and is part of the molecular chain of several proteins and enzymes.

Results also showed 1.35 g/100 g of calcium in the *A. sylvaticus*. Calcium is very important for bone mineralization, maintaining the structure and rigidity of the skeleton.³⁰

Table II Determination of minerals in A. sylvaticus						
Minerals	A. sylvaticus (mg/100 g)	Recommended Daily Intake (RDI) for adults (ANVISA, 1998)				
Iron	726.90	14 mg				
Calcium	1.35	800 mg				
Zinc	549.25	15 mg				
Cobalt	7.75	_				
Magnesium	21.19	300 mg				
Sodium	255.34	_				
Potassium	613.03	_				
Manganese	23.18	5 mg				
Copper	276.66	3 mg				

*Analyses of minerals were performed by atomic absorption spectrometry.

A. sylvaticus mushroom has also presented an important source of zinc (549.25 g/100 g). Zinc has an important physiological role, acting as an antioxidant, preventing lipid peroxidation.³¹ Zinc, found in significant concentrations in *A. sylvaticus* grown in Brazil in 2010, has been the object of studies in various researches related to the performance of this mineral in the human body. Studies have shown that children supplemented with zinc have lower incidence of diarrhea, pneumonia and malaria, when compared with children not receiving zinc.³²⁻³³

Magnesium acts as a cofactor of both enzymes responsible for various metabolic activities and in innate and acquired immune response, in addition to the important role of tissues maintenance and lymphoid cells.³⁴ It was found, 21.19 g/100 g of this mineral in the *A. sylvaticus*.

In this study, it was found high values for sodium content in *A. sylvaticus* mushroom. According to Amazonas Mala,²³ these mushrooms have significant amounts of sodium.

Copper is an essential trace element involved in multiple enzyme systems including the immune response³⁵ and high concentration is present in the *A*. *sylvaticus* mushroom (276.66 g/100 g).

In the 2005 research, the Japan Food Research Laboratories,¹⁸ also conducted an analysis of sodium (4.2 mg/100 g), iron (21.2 mg/100 g), calcium (35.7 mg/100 g), potassium (3.15 mg/100 g) magnesium (100 mg/100 g), copper (8.24 mg/100 g), zinc (6.61 mg/100 g), manganese (0.65 mg/100 g), selenium (36 g/100 g), cobalt (0.13 ppm). Neither molybdenum nor boron was detected. Comparing these results with those of the present study, one may observe the difference between results for most minerals, which come in higher concentrations in this work. According to Urben,¹⁹ this variation in minerals can be explained by the type of crop, climate, region, genetic mutations among others, which are possibly more favorable regarding the techniques used to cultivate *A. sylvaticus* mushroom today.

Borchers et al.²⁵ also observed the presence of potassium, calcium, phosphorus, magnesium, iron and zinc. In a study by Copercom,²⁶ the mineral composition of the dehydrated *A. brasiliensis* mushroom showed the following results for phosphorus, iron and calcium: 939 mg/100 g, 18.2 mg/100 g and 41.6 mg/100 g, respectively.

Oliveira et al.,¹⁴ upon studying the *A. blazei* fungus, found high levels of minerals such as potassium (2.34%), phosphorus (0.87%), calcium (0.07%), magnesium (0.08%), sulfur (0.29%), copper (61.88 mcg), zinc (86.90 mcg), iron (79.63 mcg).

Characterization of vitamins present in the Agaricus sylvaticus mushroom

Table III shows the vitamins composition in *A*. *sylvaticus* fungus according to the conditions and limi-

Determination of fat-soluble vitamins and Vitamin C in the Agaricus sylvaticus mushroom cultivated in Brazil					
Vitamins	A. sylvaticus	Recommended Dietary Allowances (RDA) for adults (ANVISA, 1998)			
Ascorbic acid (Vitamin C)	12.65 mg/100 g	60 mg			
A complex	 Retinol: 0.001 mg/100 g (Retinol acetate, retinol palmitate and retinol propionate were not detected). 	800 µg			
Vitamin D2	0.018 mg/100 g	5 mg			
E complex	 Alpha tocopherol: 0.020 mg/100 g (Tocopherol acetate, Beta tocopherol, Delta tocopherol and Gamma tocopherol were not detected) 	10 mg			
K Complex	 Menaquinone (K2): 0.001 mg/100 g [Phylloquinone (K1), Menadione (K3) and Naftoquinona were not detected (K4)]. 	80 µg			

Table III

*The determination of fat-soluble vitamins was performed in duplicate, using liquid chromatography from oil obtained in the lipid analysis of A. sylvaticus mushroom.

^{*}The wavelengths used in chromatography for the analysis of fat-soluble vitamins were mixed (varied) (λ = 460 nm for Complex A, Vitamin D2 and Vitamin K3; λ = 295 nm for Complex E; λ = 350 nm for vitamin K1 and K4; λ = 280 nm for Vitamin K2).

*The analysis for detecting Vitamin C was performed in triplicate by titration from the non fractioned aqueous of A. sylvaticus extract.

tations of the laboratories used in this study to develop the analysis.

As seen in table III, Vitamin C was detected in samples of *A. sylvaticus* analyzed in this study, which disagrees with results presented by the Japan Food Research Laboratories¹⁸ in 2005.

Vitamin C acts on cicatrizing wounds, collagen synthesis, skin lightener.³⁶ Photoprotection increases and improves the antioxidant defenses.³⁷ The recommended daily dose for maintaining Vitamin C saturation level in the body is approximately 100 mg. Higher doses are necessary in cases of infections, pregnancy and breastfeeding.³⁸ According to Lederer,³⁹ the importance of Vitamin C is associated to several types of cancer, since daily doses administered to cancer patients provided improved survival.

Vitamin A deficiency causes night blindness, rough and peeling skin, dry mucous membranes, growth inhibition, reduced resistance to infections, defects in bone development and modulation.⁴⁰ In the *A. sylvaticus* fungus Vitamin A was found only in the form of retinol (0.001 mg/100 g).

Vitamin K acts as a cofactor for carboxylation of specific glutamic acid residues to form gamma carboxyglutamic acid (Gla), amino acid found in coagulation factors, which appears related to calcium and may regulate the disposal of the mineral matrix bone as part of osteocalcin.⁴¹ In the *A. sylvaticus* mushroom, we detected the presence of Vitamin K2, menaquinone, at 0.001 mg/100 g concentration.

Vitamin E helps protect the long-chain polyunsaturated fatty acid of cell membranes and lipoproteins against oxidation in the body.⁴² Among fat-soluble vitamins, alpha tocopherol appeared in higher concentration (0.020 mg/100 g) in the A. sylvaticus mushroom. Vitamin D regulates the metabolism of calcium and phosphorus, maintaining serum calcium and phosphorus able to provide normal conditions for most metabolic functions, including bone mineralization.⁴³ It was detected 0.018 mg/100 g of Vitamin D2 in the *A. sylvaticus*.

Among the *A. sylvaticus* vitamins exhibited in the survey by the Japan Food Research Laboratories¹⁸ in 2005, the following substances were not detected in the sample: α -carotene, β -carotene and Vitamin C. However, there were findings of 1.21 mg/100 g of thiamine (Vitamin B1), 3.41 mg/100 g of riboflavin (Vitamin B2), 0.83 mg/100 g of Vitamin B6, 0.17 µg of Vitamin B12, 5.8 µg of calciferol (Vitamin D), 0.36 mg/100 g of folic acid, 39.4 mg/100 g of pantothenic acid, 201 mg/100 g of inositol and 39.9 mg/100 g of niacin.

According to Soares,⁴⁴ the accumulation of compounds such as vitamins is dependent on the handling, processing and maturity of mushroom at harvest.

Tocopherol acetate and retinol acetate, obtained only synthetically, were not detected in this sample of dehydrated *A. sylvaticus*, as shown in table II.

According to Borchers et al.,²⁵ mushrooms contain significant amounts of niacin, thiamin, riboflavin, biotin, ascorbic acid and pro-vitamins A and D.

According to Eira & Braga,⁴⁵ knowledge of the chemical composition of mushrooms is very important, and in Brazil the genetic and physiological studies, basic and applied, can be extended aiming to select more stable and productive lineages in addition to establishing more appropriate physiological conditions for the production of mushrooms in order to attain a desired standard of quality.

Clinical and experimental studies demonstrate that dietary supplementation with Agaricales mushrooms

and other medicinal fungi exert positive nutritional, medicinal and pharmacological effects and can be used as an adjuvant in cancer therapy. The mechanisms of action of bioactive compounds present in mushrooms are yet to be fully elucidated in the literature, but scientific evidence suggests that these substances are able to modulate carcinogenesis not only at early stages, but also at more advanced ones, providing benefits to individuals with various types of cancer, mainly by stimulating the immune system.⁴⁶It was observed that dietary supplementation with this medicinal fungus can significantly reduce fasting glycemia levels of colorectal cancer patients in post-surgery phase⁴⁷ and is capable of improving the life quality of these patients.⁴⁸

Conclusions

Through this study it was able to observe the fungus' rich chemical composition, highlighting the variety and quantity of minerals as well as its high protein content. There are many components of this mushroom that have medicinal properties, which are recognized as excellent antioxidants.

Results also proved that the composition of *A*. *sylvaticus* presented differences when compared to the chemical composition of other *Agaricaceae* fungi.

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