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Chemical and Antioxidant Potential of *Agaricus sylvaticus* Mushroom Grown in Brazil

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Abstract

The chemical characterization of *Agaricus sylvaticus (A. sylvaticus)* cultivated in Brazil is necessary to determine nutritional and pharmacological substances in order to guarantee its safe use as food or herbal medicine. The objective of this study was to determine the chemical composition and assess the antioxidant potential of *A. sylvaticus* fungi grown in Brazil. Through this study it was able to observe the rich chemical composition of *A. sylvaticus,* highlighting the variety and amount of minerals as well as the high protein content of this fungus. It was also observed the great antioxidant potential of the aqueous, alcoholic and ethereal *A. sylvaticus* mushroom extracts, emphasizing the alcoholic extract, which testifies the extraordinary benefits of this fungus in diet, since antioxidants prevent premature aging and various types of cancer as well. The composition of *A. sylvaticus* mushroom displayed differences when compared to the chemical composition of the same fungus in other studies and with other *Agaricales* fungi.

Keywords: Chemical composition; Medicinal mushroom; Potential antioxidant

Abbreviations: %: Percentage; Wavelengths; *A. sylvaticus* : *Agaricus sylvaticus*; ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid-diamonic; AOAC: Association of Official Analytical Chemists; BHT: di-terc-butil metil fenol; DPPH: 2.2-difenilpicril-hydrazyl; HCl: Chloridric acid; HPLC: High performance liquid chromatography; NH_4^+ : Ammonium; PUFA: Polyunsaturated fatty acids; R^2: Correlation coefficient; TBHQ: Terc butil hidroquinona

Introduction

Mushrooms are considered nutraceuticals or functional foods by many clinicians and researchers, a fact that has also stimulated the search by Brazilian producers for more advanced production techniques along with introduction of new species [1].

According to Urben [3], there is great genetic variety of native *Agaricus* genus mushrooms cultivated throughout the world. Strains produced by these mushrooms result from the kind of substrate or compost used, climatic conditions, cultivation area and genetic mutation that can occur naturally or artificially.

Mushrooms are highly nutritious foods, having high amounts of protein, equivalent to meat, eggs and milk, much higher than vegetables and fruits. They contain vitamins such as thiamine, riboflavin, ascorbic acid (Vitamin C), erbocalciferol (Vitamin D2), and a high percentage of minerals like calcium, iodine and phosphorus, besides considerable amounts of fiber [2].

Chemical studies have revealed that the high concentration of nutrients and active ingredients in mushrooms is directly related to the type of lineage used, which requires specific conditions or several factors, such as: A) nutritional factors (substances essential for development: carbon, nitrogen, vitamins and minerals), B) abiotic factors (moisture content of compost and cover, temperature, light, oxygen, chemicals in air, CO_2), C) and biotic factor (virus, bacteria, actinomycetes, fungi, nematodes, insects, mites and genetic), D) genetic factors (natural or artificial); E) processing factors (harvest, drying/dehydration and storage) [3].

Mushrooms have been used for therapeutic prevention of various diseases, in the form of drugs and/or functional foods [4]. In Brazil, despite the low consumption of mushrooms by the population, *Agaricus* genus fungi are becoming very popular due to attributed medicinal properties. There are several studies that report the effects of *A. sylvaticus* (Sun mushroom) on various diseases and these properties may also be associated to the presence of bioactive compounds with medicinal value, such as phenolic compounds, polyketides, terpenes and steroids recognized as excellent antioxidants [5].

According to Elmastas et al. [6], phenolic compounds seem to be the main component responsible for the antioxidant activity in mushroom extracts. According to Tsai et al. [7], the antioxidant properties of *Agaricus blazei* may be associated with its high concentration of tocopherols.

The aim of this study was to evaluate the chemical composition of dehydrated *A. sylvaticus* fungus with respect to protein, lipids, carbohydrates, dietary fiber, minerals, liposoluble vitamins and vitamin C as well as determine the antioxidant potential of ether, alcoholic and aqueous extracts obtained from this mushroom.

Materials and Methods

Evaluation of chemical composition

In this laboratory based experimental study, samples of dehydrated *A. sylvaticus* (Sun mushroom) mushroom were obtained from a producer in the State of Minas Gerais. Mushrooms were crushed in a Willey type grinder, Model ET-648, Brand Tecnal to allow greater extraction of components. Physical and chemical analysis was performed at the Physical Chemistry Laboratory of "Centro de Pesquisa em Alimentos", School of Veterinary Medicine (accredited

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by the Ministry of Agriculture, Livestock and Supply) and the Food Biochemistry Laboratory, School of Pharmacy, Universidade Federal de Goiás - UFG from March to June 2010.

Moisture evaluation

Moisture evaluation was performed in duplicate with dehydrated *A. sylvaticus* fungus, applying the official method for moisture rating, using a kiln at 105ºC ± 3ºC for 24 hours, established by the Ministry of Agriculture, Livestock and Supply, determined by the Association of Official Analytical Chemists [8].

This methodology quantifies the water withdrawn from the product by heating process, whereas the moisture content is calculated by the weight difference of the sample at the beginning (100%) and at the end of the process (100% -% water evaporated at 105ºC). This difference reflects the moisture of the sample under analysis.

First the sample was weighed (approximately 5g) and placed in a kiln at 105ºC until its weight remained constant. After two weightings at intervals of five hours each, weight was observed to be constant. Next the sample remained in a desiccator in order to lower the temperature (up to room temperature) and was then weighed to check moisture content.

Ash evaluation

Ash evaluation of dehydrated *A. sylvaticus* fungus was performed by calcining the sample in furnace FDG Brand, Model 3P-S 7000, at 550°C for 12 hours, according to the official method of AOAC [8]. Through this technique it is possible to determine the total ash produced using the heat in a muffle furnace, where there is total destruction of organic matter present in the sample, leaving only those minerals present.

A sample of approximately 2g of *A. sylvaticus* mushroom was weighed in a porcelain crucible, which had previously been incinerated with the aid of Bunsen burner, cooled and weighed. Then the set (sample + crucible) was incinerated in a muffle furnace, first at lower temperature and then at 550°C. After incineration, the set was removed from the flask, placed in a desiccator to cool off and weighed when it reached room temperature. The amount of ash in the sample was detected from the weight difference between the weight of the set and the weight of the empty crucible.

The mushroom ash sample served as a starting point for analyzing specific minerals.

Evaluation of minerals

To determine the minerals, an atomic absorption spectrometry was used in spectrometer GBC Brand, Model 932AA. Duplicate analyses were performed. The principle of this technique is based on measuring the absorption of electromagnetic radiation intensity, from a primary source of radiation by gaseous atoms in ground state. It was possible to search for iron, zinc, manganese, sodium, potassium, cobalt, copper, calcium and magnesium, as these tests were performed in a laboratory where there were specific cathode lamps for each of these minerals.

Protein evaluation

For protein grading the Kjedahl method was used following the AOAC [8] methodology. Total nitrogen was obtained from the sample which, through calculation was transformed into protein Nitrogen considering that each 100g of protein contains an average 16g of nitrogen. Therefore we used a 6.25 correction factor, which was multiplied by the total Nitrogen percentage of the sample, which corresponded to the protein percentages [9].

To develop this methodology we used a Nitrogen distiller Brand Tecator, Kjeltec System Model 1026. Protein analysis involved three phases. In the first phase the nitrogen in the sample was transformed into ammonium (NH_4^+) through acid digestion of organic matter, starting from 0.1 g of Degreased Dry Matter. In the second phase, separation was obtained by means of distillation and in the third phase, dosage by titration with HCl 0.02 N.

Evaluation of lipids

The amount of lipids present in the sample of the *A. sylvaticus* mushroom was obtained through continuous extraction with a Soxhlet device, Brand Gerhardt, Soxtherm Model 2000, using sulfuric ether as solvent, which has a boiling point of approximately 35ºC. After extraction, the solvent was evaporated using a Rotavapor and lipid fraction was determined gravimetrically. After 24 hours, we obtained the average weight of lipid fraction. The extracted oil was stored at 10°C for later chromatographic analysis of fat soluble vitamins.

Evaluation of total dietary fiber

The methodology for the evaluation of total dietary fiber of *A. sylvaticus* fungus was proposed by AOAC [10], whose principle is based on the sequential enzymatic digestion of dehydrated mushroom sample, in duplicate, with thermostable alpha-amylase, protease and amyloglucosidase. The digested sample was then treated with alcohol to precipitate the soluble fiber before filtering, and the residue was washed with alcohol and acetone, dried and weighed.

Carbohydrate evaluation

The evaluation of carbohydrates was calculated by the difference, using rates obtained by the analysis of moisture, fixed mineral residue, proteins and lipids, following methodology recommended by AOAC [11].

Evaluation of fat-soluble vitamins

Fat-soluble vitamins were determined by high performance liquid chromatography (HPLC), and the performance of duplicate analysis. The principle of this technique evaluates the extraction of active compounds of vitamins studied and their conversion in free form in chloroform solution for later evaluation.

For this analysis, it was used as sample the oil obtained in lipid analysis through Soxhlet extraction. It was used liquid chromatography, Gilson brand, with a stationary phase column E-18, column 10 cm/4.6 mm and particles of 5micras. For the mobile phase was used a methanol and isocratic working system with 100% of methanol and 1mL/min flow. Variable wavelengths (λ) were used for each vitamin studied, as shown in Table 3.

Vitamin C cvaluation

Vitamin C evaluation was performed in triplicate, following the Tillmans Method starting from titration of a 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 the Adolfo Lutz Institute (1995) for the Tillmans Method. To determine Vitamin C, it was obtained an aqueous, non fractioned extract of *A. sylvaticus* mushroom by diluting dried mushrooms ground in water, kept under agitation at room temperature for one hour.

Evaluation of antioxidant potential

The antioxidant potential of *A. sylvaticus* mushroom was determined following the methodology used by Borguini [12]. In

order to avoid interference of light in the sample, the experiment was conducted using material covered with aluminum foil. It was obtained the ether, alcoholic and aqueous extracts from the mushroom. First it was obtained the ether extract by diluting 2.5g of ground mushroom in 50mL of ethyl ether. From non-filtered residue and therefore **ether-insoluble**, it was obtained the alcoholic extract by adding ethanol at 1:20 ratio (residue weight: volume of alcohol). And finally, it was obtained the aqueous extract by adding water to the non-filtered residue from the previous step and also adding distilled water at 1:20 ratio (residue weight: water volume).

BHT was used as a standard antioxidant and DPPH as an oxidant. The antioxidant activity of mushroom extracts was determined by DPPH (2.2-difenilpicril-hydrazyl) described by BRAND-WILLIAMS et al. [13]. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule, and thus, is reduced in the presence of an antioxidant.

Absorbance decrease was monitored at 517nm in a spectrophotometer Model SP-220, Biospectro brand, at intervals of 0, 1, 2, 3, 4, 5, 10, 15 and 20 minutes of reaction. The values observed in the spectrophotometer were converted to a percentage scale, which indicates 0% - no inhibition of free radical production, and 100% indicates complete inhibition of the same.

Quantification of total polyphenols

Concentration of total polyphenols was determined by colorimetric method described by Singleton and Rossi [14], using the Folin Ciocalteau reagent.

For quantification of total polyphenols in the sample, a standard curve of gallic acid solution at concentrations of 0.01mg/mL to 0.06mg/mL was used. The correlation coefficient (R^2) was calculated, resulting in $R^2 = 0.99775$ to a 5% level of significance. This test was performed in triplicate, by using the ether, alcoholic and aqueous extracts of sample at the same concentrations utilized for the standard solution of gallic acid.

The reading was performed with spectrophotometer Model SP-220, brand Biospectro at 750nm.

Results

Chemical composition

Table 1 shows the results found by analyzing the chemical composition of *A. sylvaticus* dehydrated mushroom. One can observe the high protein content (41.16%), followed by carbohydrates (36.21%).

Table 2 shows values found for rating minerals in dehydrated *A. sylvaticus* fungus, including iron, zinc, calcium, cobalt, magnesium, sodium, potassium, manganese and copper. It was not possible to determine the dosage of other minerals performed in the laboratory owing to operational reasons.

The quantities of liposoluble vitamins and vitamin C found in the mushroom *A. sylvaticus* are shown in Table 3. Liquid chromatography analysis enabled the analysis of vitamin A in acetate form, palmitate and propionate in addition to its pure form; of vitamin E in acetate form, alpha, beta, delta and gamma tocopherol; of vitamin K in the K1, K2, K3 and K4 form; however, vitamin D2 was detected by titration.

Antioxidant potential

The antioxidant potential of ether, alcoholic and aqueous extracts obtained from *A. sylvaticus* mushroom is shown in Table 4.

Constituent	Composition (% in 100g)
Humidity	6.31
Ash	7.38
Protein	41.16
Lipids	6,60
Carbohydrates	36.21
Dietary fiber	2.34

* The chemical analysis was performed in duplicate.
* The methods of chemical analysis of dehydrated *A. sylvaticus* mushroom are described by AOAC: Moisture (kiln at 105ºC), 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).

Table 1: Chemical composition of dehydrated *A. sylvaticus*.

Constituent	Composition
Iron	726.90 mg/100g
Calcium	1.35 mg/100g
Zinc	549.25 mg/100g
Cobalt	7.75 mg/100g
Magnesium	21.19 mg/100g
Sodium	255.34 mg/100g
Potassium	613.03 mg/100g
Manganese	23.18 mg/100g
Copper	276.66 mg/100g

*Analyses of minerals was performed by atomic absorption spectrometry.

Table 2: Evaluation of minerals in dehydrated *A. sylvaticus*.

Vitamin	Composition	Wavelength (λ)
Ascorbic acid (Vitamin C)	12.65 mg/100g	-
Retinol acetate (Vitamin A)	0.000 mg/100g	460nm
Retinol (Vitamin A)	0.001 mg/100g	460nm
Retinol palmitate (Vitamin A)	0.000 mg/100g	460nm
Propionate, retinol (Vitamin A)	0.000 mg/100g	460nm
Vitamin D2	0.018 mg/100g	460nm
Tocopherol acetate (Vitamin E)	0.000 mg/100g	295nm
Alpha tocopherol (Vitamin E)	0.020 mg/100g	295nm
Beta Tocopherol (Vitamin E)	0.000 mg/100g	295nm
Delta Tocopherol (Vitamin E)	0.000 mg/100g	295nm
Gamma tocopherol (Vitamin E)	0.000 mg/100g	295nm
Phylloquinone (vitamin K1)	0.000 mg/100g	350nm
Menaquinone (vitamin K2)	0.001 mg/100g	280nm
Menadione (Vitamin K3)	0.000 mg/100g	460nm
Naftaquinone (Vitamin K4)	0.000 mg/100g	350nm

* The analysis of liposoluble vitamins was performed in duplicate, using liquid chromatography of the oil obtained from the lipids' analysis of *A. sylvaticus* fungus.
* The analysis for detecting vitamin C was performed in triplicate by titration from the non fractioned aqueous extract of *A. sylvaticus* mushroom.

Table 3: Composition of vitamins of *A. sylvaticus* mushroom.

Total polyphenols

The amount of polyphenols detected in the ether, alcoholic and aqueous extracts are shown in Table 5.

Discussion

In this study we observed that the protein content of *A.sylvaticus* (41.16%) is superior when compared to the protein content of beef (approximately 14.8%), as well as of other mushrooms from the *Agaricales* family [15].

In addition to the high-protein content, protein from mushroom *A. sylvaticus* has high biological value, since it exhibits all the essential

amino acids [16], as shown by research conducted by the Japan Food Research Laboratories [14] on *A. sylvaticus* grown in Brazil.

The following levels were detected at the time: 1.71g/100 g of arginine, 1.55g/100g of lysine, 0.62g/100g of histidine, 1.11g/100g of phenylalanine, 0.83g/100g of tyrosine, 1.72g/100g of leucine, 1.01g/100g of isoleucine, 0.39g/100g of methionine, 1.28g/100g of valine, 1.75g/100g of alanine, 1.25g/100g of glycine, 1, 26g/100g of proline, 5.73g/100g of glutamic acid, 1.20g/100g of serine, 1.21g/100g of threonine, 2.35g/100g of aspartic acid, 0.43g/100g of tryptophan and 0,36g/100g of cystine.

Because they are high-protein food, mushrooms are highly recommended for those who need a high protein diet, or for those whose diet has restrictions on lipids. This fact is of great importance regarding public health, since research reveals that the Brazilian population includes a large number of overweight or obese individuals. This is certainly already causing public health concern, upon considering a population whose consumption profile has considerably changed, especially during the 80's, due to economic factors and the related social consequences [18].

According to results on the amounts of protein and lipids in the present study, *A. sylvaticus* mushroom can also be suggested as an important alternative health food.

In the 2005 survey conducted by the Japan Food Research Laboratories on the *A Sylvaticus* grown in Brazil, values found for dehydrated mushroom were 4.4 g/100g of moisture, 39.4 g/100g of protein, 3.0g/100g of lipid, 45.6g/100g of carbohydrate and 7.6/100g of minerals. Comparing the above results with the present study, *A. sylvaticus* mushroom grown in Brazil in 2010 in dried state, shows higher values of moisture content (6.31%), lipids (6.60%) and protein (41.16%), which can be explained if taking into account differences in farming technique, region, climate, genetic mutations [3], conditions which are probably better in the areas where the mushroom is currently cultivated.

In a study by Copercon, cited by Eira [19], the chemical composition of other mushrooms of the genus *Agaricus*, *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.

The present study revealed 2.34% value of dietary fiber. According to Novaes and Novaes [15], the dietary fibers contained in mushrooms can absorb toxic, harmful and carcinogenic substances. Countless studies show fibers being associated to lower incidence of colorectal cancer, since it accelerates faecal excretion by laxative action, reducing the time spent in the intestines.

Extract	Antioxidant potential (%)
Alcoholic	75.6
Ethereal	14.6
Aqueous	14.6

* The antioxidant potential of *A. sylvaticus* mushroom was observed from spectrophotometric analysis of three extracts from the sample. As oxidant we used the DPPH as standard.

Table 4: Antioxidant potential of ether, alcoholic and aqueous of *A. sylvaticus* fungus extracts.

Extract	Total polyphenols (%)
Ethereal	4.11
Alcoholic	9.43
Aqueous	0.98

* Total polyphenols research was performed using the Folin-Ciocalteou in spectrophotometer at 750nm.

Table 5: Quantification of total polyphenol of ether, alcoholic and aqueous extracts of *A. sylvaticus* fungus.

With respect to the lipid content, we detected 6.60% of this nutrient in the *A. sylvaticus* fungus. According to Borchers et al. [20], 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 and Novaes [15], crude fat mushrooms consists of several classes of lipids, including free fatty acids, mono- di- and triglycerides, sterols, terpenoids and phospholipids, especially lecithin.

The Japan Food Research Laboratories also performed analysis of sodium (4.2mg/100g), iron (21.2mg/100g), calcium (35.7mg/100 g), potassium (3.15mg/100g) magnesium (100mg/100g), copper (8.24 mg/100 g), zinc (6.61mg/100g), manganese (0.65mg/100 g), selenium (36µ g/100g), and cobalt (0.13ppm). Neither molybdenum nor boron was detected. Comparing these results with this study, we can observe the discrepancy between results for the most researched minerals, which come in higher concentrations in this work. According to Urben [3], this variation in minerals can also be explained by the type of crop, climate, region, and genetic mutations, among others, found more favorable in techniques used at present to cultivate the genus *A. sylvaticus* mushroom.

According to [16], mushrooms have significant amounts of sodium. The presence of potassium, calcium, phosphorus, magnesium, iron and zinc was also observed by Borchers et al. [20].

In a study by Copercon, cited by Eira [19], the mineral composition of the dehydrated mushroom *A. brasiliensis* showed the following results for phosphorus, iron and calcium: 939mg/100g, 18.2mg/100g and 41.6mg/100g, respectively.

Olivera et al. [18], studying the fungus *A. blazei,* 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).

Among the vitamins exhibited by *A. sylvaticus* surveyed by the Japan Food Research Laboratories in 2005, the following substances were not detected in the sample: α-carotene, β-carotene and Vitamin C. However, values found were 1.21mg/100g of thiamine (Vitamin B1), 3.41mg/100g of riboflavin (Vitamin B2), 0.83mg/100g of Vitamin B6, 0,17µg of Vitamin B12, 5,8µg of calciferol (Vitamin D), 0.36mg/100g of folic acid, 39.4mg/100g of pantothenic acid, inositol 201mg/100g and 39.9mg/100g of niacin.

As seen in Table 3, vitamin C was detected in samples of *A. sylvaticus* analyzed in this study, which disagrees with the results presented by the Japan Food Research Laboratories [17]. According to Lederer [21], the importance of vitamin C is associated with several types of cancer, and daily doses administered to patients with cancer have improved their survival.

Among the surveyed liposoluble vitamins, alpha tocopherol within the D complex, retinol, within the A complex and menaquinone from K Complex were detected. According to Soares [22], the accumulation of these compounds is dependent on the handling, processing and maturity of mushroom at harvest.

Because they are obtained synthetically, tocopherol acetate and retinol acetate were not detected in samples of dehydrated *A. sylvaticus* mushroom. According to Borchers et al. [20], mushrooms contain

significant amounts of niacin, thiamin, riboflavin, biotin, ascorbic acid and pro-vitamins A and D. According to Eira and Braga [23], knowledge of the chemical composition of mushrooms is very important, and in Brazil the genetic and physiological studies, basic and applied, can be expanded aiming at selecting more stable and productive lineages, establishing more appropriate physiological conditions for the cultivation of mushrooms so as to attain the desired standard of quality.

According to Silva et al. (24), despite the high biodiversity of mushrooms found in Brazil and great exploitation potential, there is little data on the antioxidant activity of mushroom extracts, since antioxidants have the ability to scavenge free radicals, which are harmful to human health [25].

Antioxidants are able to slow oxidation rate, inhibiting free radicals and preventing the onset of diseases, thus contributing to greater longevity, making the balance between free radicals and the antioxidant defense system essential [26].

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 found 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 at more advanced phases of disease progression as well, providing benefits to individuals with various types of cancer, mainly by stimulating the immune system [27].

Regarding antioxidant activity it was observed that the alcoholic extract of the mushroom *A. sylvaticus* has great antioxidant potential (74.6%), suggesting that most antioxidant compounds present in this mushroom can be more easily diluted in alcohol. However, the aqueous and ether fractions showed lower antioxidant potential (14.6% each) when compared to alcoholic fraction. The aqueous fraction presented reduced antioxidant potential (14.6%) compared to results reported by Percario et al. [28] for the fungus in liquid suspension (50%), since in this work, antioxidant compounds had already been extracted by ether and by alcohol.

Polyphenols make a heterogeneous group, composed of several classes of substances with antioxidant capacity, among which phenolic acids and flavonoids stand out. The antioxidant activity of polyphenols is mainly due to its reducing properties, whose intensity of antioxidant activity exhibited by these phytochemicals is notably differentiated because it depends fundamentally on the number and position of hydroxyl groups present in the molecule [29].

In this study we determined the amount of total polyphenol for the etheric, alcoholic and aqueous extracts. We noticed that the largest amount of alcoholic extract is concentrated in polyphenols (9.43mg/100g) followed by etheric extract (4.11mg/100g), and aqueous extract (0.98mg/100g). The use of ethanol made possible the extraction of a higher content of polyphenols, since the alcoholic extract of the *A. sylvaticus* sample exhibited higher total phenolic content than the aqueous and ethereal which hold lower levels of these constituents.

Aiming to evaluate the antioxidant capacity of the *A. sylvaticus* mushroom in different forms of preparation (liquid suspension, fresh, dry and tablets), Percario et al. [28] assessed the ability of samples to inhibit *in vitro* the formation of free radicals by ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid-diamonic) over a period of 90 seconds, resulting in decreased absorbance at 600nm.The authors observed excellent antioxidant activity (%) in all forms of preparation of *A. sylvaticus* at concentrations of 1mg sample. The authors emphasized that the temperatures used in the preparation of the samples were 60°C for the dried mushroom and liquid suspension, since high temperatures can inactivate most molecules with antioxidant properties present in *A. sylvaticus* According to the authors, these molecules are easily degraded when exposed to industrial processes, which reduces their antioxidant capacity. According to Barros et al. [30], the cooking processes are responsible for the reduction of nutrients with antioxidant capabilities in several mushrooms analyzed in Portugal.

Percario [28] researched different molecules with antioxidant capacity in *A. sylvaticus* fungus, and found results of 72mg/g for β-Glucan in the liquid suspension and 14.1mg/g in tablet form. For flavonoids, values of 0.88mg/g were found in liquid suspension and 0.63mg/g in tablet form. For total phenols, values were 0.1mg/g for liquid suspension and 3.4mg/g for tablet form. The author suggested that the antioxidant activity of *A. sylvaticus* mushroom is due to the entirety of molecules it contains, and not a specific component only.

In a study performed by Silva et al. [24] the antioxidant potential of different extracts of the mushroom *A. blazei* was evaluated by the DPPH method. The authors also observed a higher antioxidant activity (28.6%) in methanol extract: aqueous (1:1), with extraction time of six hours. Results displayed in the present work, confirmed that the best antioxidant activity for *Agaricus sylvaticus* extract was in the alcoholic fraction (74.6%), which shows that components with antioxidant properties of this mushroom are more easily soluble in alcohol.

Some authors utilized the researched mushroom extracts as ingredients in some foods in order to find out the antioxidant effect in processed products. Silva et al. [24] added the methanol: water extract (1:1) to soybean oil and obtained good results. Results showed effective protection (20.4 h of oxidative stability), and the activity of *A. blazei* extract was more efficient than the synthetic antioxidant BHT (100mg/kg) and less efficient than the TBHQ (50mg/kg).

Silva et al. [24], evaluating the *A. blazei* mushroom, obtained concentration of 15mg/g of total phenolic compounds in methanol extract: water extract (1:1). The content of total phenolic compounds present in *A. blazei* was also assessed by Tsai et al. [7], who obtained 5.67mg/g of phenolic compounds in the aqueous extract of this mushroom. In this study, the values of total polyphenols were lower. The alcoholic extract of the mushroom *A. sylvaticus* showed 9.43mg/100g of phenolic compounds. The aqueous and ether extracts showed 4.11 and 0.98mg/100g respectively.

Conclusion

Through this study we were able to observe the rich chemical composition of *A. sylvaticus,* highlighting the variety and quantity of minerals and the high protein content of this mushroom. It was also found that the chemical composition of the mushroom showed differences when compared to the composition of the same mushroom in other studies and other mushrooms of the *Agaricales* genus.

It was also observed the great antioxidant potential of aqueous, alcoholic and ethereal extracts of the *A. sylvaticus* mushroom, emphasizing the alcoholic extract, which demonstrated the extraordinary benefits of this mushroom in diet, considering that antioxidants prevent against premature aging and various types of cancer.

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