Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.71859

Chemical analysis of wild edible mushrooms from the South-Western Amazon

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ABSTRACT. Edible mushrooms are widely studied because they have nutritional and medicinal properties, being considered a good source of protein, with an amount comparable to animal protein and low in fat. Due to the presence of specific bioactive compounds in mushrooms, they have a high therapeutic value in strengthening the immune system and preventing diseases and can be considered highly functional foods. Thus, the objective of this work was to evaluate the nutritional composition of wild mushrooms from the South-western Amazon. Two fungal samples that showed low cytotoxicity against HepG2 in a previously done assay were used. To obtain mycelium, the isolated mushrooms were grown in Petri dishes with 20 mL of Potato-Dextrose-Agar and incubated at 28 °C for 14 days. After this period, the mycelium was transferred to Erlenmeyer flasks containing 200 mL of Potato-Dextrose medium in a 10% proportion, for growth for another 14 days at 28 °C, without agitation. Then, the mycelium was separated from the liquid medium and dried for 24 hours in an oven at 37 °C, and then weighed and ground to do chemical analysis. Moisture, ash, lipids, proteins and carbohydrates from mushrooms 5.332 *Oudemansiella cubensis* and 5.358 *Hohenbuehelia* sp. were analyzed according to the methodologies of Instituto Adolfo Lutz [IAL] in triplicates. As a result, mushroom 5.332 *O. cubensis* had 11.12% moisture, 33.75% lipids, 2.90% ash, 47.27% protein and 9.97% carbohydrates, while mushroom 5.358 *Hohenbuehelia* sp. had 5.14% moisture, 26.38% lipids, 3.11% ash, 59.11% proteins and 6.26% carbohydrates. The Amazonian mushrooms analyzed in this work are rich in proteins and lipids, however they have a low carbohydrate content and can be considered potential sources of functional ingredients. This study contributes to the first report on the nutritional analysis of *Oudemansiella cubensis* and *Hohenbuehelia* sp.

Keywords: Agaricomycetes; functional food; *Hohenbuehelia* sp.; *Oudemansiella cubensis*.

Received on April 04, 2023
Accepted on July 05, 2024

Introduction

Edible mushrooms are widely studied for their nutritional and medicinal properties (Yamauchi et al., 2019). About 2,000 species of mushrooms have food potential, of these, only 25 species have accepted food functionalities (Furlani & Godoy, 2007).

Regarding their nutritional composition, they are considered a good source of protein, with a quantity comparable to animal protein and low in fat. Based on dry weight the amount of protein can range from 10-40%, carbohydrate content ranges from 3-21%, fiber content ranges from 3-35% and fat content ranges from 0.7% to 9.7% (Smith et al., 2002; Novaes, 2007). In addition, they provide a significant amount of vitamins (B1, B2, B12, C and D) and mineral elements (Ca, K, Mg, Na, P, Cu, Fe, Mn and Se) (Mattilda et al., 2001).

Due to the presence of specific bioactive compounds in mushrooms, they have a high therapeutic value in strengthening the immune system and preventing diseases such as hypertension, obesity, hyperlipidemia, hypercholesterolemia, diabetes, cancer and neurodegenerative diseases, and are considered a good source of prebiotic substances (Rathore et al., 2017; Roncero-Ramos & Delgado-Andrade, 2017; Sawangwan et al., 2018). Thus, they can be considered highly functional foods, which provided a wide advance in the commercialization and appreciation of mushrooms in the area of nutraceutical products (Rathore et al., 2017).

Some species are usually found in human food and only part of them are cultivated commercially (Valverde et al., 2015), with emphasis on the species *Agaricus bisporus* (champignon-de-Paris), *Lentinula edodes* (shiitake) and *Pleurotus* sp. (oyster mushroom) (Guillamón et al., 2010). The species native to Brazil, *Agaricus*

brasiliensis popularly known as sun mushroom, is commercially cultivated and has attracted worldwide attention for being one of the mushrooms with the highest commercial value in the market (Wasser et al., 2002).

In the Amazon, edible wild mushroom species are known, such as the native species *Lentinula raphanica* (Oliveira et al., 2022), also reported as potential food by the Yanomani peoples in Roraima, which still feed on the following wild species, *Panus neostrigosus*, *Panus strigellus*, *Panus velutinus*, *Lentinus bertieri*, *Lentinus crinitus*, *Pleurotus djamor*, *Polyporus tricholoma* and *Polyporus philippinensis* (Sanuma et al., 2016).

Edible mushrooms in general are considered important biotechnological products, as they are a good source of proteins, vitamins, minerals and biologically active substances, and can be used to formulate healthier food products (Liu et al., 2018; Perez-Montes et al., 2021). Thus, the objective of this study was to evaluate the nutritional composition of wild Amazonian mushrooms.

Material and methods

Fungal samples

The fungal samples used for growth and chemical analysis were the isolates that had low cytotoxicity against HepG2 in vitro assay. Among the samples that showed low cytotoxicity, those mushrooms that had large basidiomes with a soft texture and pleasant smell were selected.

Obtaining mycelium through submerged cultivation

To obtain mycelium, the isolated mushrooms were grown in Petri dishes with 20 mL of Potato-Dextrose-Agar (PDA) and incubated at 28 ºC for 14 days. After this period, the mycelium was transferred to Erlenmeyer flasks containing 200 mL of Potato-Dextrose (BD) medium in a 10% proportion, for growth for another 14 days at 28 °C, without agitation (Viecelli et al., 2010).

After that, the mycelium was separated from the liquid medium and dried for 24 hours in an oven at 37 °C, and then weighed and ground to do chemical analysis.

Chemical analysis

Chemical analyses were done at the *Unidade de Tecnologia de Alimentos* (UTAL) at UFAC, according to the methodologies of the Instituto Adolfo Lutz (1985). Moisture, ash, lipids, proteins and carbohydrates were analyzed in triplicates.

Moisture

Humidity corresponds to the loss in weight suffered by the product when heated under conditions in which water is removed. Direct heating of the sample to 105 °C is the most common procedure.

0.25g of the sample was used, heated at 105 °C for 3 hours, after which the sample was cooled in a desiccator at room temperature and then weighed.

The moisture content was determined by calculating: $\frac{100\ x\ N}{P}$, where N = number of grams of moisture (loss of mass in g) and P = number of grams of the sample.

Ashes

Waste from incineration or ash is the name given to the residue obtained by heating a product at a temperature close to 550-570 °C.

0.25g of the sample was weighed, heated in an exhaust hood in order to remove all organic matter. After that, the sample was placed in a muffle and heated to 550 °C for incineration for 3 hours. After cooling, the sample was placed in a desiccator until room temperature and weighed.

The ash content was determined by calculating: $\frac{100\ x\ N}{P}$, where N = number of grams of ash, and P = number of grams of the sample.

Lipids (Ethereal extract)

The determination of lipids was done using a Soxhlet-type extractor device, followed by removal by distillation of the solvent used.

0.25g of the sample was weighed in a Soxhlet cartridge. In the Soxhlet-type extractor apparatus, hexane was added in sufficient quantity for two Soxhlets. Under heating on an electric plate, the extraction was continuous for

48 hours. The cartridge was removed and the hexane distilled with the extracted residue was transferred to an oven at 105 °C, and kept for 30 min. The sample was cooled in a desiccator to room temperature and weighed.

The lipid content was determined by calculating: $\frac{100\ x\ N}{P}$, where N = number of grams of lipids, and P = number of grams of the sample.

Proteins

Protein determination is based on nitrogen determination, usually done by Kjeldahl digestion process. In this work, the classic Kjeldahl method was used, which occurs through three stages: digestion, distillation and titration.

0.25 g of the sample was used. For digestion, the samples were transferred to test tubes, where 7.5 mL of sulfuric acid and 1 g of catalytic mixture were added. The tubes were heated at approximately 420 °C for 3 hours. For the distillation step, 125 mL Erlenmeyer flasks containing 25 mL of boric acid and approximately 10 drops of the mixed protein indicator were prepared, these Erlenmeyer flasks were connected to the distillation set, together with the test tubes containing the digested samples. 25 mL of distilled water and 25 mL of 30% sodium hydroxide solution were added to the tubes containing the digested sample. The material was distilled to about 75-100 mL of distillate. For the titration, a solution of hydrochloric acid was used, where the greater the volume of hydrochloric acid spent in the titration, the greater the amount of nitrogen present in the sample.

The total protein content was determined by calculating: $\frac{V\ x\ 0{,}14\ x\ f}{P}$, where V = difference between the number of mL of hydrochloric acid used in the titration, P = number of grams of the sample and f = conversion factor (4.38).

Carbohydrates

Carbohydrate content was calculated by difference. For the calculation, the five determinations were added: moisture (%), ash (%), lipids (%) and protein (%). The total was subtracted from the whole (100%) and the result represents the carbohydrate content.

Results

Chemical analysis of mycelia from mushrooms 5.332 *Oudemansiella cubensis* and 5.358 *Hohenbuehelia* sp. were made (Figure 1). As a result, the mushroom 5.332 *O. cubensis* presented 11.1% moisture, 33.8% lipids, 2.9% ashes, 47.3% proteins and 10.0% carbohydrates, while the mushroom 5.358 *Hohenbuehelia* sp. had 5.1% moisture, 26.4% lipids, 3.1% ash, 59.1% proteins and 6.3% carbohydrates (Table 1).

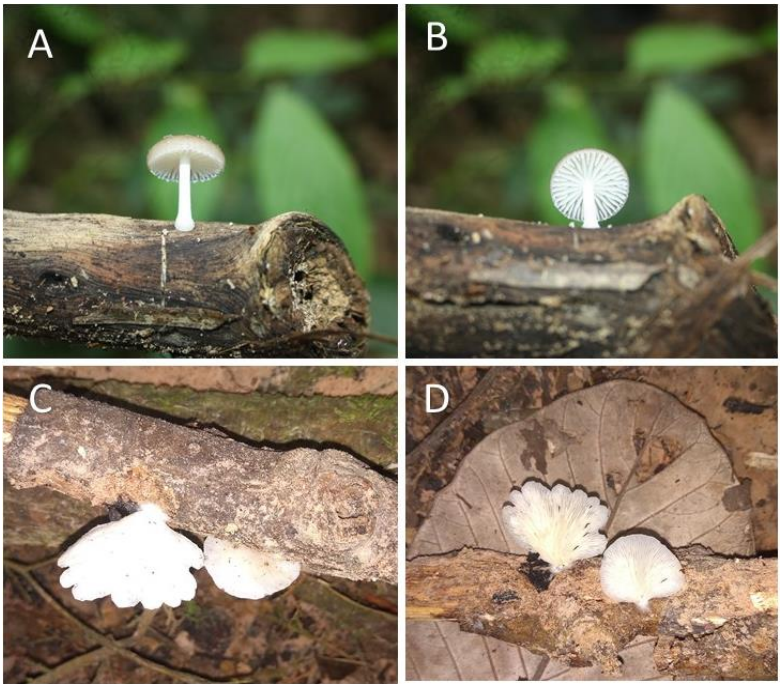

Figure 1. Basidiome of wild Amazonian mushrooms chemically analyzed in this study. A-B: 5.332 *Oudemansiella cubensis*; C-D: 5.358 *Hohenbuehelia* sp.

Table 1. Nutritional composition (g 0.25 g^{-1}) of mushroom mycelia 5.332 *Oudemansiella cubensis* and 5.358 *Hohenbuehelia* sp. Data are expressed as mean ± SD (n = 3).

Registration N°.	Chemical analysis (%)				
	Moisture	Lipid	Ash	Protein	Carbohydrate
5.332 *Oudemansiella cubensis*	11.12 ± 0.44	33.75 ± 10.76	2.90 ± 0.77	42.27 ± 3.23	9.97 ± 6.27
5.358 *Hohenbuehelia* sp.	5.14 ± 1.68	26.38 ± 9.41	3.11 ± 0.37	59.11 ± 8.69	6.26 ± 1.84

Discussion

It is necessary to emphasize that although the results of this work refer to mycelial analysis and not to the basidioma, the technique of submerged cultivation of mushrooms is adequate to provide a significant increase in the production of nutraceutical and pharmaceutical agents, which in turn are important substances extracted of the mycelium or its broth (Smith et al., 2002). A study done with the species *Lentinus sajor-caju* showed that the growth in liquid culture medium presents results of biomass and nutritional value comparable to those of the fruiting bodies of the same species (Confortin et al., 2008).

Mushrooms 5.358 *Hohenbuehelia* sp. and 5.332 *O. cubensis* had a high protein content present in their mycelia, 59.1 and 42.3%, respectively. Mushrooms can produce a wide variety of bioactive proteins and peptides, such as lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial proteins, ribonucleases and laccases with antitumor, antiviral, immunomodulatory and antimicrobial activities (Xu et al., 2011).

In a study where the chemical composition of a wild species of *Oudemansiella canarii* was analyzed, it was demonstrated that the mushroom had protein contents ranging from 16-18% (Xu et al., 2016). Other studies analyzing wild mushroom species have shown that *Tricholoma portentosum* and *T. terreum* species had protein contents of approximately 16%, and *Lentinus crinitus* had 14.4%, (Diez & Alvarez, 2001; Dávila et al., 2020). It was observed that the species reported in the studies had a low protein content in relation to the species analyzed in the present work, as well as when compared with the protein content of domesticated species and usually used for food, such as the species *Agaricus bisporus* (28.5% protein) and *Lentinula edodes* (19% protein) (Furlani & Godoy, 2007).

The lipid contents present in the analyzed mushrooms were 26.4% and 33.8%. Although the amount of lipids present in mushrooms is low, in many cases (Alam et al., 2007; Sales-Campos et al., 2013; Wang et al., 2014) a high lipid content was observed in this work. Essential fatty acids are a substantial part of the lipid content of mushrooms, such as linoleic and oleic acids, demonstrating great nutritional importance when compared to peanuts and other foods rich in proteins and fats, such as olive oil and meat varieties (Sande et al., 2019; Çayan et al., 2020)

Mushrooms are known to have a high carbohydrate content, mainly represented by polysaccharides. In this study, the carbohydrate contents were 9.97% and 6.26%. In one study, the chemical composition of 37 wild mushrooms was analyzed, carbohydrate contents ranging from 38.4% to 5.3% (Ao & Deb, 2019), demonstrating that the amount of carbohydrate can vary from species to species. Mushroom polysaccharides are highly bioactive, making mushrooms interesting functional foods, in addition to being safe, because they are a good source of prebiotic substances, containing short-chain sugars such as glucose, galactose, fructose and N-acetylglucosamine (Patel & Goyal, 2011) which are non-digestible carbohydrates that stimulate the growth of beneficial microorganisms in the body (Sawangwan et al., 2018).

Conclusion

The Amazonian mushrooms analyzed in this study are rich in proteins and lipids, however they have a low carbohydrate content and can be considered potential sources of functional ingredients. This study contributes to the first report on the nutritional analysis of *Oudemansiella cubensis* and *Hohenbuehelia* sp.

Acknowledgment

We extend our gratitude to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for providing a scholarship to the author Geyse Souza Santos, and to the *Fundação de Amparo à Pesquisa do Estado do Acre* (FAPAC) for awarding a scholarship to the author Bruno Jhosef Freires de Souza and for funding the projects *Princípios Bioativos de Basidiomicetos Amazônicos e Potencial alimentar de cogumelos (Agaricomycetes, Basidiomycota) Amazônicos*.

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