

Main biocompounds profile in brazilian strains of medicinal mushroom Reishi**Perfil dos principais biocompostos em cepas brasileiras de cogumelos medicinais Reishi**

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Charline Soares dos Santos Rolim

Graduanda em Eng. de Alimentos pela Faculdade de Ciências Agrárias

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil

E-mail: charlinerolim@gmail.com

Leonardo do Nascimento Rolim

Doutor em Microbiologia pela UFPE

Instituição: Centro Universitário FAMETRO

Endereço: Av. Constantino Nery, 3204 - Chapada, Manaus - AM, Brasil

E-mail: leonardorolim@yahoo.com.br

Régis Tribuzy de Oliveira

Mestre em Ciências e Fisiologia do Exercício pela UTAD / Vila Real, Portugal

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil

E-mail: educacaofisica.regis@gmail.com

Maria Letícia de Sousa Gomes

Graduanda em Eng. de Alimentos pela Faculdade de Ciências Agrárias

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil

E-mail: m.leticiaep@hotmail.com

Gabriel Alexandre Silva Martins

Graduando em Eng. De Alimentos pela Faculdade de Ciências Agrárias

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil

E-mail: alexandre240527@gmail.com

Eyde Cristianne Saraiva-Bonatto

Doutora em Planejamento de Sistemas Energéticos pela UNICAMP

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil

E-mail: eydesaraiva@ufam.edu.br

Maria das Graças Gomes Saraiva

Mestre em Doenças Tropicais e Infecciosas

Instituição: Fundação de Medicina Tropical Doutor Heitor Vieira Dourado

Endereço: Av. Pedro Teixeira, s/n - Dom Pedro, Manaus - AM, Brasil
E-mail: gracag.saraiva@gmail.com

Carlos Victor Lamarão

Doutor em Biotecnologia pela UFAM

Instituição: Universidade Federal do Amazonas

Endereço: Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus - AM, Brasil
E-mail: victorlamarao@ufam.edu.br

ABSTRACT

This article aims to analyze Reishi mushrooms (*Ganoderma lucidum*) native to the Brazil, considering the proposal as another stimulus to the development and preservation of the tropical forests. The Reishi has been used for thousands of years in traditional Chinese medicine to treat several diseases. The nutritive quality of the mushrooms cultivated in Jun-Cao overcomes the nutritional valued of mushrooms cultivated through classical methods. Proteins, total sugars, phenols and polysaccharides (β -glucans) were compared in two non-exploited Brazilian strains of this fungus with two commercial Chinese strains. In general, the Brazilian strains showed proteins, carbohydrates and phenol concentrations similar to Chinese varieties. The results suggest that Brazilian strains have similar biochemical potential as Chinese varieties.

Keywords: Amazon rainforest, *Ganoderma lucidum*, fungal biochemistry, Jun-Cao Technology.

RESUMO

Este artigo tem como objetivo analisar os cogumelos Reishi (*Ganoderma lucidum*) nativos do Brasil, considerando a proposta como mais um estímulo ao desenvolvimento e preservação das florestas tropicais. O Reishi tem sido usado há milhares de anos na medicina tradicional chinesa para tratar várias doenças. A qualidade nutritiva dos cogumelos cultivados em Jun-Cao supera a valor nutricional dos cogumelos cultivados por métodos clássicos. Proteínas, açúcares totais, fenóis e polissacarídeos (β -glucanos) foram comparados em duas linhagens brasileiras não exploradas desse fungo com duas linhagens comerciais chinesas. De maneira geral, as linhagens brasileiras apresentaram concentrações de proteínas, carboidratos e fenóis semelhantes às variedades chinesas. Os resultados sugerem que as cepas brasileiras têm potencial bioquímico semelhante ao das variedades chinesas.

Palavras-chave: Floresta Amazônica, *Ganoderma lucidum*, bioquímica de fungos, Tecnologia Jun-Cao.

1 INTRODUCTION

Currently, one of the greatest Brazilian scientific challenges is to plan a territorial management system for the Amazon that takes into account both the conservation of its extraordinary natural resources and the promotion of the social and economic development of its inhabitants. In this context, fungiculture contributes to maintaining ecological balance and reducing impacts on the environment, being one of the best ways to eliminate waste and convert it into organic products of high gastronomic and nutritional value (MELLO, 2015).

The Amazon has favorable characteristics for the development of fungiculture because it has the native diversity of edible mushroom species and abundant lignocellulosic substrates. The Amazon rainforest has been a major target of national and international scientific interest due to the great diversity of fungi easily seen even in short walks through the forest, especially after the rain (VIEIRA et al., 2005).

Ganoderma lucidum is a basidiomycete from the Aphyllophorales order. Its fruiting body is named “Lingzhi” in China and “Reishi” in Japan. This mushroom has been traditionally used in Chinese medicine to promote vitality and to treat a wide range of diseases such as inflammation, diabetes, heart problems, cholesterol, and cancer (MUSZYNSKA et al., 2018; ZHAO; HE, 2018; ZHANG et al., 2019).

Molecular analysis has identified a number of bioactive compounds such as triterpenoids, proteins, steroids, alkaloids, enzymes, organic acids, lactones, mineral salts and fatty acids. Polysaccharides (specially β -d-glucans) are known to be anti-carcinogenic (ZHANG et al., 2016; IQBAL et al., 2018; ZHAO et al., 2018).

Among the various cultivation methods that have been developed, the Chinese Jun-Cao technique (Jun = mushroom; Cao = grass) has been highlighted. The main characteristic in the Jun-Cao is the substitution of wood or fodder for grass and agricultural residues. The nutritive quality of the mushrooms cultivated through Jun-Cao overcame the nutritional value of mushrooms cultivated through classical methods (XI et al., 2012; ROLIM et al., 2014).

As with other mushrooms of commercial interest, the cultivation of Reishi through Jun-Cao may be an alternative for producers willing to make recyclable cultivations for which the prime matter may be obtained from materials that are easily found, such as grass, sugar cane bagasse, lime, sugar, etc. Besides that, after mushrooms are harvested, the substrate may be used as food for animals or fertilizer (URBEN et al., 2017). The purpose of this study was to compare the biochemical profile (sugars, proteins, β -glucans and phenols) of non-exploited Brazilian *Ganoderma lucidum* strains with commercial Chinese varieties using the Jun-Cao technique.

2 MATERIAL AND METHODS

2.1 Reishi strains

Four *Ganoderma lucidum* strains were used: two Brazilian, collected in the Amazonas state (identified as CC-144 and CC-157) and two Chinese (CC-22 and CC-63), all located in the Germplasm Bank of Mushrooms for Human Use of Embrapa Genetics Resources and

Biotechnology (Embrapa Cenargen), Brasilia, Federal District, Brazil. The “CC” initials means “Cenargen Collection”.

2.2 Cultivation

Cultures were seeded on Petri dishes containing PDA medium (Potato-Dextrose-Agar) and incubated at 28 °C to 30 °C until mycelia covered the agar surface after about 7 to 10 days. The strains were then transferred to a grass substrate with grass composed of: elephant grass (*Pennisetum purpureum*) 39%, mango tree fodder (*Mangifera indica*) 10%, rice bran (*Oryzae sativa*) 10%, sugar cane waste (*Saccharum officinarum*) 10% and agricultural lime 2%. After three months of cultivation, the crop was harvested, dehydrated, powdered and sent for biochemical analysis.

2.3 Total sugars

Sugars were extracted and analyzed as described by Ajlouni et al. (1995). The dry extract (3 g) was added to ethanol 80% (50 ml), and the suspension shaken for 45 minutes at room temperature and then filtered (Whatman #4 filter paper). The residue was washed five times with 5 mL of ethanol 80%, filtered again, dried and then solved in de-ionized water to a final volume of 10 ml. The aqueous extract was shaken and filtered using an Acrodisc-CR 0.45 µm and then subjected to HPLC (high performance liquid chromatography).

2.4 Proteins

Protein analysis was carried out using the classical Lowry Test (LOWRY et al., 1951). Initially, 10 g of the *Ganoderma lucidum* powder was added to 400 ml of water at 100 °C and left for 10 minutes. The mixture was filtered and 1 ml of the supernatant was added to 5 ml of the reagent C, made by mixing 0.5 ml of the solution B1 with 0.5 ml of the solution B2 and 49 ml of the solution A. Solution A was obtained by diluting 20 g of Na₂CO₃ in 1 L of NaOH 0.1 N. For solution B1, 10 g of CuSO₄ · 5H₂O was diluted in 1 L of H₂O. Solution B2 was made from 20 g of sodium (or potassium) tartrate diluted in 1 L of H₂O. The mixture of 1 ml of the supernatant with the reagent C was shaken and left to rest for 10 minutes. Then, 0.5 ml of Folin-Ciocalteu reagent (diluted 1:2 in water) was added to the mixture and it was shaken again. After 30 minutes the solution was analyzed in a spectrophotometer at 500 nm.

2.5 Phenol

Phenol was measured through the usual Phenol Test (SWAIN; HILLS, 1959). As for the protein analysis, 10 g of the Reishi powder was added to 400 ml of water previously warmed at 100 °C and left for 10 minutes. Then, 1 ml of the supernatant was mixed in 0.5 ml of Folin-Ciocalteu reagent (diluted 1:2 in water) and shaken. After 30 minutes, 1 ml of saturated sodium carbonate was added to the mixture, and then water was added until the total volume was 10 ml. This solution was kept resting for 1 hour and evaluated using a spectrophotometer at 500 nm.

2.6 β -Glucans

In order to evaluate β -Glucans, the method from Mizuno et al. (1998) was adopted, in which the mushroom dry powder was homogenized with liquid nitrogen in a Warning blender and then lyophilized. The lyophilized mushrooms (10 g) were extracted 6 times with 80% ethanol (100 ml) at 80 °C for 6 h. The residues after extraction with ethanol were extracted 4 times with hot water (200 ml) for 4 h and the filtered. The solution was lyophilized and stored. The fraction lyophilized (100 mg) was dissolved in 100 ml water. The solution was added to 5% trichloroacetic acid to precipitate the proteins and then centrifuged for 15 min at 120 g. The supernatant was concentrated to a small volume by evaporation and then lyophilized. After, 5 mg was submitted to chromatography on a DEAE-Sepharose CL-6B column (2.6 x 34 cm) with a stepwise elution of 300 ml of phosphate buffer 1 M (pH 7.2), 400 ml of the same buffer containing 0.25 M NaCl, and the 300 ml of the same buffer containing 1 M NaCl. The active fraction was further separated by a Sepharose 6B column (1.6 x 94 cm) equilibrated with 1 M phosphate buffer (pH 7.2). The column was eluted with the same buffer. The final sample was evaluated by spectrophotometer 500 nm.

2.7 Statistical analysis

The one-factor ANOVA was used to determine whether the difference in the concentrations of sugars, proteins, phenols and β -glucan from the different samples differed significantly. Tukey's test was applied *a posteriori* in order to determine which strains generated this statistical difference. The CC-22, a well known commercial strain used in China, was taken as a reference standard. All results were based on an average of five repetitions per strain. The statistical software used was SISVAR 5.3 for Windows.

3 RESULTS

The one-factor ANOVA showed significant variations between the four strains and the Tukey test revealed significant differences between Chinese and the Brazilian strains in some aspects (Table 1).

Table 1. Comparison of biochemical elements contained in four strains of *Ganoderma lucidum* cultivated by the Jun-Cao technique

Strain	Composition (mg/g)			
	Total sugar	β -Glucans	Proteins	Phenols
CC-22	583.6 (a2)	48.12 (b1)	1.64 (c1)	0.473 (d1)
CC-63	421.74 (a1a2)	40.52 (b1)	1.28 (c1)	0.457 (d1)
CC-144	290.16 (a1)	93.16 (b2)	1.86 (c1)	0.451 (d1)
CC-157	304.62 (a1)	56.54 (b1)	1.70 (c1)	0.513 (d2)

Means followed by the same letter define the same biochemical analysis between different samples. Equal numbers indicate that there is not statistically significant difference in the strains when compared under the same parameter. (Turkey, 5%; total sugar: LSD* = 199.13 and CV** (%) = 19.03; β -glucans: LSD = 33.43 and CV (%) = 21.45; proteins: LSD = 0.61 and CV (%) = 14.43; phenols: LSD = 0.04 and CV (%) = 3.17). *LSD (Least Significant Difference); **CV (Coefficient of variation).

The Chinese strains CC-22 and CC-63 contain a higher concentration of total sugars when compared to Brazilian varieties CC-144 and CC-157. *Ganoderma lucidum* CC-22 presented better sugars concentration than the other ones (average of 583.6 mg g⁻¹), while the Brazilian strains showed lower concentrations. CC-63 presented an average value of 421.74 mg g⁻¹, whereas CC-144 and CC-157 had average concentration of 290.16 and 304.62 mg g⁻¹, respectively.

The Brazilian strain CC-144 showed the highest concentration of β -glucans (93.16 mg g⁻¹), being the difference statistically significant when compared to other three strains. The Chinese strains CC-22 and CC-63, and the Brazilian CC-157 presented, respectively 48.12, 40.52 and 56.54 mg g⁻¹, with no significant difference among them.

Out of the four tested strains, CC-144 had a significantly higher protein concentration (average of 1.86 mg g⁻¹) than the lowest, CC-63 (1.28 mg g⁻¹). CC-22 and CC-157 presented, respectively, 1.64 and 1.70 mg g⁻¹. The statistical analysis showed significant difference only among CC-63 and others strains.

The strain CC-157 had the highest concentration of phenols among the tested strains (0.513 mg g⁻¹), while CC-144 had the lowest (0.451 mg g⁻¹). CC-22 and CC-63 presented, respectively, 0.473 and 0.457 mg g⁻¹. Significant difference about concentration of phenols was present only among CC-157 and others strains.

4 DISCUSSION

To Huihua et al. (2013), Reishi shows variations in biochemical composition. The variability may be due to differences in the origin, strain and cultivation conditions. Mycelia samples provided higher sugars concentration than fruiting body and spores, probably due to its incorporation from the culture media, as sugars are more available in this situation (*in vitro* growth) than in *in vivo* conditions (BARROS et al., 2008).

The sugar concentrations of the four *Ganoderma lucidum* strains were similar to those found in other edible mushrooms (BISHOP et al., 2015; TAOFIQ et al., 2017). The carbohydrate content was lower than that reported in an earlier study (OGBE; OBEKA, 2013) but the amount was higher than that reported in another study on Reishi. The same range was observed in the case of other mushrooms such as *Agaricus bisporus*, the most popular edible mushroom, *Calocybe indica* and *Lentinus edodes* (PUSHPA; PURUSHOTHOMA, 2010).

Kim et al. (2009) tried to establish a link between the total carbohydrates in edible and medicinal mushrooms, explaining that medicinal mushrooms tend to possess half of the carbohydrate concentration present in edible mushrooms, but this was not observed in the *Ganoderma lucidum* strains studied in this work. In the total carbohydrates observed, 10 to 15% were represented by soluble sugars, and the other part was divided into structural carbohydrates (e.g. chitin), glucogen and other polysaccharides, such as β -glucans.

The β -glucans result is in agreement with the previous research in which polysaccharides are isolated from Reishi (MA et al., 2018; ZHANG et al., 2019). Sugars, as the carbon sources, can influence mycelium growth and polysaccharide production (HSIEH et al., 2006). It is known that lactose exerts more favorable impact on cell growth and production of intercellular polysaccharide than glucose. The effect of different carbon sources on the biomass production in *Ganoderma lucidum* was reported by Avtonomova et al. (2006), Skalicka-Wozniak et al. (2012) and Sargowo et al. (2018).

Baabitskaia et al. (2005) maintain that polysaccharide production by *Ganoderma lucidum* depends on such conditions as the initial pH of the substrate, C:N ratio and incubation temperature. The influence of substrate pH on polysaccharide production by Reishi was also reported by Kim et al. (2006). According to Doczekalska and Zborowska (2010), wood of various species differs with regard to the content of cellulose, lignin, hemicellulose, sugars, protein as well as other substances and shows differences in pH. It is possible that these properties led to differences in the polysaccharide content of the fruiting bodies of *Ganoderma lucidum* obtained from the substrates used.

Edible mushrooms often possess a high concentration of proteins, around 19 to 35% of their dry weight, whereas *Ganoderma lucidum* showed lower amounts, between 7 and 8% as is typical for medicinal species (ERJAVEC et al., 2012). Low protein concentration in medicinal mushrooms was previously reported (MAU et al., 2001). On the other hand, carbohydrates, fibers and mineral salts are found in high levels among medicinal mushrooms (TAOFIQ et al., 2017; SALTARELLI et al., 2019).

Natural antioxidants have been proved to be effective protectors of body from the adverse effects of free radicals caused oxidative stress. Mushrooms are found to be rich source of these antioxidants with immense antiradical activity (SARGOWO et al., 2018). Reishi with wider medicinal values also contains rich antioxidants. Kim et al. (2008) reported a lower amount of phenolic compounds in a sample of *G. lucidum* from Korea (0.162 mg/g), as also the presence of different phenolic compounds, such as other phenolic acids and derivatives (gallic acid, protocatechuic acid, 5-sulfosalicylic acid and pyrogallol).

Several mushrooms have been reported to contain high levels of these compounds. In a study with several mushrooms 63 mg g⁻¹ of phenolics from hot water extracts of *Ganoderma lucidum* was reported (ABDULLA et al., 2012). Low level of phenolics might be due the solvent. It is reported that the polarity of the extraction solvent affect the level of phenolics. Higher extraction yields of phenolics were noted with increased polarity (PUTTARAJU et al., 2006).

Tsai et al. (2009) have stated that the total phenol concentration found in edible mushrooms is around 5 mg g⁻¹, so the levels in Reishi were an order of lower magnitude. It is important to mention that phenols found in mushrooms possess antioxidant properties (ZHANG et al., 2016). Due to their characteristics on combating free radicals and their role in reducing ferrous ions, phenols may be considered not only good antioxidants, but also good anti mutants and anti carcinogenics (COR et al., 2014). Tsai et al. (2009) also describe a relationship between the phenolic compounds of edible mushrooms and their antioxidant action, trying to establish the way these biochemical compounds act. In addition, Dubost et al. (2007) developed good correlation between the superoxide radical and the phenols absorbance capacity. However it is not possible to establish the specific amount of phenols that would be enough for a satisfactory antioxidant activity in organisms.

5 CONCLUSION

The results show the Amazonian strains as a good choice for fungiculture, due to their biochemical potential, although they are not known commercially. Moreover, this work contributes to establishing a profile for the Brazilian Reishi (*Ganoderma lucidum*) strains, serving as a stimulus for further studies.

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