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# Growth and proximate composition of *Pleurotus ostreatus* cultivated on green bocaiuva pulp substrates with different nitrogen sources

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**ABSTRACT.** The aim of this work was to evaluate the growth and the proximate composition of the mycelium-based bocaiuva pulp with the edible mushroom *Pleurotus ostreatus* on green bocaiuva flour added with different sources of nitrogen (urea, ammonium nitrate and sulfate ammonia). Growth was monitored by kinetics. At the end, the proximate composition of the best three treatments (dehydrated green bocaiuva pulp and water, T1; dehydrated green bocaiuva pulp and ammonium nitrate, T3; and green bocaiuva pulp/wheat bran and ammonium nitrate, T7) was determined. Ammonium nitrate was the nitrogen source that showed the greatest growth in both substrates (T3:8.33 cm and T7:7.67 cm) in relation to the other treatments (4.67 to 7.17 cm), with emphasis on the green bocaiuva pulp. The substrate with green bocaiuva pulp and water was the one that showed the highest growth (7.50 cm), which was close to the treatment with mixed substrate and ammonium nitrate (7.67 cm). The treatment with the green bocaiuva pulp and ammonium nitrate (T3) was highlighted due to its significant increase in proteins (9.42 g 100 g<sup>-1</sup>) and fibers (5.21 g 100 g<sup>-1</sup>), and decrease in carbohydrates (9.52 g 100 g<sup>-1</sup>), in comparison to the other treatments T7 (8.94, 2.16, and 5.99 g 100 g<sup>-1</sup>, respectively) and T1 (2.78, 4.33, and 2.28 g 100 g<sup>-1</sup>, respectively). The product obtained from the growth of *P. ostreatus* in green bocaiuva pulp presents promising perspectives to be utilized as raw material for the development of new food products with added nutritional value.

**Keywords:** *Acrocomia*; edible mushroom; nitrogen source; proximate composition and solid-state cultivation.

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## Introduction

The consumption of fruits and vegetables has been stimulated in many countries because it is associated with a lower incidence of mortality from chronic non-communicable diseases (NCDs) (Miller et al., 2017; Rosário et al., 2018; Uddin, Lee, Khan, Tremblay, & Khan, 2020). Among them, cardiovascular diseases, diabetes, cancer and chronic respiratory diseases are prevalent. They are accentuated by smoking, physical inactivity, inadequate diet and alcohol use (Duncan et al., 2012; Pengpid & Peltzer, 2017). On the other hand, some of these diseases can be minimized or even prevented by changing eating habits, e.g. incorporating the daily consumption of fruits and vegetables (Raigond et al., 2018; Yuan et al., 2018; Jezewska-Zychowicz, Gębski, Plichta, Guzek, & Kosicka-Gębska, 2019) with high content of vitamins, minerals, dietary fiber (Fayet-Moore, Petocz, McConnell, Tuck, & Mansour, 2017; Arun, Thomas, Reshmitha, Akhil, & Nisha, 2017; Volpe 2019), and low glycemic indexes (Obboh et al., 2015; Raigond et al., 2018).

In the Brazilian savanna biome are found species of native or adapted fruit plants with nutritional and therapeutic properties, depending on the primary and secondary compounds derived from the plant's metabolism, as well as, by the peculiar aroma and flavor that make them attractive for consumption (Reis & Schmieles, 2019). Among these species *Acrocomia* sp. stands out, whose fruits are commonly known as bocaiuva, macauba or macaiba (Vianna, Berton, Pott, Guerreiro, & Colombo, 2017). These fruits provide mature pulp rich in lipids, carbohydrates, fibers (Bora & Rocha, 2004; Ramos, Ramos Filho, Hiane, Braga Neto, & Siqueira, 2008; Orsi, Nishi, Carvalho, Brandão, Carvalho, & Asquiere, 2015), copper, iron, manganese, potassium and zinc (Ramos et al., 2008; Gonçalves et al., 2020) being able to supply the nutritional needs of human beings through in natura or processed ingestion (Ramos et al., 2008; Oliveira et al., 2020). In addition,

the pulp is rich in  $\beta$ -carotene (Ramos et al., 2008; Coimbra & Jorge, 2011; Orsi et al., 2015) and  $\alpha$ -tocopherol (Coimbra & Jorge 2011), which are important carotenoids due to their antioxidant action and anti-inflammatory effect (Costa, Buccini, Arruda, Favaro, & Moreno, 2020; Lescano, Iwamoto, Sanjinez-Argandoña, & Kassuya, 2015). Most scientific studies on the nutritional and functional properties of the genus *Acrocomia* refer to the species *A. aculeata*. Studies with other species, e.g. *A. totai* are scarce and indispensable to confirm their properties, increase their value and contribute to the preservation of palm trees, as they are often deforested for agricultural purposes in the region.

The bociuiva pulp has important nutritional, sensory and functional characteristics for health, thus being considered a promising source for the food industry (Orsi et al., 2015; Valério, Celayeta, & Cren, 2019). The pulp flour from the fruit of green *A. aculeata* is rich in soluble fibers and has been shown to reduce body weight, the lipid activity in the fractions of total cholesterol, LDL-cholesterol, and triglycerides, and the fasting glucose in male mice (Giunco, 2018). However, it is a fruit little used in human food, mainly in the green maturation stage. Therefore, its consumption can be favored from the bioconversion of the green bociuiva pulp by edible mushrooms in order to obtain food products with low carbohydrate content and enriched in proteins and fibers, that are accepted by most of the consumers (Ritota & Manzi 2019).

Mushrooms can contribute to the prevention of some diseases, such as hypertension (Vaz et al., 2011), cholesterol (Wei, Yue, Zhang, & Lu, 2018; Liu, Wang, Mei, Li, & Liang, 2019), diabetes (Asrafuzzaman et al., 2018; Liu et al., 2019; Khatun, Sato, & Konishi, 2020; Balaji et al., 2020), stress (Akata, Ergonul, & Kalyoncu, 2012), obesity (Khatun et al., 2020) and acting as an appetite suppressant (Sheng et al., 2019).

*Pleurotus ostreatus* is an edible oyster mushroom that has a light color (white, gray or brown) and a basidiocarp with a fleshy leaf shape (Jonathan & Esho, 2010). *Pleurotus* are a source of nutrients, mainly proteins, minerals and vitamins B, C and D (Panjikkaran & Mathew, 2013). This mushroom contains 20-35% protein (dry weight) and low levels of essential lipids and amino acids (Li, Zhang, Li, Li, & Sun, 2017; Lavelli, Proserpio, Gallotti, Laureati, & Pagliarini, 2018). Due to its low caloric value it can be included in diets with controlled calorie intake (Jaworska & Bernás, 2009).

Pectocellulosic substrates, such as peels, pulps and fruit pomace, present favorable conditions for the cultivation of edible mushrooms, as they have a lower carbon: nitrogen ratio and high concentrations of simple sugars, which facilitates their availability to the fungus (Rivas, Pereira Filho, Santos, & Rosa, 2010; Cardoso, Demenjour, & Paz, 2013; Silva, Lacerda, Leite, & Fonseca, 2014; Silva et al., 2020). The adaptation of *Pleurotus* species to new substrates represents one of the main bioconversion processes in food products with added value and nutritional quality (Mbassi, Mobou, Ngome, & Sado, 2018; Ritota & Manzi, 2019). Therefore, the use of fruit substrates for the cultivation of edible mushrooms makes it a viable alternative for the nutritional enrichment of these products and the enhancement of species within a productive chain, with permanent and sustainable production (Carrasco-González, Serna-Saldívar, & Gutiérrez-Uribe, 2017; Ritota & Manzi, 2019).

Thus, the aim of this work was to evaluate the growth and nutritional properties of the myceliated bociuiva pulp with the edible mushroom *P. ostreatus* cultivated on green bociuiva flour added with different sources of nitrogen, in order to obtain a product with a low content of carbohydrates and rich in proteins and fibers.

## Material and methods

### Microorganism and substrate

*Pleurotus ostreatus* URM 4072 was obtained from the Fungal Culture Collection of the *Universidade Federal de Pernambuco*. The cells were received lyophilized and, after reactivation, kept in a medium inclined potato dextrose agar (PDA) immersed in mineral oil and stored at 4°C (Fonseca, Gandra, Sclowitz, Antunes, & Costa, 2009; Silva, Lacerda, & Fonseca, 2013). The strain was grown in Petri dishes containing Sabouraud 4% glucose agar medium at 30°C for 10 days to reactivate the mycelial growth.

The substrates utilized were the dehydrated green bociuiva pulp and the mixture of dehydrated green bociuiva pulp (50%) and wheat bran (50%). The bociuiva fruits (*Acrocomia totai* Mart) were collected in the municipality of Bela Vista-MS (56° 24'12.6" West longitude, 21° 56'15.9" South latitude, and 153 m altitude), Brazil.

After harvested, they were washed and sanitized with sodium dichloroisocyanurate 0.66% (w v<sup>-1</sup>) solution (Sumaveg de Diversery Lever) with 200 ppm of active chlorine for 15 min. Then, the fruits were manually peeled and mechanically pulped in a device to pulp bociuiva fruits (Chuba, Silva, Santos, & Sanjinez-

Argandoña, 2019). The pulp obtained was dehydrated in a circulation oven, with drying air speed of  $1 \text{ m s}^{-1}$  at  $50^\circ\text{C}$  for 24h. Wheat bran was purchased from a local market in the city of Dourados-MS.

### Inoculation, cultivation and mycelial growth

The experiments were carried out with 2 pulp combinations: dehydrated green bocaiuva pulp (BP) or dehydrated green bocaiuva pulp plus wheat bran (BPWB) in a 1: 1 w w<sup>-1</sup> proportion. For each combination of pulps, experiments were carried out without the addition of a nitrogen source, but only distilled water, or with the addition of 2% external nitrogen sources: urea (U), ammonium nitrate (AN), and ammonium sulfate (AS), resulting in eight treatments (Table 1). Each treatment was placed into 50 mL test tubes, sterilized in an autoclave at  $121^\circ\text{C}$  for 20 min. and inoculated with 5 cm discs of myceliated agar. The tubes were incubated at  $30^\circ\text{C}$  and the axemic growth of the fungus was measured every 2 days with a calibrated ruler (Ilyas & Avin, 2018).

**Table 1.** Treatments for growth experiments.

Treatment	Pulp mixture	External N source
T1	BP	-
T2	BP	U
T3	BP	AN
T4	BP	AS
T5	BP/WB	-
T6	BP/WB	U
T7	BP/WB	AN
T8	BP/WB	AS

BP: green bocaiuva pulp; BP/WB: green bocaiuva pulp/wheat bran; U: urea; AN: ammonium nitrate; AS: ammonium sulfate.

### Proximate composition

The proximate composition was determined for the dehydrated green bocaiuva pulp (BP), the wheat bran (WB), the mixture of green bocaiuva pulp and wheat bran (BP/WB) and the three best experiments with mycelial growth. The moisture content was determined by using an oven with air circulation at  $70^\circ\text{C}$  (Method n° 44-15.02, American Association of Cereal Chemists [AACC], 2010), the mineral residue by weighing the residues from muffle incineration at  $550^\circ\text{C}$  (Method n° 08-01.01, AACC, 2010), the proteins by the Kjeldahl procedure (Method n° 2001.11, AOAC 2005), the total lipids by using the Soxhlet extractor (Method n° 30-25.01, AACC, 2010), and the crude fiber by the gravimetric method using a fiber determiner (Method n° 978.10, Association of Officiating Analytical Chemists [AOAC] 2005). The determination of the carbohydrates was performed by difference (= total - moisture - minerals - lipids - proteins - fibers), according to Silva et al., 2014.

### Energetic value and proximate composition variation (PCV)

The energy value was calculated using the Atwater coefficients that consider  $4 \text{ kcal g}^{-1}$  of sample for proteins and carbohydrates and  $9 \text{ kcal g}^{-1}$  of sample for lipids (Merril & Watt, 1973). The proximate composition variation of the cultivated media for proteins, lipids and fibers was calculated by the percentage difference between the concentration presented in the cultivated medium (greater accumulation) and treated medium (inoculated) (Equation 1), according to Fonseca et al. (2009).

$$\text{PCV (\%)} = \left( \frac{\text{Final content (\%)}}{\text{Initial content (\%)}} \times 100 \right) - 100 \quad (1)$$

### Statistical analysis

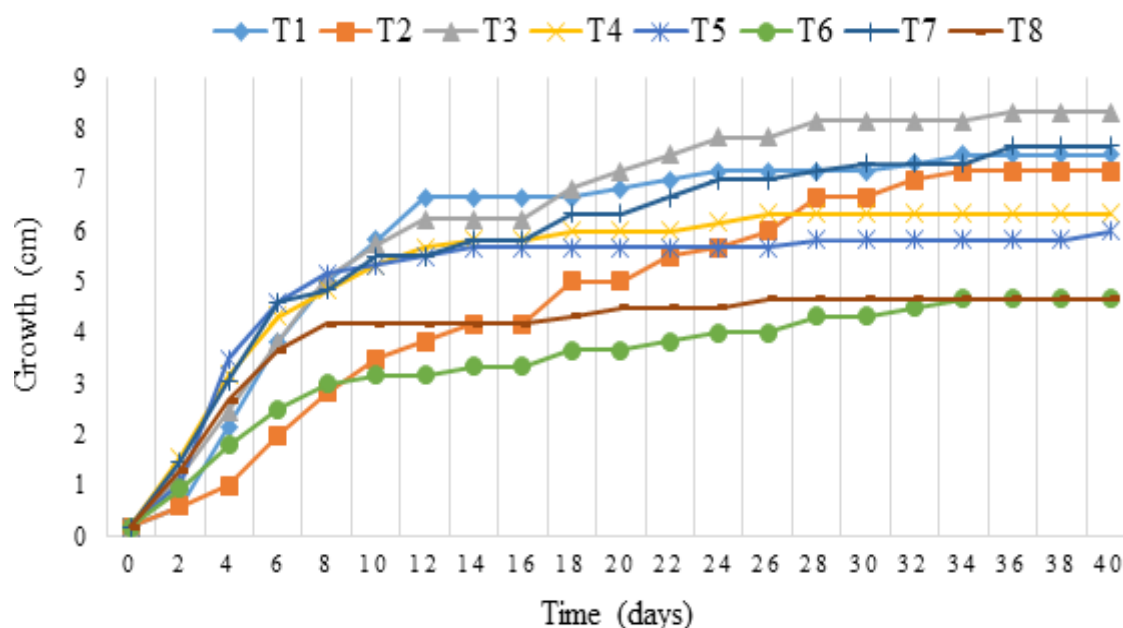
The experiments were carried out in triplicate and the results expressed as mean and standard deviation. Analysis of variance (ANOVA) and Tukey's multiple comparison test ( $p < 0.05$ ) were calculated using Statistica version 8.0 software (StatSoft, Inc, Tulsa, USA).

## Results and discussion

### Mycelial growth

Figure 1 shows the growth curves of *P. ostreatus* in dehydrated green bocaiuva pulp and the mixture of dehydrated green bocaiuva pulp and wheat bran substrates without the addition of a nitrogen source and with different nitrogen sources (urea, ammonium nitrate, and ammonium sulfate) for 40 days. In all treatments,

colonization was observed after two days of inoculation, as reported elsewhere (Patel, Gupte, & Gupte, 2009). The growth was evident up to the 30<sup>th</sup> day. After that, a stationary phase was observed.



**Figure 1.** Growth curves of *Pleurotus ostreatus*. T1: dehydrated green bocaiuva pulp (BP) and water; T2: dehydrated green bocaiuva pulp (BP) and Urea (U); T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN); T4: dehydrated green bocaiuva pulp (BP) and ammonium sulfate (AS); T5: green bocaiuva pulp/wheat bran (BP/WB) and water; T6: green bocaiuva pulp/wheat bran (BP/WB) and urea (U); T7: green bocaiuva pulp/wheat bran (BP/WB) and ammonium nitrate (AN); T8: green bocaiuva pulp/wheat bran (BP/WB) and ammonia sulphate (AS).

Ammonium nitrate was the nitrogen source that showed the greatest growth in the two substrates (T3: 8.33 cm and T7: 7.67 cm) compared to the other treatments (4.67 to 7.17 cm), especially for the green bocaiuva pulp. In relation to the control treatments, it can be observed that the substrate with green bocaiuva pulp and water showed the greatest growth (7.50 cm), which was close to that observed for the treatment with ammonium nitrate and a mixture of green bocaiuva pulp and wheat bran (T7: 7.67 cm). There was no significant difference ( $p < 0.05$ ) in relation to all treatments after 30 and 40 days of cultivation.

The type of substrate utilized influences on the chemical, functional and sensory characteristics of the mushrooms (Mbassi et al., 2018; Lavelli et al., 2018; Pazza, Zardo, Klein, Cas, & Bernardi, 2019; Valenzuela-Cobos et al. 2019). *Pleurotus* sp. extract the nutrients from the substrate through the mycelium, obtaining substances necessary for its development, such as carbon, nitrogen, vitamins and minerals (Lavelli et al., 2018; Bellettini et al., 2019). The nutritional content of the substrates can be improved by supplementing it with nitrogen (Nunes et al., 2012).

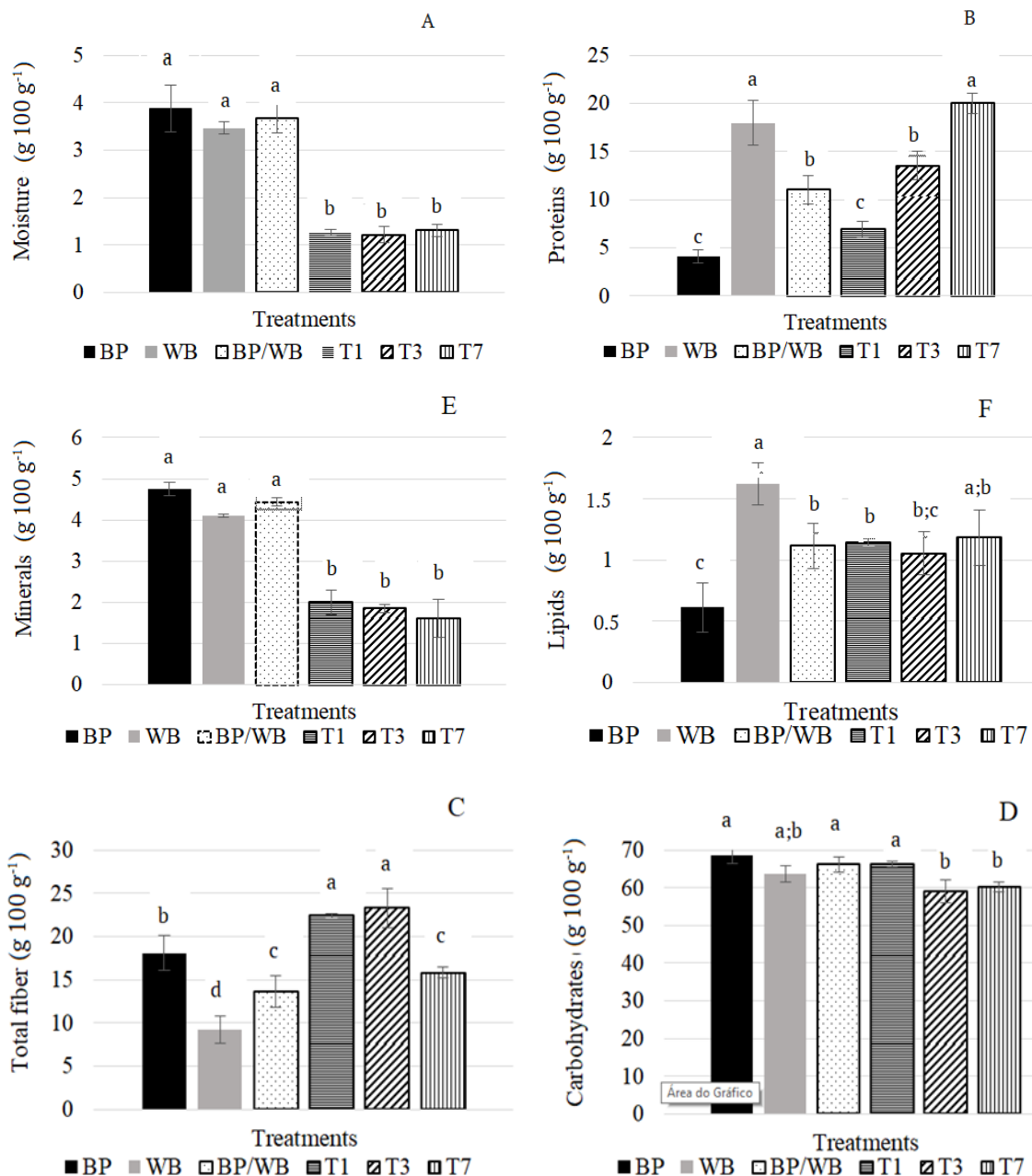
The nitrogen source is an important factor in the synthesis of proteins, nucleic acids, purines, pyrimidines and polysaccharides (Drozdowski et al., 2010; Abdullah, Lau, & Ismail, 2015). Some studies have pointed out that nitrogen supplementation can increase productivity yield, but up to a certain level, as high nitrogen values can inhibit the fructification of *Pleurotus* sp. (Silva, Dias, Siqueira, & Schwan, 2007). Thus, supplementation with 2% ammonium nitrate in the green bocaiuva pulp-based substrate was efficient for the mycelial growth of *P. ostreatus*.

### Proximate composition

The results obtained for the proximate composition of wheat bran, dehydrated green bocaiuva pulp and the three best treatments with dry mycelial growth are shown in Figure 2. It is observed that the wheat bran, the green bocaiuva pulp and the treatments presented moistures of  $1.22 \pm 0.17$  to  $3.88 \pm 0.49$  g 100 g<sup>-1</sup> (Figure 1A). According to the Brazilian legislation it should not exceed 15 g 100 g<sup>-1</sup> (Agência Nacional de Vigilância Sanitária [Anvisa], 2005a).

The type of substrate and the nitrogen source utilized for the cultivation of *Pleurotus* sp. influenced directly on the final proximate composition of the products. The main proximate components that had a significant increase ( $p < 0.05$ ) were proteins and fibers for all treatments, in relation to the initial composition, without miceliation (BP). It also underlines the treatment with green bocaiuva pulp and ammonium nitrate (T3) due to its significant increase in proteins (9.42 g 100 g<sup>-1</sup>) (Figure 2B) and fibers (5.21 g 100 g<sup>-1</sup>) (Figure 2C), in

comparison to the other T7 treatments (8.94 and 2.16 g 100 g<sup>-1</sup>, respectively) and T1 (2.78 to 4.33 g 100 g<sup>-1</sup>, respectively) (Figure 2B and 2C). The variation in protein content can be explained by the addition of a nitrogen source (ammonium nitrate) in the substrate. In accord with it, it was reported elsewhere that the protein enrichment of pineapple peels by using *Trichoderma viride* was higher, when there was a better aeration and the addition of ammonium sulfate as nitrogen source (Aruna, 2019).



**Figure 2.** Proximate composition for different cultivation treatments with *Pleurotus ostreatus*.

BP: dehydrated green bocaiuva pulp without increment; WB: wheat bran without increment; BP/WB: green bocaiuva pulp/wheat bran without increment; T1: dehydrated green bocaiuva pulp (BP) and water; T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN); T7: green bocaiuva pulp/wheat bran (BP/WB) and ammonium nitrate (AN).

The increase in fiber content during the solid state bioprocess may be associated with the fungal cell wall, as it is composed of chitin fibers, which is a polysaccharide consisting of a long chain of N-acetylglucosamine, insoluble in water (Garcia-Rubio, Oliveira, Rivera, & Trevijano-Contador, 2020). The cell wall of filamentous fungi contains chitin in the proportion of 10 to 20%. These structures withstand great pressure and, thus, become responsible for the integrity of the cell wall. When chitin synthesis is interrupted, the cell wall becomes disorganized and the fungal cell undergoes deformations and osmotic instability (Bowman & Free, 2006).

It was demonstrated that the soluble dietary fiber in nejayote increased 45% after solid state cultivation with *P. ostreatus* Perla, improving the bioavailability of the fiber source as a functional ingredient (Acosta-Estrada, Villela-Castrejón, Perez-Carrillo, Gómez-Sánchez, & Gutiérrez-Urbe, 2019).

The use of *P. ostreatus* in the bioconversion of green pulp is an alternative to produce improved foods with added value for disease prevention due to the enrichment of the nutrient content by the fungus.

According to technical regulations, T1 can be considered as a protein source and T3 and T7 as high protein content sources, all with high fiber content (Agência Nacional de Vigilância Sanitária [Anvisa], 2012). It is recommended the daily consumption of 50 g of protein by adults and 34 g by children up to 10 years old (Agência Nacional de Vigilância Sanitária [Anvisa], 2005b). In this sense, the intake of 100 g of T3 provides about 27 to 40% of the daily protein requirement. It also provides about 93% of the daily recommendation of fiber for adults by FAO (United Nations Food and Agriculture Organization) and WHO (World Health Organization), being that at least 25 g of fibers per day is considered enough to prevent chronic diseases (World Health Organization and Food/Agriculture Organization [WHO/FAO], 2003).

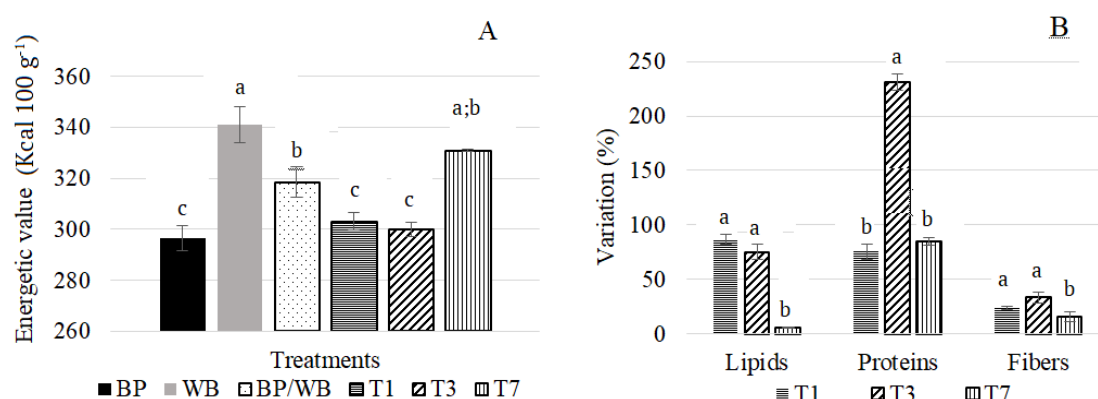
Regarding the carbohydrate content, there was a significant reduction for all treatments (Figure 2D) being greater in T3 (9.52 g 100 g<sup>-1</sup>), followed by T7 (5.99 g 100 g<sup>-1</sup>) and T1 (2.28 g 100 g<sup>-1</sup>), in relation to the initial composition, without miceliation (BP). The carbohydrates consumed were used as sources of carbon and energy for growth and synthesis of other compounds (Fonseca et al., 2009; Silva et al., 2013).

The minerals showed a decrease in the contents for all treatments compared to the initial characterization (2.75, 2.89, and 2.81 g 100 g<sup>-1</sup> for T1, T3, and T7, respectively) (Figure 2E). This decrease may be due to leaching, as a result of microbial activities that some minerals are lost or used during solid state fermentation by *P. ostreatus* (Adebayo, Ogidi, & Akinyele, 2019). The range of values found in the present study (1.62 to 2.00 g 100 g<sup>-1</sup>) is below to that reported by Silva et al. (2014), with minerals reaching up to 10.91 g 100 g<sup>-1</sup>. These contents can vary depending on the fungal species and the substrate utilized.

The treatments had an increase of 0.06 to 0.53 g 100 g<sup>-1</sup> of lipids in relation to the initial characterization (Figure 2F). The increase in lipids may have occurred due to the production of enzymes during mycelial growth, as these lipids are intended for the construction of the cell wall of fungi (Fonseca et al., 2009; Athenaki et al., 2017) and it is also associated with the fact that some fungi species are able to accumulate lipids during the bioprocess (Dulf, Vodnar, & Socaciu, 2016; Dulf, Vodnar, Dulf, & Pinte, 2017; Araújo et al., 2020).

### Energetic value and proximate composition variation

It can be observed that the treatments with green bocaiuva pulp (T1 and T3) had lower energy value in relation to the experiment with the mixture of bocaiuva pulp and wheat bran (T7) (Figure 3A). This behavior may be related to the increase in the protein content, indicating that the microorganism efficiently utilized the source of exogenous nitrogen to the bioconversion of carbohydrates and some fibers into protein.



**Figure 3.** Energetic value and composition variation for lipids, proteins and fibers for different cultivation treatments with *Pleurotus ostreatus*. BP: dehydrated green bocaiuva pulp without increment; WB: wheat bran without increment; BP/WB: green bocaiuva pulp/wheat bran without increment; T1: dehydrated green bocaiuva pulp (BP) and water; T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN); T7: green bocaiuva pulp/wheat bran (BP/WB) and ammonium nitrate (AN).

An expressive positive protein variation (enrichment) was observed for all treatments, mainly for T3 with 231.12%, followed by T7 (84.44%) and T1 (75.17%) (Figure 3B). These results demonstrate that T3 was superior

in terms of protein enrichment than e.g. the fruit residues of *Caryocar brasiliense* (160.04%), *Annona crassiflora* (143.31%), *Campomanesia pubescens* (102.42%), and the T1 (75.17%) presented itself higher to the substrate *Acrocomia aculeata* (67.88%) with *Lichtheimia ramosa* (Silva et al., 2014). Regarding the lipid variation, it can be observed that T1 presented a higher percentage (86.76%) than that observed for T3 (75.08%) and T7 (5.57%). This enrichment was greater than that reported by Fonseca et al. (2009) who obtained 66.70% of variation using *P. ostreatus* in cultivations containing a mixture of rice bran, rice straw and sof rush. The fibers had a variation of 15.88, 24.00, and 33.51%, for T7, T1 and T3, respectively. In the study by Zusman et al. (1997) it can be observed increases up to 78% in the fiber content in *P. ostreatus* fungi grown on corn cobs.

## Conclusion

The addition of ammonium nitrate as nitrogen source in the T3 treatment contributed to a better mycelial growth and improved the bioconversion of carbohydrates from the green pulp of *P. ostreatus* into proteins, fibers and lipids, which is promising for the development of new food products with nutraceutical added value.

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