Production of commercial and Amazonian strains of *Pleurotus ostreatus* in plant waste

Produção de linhagens comerciais e amazônicas de *Pleurotus ostreatus* em resíduos de plantas

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ABSTRACT

Pleurotus spp. have the ability to grow on a wide variety of lignocellulosic materials, which opens up a range of options to be explored. The use of locally available residues and native strains can reduce production costs, thus making fungiculture a potential economic activity in developing regions, such as northern Brazil. The aim of this research was to compare the production and nutritional characteristics of different strains of *P. ostreatus*, cultivated on lignocellulosic residues available in Amazonas state, Brazil. The

native (474 and 1467) and commercial (542 and 885) strains were cultivated in marupá (*Simarouba amara*) and pine (*Pinus* sp.) sawdust, açaí (*Euterpe oleracea*) seeds and elephant grass (*Pennisetum purpureum* Schum.) straw. Productivity was evaluated according to biological efficiency, yield and loss of organic matter. Some physicochemical and nutritional parameters were also evaluated. In general, among the residues evaluated for mushroom cultivation, the elephant grass substrates (EG) showed better physicochemical properties in terms of moisture, ash content (minerals), hemicellulose and lignin. Additionally, the EG-based substrate resulted in the best productive parameters (yield and EB) for all strains studied, with emphasis on strain 1467, which is native to the Amazon. The basidiocarps of the native strains (474 and 1467) presented a nutritional composition that is similar to the commercial strains (885 and 542), and the mushrooms grown in EG-based residue, in general, presented the best nutritional compositions. Thus, strain 1467 of *Pleurotus ostreatus*, native to the Amazon, shows promise for commercial purposes, when cultivated in elephant grass residue.

Keywords: edible mushrooms, bioconversion, biological efficiency, solid fermentation, alternative substrates, basidiomycetes.

RESUMO

Pleurotus spp. têm capacidade de crescer em uma grande variedade de materiais lignocelulósicos, abrindo um leque de opções para serem explorados. A utilização de resíduos disponíveis localmente e de linhagens nativas podem reduzir os custos de produção, colocando a fungicultura como uma atividade econômica em regiões em desenvolvimento. O objetivo desta pesquisa foi comparar a produção e características nutricionais de diferentes linhagens de *P. ostreatus*, cultivadas em resíduos lignocelulósicos disponíveis localmente. As linhagens nativas (474 e 1467) e comerciais (542 e 885) foram cultivadas em serragem de marupá (MS) e pinus (PS), sementes de açaí (AS) e palha de capim-elefante (EG). A produtividade foi avaliada a partir da eficiência biológica (EB), rendimento e perda de matéria orgânica (PMO). Alguns parâmetros físico-químicos e nutricionais também foram avaliados. Em geral, dentre os resíduos avaliados para o cultivo de cogumelos, os substratos à base de EG apresentou melhores propriedades físico-químicas quanto à umidade, conteúdo de cinzas (minerais), hemicelulose, lignina. Adicionalmente, o substrato EG resultou nos melhores parâmetros produtivos (rendimento e EB), para todas as linhagens estudadas, com ênfase para 1467, nativa da Amazônia. Os basidiomas das linhagens nativas (474 e 1467) apresentaram composição nutricional semelhantes às linhagens comerciais (885 e 542), sendo os cogumelos cultivados em resíduo de EG, em geral, os que apresentaram as melhores composições nutricionais. Desta maneira, a linhagem 1467 de *Pleurotus ostreatus*, nativa da Amazônia, mostra-se promissora para fins comerciais, quando cultivada em resíduo de capim-elefante.

Palavras-chave: cogumelos comestíveis, bioconversão, eficiência biológica, fermentação sólida, substratos alternativos, basidiomicetos.

1 INTRODUCTION

The Amazon has a vast biodiversity of living organisms, including fungi (Pereira et al., 2017). In nature, fungi play a fundamental role in nutrient cycling, mainly in the

degradation of lignin and cellulose from plant substrates (Soares et al., 2014). Among these fungi, *Pleurotus* is one of the genera of macrofungi that occur in the Amazon Rainforest; however, little is known about the diversity and potential of native strains, which have several different genotypes and are adapted to local growth conditions, thus allowing cultivation at different times of the year (Yamashita and Hirose, 2016; Melanouri et al., 2022).

The genus *Pleurotus* is the second most cultivated mushroom in the world due to its adaptability in nature or in artificial development since it presents low demands on the cultivation substrate, and is able to be produced in different temperature conditions and in the most varied lignocellulosic materials (Melanouri et. al., 2022).

The main residues used in mushroom cultivation are wheat and rice straw, cotton husk, corn cob, rice and wheat bran (Jeznabadi et al., 2016). However, other alternative substrates have also been applied in order to maximize mushroom production. Additionally, using locally available waste products not only reduces mushroom production costs, but also helps in the recycling of locally discarded vegetable waste, thus reducing their environmental impacts and making this a sustainable practice (Sadh et al., 2018).

Among some of the residues available in the state of Amazonas, Brazil, which come from agro-industrial and wood processing and have potential for use as a substrate in mushroom cultivation, elephant grass (*Pennisetum purpureum* Schum.), pine (*Pinus* sp.), marupá (*Simarouba amara*) and açaí (*Euterpe* sp.) can be cited. Elephant grass is an important forage plant for animal feed, is capable of growing in tropical and subtropical regions, and is characterized by having a high content of dry matter, carbohydrates, fiber and proteins, with high mass production per area (Haldar and Purkait, 2020; Monção et al., 2020).

The pine, despite not being a native species of the Amazon, is processed by local industries in the manufacture of pallets, boxes and doors. It is an essential wood species for several areas of the timber industry and is widely planted or used throughout Brazil (Lima et al., 2016). Marupá is a woody species native to the Amazon that can be an alternative option for the wood industries, for example, for the manufacture of furniture, and the interest has grown in this species and, consequently, its use (Souza et al., 2020). Açaí is a traditional palm from the northern region of Brazil, much appreciated for the pulp of its fruit, which provides several food products; however, it presents many losses during the processing of this pulp, with about 93% of the fruit correspond to the seed,

which is discarded and generates a high volume of waste (Maranho and Paiva, 2012; Lima et al., 2021).

As such, determining suitable substrates, added to the use of adapted fungal strains with high performance, can lead to a profitable cultivation (Melanouri et al., 2022). Thus, this study aimed to cultivate different strains of *Pleurotus ostreatus*, both native to the Amazon and commercial strains, in different regional residues, and evaluate production parameters in order to enable greater use of Amazonian species, mainly for commercial purposes, as well as verify the nutritional quality of these mushrooms.

2 MATERIAL AND METHODS

2.1 BIOLOGICAL MATERIAL

The native (codes 474 and 1467) and commercial (codes 542 and 885) strains of *Pleurotus ostreatus* were acquired from the Collection of Microorganisms of Agrosilvicultural Interest, at the National Institute for Amazonian Research (INPA). Marupá (*Simarouba amara*) sawdust – MS, pine sawdust (mixture of *Pinus elliottii* and *P. taeda*) – PS, açaí (*Euterpe precatoria*) seeds – AS and elephant grass (*Pennisetum purpureum*) straw – EG were obtained from agro-industries and wood industries in the city of Manaus (3° 06′ 06″ S, 60° 01′ 29″ W), state of Amazonas, Brazil. The residues were dried in the open air, milled and then stored until use in the cultivation of the mushrooms.

2.2 CULTIVATION OF MUSHROOMS

P. ostreatus strains in PDA medium (potato dextrose agar) were used as a pre-inoculum in the production of spawns, which was prepared using residues supplemented with rice, wheat and corn bran (75:20:5), humidified and adjusted to pH 6.0 by adding $CaCO_3$, and then incubated at 25 °C. Mushroom cultivation was carried out in polypropylene bags and using the respective spawns as inoculum. The bags were incubated at 25 °C, 90% air humidity and under a 12-hour photoperiod. The basidiocarps produced were collected, lyophilized and ground.

2.3 PHYSICOCHEMICAL AND NUTRITIONAL PARAMETERS

The moisture content was obtained by drying the samples at 105 ºC, and the ash content by the calcination of the samples at 550 ºC for 4 h (IAL, 2008). Carbon (Walkley-Black method) was determined by mixing the samples in a $K_2Cr_2O_7$ and H_2SO_4 solution,

with subsequent heating at 60 °C for 10 minutes, followed by the addition of an indicator and H_3PO_4, and then titrated with ammoniacal ferrous sulfate solution (Tedesco et al., 1995).

In the nitrogen analysis (Kjeldahl's method), the samples were digested in H_2SO_4 at 350 °C), distilled in NaOH, collected in an H_3BO_3 solution containing indicators, and titrated with HCl solution (Tedesco et al., 1995). The protein content was obtained by multiplying the N value by the conversion factor 4.38 for mushrooms and 6.25 for substrates (Furlani and Godoy, 2005). The lipids were quantified using extraction in a chloroform, methanol, water and Na_2SO_4 solution. Afterwards, the samples were filtered, dried until the evaporation of the chloroform and then weighed (IAL, 2008).

For the quantification of fiber, the samples were digested in a neutral detergent solution and dried at 105 °C. Subsequently, the samples were digested in an acid detergent solution and dried at 105 °C (Van Soest, 1963). Then, the samples were submitted to lignin determination, with incubation in $KMnO_4$ for 2 h, followed by incubation in demineralizing solution for 30 min. After this step, the materials were dried at 105 °C and calcined at 550 °C (Van Soest and Wine, 1968). The total fiber value was obtained from the sum of all fiber classes obtained. Available carbohydrates were obtained by subtracting the moisture, protein, lipid, ash and fiber contents. While the total carbohydrates (TC) were determined from the subtraction of the moisture, protein, lipids and ash contents. Energy values (Kcal/100g) were calculated using the Atwater conversion factor (Nepa, 2011).

2.4 PRODUCTION PARAMETERS

Biological efficiency (BE) was calculated from the ratio between the fresh mass of the basidiocarps and the dry mass of the initial substrates. The yield, based on the ratio between the fresh mass of the basidiocarps and the fresh mass of the substrates after cultivation and the loss of organic matter (LOM), was calculated by the difference between the initial and residual dry mass (spent) of the substrates divided by the initial dry mass of the substrates (Sales-Campos and Andrade, 2011).

2.5 STATISTICAL ANALYSIS

The experiment was arranged in a completely randomized design, in a 4x4 factorial scheme, corresponding to 4 fungal strains (474, 542, 885 and 1467) of *P. ostreatus* and 4 residues (marupá, pine, açaí and elephant grass), with 11 repetitions.

Physicochemical and nutritional analyses were performed in three replications. All data were submitted to ANOVA and compared using Tukey's test ($p<0.05$) using the Sisvar 5.6 program (Ferreira, 2019).

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL PARAMETERS OF SUBSTRATES

The physicochemical parameters were determined in the initial and post-cultivation (spent) substrates of the native and commercial strains of *P. ostreatus*. The moisture, a factor of fundamental importance in mushroom cultivation, was higher in the elephant grass (EG) substrate (74.70%), and remained around 70% for most other substrates, except for the substrate composed of açaí (AS) residue, which presented a moisture content of 52.44% (Table 1). This value is within the range described by Chang and Miles (2004), in which the moisture levels of mushroom growing substrates should vary from 50 to 75% so that they can help in the development of the basidiocarps but not affect the oxygen supply and, consequently, not cause mushroom mortality. However, the 60-65% moisture range is considered the most desirable in solid mushroom cultivation (Mahari et al., 2020).

For Gaitán-Hernández et al. (2017), under indoor cultivation conditions, the moisture content of the substrate should vary between 70 and 80% in order to promote the absorption of nutrients by the fungal hyphae and facilitate the occurrence of metabolic reactions involved in the formation and development of hyphae, which comprises the phase of colonization of the substrate and, later, the formation of basidiocarps.

In the post-cultivation substrates (spent), there was no pattern in the percentage of moisture. In general, the pine sawdust (PS) showed a reduction in moisture content, while the AS substrate showed an increase in the percentage of moisture, after the cultivation of all strains (Table 1).

The ash content demonstrates the mineral contribution of the material, and corresponds to the fixed mineral residue that is obtained after the decomposition of all organic components (Sales-Campos et al., 2011). In this study, the EG substrate had the highest ash content, both in the initial (8.18%) and in the spent substrate, with emphasis on strain 1467, which was cultivated in EG (19.36%) (Table 1). Additionally, an increase in the percentages of ash was observed in all spent substrates when compared to the initial substrates (Table 1). The increase in ash content in spent substrates may be related to the bioconversion process that occurs during growth. With the constant use of organic matter,

which is consumed during cultivation, inorganic elements become more evident, increasing the ash content (Singh, 2000).

As for the carbon content, the initial substrates of marupá sawdust (MS) and pine sawdust (PS) presented the highest percentages, while the highest nitrogen content was obtained in the initial substrate of AS, and the C:N ratio was higher in the initial PS substrate (Table 1).

Carbon is one of the fundamental elements for the growth of basidiocarps, and is used in the synthesis of macromolecules and as a source of energy (Anike et al., 2016). In this sense, the AS substrate, which presented the lowest carbon content among all the evaluated substrates, possibly provided the lowest energy, which may be one of the factors responsible for its low production.

In the spent substrates, there was a reduction in the levels of carbon for most substrates, compared to the values present in the initial substrates (Table 1). This gradual reduction in the carbon content of the substrates after mushroom cultivation occurs due to the accumulation of carbon in the process of basidiocarp formation itself (formation of structures and cellular composition), in addition to the loss that occurs during the process of cellular respiration. (Chanakya et al., 2015).

Analyzing the C% between the strains, the highest levels were observed in the spent of the cultures of 474 strain in the substrates EG and MS (Table 1). For strain 542, MS obtained the most significant content (Table 1). The same occurred for 885, with 47.52% for the MS substrate, while the substrates MS and PS exhibited the highest carbon values after the cultivation of strain 1467 (Table 1). Thus, the spent marupá sawdust (MS) substrate stands out, since it obtained the highest carbon contents, regardless of the *P. ostreatus* strain evaluated.

As for nitrogen content, there was an increase in N% in the spent substrates, except for strain 542 in PS (Table 1). The increase in the concentrations of N in substrates after mushroom cultivation is commonly reported, and is a fact that can be explained by different hypotheses, such as the excretion of hydrolytic enzymes in the culture medium during fungal metabolism, the presence of fungal mycelium in the final substrate or even by the possibility that some species of macrofungi fix atmospheric nitrogen when associated with diazotrophic organisms, as already reported for several species of *Pleurotus* (Jayasinghearachchi and Seneviratne, 2004; Andrade et al., 2013).

In the C and N values obtained, there was a narrowing of the C:N ratio in all spent substrates, with the highest C:N ratio obtained for strain 542 in MS and PS (Table 1)

Table 1. Moisture, ash, carbon (C), nitrogen (N), C:N ratio, proteins and lipids of the initial and post-cultivation (spent) substrates of native (1467 and 474) and commercial (542 and 885) strains of *Pleurotus ostreatus*, cultivated in different local lignocellulosic wastes. AS: açaí seeds; EG: elephant grass; MS: marupá sawdust; PS: pine sawdust.

			Moisture (%)	Ashes (%)	C (%)	N (%)	C/N	Proteins (%)	Lipids (%)
Initial substrate		AS	52.44 d ± 0.62	5.69 b ± 0.53	44.12 c ± 0.92	1.44 a ± 0.02	30.61 d ± 1.06	9.01 a ± 0.13	2.17 c ± 0.33
		EG	74.70 a ± 0.21	8.18 a ± 0.24	46.95 b ± 1.09	1.03 b ± 0.02	45.55 c ± 0.40	6.44 b ± 0.11	3.61 b ± 0.13
		MS	72.51 b ± 0.24	3.68 c ± 0.38	53.60 a ± 1.31	0.51 d ± 0.01	104.74 a ± 3.25	3.20 d ± 0.04	1.05 d ± 0.28
		PS	71.43 c ± 0.17	3.34 c ± 0.13	53.05 a ± 0.52	0.64 c ± 0.02	83.36 b ± 2.41	3.98 c ± 0.16	5.91 a ± 0.27
Spent sbustrate	474	AS	72.20 Aabcde ± 5.92	8.38 Bde ± 0.58	41.04 Cef ± 1.53	2.17 Aa ± 0.04	18.94 Dg ± 1.07	13.55 Aa ± 0.26	0.81 Be ± 0.38
		EG	74.85 Aabcd ± 3.58	13.14 Ab ± 0.12	47.05 Aab ± 0.62	1.71 Bb ± 0.03	27.54 Cf ± 0.47	10.67 Bc ± 0.21	1.19 Bde ± 0.03
		MS	72.98 Aabcde ± 1.72	6.75 Cgh ± 0.06	46.24 Aabc ± 0.00	0.97 Ce ± 0.03	47.90 Bde ± 1.32	6.03 Cg ± 0.17	0.83 Be ± 0.13
		PS	56.24 Bf ± 2.81	6.33 Ch ± 0.06	43.65 Bcde ± 1.49	0.75 Df ± 0.05	58.27 Abc ± 6.26	4.71 Dh ± 0.33	3.33 Ab ± 0.14
	1467	AS	78.53 Aab ± 3.55	7.45 Bfg ± 0.06	44.45 Abbcd ± 0.66	1.56 Ac ± 0.01	28.43 Cf ± 0.35	9.77 Ad ± 0.03	0.92 Be ± 0.21
		EG	74.03 Aabcd ± 5.32	19.36 Aa ± 0.26	42.58 Bdef ± 0.68	0.70 Bfg ± 0.03	60.14 Bb ± 1.45	7.41 Bf ± 0.00	1.42 Bcde ± 0.35
		MS	79.55 Aa ± 4.24	5.44 Dj ± 0.32	44.62 ABabcd ± 0.36	0.69 Bfg ± 0.02	65.00 Bb ± 2.98	4.30 Chi ± 0.16	1.03 Bde ± 0.28
		PS	34.95 Bg ± 4.44	6.19 Chi ± 0.24	46.47 Aabc ± 0.60	0.57 Cgh ± 0.03	81.83 Aa ± 3.59	4.39 Chi ± 0.02	3.64 Aab ± 0.24
	885	AS	73.05 Aabcde ± 3.43	6.46 Bh ± 0.24	41.16 Cef ± 1.13	1.55 Ac ± 0.09	26.55 Cfg ± 1.16	9.70 Ad ± 0.54	0.93 Ce ± 0.06
		EG	68.03 ABcde ± 11.34	12.19 Ac ± 0.45	44.27 Bbcd ± 1.51	1.56 Ac ± 0.05	28.45 Cf ± 1.07	9.73 Ad ± 0.29	1.73 Bcd ± 0.36
		MS	65.90 Bde ± 10.78	5.50 Cij ± 0.08	47.52 Aa ± 1.45	0.92 Be ± 0.05	51.74 Acd ± 3.09	5.75 Bi ± 0.31	1.08 Cde ± 0.08
		PS	42.33 Cg ± 3.47	6.52 Bh ± 0.14	41.91 Cdef ± 0.98	0.98 Be ± 0.01	42.49 Be ± 1.43	6.17 Bg ± 0.10	3.87 Aab ± 0.14
	542	AS	76.35 Aabc ± 10.44	8.87 Bd ± 0.15	34.20 Cg ± 0.03	1.84 Ab ± 0.09	18.58 Cg ± 0.93	11.53 Ab ± 0.58	1.26 Cde ± 0.06
		EG	64.37 Bef ± 8.62	18.65 Aa ± 0.22	39.63 Bf ± 0.29	1.40 Bd ± 0.02	28.43 Bf ± 0.66	8.71 Be ± 0.14	2.00 Bc ± 0.17
		MS	70.07 ABbcde ± 3.73	6.21 Dhi ± 0.07	46.38 Aabc ± 1.42	0.62 Cfgh ± 0.02	75.35 Aa ± 5.35	3.85 Di ± 0.15	1.03 Ce ± 0.09
		PS	40.82 Cg ± 3.70	8.04 Cef ± 0.10	40.90 Bef ± 0.86	0.54 Ch ± 0.00	75.30 Aa ± 1.12	4.63 Chi ± 0.02	4.27 Aa ± 0.41

Means ± standard deviation followed by uppercase letters compare post-cultivation substrates within each strain and lowercase letters compare all substrates and strains, separated into initial and post-cultivation substrates of mushrooms.

Investigations into the C:N ratio are important, since it plays an important role in mushroom growth, and physicochemical properties (size, porosity and gas exchange) influence mycelial colonization rate and substrate bioconversion in the mushroom (Cueva et al., 2017).

In nature, *Pleurotus* species grow on wood, which normally presents a C:N ratio ranging from 350-500:1. However, to achieve the desired productivity in a cultivation, this ratio must be more equal, with a ratio of 19:1 to 22:1 up to a maximum of 70-80:1 (Chang and Miles, 2004; Mahari et al., 2020), which are values that are close to those found for the AS and EG initial substrates.

The nitrogen present in the substrates is essential for protein synthesis in the basidiocarps; however, this nitrogen-protein-mushroom ratio is close and levels ranging from 0.65 to 1.30% stimulate protein synthesis, while values between 1.75 and 2.20% inhibit fungal colonization in the substrate (Cueva et al., 2017). The AS substrates (initial and spent) and the basidiocarps produced on this substrate showed the highest content of N and proteins, thus corroborating the influence of nitrogen on protein synthesis. Furthermore, nitrogen is used not only in protein synthesis, but also in cellular activities (Anike et al., 2016).

As observed for ash, the protein content increased in spent substrates, with the AS substrate showing the highest percentages, both in the initial substrate and after mushroom cultivation (Table 1). On the other hand, lipid levels decreased in spent substrates when compared to initial substrate concentrations, with PS substrates showing the highest concentrations (Table 1). These higher levels of lipids found in the PS substrate may be related to the presence of greases, resins and essential oils (Vogelmann et al., 2015).

Regarding the fibrous constitution of the materials, the MS initial substrate had the highest cellulose content (Table 2), while AS and EG showed the highest values of hemicellulose, with 30.85 and 32.39%, respectively (Table 2). Whereas, the lignin content was prominent in the MS and PS substrates (Table 2).

Lignin is an unwanted resistant material, as it has an inhibitory effect on microorganisms, and acts as a physical barrier to cellulose degradation (Shin et al., 2019). Corroborating this, the EG substrate, which corresponds to the substrate with the lowest lignin content, showed the highest productivity rate for all strains tested. Thus, high concentrations of lignin in the substrate can be a limiting factor in the productive yield of

mushrooms. On the other hand, hemicellulose is more easily degraded compared to cellulose, since it is more easily available to the fungus (Annepu et al., 2019).

There is some conjecture about the relationship between the contents of cellulose, hemicellulose, lignin and mushroom production. Öztürk and Atila (2021) observed a correlation between cellulose degradation, yield and biological efficiency. In fact, strains 1467 and 542 cultivated in EG, with higher BE (102.93% and 86.18%, respectively), showed a significant reduction in cellulose content when compared to the other residues.

Table 2. Total fibers, cellulose, hemicellulose, lignin, total carbs (carbohydrates), available carbs (carbohydrates) and energy values of the initial and final substrates of *Pleurotus ostreatus* native and commercial strains, cultivated in different local lignocellulosic wastes. AS: açaí seeds; EG: elephant grass; MS: marupá sawdust; PS: pine sawdust; ASM: mushroom from açaí seeds; EGM: mushroom from elephant grass; MSM: mushroom from marupá sawdust; PSM: mushroom from pine sawdust.

			Total fibers (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Total carbs (%)	Disponible carbs (%)	Energy (kcal 100 g–1)
Initial substrate		AS	36.77 bc ± 1.17	38.63c ± 1.23	30.85 a ± 3.99	6.41b ± 0.12	78.78 c ± 0.73	42.01 a ± 1.63	223.68 a ± 7.53
		EG	35.63 c ± 0.33	37.43c ± 0.35	32.39a ± 0.40	4.90c ± 0.73	78.03 c ± 0.43	42.40 a ± 0.14	227.86 a ± 1.21
		MS	53.12 a ± 3.45	55.62a ± 3.61	23.13b ± 3.88	10.81a ± 0.33	87.56 a ± 0.25	35.45 b ± 3.58	160.04 b ± 6.60
		PS	41.05 b ± 0.19	44.03b ± 0.20	23.04b ± 0.48	10.00a ± 0.22	81.53 b ± 0.46	40.48 a ± 0.48	231.01 a ± 1.01
Spent substrate	474	AS	35.69 Dfg ± 0.27	38.91 Cfg ± 0.30	21.86 Aa ± 0.89	4.54 BCgh ± 0.24	68.95 Bf ± 0.39	33.8 Aghi ± 0.63	194.61 Adef ± 5.05
		EG	38.09 Cef ± 0.46	40.81 Cef ± 0.49	14.97 Bef ± 0.48	3.08 Ch ± 0.06	68.35 Bf ± 0.40	30.25 Bi ± 0.72	174.42 Bgh ± 2.31
		MS	48.16 Ab ± 0.52	51.40 Aa ± 0.23	13.06 Cgh ± 0.17	6.52 Afg ± 0.54	79.05 Ac ± 0.50	30.65 Bi ± 0.99	154.18 Ci ± 3.05
		PS	44.24 Bc ± 0.21	47.01 Bbc ± 0.55	9.04 Di ± 0.42	5.83 Abg ± 0.45	78.72 Ac ± 0.74	34.49 Afgh ± 0.81	186.74 Afg ± 3.20
	1467	AS	30.09 Ch ± 1.51	30.40 Cij ± 0.64	19.79 Ab ± 0.21	21.93 Aa ± 0.48	78.24 Bc ± 0.32	48.15 Aa ± 1.21	239.99 Aa ± 7.50
		EG	26.88 Di ± 0.98	28.35 Cj ± 1.04	16.53 Bde ± 0.96	8.43 Def ± 0.51	66.60 Cg ± 0.16	39.73 Bbcd ± 1.14	201.33 Bcdef ± 6.27
		MS	47.13 Abc ± 0.46	50.62 Aa ± 0.49	14.63 Cfg ± 0.18	13.69 Bc ± 0.52	82.34 Ab ± 0.61	35.21 Cefg ± 0.56	167.29 Chi ± 3.05
		PS	40.71 Bde ± 0.37	43.69 Bde ± 0.39	11.81 Dh ± 0.27	10.63 Cde ± 0.45	78.94 Bc ± 0.32	38.24 Bcde ± 0.42	203.33 Bcde ± 2.98
	885	AS	48.21 Bb ± 0.97	49.89 Aab ± 1.00	18.56 Abc ± 0.21	6.50 Afg ± 0.33	79.53 Bc ± 0.53	31.32 Chi ± 0.65	172.50 Cgh ± 3.64
		EG	34.47 Dg ± 0.68	33.42 Ch ± 0.66	17.47 Acd ± 1.27	5.37 Agh ± 0.33	74.53 Cd ± 0.91	40.06 Abcd ± 0.84	214.79 Abc ± 1.07
		MS	52.39 Aa ± 1.25	51.62 Aa ± 1.23	12.64 Bh ± 0.06	6.81 Afg ± 1.34	82.55 Ab ± 1.04	30.16 Ci ± 1.56	153.69 Di ± 4.48
		PS	41.19 Cd ± 1.56	44.20 Bcd ± 1.68	12.41 Bh ± 0.02	6.95 Afg ± 1.52	75.50 Cd ± 0.59	34.31 Bfgh ± 2.11	196.70 Bdef ± 9.07
	542	AS	33.96 Bg ± 1.28	36.52 Bg ± 1.38	19.78 Ab ± 0.53	12.06 BCcd ± 1.80	71.34 Ce ± 0.54	37.38 Cdef ± 1.64	206.97 Ccd ± 5.11
		EG	20.34 Dj ± 0.16	22.05 Dk ± 0.17	3.46 Dj ± 0.43	18.60 Ab ± 0.88	62.87 Dh ± 0.13	42.53 Bb ± 0.29	222.95 Bb ± 2.26
		MS	44.23 Ac ± 0.77	45.78 Acd ± 0.80	11.79 Bh ± 0.60	11.58 Ccd ± 0.96	85.50 Aa ± 0.16	41.7 Bbc ± 0.71	189.81 Def ± 2.94
		PS	30.92 Ch ± 1.87	32.39 Chi ± 1.96	8.25 Ci ± 0.35	13.82 Bc ± 0.81	78.52 Bc ± 0.67	47.59 Aa ± 1.81	247.35 Aa ± 8.21

Means ± standard deviation followed by uppercase letters compare post-cultivation substrates within each strain and lowercase letters compare all substrates and strains, separated into initial and post-cultivation substrates of mushrooms.

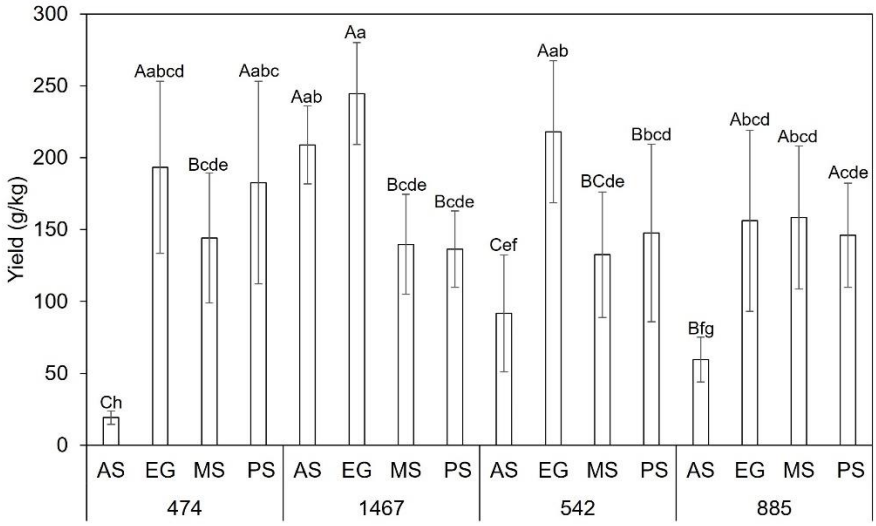
In regards to yield, this same cellulose reduction ratio is observed in strain 1467 EG, strain 542 EG and strain 1467 AS, but not in strain 474 PS and strain 474 EG. Characteristics, such as the accessibility of these fibrous components in the substrate and the enzymatic contribution present in the cultivated fungus, may be some of the factors responsible for a greater EB in certain substrates and when using certain strains.

As for total fibers, the initial and spent substrates based on MS and PS had the highest levels (Table 2), with the high fiber content being characteristic of wood residues (Aguiar et al., 2021). The type of wood, its metabolites and the availability of its fibrous constituents, such as cellulose, are essential for successful cultivation (Alfianti et al, 2021). Thus, it is not enough for the material to be rich in nutrients, they must also be available for use.

3.2 PRODUCTIVE PARAMETERS

Comparing all the combinations of strains and substrates, the yield was statistically different only for strains 474, 542 and 885, when grown on AS substrate (Figure 1). Under these conditions, it can be inferred that these three strains did not obtain a yield considered commercially profitable, since to be considered a substrate with good productivity, the minimum yield must be 100 g (mushroom) /kg (substrate), are those that exceed this value are desirable (Siqueira et al., 2011).

Figure 1. Yield of native and commercial strains of *Pleurotus ostreatus* cultivated in different regional lignocellulosic wastes. AS: açaí seeds; EG: elephant grass; MS: marupá sawdust; PS: pine sawdust. Bars with the same lowercase letter for all combinations (strains and substrates) and with the same uppercase letter, when comparing each strain in all substrates, do not differ statistically from one another as per the Tukey test at a level of significance of 5%.



In the other experimental conditions, there was a tendency towards higher yields among the different strains (1467, 474 and 542) when cultivated in EG (Figure 1). In this sense, it is important to emphasize that the presence of sugars and other readily available components in the residue are essential for greater productivity (Cardoso et al., 2013), while materials that are difficult to decompose by the fungus can generate the reverse effect (Pedra and Marino, 2006), which can be seen in the characteristics of the materials selected for this cultivation.

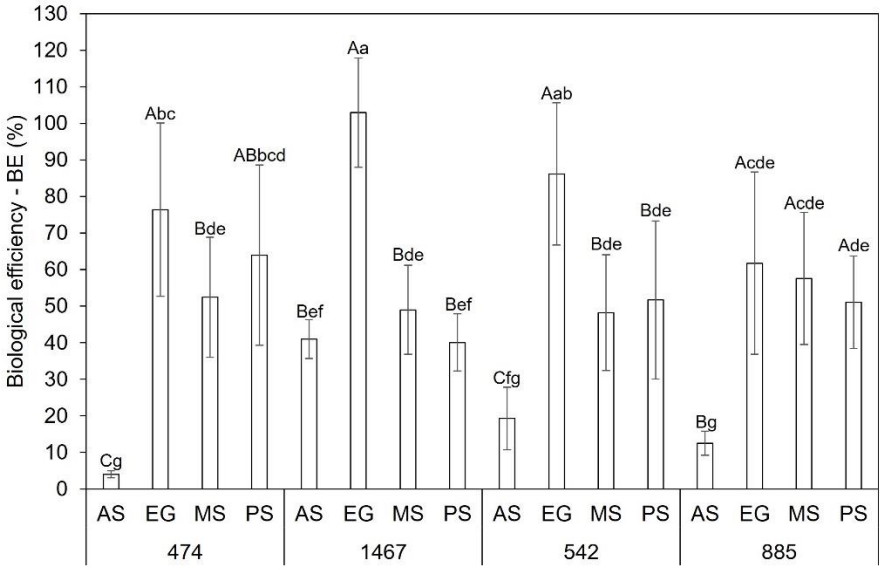
The highest values found are similar to those obtained by Otieno et al. (2022), for *P. ostreatus*, which showed yields of 53 g of mushroom in 100 g of dry orange peel and 55 g of mushroom in 100 g of dry watermelon peel, which would correspond to approximately 400 g of dry substrate in the present study (1 kg of residue with 60% moisture), which produced 212 g/400 g and 220 g/400 g.

The variation in productivity in the substrates can demonstrate the presence of nutrients in greater quantity or even of nutrients of superior quality in the substrates (Gume et al., 2013). It is noted that, of the 4 strains of *P. ostreatus*, 3 did not show good yields from açaí residue, which may reflect its nutritional composition, since it was not suitable for most of the strains tested.

Biological efficiency (BE) expresses the ability of the fungus to convert the substrate into basidiocarps and, as observed for yield, there is also a desired BE value in order to be considered an economically profitable cultivation (Sadar et al., 2017). For the

cultivation of *P. ostreatus*, those that reach at least 40% BE are considered economically viable substrates (Ríos et al., 2010; Gume et al., 2013). Thus, of the 16 combinations of substrates and strains, only 5 are not suitable for cultivation (474 AS, 1467 AS, 1467 PS, 542 AS and 885 AS), since the BE was below or very close to 40% (Figure 2).

Figure 2. Biological efficiency (BE) of native and commercial strains of *Pleurotus ostreatus* cultivated in different regional lignocellulosic wastes. AS: açaí seeds; EG: elephant grass; MS: marupá sawdust; PS: pine sawdust. Bars with the same lowercase letter for all combinations (strains and substrates) and with the same uppercase letter, when comparing each strain in all substrates, do not differ statistically from one another as per the Tukey test at a level of significance of 5%.



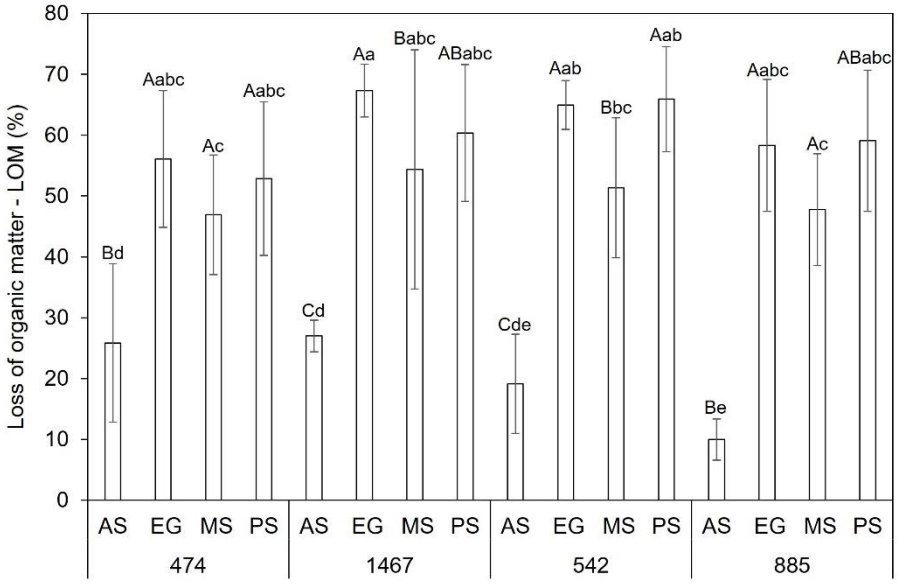
Piña-Guzmán et al. (2016) report that BE must present values close to 100% in order to obtain high mushroom productivity. In this study, the highest biological efficiencies obtained were for 1467 EG (102.93%) and 542 EG (86.18%), which correspond to a native and commercial strain, respectively (Figure 2). The results for BE in this study corroborate Toro et al. (2018) who evaluated different strains of *Pleurotus* spp. grown on *Yucca schiedigera* residues, for which they obtained BE values ranging from 107.7 to 125.6%. Koutrotsios et al. (2017) observed BE values ranging from 43.89 to 109.38% for other species of *Pleurotus* spp., when grown on wheat grains. Comparing BE among the cultivation substrates studied herein, it was found that EG residue provided a higher BE for all strains evaluated. While, among the strains, 1467 (native strain from the Amazon) showed better performance (Figure 2).

In a study evaluating the effect of different substrates (wheat, rice and soybean meal) on the production of *Pleurotus* spp., BE varied from 67.23 to 236.32%, indicating that it was significantly affected by the combination and supplementation of substrates

(Jeznabadi et al., 2017). This fact can be explained by the structural differences between the substrates, including the composition and structures of the fibers (hemicellulose, cellulose and lignin), as these characteristics influence the development and mycelial penetration into the substrate, which reflects on the formation of the basidiocarps (Jeznabadi et al., 2016; 2017).

The loss of organic matter (LOM) showed the same pattern described for yield and BE, in which fungi grown on the AS substrate exhibited the lowest LOM. Conversely, fungi grown on the EG and PS substrates had the highest LOM, especially strain 1467 (Figure 3).

Figure 3. Loss of organic matter (LOM) of native and commercial strains of *Pleurotus ostreatus* cultivated in different regional lignocellulosic wastes. AS: açaí seeds; EG: elephant grass; MS: marupá sawdust; PS: pine sawdust. Bars with the same lowercase letter for all combinations (strains and substrates) and with the same uppercase letter, when comparing each strain in all substrates, do not differ statistically from one another as per the Tukey test at a level of significance of 5%.



A correlation between BE, yield and LOM can be observed for the two treatments with the highest BE, which also showed some of the highest LOM and yield. However, one of the highest yields obtained, in the combination of the 1467 strain cultivated in AS (208.91 g/Kg), a low LOM (27%) was observed (Figures 1 and 3), indicating that there is not always a relationship between productivity and LOM. An explanation for this low ratio may be the loss of CO_2 and H_2O during fungal metabolism, which does not necessarily indicate the degradation of lignocellulosic material for the formation of basidiocarps (Carvalho et al., 2012).

3.3 NUTRITIONAL PARAMETERS OF MUSHROOMS

In the basidiocarps, the moisture ranged from 73.46% (1467 MS) to 91.08% (474 PS), and most mushrooms presented moisture in the range of 80%. These values obtained are within or close to the expected moisture in mushrooms, which range from 85 to 95% (Singh and Singh, 2021).

The average ash value (indicative of minerals) was 6%, and the basidiocarps of strain 474 presented the lowest (3.48%) and highest (8.70%) ash content when cultivated in AS and PS, respectively (Table 3). Singh and Singh (2021) highlight that mushrooms have a significant amount of minerals, evidenced by the amount of ash and describe that the ash content in *Pleurotus* species varies from 5 to 15% of its dry base, which is within the range found for the different strains of this study.

The analyses of the centesimal composition of the mushrooms produced showed carbon and nitrogen contents of around 40% and 5%, respectively, for all the conditions evaluated, which resulted in an average C:N of 8.6 (Table 3). Furthermore, mushrooms from cultivation in the EG substrate showed the highest C:N, which may justify the best production parameters for this experimental condition (Table 3).

The protein content of the basidiocarps varied from 13.27 to 30.07%, with the AS substrate providing the highest protein content for all the evaluated mushroom strains, thus corroborating the results obtained for the initial substrates, in which the açaí residues exhibited the highest protein content (Table 3). The lipid contents of the mushrooms ranged from 3 to 4.98%, while the fungi grown on the EG substrate had some of the highest levels of total and available carbohydrates (Table 3). Usually, mushrooms of species of the genus *Pleurotus* are reported to have protein concentrations of up to 27 g/100 g on a dry weight basis and lipids ranging from 1.1 to 8.3% on a dry weight basis, while carbohydrates, which correspond to the main constituents of the dry weight of a mushroom, vary from 46.6 to 81.8% (Singh and Singh, 2021).

Table 3 – Moisture, ash, carbon (c), nitrogen (n), C:N ratio, proteins and lipids of the basidiocarps of *Pleurotus ostreatus* native and commercial strains, cultivated in different regional lignocellulosic waste. ASM: mushroom from açaí seeds; EGM: mushroom from elephant grass; MSM: mushroom from marupá sawdust; PSM: mushroom from pine sawdust. Means followed by the same lowercase letter in the columns and same uppercase letter in columns, when comparing each strain in all substrates, do not differ statistically from one another by Tukey at 5% significance.

Basidiomes		Moisture (%)	Ashes (%)	C (%)	N (%)	C:N	Proteins (%)	Lipids (%)
474	ASM	82.32 Babcd ± 4.16	3.48 Dg ± 0.00	41.60 Aabc ± 0.41	5.61 Ad ± 0.05	7.41 Dhi ± 0.01	24.60 Ad ± 0.22	3.79 Abcd ± 0.21
	EGM	89.10 ABab ± 2.56	7.29 Cb ± 0.17	41.33 ABabcd ± 0.23	3.28 Dhi ± 0.01	12.60 Ab ± 0.05	14.37 Dhi ± 0.03	4.23 Aabc ± 0.20
	MSM	90.58 Aab ± 1.81	7.93 Bb ± 0.06	40.00 Bbcde ± 1.03	4.99 Be ± 0.07	8.01 Cg ± 0.09	21.87 Be ± 0.32	4.40 Aab ± 0.31
	PSM	91.08 Aa ± 2.74	8.70 Aa ± 0.12	42.01 Aab ± 0.74	4.60 Cf ± 0.05	9.14 Bd ± 0.24	20.13 Cf ± 0.21	4.02 Aabc ± 0.60
1467	ASM	84.52 Aabc ± 6.51	5.55 Bdef ± 0.25	40.54 Abcd ± 0.73	6.21 Bc ± 0.13	6.53 Cj ± 0.06	27.20 Bc ± 0.58	2.15 Be ± 0.26
	EGM	85.55 Aabc ± 6.05	6.12 Acd ± 0.05	37.80 Bf ± 0.71	4.04 Dg ± 0.12	9.35 Ad ± 0.16	17.70 Dg ± 0.54	2.26 Be ± 0.27
	MSM	73.46 Bd ± 8.65	6.21 Acd ± 0.09	38.03 Bef ± 1.24	6.53 Ab ± 0.24	5.83 Dk ± 0.13	28.60 Ab ± 1.07	3.32 Acd ± 0.31
	PSM	86.91 Aabc ± 4.38	6.21 Acd ± 0.11	40.66 Abcd ± 1.10	5.09 Ce ± 0.07	7.98 Bg ± 0.24	22.31 Ce ± 0.31	3.00 Ade ± 0.13
885	ASM	80.45 Abcd ± 8.58	5.68 ABcdef ± 0.18	40.89 Aabcd ± 0.10	6.86 Aa ± 0.06	5.96 Dk ± 0.04	30.07 Aa ± 0.27	3.66 Bbcd ± 0.31
	EGM	85.74 Aabc ± 8.61	6.15 Acd ± 0.50	39.27 Bcdef ± 0.57	3.58 Dh ± 0.04	10.98 Ac ± 0.19	15.67 Dh ± 0.19	4.12 Babc ± 0.14
	MSM	85.20 Aabc ± 6.95	5.25 BCef ± 0.19	40.13 Aabcd ± 0.72	5.20 Be ± 0.18	7.72 Cgh ± 0.40	22.77 Be ± 0.80	4.98 Aa ± 0.32
	PSM	87.88 Aab ± 4.65	5.11 Cf ± 0.43	37.89 Cf ± 0.62	4.40 Cf ± 0.05	8.61 Bef ± 0.06	19.27 Cf ± 0.22	3.75 Bbcd ± 0.07
542	ASM	77.06 Bcd ± 8.31	6.09 Acd ± 0.02	39.87 Ccdef ± 0.41	5.78 Ad ± 0.02	6.90 Dij ± 0.06	25.30 Ad ± 0.07	3.83 Abcd ± 0.73
	EGM	81.94 ABabcd ± 6.22	6.42 Ac ± 0.58	41.95 ABab ± 0.66	3.03 Di ± 0.01	13.85 Aa ± 0.24	13.27 Di ± 0.03	4.18 Aabc ± 0.20
	MSM	85.84 Aabc ± 3.79	6.03 Acd ± 0.08	42.81 Aa ± 0.00	5.26 Be ± 0.01	8.14 Cfg ± 0.01	23.03 Be ± 0.03	4.52 Aab ± 0.27
	PSM	81.69 Ababcd ± 4.73	5.96 Acde ± 0.14	41.27 BCabcd ± 0.23	4.61 Cf ± 0.10	8.95 Bde ± 0.23	20.20 Cf ± 0.43	4.19 Aabc ± 0.21

Means ± standard deviation followed by uppercase letters compare basidiocarps within each strain and lowercase letters compare basidiocarps between all substrates and strains.

The total fiber content in species of the genus *Pleurotus* ranges from 7.5 to 27.6% on a dry weight basis (Singh and Singh, 2021). Except for 542 AS and 542 EG, which presented a content of 4.60 and 6.84% of total fiber, respectively, the other 14 combinations of strains and cultivation substrates are within this mentioned range (Table 4). Additionally, among all combinations, the highest total fiber content in mushrooms was obtained for 1467 PS (23.05%), which was the cultivation substrate with the second highest total fiber content (Table 4).

Table 4 – Ash, total fibers, proteins, lipids, total carbs (carbohydrates), disponible carbs (carbohydrates) and energy values of the basidiomas of native and commercial strains of *Pleurotus ostreatus* cultivated in different local lignocellulosic wastes. ASM: mushroom from açaí seeds; EGM: mushroom from elephant grass; MSM: mushroom from marupá sawdust; PSM: mushroom from pine sawdust.

Basidiomes		Total fibers (%)	Total carbs (%)	Disponible carbs (%)	Energy (kcal 100 g–1)
474	AS	12.59 Acdefg ± 0.82	63.30 Bcd ± 0.78	50.71 Abcde ± 1.29	335.23 Aab ± 2.90
	EG	12.09 Adefg ± 0.96	65.45 Abc ± 0.21	53.37 Abc ± 0.22	309.02 Bcde ± 4.49
	MS	13.01 Abcde ± 0.64	56.77 Cfgh ± 0.74	43.76 Cgh ± 1.29	302.03 Bde ± 2.57
	PS	9.33 Bgh ± 0.23	57.07 Cfg ± 0.88	47.73 Bdefg ± 0.81	307.67 Bcde ± 4.71
1467	AS	12.79 Cbcdef ± 1.20	56.65 Cfgh ± 0.62	43.86 Bgh ± 1.74	303.56 Ade ± 5.03
	EG	15.24 BCbcd ± 2.11	67.67 Aab ± 0.66	52.44 Abc ± 1.59	300.94 Ade ± 10.09
	MS	16.22 Bb ± 1.56	54.18 Dhi ± 1.24	37.96 Ci ± 2.75	296.12 Ae ± 6.26
	PS	23.05 Aa ± 1.52	61.83 Bde ± 0.57	38.78 Ci ± 1.63	271.32 Bf ± 5.72
885	AS	12.23 ABcdefg ± 0.99	53.62 Di ± 2.40	41.40 Chi ± 2.99	318.76 Abcd ± 11.85
	EG	13.79 Abcde ± 0.30	69.86 Aa ± 0.37	56.07 Ab ± 0.62	324.09 Abc ± 3.15
	MS	10.69 Befg ± 1.54	56.41 Cghi ± 1.22	45.72 Bfgh ± 0.39	318.87 Abcd ± 6.00
	PS	13.56 Abcde ± 1.61	61.30 Bde ± 0.66	47.75 Bdefg ± 2.16	301.84 Bde ± 9.81
542	AS	4.60 Ci ± 0.12	56.55 Dgh ± 0.81	51.96 Bbcd ± 0.52	343.57 Aa ± 4.07
	EG	6.84 Bchi ± 1.39	70.43 Aa ± 0.74	63.59 Aa ± 0.69	345.00 Aa ± 6.06
	MS	9.34 Bfgh ± 0.29	59.44 Cef ± 0.23	50.09 BCcdef ± 0.31	333.18 Aab ± 0.31
	PS	15.64 Abc ± 0.24	62.50 Bd ± 0.27	46.86 Cefg ± 0.99	305.96 Bcde ± 0.53

Means ± standard deviation followed by uppercase letters compare basidiocarps within each strain and lowercase letters compare basidiocarps between all substrates and strains.

The efficiency in converting lignocellulosic residues into basidiocarps depends mainly on the structural and/or nutritional composition of the substrate used in mushroom cultivation, as well as on factors that are intrinsic to the fungus species (genetic factors, including their lineages). Therefore, it is essential to carry out studies aimed at selecting highly productive strains and substrates that provide high productivity (Koutrotsios et al., 2017).

4 CONCLUSION

When cultivated in elephant grass residue, strain 1467 of *Pleurotus ostreatus*, which is native to the Amazon, presented the best productive performance and showed satisfactory nutritional quality, and is promising for commercial purposes. However, investigations into new lignocellulosic

residues are recommended, aiming to maximize and optimize mushroom production, as well as nutrient contents in order to provide a product with high nutritional content.

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