Conjecturas

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Physico-chemical characterization of lignocellulosic wastes used in the cultivation of *Pleurotus ostreatus*

Caracterização físico-química de resíduos lignocelulósicos utilizados no cultivo de *Pleurotus ostreatus*

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ABSTRACT

The choice of residue to be used in the cultivation of a fungal species must take into account the structural and chemical composition of the material in order to be successful in its cultivation. Thus, this study evaluated the physical and chemical characteristics of pineapple and açaí residues and their alterations after the cultivation of two *Pleurotus ostreatus* strains (474 and 542). The fungi were cultivated in pineapple, açaí, and pineapple + açaí residues, which were added to a mixture of brans, and $CaCO_3$ in the proportion of 78:20:2 w/w/w. The physical and chemical parameters and proximate composition of the residues, and the initial and spent substrates were determined, in addition to being analyzed using scanning electron microscopy and X-ray diffraction. In general, among the residues tested, the pineapple substrate showed more interesting nutritional characteristics for fungiculture, as well as structural conformation and greater availability of nutrients. The açaí substrate was not suitable for fungal cultivation due to its structural characteristics and high tannin content. In this sense, knowing the characteristics of the material used as a support for mushroom growth is of paramount importance in order to achieve successful cultivation.

Keywords: Structural features; Lignocellulosic degradation; Fungiculture; Productivity.

RESUMO

A escolha do resíduo a ser utilizado no cultivo de uma espécie fúngica deve levar em consideração a composição estrutural e química do material para que haja sucesso no seu cultivo. Assim, este estudo avaliou as características físicas e químicas dos resíduos de abacaxi e açaí e suas alterações após o cultivo de duas linhagens de *Pleurotus ostreatus* (474 e 542). Os fungos foram cultivados em resíduos de abacaxi, açaí e abacaxi + açaí, que foram adicionados a uma mistura de farelos, e $CaCO_3$ na proporção de 78:20:2 p/p/p. Foram determinados os parâmetros físico-químicos e a composição centesimal dos resíduos e os substratos inicial e residual, além de serem analisados por microscopia eletrônica de varredura e difração de raios-X. De maneira geral, dentre os resíduos testados, o substrato de abacaxi apresentou características nutricionais mais interessantes para a fungicultura, além de conformação estrutural e maior disponibilidade de nutrientes. O substrato de açaí não foi adequado para o cultivo de fungos devido às suas características estruturais e alto teor de tanino. Nesse sentido, conhecer as características do material utilizado como suporte para o crescimento de cogumelos é de suma importância para o sucesso do cultivo.

Palavras-chave: Características estruturais; Degradação lignocelulósica; Fungicultura; Produtividade.

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INTRODUCTION

Lignocellulosic residues, such as rice and wheat straw/bran, sugarcane bagasse, sawdust, corncobs and straw, and coffee husks, have been used as substrates for mushroom cultivation, including in the cultivation of *Pleurotus* species, due to their low costs and accessibility (Otieno *et al*., 2022). Among the lignocellulosic residues of incipient use in the Amazon region are those from the processing of pineapple (*Ananas comosus* (L.) Merril) and açaí *(Euterpe* spp.) (Correa *et al*., 2019; Hamzah *et al*., 2021).

Pineapple is one of the most representative products of Brazilian national fruit production. Brazil being one of the largest pineapple producers in the world and the state of Amazonas one of the largest national producers (Pinheiro *et al*., 2020; Sousa *et al*., 2020). In pineapple plants, only about 22.5% of the fruit is used and, of the remainder, 4.5% corresponds to the skin and 73% to the vegetative part (leaves, stem and crown) (Carvalho *et al*., 1991; Paula; Faria Júnior, 2019).

Açaí is a crop of economic and social value in the Amazon region. In the national scenario in Brazil, the state of Amazonas is the second largest producer of açaí and, in addition to supplying the Brazilian market, it also exports to other countries such as France and Switzerland (Melo; Costa; Silva, 2021). However, the edible part of the fruit corresponds to only 17% of its weight, and thus its cultivation generates a considerable amount of waste, composed of fibers and seeds (Barbosa *et al.*, 2019; Boeira *et al.*, 2020).

Residues from agro-industrial activities have a great potential for use and many are underused. In this sense, studies related to the use of plant residues in mushroom cultivation may demonstrate an improvement in productive yield, in addition to contributing to the nutritional and chemical composition of the mushroom produced (Tavarwisa *et al*., 2021; Suwannarach *et al*., 2022). Moreover, the use of lignocellulosic residues as cultivation substrates implies the reduction of environmental impacts generated by the residues, in addition to bringing economic benefits, such as the fungiculture itself (Gowda; Manvi, 2020).

Choosing a suitable substrate is essential for successful mushroom cultivation, since characteristics, such as carbon content, nitrogen and C/N ratio (Sales-Campos; Andrade, 2011; Mahari *et al*., 2020), lignin, hemicellulose and cellulose (Annepu *et al.*, 2019; Shin *et al*., 2019) and the availability, composition and structure of fibrous components (Alfianti *et al.*, 2021), are essential for productivity. These factors can also influence mycelial development (Ivarsson *et al*., 2021) and basidiocarp formation

(Jeznabadi; Jafarpour; Eghbalsaied, 2016; Jeznabadi *et al*., 2017); therefore, it is essential to discover the physicochemical characteristics of lignocellulosic residues in order to choose a suitable growing substrate for a particular fungal species.

Another point to be highlighted is that, generally, the residues used in mushroom cultivation tend to be fibrous products of low digestibility, with low nutritional contents and a complex fibrous structure, which makes it difficult to use them for other purposes. One way to improve the nutritional quality and digestibility of the residue is by treatment with basidiomycetes, which degrade cellulose, hemicellulose and lignin and enrich the post-culture substrate, cause increments in protein and N, and alterations in the fibrous constitution, as well as promoting the accessibility of nutrients, making it more suitable for other uses such as in animal feed and as an organic fertilizer (Gonçalves *et al*., 2010). Thus, the present study aimed to evaluate the physical and chemical characteristics of lignocellulosic residues and their alterations after the cultivation of two *Pleurotus ostreatus* strains (474 and 542) in order to infer about the most suitable residue for the production of this fungus.

MATERIAL AND METHODS

Açaí seeds (*Euterpe* sp.) and pineapple crowns (*Ananas comosus* (L.) Merril) were acquired in the city of Manaus, Amazonas (3° 06′ 06″ S, 60° 01′ 29″ W), dried, crushed, and stored for fungal cultivation. Two strains of *Pleurotus ostreatus* (474 and 542), obtained from the Collection of Microorganisms of Agrosilvicultural Interest (Instituto Nacional de Pesquisas da Amazônia - INPA), were used for cultivation.

Spawn production was carried out in flasks containing 78% açaí seeds (AS), pineapple crown (PC) or açaí seeds + pineapple crowns (AS+PC, 1:1 m/m) supplemented with a 20% mixture of rice, wheat and corn bran (60:20:20 w/w/w) and 2% of $CaCO_3$ (w/w). The flasks were autoclaved at 121 ºC, and the mycelium inoculum (5% w/w) of the strains in PDA (potato dextrose agar) media were transferred from Petri dishes. After incubation (25 ºC) and growth, 5% of the spawn was transferred to the cultivation bags containing each substrate (1 kg), prepared in the same way as described for the spawn formulation. The bags were incubated at 25 ºC and subjected to 90% humidity and a photoperiod of 12 hours after colonization (Aguiar *et al*., 2022).

The evaluation of the proximate composition was carried out on the residues, initial substrates (pre-cultivation) and spent mushroom substrate (post-cultivation

substrates) of the mushrooms (spent). Initially, the moisture content was obtained by drying the samples at 105 ºC and pH was determined using a potentiometer (IAL, 2008).

To obtain the total tannin content, the residues were previously dried for 16 hours at 60 ºC, with subsequent extraction in a solution of ethanol, methanol and chloroform for 10 days in the absence of light (Pansera *et al*., 2003). For quantification, the extracts (or tannic acid) were incubated with distilled water, Folin-Ciocalteu reagent and Na_2CO_3 solution (10%); then, the reaction mixture was read at 725 nm in a microplate reader.

In the carbon analysis (Walkley-Black), the residues were titrated with ammoniacal ferrous sulfate, after being previously heated (60 ºC for 10 minutes) with potassium dichromate and sulfuric acid and having a diphenylamine indicator and phosphoric acid added. For nitrogen determination (Kjeldahl), the samples were digested with sulfuric acid in a digester block (350 ºC), distilled with sodium hydroxide, collected in a boric acid solution with indicators and titrated with hydrochloric acid solution (Tedesco *et al.*, 1995). The protein content was obtained by multiplying the value of N by the conversion factor 6.25 (Furlani; Godoy, 2005).

For the quantification of total fibers, the samples were digested in solutions of sulfuric acid and sodium hydroxide, dried at 105 °C and calcined at 550 ºC (IAL, 2008). Neutral detergent fiber (NDF) content was obtained from the digestion of samples in a neutral detergent solution and dried at 105 ºC. Then, samples were digested in acid detergent solution (ADF) and dried at 105 ºC (Van Soest, 1963). The lignin content was determined from samples from the NDF and ADF, that were in contact with $KMnO_4$ for 2 h, followed by incubation for 30 minutes in the demineralizing solution. Subsequently, the samples were dried at 105 ºC and calcined at 550 ºC (Van Soest; Wine, 1968). Ashes were determined from calcination at 550 ºC for 4 h (IAL, 2008).

For the lipid content, the samples were subjected to extraction in chloroform, methanol, water and sodium sulfate solution, filtered, and dried until the evaporation of the chloroform (IAL, 2008). Finally, the available carbohydrate was obtained by difference, from the subtraction of the moisture, protein, lipid, ash and fiber contents, from a total of 100, while the total carbohydrate content was obtained by subtracting the moisture, protein, lipid and ash content from a total of 100 (NEPA, 2011).

Scanning electron microscopy (SEM) and X-Ray diffraction (XRD) analyses were performed on the residues and the initial and spent mushroom substrates. In the SEM analyses, the samples were observed under a microscope (Phillips XL - 30ESEM) using

a 15 kV electron beam. XRD was evaluated using a diffractometer (XRD-6000, Shimadzu), copper Kα radiation, with a voltage of 30.0 kV and an electrical current of 15 mA, at a rate of 2.0 degrees per minute, for a continuous scan of 2θ with range of 4.0 – 70.0 ºC. The fiber crystallinity index was calculated according to Segal *et al.* (1959).

The experiment consisted of two *P. ostreatus* strains (474 and 542) and three substrates (AS, PC, AS+PC), in a completely randomized design, in a 2 x 3 factorial scheme, with 14 repetitions (each repetition as a grow bag). Physicochemical analyses were performed in triplicate. Data were submitted to ANOVA and then compared using the Scott-Knott test ($p>0.05$), using the Sisvar 5.6 software (Ferreira, 2019).

RESULTS AND DISCUSSION

The initial substrates presented humidity that varied between 63.34% and 67.17%. In the spent mushroom substrates, the moisture varied from 29.55% (strain 542 in açaí residues) to 55.84% (strain 542 in pineapple residues) (Table 1). Additionally, the pineapple-based substrate presented the highest moisture content both when grown with strain 542 and when grown with strain 474 (Table 1).

Moisture is responsible for affecting the availability of nutrients in the cultivation substrate, as well as access to oxygen by the fungus, in addition to favoring the presence of contaminants when in excess. Thus, the substrate moisture must be in a range of 50 to 75% in order to benefit mushroom cultivation (Chang; Miles, 2004), with a range of 60 to 65% being ideal (Mahari *et al*., 2020).

Analyzing the pH, all residues and substrates showed acidic values, ranging from 6.00 to 6.24 for the residues and from 6.59 to 6.69 for initial substrates (Table 1). Due to the presence of short-chain fatty acids, lignocellulosic residues commonly have acidic pH (Xiros; Shahab; Studer, 2019; Solanilla-Duque; Salazar-Sánchez; Herrera, 2021). After cultivation, a decrease in pH values was observed for all (Table 1). Acidification of the pH after cultivation may be due to the production of secondary metabolites during growth, such as low molecular weight organic acids (Liu *et al*., 2021). Kainthola *et al.* (2019) evaluated the growth of *P. ostreatus*, *Phanerochaete chrysosposrium* and *Ganoderma lucidum* in rice straw, and found a decrease in pH from 7.5 to 5.5 and attributed this to the ability of white rot fungi to degrade the lignocellulosic wall in plant biomass.

Thus, if the pH is not suitable for cultivation, even if the conditions of temperature and amount of nutrients in the substrate are ideal, a decrease in growth or a complete

inhibition of the fungus may occur (Sánchez; Royse, 2001). Therefore, the range of pH values suitable for growth can vary from 5 to 7, though optimal values should be from 5.0 to 6.5 for mycelial growth and from 6.5 to 7.0 for basidiome (Mahari *et al*., 2020).

Table 1 – Physicochemical parameters of residues, initial substrates and spent mushroom substrates of two *Pleurotus ostreatus* strains (474 and 542).

		Moisture (%)	pH	Tannins (µg/mg)	Carbon (%)	Nitrogen (%)	C/N	Ashes (%)
Residues	PC	6.48^{c} ±0.07	6.00^{c} ±0.01	8.51^{c} ±0.30	43.26^{a} ±0.41	0.56^{a} ±0.00	77.38^{c} ±0.74	5.22^{a} ±0.21
	AS	11.88^{a} ±0.14	6.24^{a} ±0.01	13.88^{a} ±0.51	38.61^{c} ±0.72	0.19^{c} ±0.00	207.11^{a} ±3.89	1.22^{c} ±0.12
	PC+AS	9.45^{b} ±0.11	6.12^{b} ±0.01	11.21^{b} ±0.31	41.35^{b} ±0.41	0.37^{b} ±0.00	110.98^{b} ±1.10	3.12^{b} ±0.06
Initial substrates	PC	63.34^{b} ±0.44	6.59^{c} ±0.01	-	44.85^{a} ±0.33	0.65^{a} ±0.01	68.74^{c} ±0.51	11.62^{a} ±0.34
	AS	67.17^{a} ±1.91	6.69^{a} ±0.01	-	42.34^{b} ±0.58	0.37^{c} ±0.00	113.59^{a} ±1.59	4.07^{c} ±0.02
	PC+AS	64.43^{b} ±1.16	6.61^{b} ±0.01	-	41.56^{b} ±0.49	0.47^{b} ±0.01	89.24^{b} ±1.05	5.29^{b} ±0.18
Spent substrates	474 PC	54.85^{Aa} ±3.22	5.75^{Bb} ±0.01	-	39.05^{Dc} ±0.33	0.84^{Ba} ±0.00	46.54^{Ec} ±0.38	10.29^{Ba} ±0.17
	474 AS	38.20^{Bb} ±4.34	5.79^{Aa} ±0.01	-	41.37^{Bb} ±0.41	0.56^{Ec} ±0.00	74.01^{Ba} ±0.61	7.59^{Ec} ±0.04
	474 PC+AS	40.43^{Bb} ±2.19	5.50^{Ec} ±0.01	-	42.15^{Aa} ±0.41	0.65^{Db} ±0.00	64.62^{Cb} ±0.54	9.39^{Cb} ±0.24
	542 PC	55.84^{Aa} ±5.12	5.52^{Db} ±0.01	-	36.54^{Ec} ±0.00	0.93^{Aa} ±0.00	39.18^{Fc} ±0.02	13.38^{Aa} ±0.19
	542 AS	29.55^{Dc} ±4.22	5.50^{Ec} ±0.01	-	42.34^{Aa} ±0.00	0.47^{Fc} ±0.00	90.83^{Aa} ±0.03	8.32^{Dc} ±0.09
	542 PC+AS	37.75^{Cb} ±7.46	5.64^{Ca} ±0.01	-	40.21_{Cb} ±0.33	0.65^{Cb} ±0.00	61.58^{Db} ±0.53	10.32^{Bb} ±0.01

PC: Pineapple crowns; AS: Acai seeds; PC + AS: Pineapple crowns + Acaí seeds (1:1 w/w). Averages followed by lowercase letters compare all residues and all initial and spent mushroom substrates. Capital letters compare spent mushroom substrates within each strain. Averages were compared using the Scott-Knott Test ($p<0.05$).

The tannin content in the residues is one of the variables that may have influenced the mushroom production, since the açaí residue had a higher tannin content (Table 1). The high tannin content in residues is undesirable in mushroom cultivation, as it is an anti-nutritional factor that is harmful to mycelial development and, consequently, affects the nutritional quality of the basidiomes (Ivarsson *et al*., 2021).

As for carbon, the three types of residue (PC, AS and PC+AS) and the pineapple-based initial substrate (PC) showed the highest levels; though, after fungal growth, the pineapple-based initial substrate (PC) exhibited the greatest reductions in their content

(Table 1). Carbon is essential as an energy source in development, and participates in the formation of fungal cell structures and chemical reactions (Chang; Miles, 2004; Anike *et al*., 2016). Thus, the PC-based substrate provided greater energy input for the cultivation of the fungal strains studied, which may be one of the reasons for its higher productivity.

As with the carbon content, nitrogen was also higher in the three types of residue and PC-based initial substrate and, after the growth of the fungi, there was an increase in the nitrogen levels in all spent mushroom substrates (Table 1). The C/N ratio in the residues ranged from 77:1 to 207:1, while, in the initial substrates, it ranged from 68:1 to 113:1, with the highest ratios observed for the açaí (AS) residue (Table 1). After mushroom cultivation, a reduction of about 30% of C/N was observed for all spent mushroom substrates (Table 1). The values obtained for the residue and PC-based substrate are in the most suitable ranges for the cultivation of *Pleurotus* spp.

The increase in nitrogen content in all the substrates after mushroom cultivation may be associated with the presence of the mycelium of the fungus, resulting from its growth and development, since the fungus normally has a greater amount of nitrogen in relation to substrates, in addition to the excretion of hydrolytic enzymes from fungal metabolism (Andrade *et al.*, 2013; Oztürk and Atila, 2021). There is also the possibility of fixing atmospheric nitrogen through the association of *Pleurotus* sp. with diazotrophic organisms (Jayasinghearachchi; Seneviratne, 2004).

Pleurotus spp. can grow in different wood species in nature, with a C/N ratio of 350 to 500:1; however, for high productivity, they need a greater amount of N (Chang; Miles, 2004; Sales-Campos; Andrade, 2011). The nitrogen content and the C/N ratio of the substrate correspond to one of the essential factors for mushroom cultivation. According to Mahari *et al*. (2020), the ideal C/N ratio should vary between 19:1-22:1. However, an initial C/N ratio of around 70-80:1 is also efficient for a productive crop (Chang; Miles, 2004).

Another characteristic that may also have influenced production is the ash content, since the three types of residue and the PC-based initial substrate had the highest ash contents, with 5.22% and 11.62%, respectively, while the three types of residue and the AS-based initial substrate exhibited the lowest ash contents (Table 1). Thus, PC provided greater mineral supply to the mushrooms.

After mushroom cultivation, an increase in ash content was observed in spent mushroom substrates, except for in strain 474 when cultivated in PC (Table 1). This

increase is caused by the process of consumption of the substrate, since the organic matter is degraded by the fungus during cultivation, releasing inorganic elements in the medium, represented by ash (Singh, 2000).

Grimm *et al.* (2021) cultivated *Pleurotus ostreatus* in substrates with different concentrations of residues from the paper industry, and noticed that fungal development was affected by the ash content of the substrates. Thus, the substrates with higher ash contents provided a shorter collection time of the first productive flush, in addition to presenting faster mycelial development. Therefore, the ash content of a material provides an indication of its mineral, as it corresponds to the fixed mineral residue, which is obtained after the decomposition of the organic components (Sales-Campos *et al.*, 2010).

As for the total fibers, the three types of residue and AS-based initial substrate showed the highest levels. A decrease of 28% and 22% in AS-based substrate occurred after the cultivation of strains 474 and 542, respectively (Table 2). Otieno *et al.* (2022) used various fruit residues as a substrate for the cultivation of different *Pleurotus* strains and observed higher total fiber contents for wheat straw, with a value of 41.60%.

The profile of the lignocellulolytic composition of the residues shows that the substrate most degraded by the strains was PC substrate, with a reduction of over 50% of the cellulose, hemicellulose and lignin contents after mushroom cultivation. On the other hand, the AS-based substrate showed the lowest consumption of these lignocellulosic compounds by strains 474 and 542 (Table 2). In general, the lignocellulosic composition of waste is 35-50% cellulose, 20-35% hemicellulose and 10-25% lignin, varying according to the origin and type of material (Santos *et al*., 2012; Gong *et al*., 2019).

Regarding the protein content, as observed for nitrogen, the residue and initial and spent mushroom PC-based substrates had the highest protein content (Table 2). Additionally, in the formulation of the initial substrates, there was a contribution in the protein content that was probably from the supplementation, as well as after the cultivation of strains 474 and 542. It is possible that this was due to the presence of fungal hyphae (Table 2). The protein present in the substrate is essential for adequate enzymatic activity of the fungus, both for the production of the enzymes responsible for substrate degradation and for those related to mycelial development (Atoji-Henrique *et al*., 2017).

The PC residue presented the highest lipid content; however, after the formulation of the initial substrates, the pineapple crowns + açaí seeds (PC + AS) provided the highest lipid supply (Table 2). After mushroom cultivation, it was observed that strain 474

consumed a greater amount of lipids from the substrates when compared to strain 542 (Table 2).

Table 2 – Centesimal composition of residues, and initial and spent mushroom substrates of two *Pleurotus ostreatus* strains (474 and 542).

		Total fibers (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Total protein (%)	Lipids (%)	Total carbs (%)	Available carbs (%)
Residues	PC	29.58^{c} ±0.43	36.70^{c} ±1.13	29.24^{a} ±0.73	6.49^{a} ±1.26	3.49^{a} ±0.00	4.11^{a} ±0.01	80.69^{c} ±0.27	51.11^{a} ±0.16
	AS	42.90^{a} ±0.37	62.88^{a} ±1.63	30.27^{a} ±0.49	4.46^{a} ±0.85	1.16^{c} ±0.00	3.23^{b} ±0.06	82.50^{a} ±0.20	39.61^{c} ±0.57
	PC+AS	35.62^{b} ±0.10	45.40^{b} ±1.82	29.83^{a} ±0.47	5.52^{a} ±0.34	2.33^{b} ±0.00	3.21^{b} ±0.00	81.88^{b} ±0.06	46.26^{b} ±0.04
Initial substrates	PC	28.30^{c} ±0.30	37.78^{b} ±0.98	26.36^{a} ±0.49	7.01^{a} ±0.75	4.08^{a} ±0.00	3.39^{b} ±0.11	77.77^{c} ±0.45	49.47^{a} ±0.14
	AS	40.96^{a} ±0.53	48.96^{a} ±2.64	25.17^{a} ±1.56	5.42^{a} ±0.18	2.33^{c} ±0.00	3.41^{b} ±0.35	84.56 a ±0.11	43.59^{b} ±0.64
	PC+AS	32.82^{b} ±0.24	49.96^{a} ±3.25	23.06^{b} ±1.07	5.22^{a} ±0.40	2.91^{b} ±0.00	4.20^{a} ±0.13	82.49^{b} ±0.48	49.68^{a} ±0.24
Spent substrates	474 PC	11.69^{Fc} ±0.49	18.95^{Cc} ±1.57	10.38^{Cb} ±0.27	2.86^{Ab} ±0.25	5.24^{Ba} ±0.00	4.52^{Aa} ±0.08	72.85^{Dc} ±0.07	61.16^{Aa} ±0.56
	474 AS	29.47^{Ba} ±0.35	41.79^{Aa} ±1.52	16.82^{Ba} ±0.46	4.01^{Aa} ±0.23	3.49^{Ec} ±0.00	1.93^{Ec} ±0.05	82.62^{Aa} ±0.13	53.15^{Bb} ±0.22
	474 PC+AS	27.20^{Db} ±0.37	35.48^{Bb} ±2.31	16.65^{Ba} ±2.05	3.53^{Ab} ±0.07	4.07^{Db} ±0.00	2.27^{Db} ±0.06	80.09^{Bb} ±0.05	52.90^{Bb} ±0.49
	542 PC	16.59^{Ec} ±0.01	8.58^{Db} ±0.35	16.10^{Bb} ±2.44	3.43^{Aa} ±0.46	5.83^{Aa} ±0.00	3.72^{Ba} ±0.20	70.24^{Ec} ±0.35	53.65^{Ab} ±0.34
	542 AS	31.78^{Aa} ±0.26	40.84^{Aa} ±0.52	22.40^{Aa} ±0.79	3.61^{Aa} ±0.36	2.91^{Fc} ±0.00	3.42^{Cb} ±0.19	78.51^{Cb} ±0.25	50.17^{Dc} ±0.31
	542 PC+AS	27.96^{Cb} ±0.12	38.75^{Aa} ±2.03	16.73^{Bb} ±0.91	3.66^{Aa} ±0.27	4.08^{Cb} ±0.00	1.92^{Ec} ±0.04	80.15^{Ba} ±0.28	52.19^{Cb} ±0.17

PC: Pineapple crowns; AS: Acai seeds; PC + AS: Pineapple crowns + Acaí seeds (1:1 w/w). Averages followed by lowercase letters compare all residues and all initial and spent mushroom substrates. Capital letters compare spent mushroom substrates within each strain. Averages were compared using the Scott-Knott Test ($p<0.05$).

Peter *et al.* (2019) used cassava peels, banana leaves, sawdust, yam peels and groundnut shells in the cultivation of *P. ostreatus*, and obtained mushrooms that ranged from 1.83 to 4.62% lipids. This variation is believed to be linked to the nature of the substrate used. The use of lipids as an energy source by the fungus is indicative of a preference for the soluble fraction of the substrate, which is more easily metabolized (Hidayat *et al.*, 2022). According to Yulistiani, Puastuti and Wina (2012), mushrooms primarily use the soluble sources of the medium as an energy source for their metabolism.

The total carbohydrates of the residues varied from 80.69% for the PC to 82.50% for the AS, with the AS residue having the highest amount. Similar behavior was observed

for the initial substrates produced with these residues. Analyzing the spent substrate, it is possible to notice a reduction in the total carbohydrate content in all substrates (Table 2).

As for available carbohydrates, the residue, PC-based initial substrate and PC-based spent mushroom substrate showed greater availability of carbohydrates, with an increase in the amount of available carbohydrates in the spent mushroom substrates of strains 474 and 542 (Table 2). One of the basic criteria for the formulation of a good substrate for mushroom cultivation is to present an adequate carbohydrate and nitrogen content in order to provide energy and structural support for the growth, as substrates with high nutritional content shorten the propagation time of the mycelium and accelerate substrate colonization (Ogundele; Abdulazeez; Bamidele, 2014; Khoo *et al*., 2022). Increases in available carbohydrates, ash and others, such as nitrogen, are interesting for the reuse of these substrates in animal feed (Ivarsson *et al.*, 2021), in a new mushroom cultivation (Lisiecka, Prasad; Jasinska, 2021), or as a bio-fertilizer (Carmo *et al*., 2021).

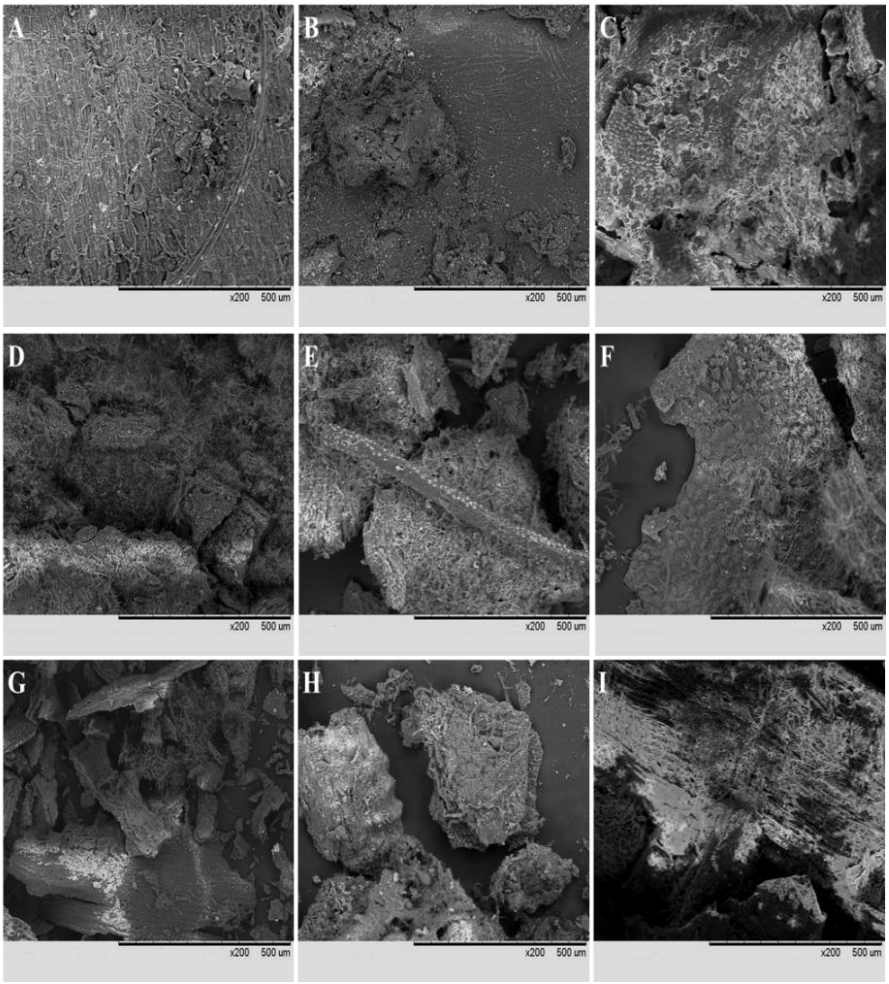
The aspects of the lignocellulosic fibers of the initial and spent mushroom substrates of strains 474 and 542 were obtained using scanning electron microscopy (SEM). It was observed that the initial substrates of pineapple crowns (PC), açaí seeds (AS) and PC + AS did not present consistent structural arrangements, which is associated with the heterogeneity of biomass (Figure 1). In addition, the structural irregularity of the residue may be related to the process used to grind the substrate, as well as the friction between the particles (Costa *et al*., 2015; Barbosa *et al*., 2019).

As for the characteristics reported in the literature, açaí seeds have a porous surface and an irregular structure (Figure 1B), with fibers of different shapes and dimensions, as well as small silica spheres (Figure 1E) (Mesquita *et al*., 2018; Martins *et al*., 2021). Oliveira *et al.* (2019) describe these silica spheres as granular structures found on the external and internal surfaces of pit channels in açaí seeds. The presence of silica in plant biomass increases fiber rigidity, and protects against predators, in addition to increasing resistance to water stress and temperature fluctuations (Silva; Potiguara, 2008).

The pineapple crown presents flattened fragments arranged mainly towards the smooth surface and has extractives, such as waxes (Figure 1A) (Pereira; Ornaghi Júnior; Coutinho, 2020). After mushroom cultivation, it was possible to observe the presence of pores with surface roughness in the growth substrates of strain 474 (Figures 1 D, E and F), while the growth substrates of strain 542 showed irregular surfaces with aggregates and porosity (Figures 1 G, H and I).

The structural modification of the spent mushroom substrates in relation to the initial substrates is evident, with differences between the degradation promoted by strains 474 and 542. Among them, the presence of smaller particles in 542-PC (Figure 1G), compared to 474-PC (Figure 1D) and more pores in 542-AS (Figure 1H) in relation to 474-AS (Figure 1E), as well as the high degree of fiber fragmentation in 542 PC+AS (Figure 1I) suggest that the degradation promoted by strain 542 was superior.

Figure 1 - Scanning electron microscopy (SEM) of the initial substrates and spent mushroom substrates of strains 474 and 542 of *Pleurotus ostreatus* (200x magnification).



Initial substrates: pineapple crown (A), açaí seeds (B) and pineapple crown + açaí seeds (C). Spent mushroom substrates: 474-pineapple (D), 474-açaí (E), 474-pineapple+açaí (F), 542-pineapple (G), 542-açaí (H) and 542-pineapple+açaí (I).

Regarding the substrates used, the most visible signs of degradation were found in spent mushroom substrates based on pineapple (Figures 1D and 1G) and açaí (Figures 1E and 1H), as there was degradation of the plant cell wall, with an increase in the

cellulose contact, resulting from particle size regression. Similar results were observed by Palangi *et al.* (2022), who cultivated *Agaricus bisporus* in plant biomass, and found a reduction in the size of fibers. The presence of highly irregular pores on the surface of the material and loosening of the fibers, observed after mushroom cultivation, is reported as a possible sign of substrate delignification caused by the fungal development itself, and is associated with the action of delignifying enzymes (Rezende *et al*., 2011; Grimaldi *et al*., 2015).

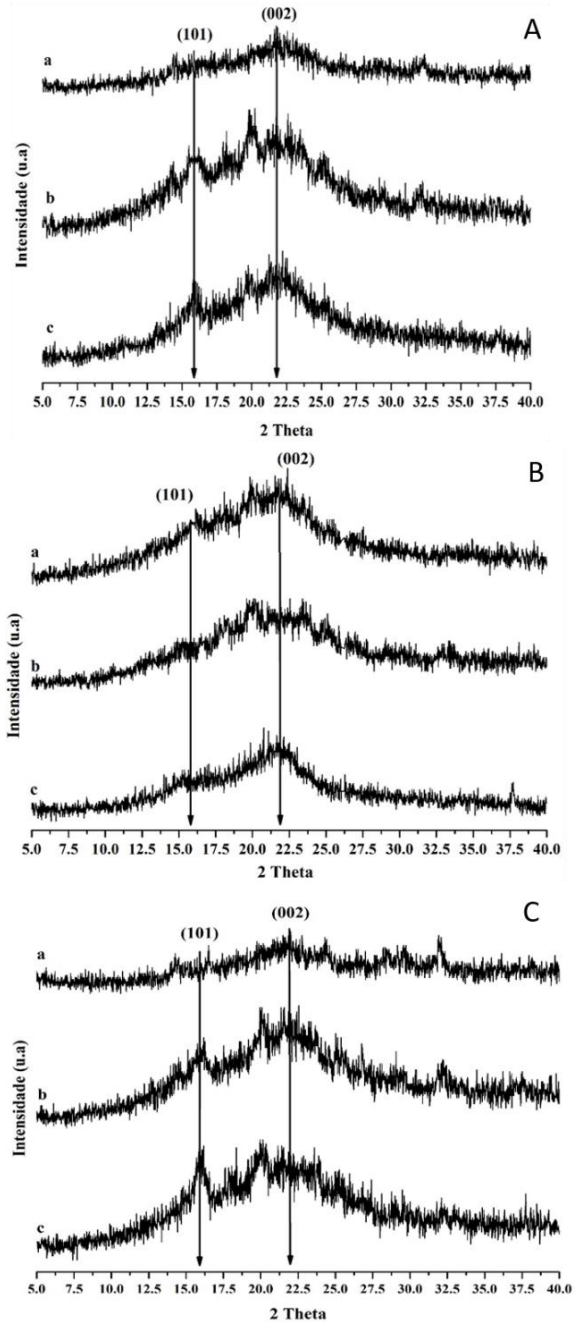
The increase in irregular shapes observed in spent mushroom substrates (Figure 1D - I) may be related to the production of lignocellulolytic enzymes by *P. ostreatus*. A similar result was obtained by Faria *et al.* (2020) who evaluated the degree of degradation of sugarcane bagasse after fermentation of *Humicola grisea* var. *thermoidea*, and detected an increase in cracking, spacing, fragmentation, and loosening of lignocellulosic fibers, and associated this to the action of cellulases and xylanases.

Sivaramakrishnan *et al.* (2021) observed that the surface of rice bran untreated with *Rhizopus oligosporus* is smooth when compared to pre-treated rice bran. The fungal pretreatment led to the formation of cracks, erosion and the appearance of rough structures, which suggests that these modifications are associated with the delignification and depolymerization of the plant biomass.

The X-ray diffraction (XRD) technique was used to compare the degree of crystallinity of the sample before and after the cultivation of strains 474 and 542 of *P. ostreatus* (Figure 2). Cellulose can exist in a highly structured form, known as microcrystalline cellulose, or in disordered regions, known as amorphous cellulose (Okolie *et al*., 2021). Furthermore, the amorphous region found in lignocellulose may be composed of hemicellulose, lignin, pectin and extractives (Solikhin *et al*., 2018).

When analyzing the diffractograms (XRD) of the initial and spent mushroom substrates of different strains of *P. ostreatus* (474 and 542), peaks were revealed in similar regions. For 2θ, at approximately 16°, crystallographic plane 101 was observed, which is characteristic of the amorphous region of cellulose, and for $2\theta = 22°$, the 002 crystallographic plane was observed, which is characteristic of the crystalline region of cellulose (Figure 2) (Suman *et al*., 2022). Lu *et al.* (2022) found a similar diffraction pattern for maize stem lignocellulosic residue.

Figure 2 - Diffractograms of the initial substrates (A) and spent mushroom substrates of *P. ostreatus* strains 474 (B) and 542 (C). Pineapple crowns (a), açaí seeds (b) and pineapple crowns + açaí seeds (c).



When comparing the crystallinity index (CI) of the initial substrates (Table 3), it is possible to observe that the lowest degree of crystallinity was detected in the pineapple residue. This indicates that this substrate is more easily hydrolysable, since the presence of highly crystalline regions results in a slower rate of enzymatic metabolism (Karimi; Taherzadeh, 2016). Zhao, Zhang and Liu (2012) confirm that crystalline cellulose is more

recalcitrant to microbial and enzymatic attacks when compared to amorphous attacks. The other initial substrates had a CI of around 40%, which similar to that found in cornhusks (41.2%) (Zhang *et al*., 2021).

Table 3 – Crystallinity Index (CI) by X-ray diffraction (XRD) of the initial substrates and spent mushroom substrates of strains 474 and 542 of *P. ostreatus.*

	Substrates	CI (%)
Initial substrates	PC	31.25
	AS	44.23
	PC + AS	41.07
Spent mushroom substrates	474 PC	23.81
	474 AS	24.29
	474 PC + AS	10.42
	542 PC	27.27
	542 AS	29.23
	542 PC + AS	16.67

PC: Pineapple crowns; AS: Açaí seeds; PC + AS: Pineapple crowns + Açaí seeds (1:1 w/w).

The CI, due to the relationship between the crystalline phase around 2θ = 22º and the amorphous phase at 2θ between 18° and 19º, was reduced in the spent mushroom substrates from the two *P. ostreatus* strains studied. This suggests a possible change in crystallinity after enzymatic hydrolysis. Bala and Singh (2019) observed that enzymatic hydrolysis, after saccharification of the residue, caused a decrease in the CI of the cellulose. Hu *et al.* (2021) found a reduction in the crystallinity of residues after fermentation of *P. geesteranus*, and attributed this fact to the degradation and absorption of cellulose as a source of nutrients by the fungus.

CONCLUSION

The pineapple-based substrate was the most suitable for mushroom cultivation since it has a lower tannin and cellulose content, which facilitates the degradation of the residue, in addition to providing greater energy input for the fungus.

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