Bioconversion of waste of macauba as indication of use for input in animal feeds

Bioconversão de resíduos de macaúba como indicação de uso para insumo em ração animal

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Abstract

Acrocomia aculeata, belonging to the Arecaceae family, a palm tree known locally as macauba or bocaiuva, displays potential for obtaining vegetable oil with application in the production of biofuels, food and pharmaceuticals. The objective of this work was to improve the nutritional aspects of macauba residues by myceliation by the fungi *Pleurotus ostreatus* and *Pleurotus sajor-caju*. The extraction of its oils (from the pulp and the kernel) generates residue, the most interesting being the bran or cakes. In this study, macauba's kernel and pulp bran, as well as their mixture with parts of the fruit, such as the epicarp, were submitted to the bioconversion treatment with the fungi *P. ostreatus* and *P. sajor-caju*. The composition of ash, crude protein, neutral and acid detergent fiber, lignin, cellulose, crude fiber, ether extract and in vitro digestibility were determined, in comparison to the control samples. In all samples there was a considerable increase in ash, protein and digestibility when compared to the control. Among the results obtained, the sample of the mixture of pulp bran and kernel in a 1:1 ratio stands out, which went from roughage to concentrate and was classified as protein. In the kernel bran-pulp bran (1:1) sample, production was carried out until the maturation phase with the fungus *P. ostreatus*. Fruiting occurred 80 days after the initial mycelium. The biological efficiency of this culture was 34.42%, and the value found for the crude protein of the mushroom produced was 33.11%.

Keywords: Agroindustrial co-products. Bioconversion. Edible fungi. Feed animals. Macauba bran.

Resumo

Acrocomia aculeata*, pertencente à família Arecaceae, palmeira conhecida localmente como macaúba ou bocaiúva, apresenta potencial para obtenção de óleo vegetal com aplicação na produção de biocombustíveis, alimentos e fármacos. O objetivo deste trabalho foi melhorar os aspectos nutricionais de resíduos de macaúba por meio da miceliação pelos fungos* Pleurotus ostreatus *e* Pleurotus sajor-caju*, visando seu uso em*

alimentaçãoo animal. A extração de seus óleos (da polpa e da amêndoa) gera resíduo, sendo o mais interessante o farelo ou bolos. Neste estudo, o caroço e o farelo da polpa da macaúba, bem como sua mistura com partes do fruto, como o epicarpo, foram submetidos ao tratamento de bioconversão com os fungos P. ostreatus *e* P. sajor-caju*. A composição de cinzas, proteína bruta, fibra em detergente neutro e ácido, lignina, celulose, fibra bruta, extrato etéreo e digestibilidade in vitro foram determinadas, em comparação com as amostras controle. Em todas as amostras houve aumento considerável de cinzas, proteína e digestibilidade quando comparadas ao controle. Entre os resultados obtidos, destaca-se a amostra da mistura de farelo de polpa e amêndoa na proporção de 1:1, que passou de volumoso para concentrado e foi classificada como proteína. Na amostra farelo-polpa de amêndoa (1:1), a produção foi realizada até a fase de maturação com o fungo* P. ostreatus*. A frutificação ocorreu 80 dias após o micélio inicial. A eficiência biológica desta cultura foi de 34,42%, e o valor encontrado para a proteína bruta do cogumelo produzido foi de 33,11%.*

Palavras-chave: *Coprodutos agroindustriais. Bioconversão. Fungos comestíveis. Alimentação animal. Farelo macaúba.*

Introduction

Macauba (*Acrocomia aculeata*) is a palm tree native to tropical America, widely distributed in the Brazilian savannah. Its fruits stand out for their high production of vegetable oil, which can be extracted from both the pulp and the kernel. The extracted oils, as well as the fruits in natura, have great pharmacological and nutritional importance (Silva et al., 2019; Moreira et al., 2022). The bran obtained from the extraction of oil from macaúba's pulp and kernel are rich in important residual nutritional properties, such as vitamins, antioxidants and minerals. These bran can also be nutritionally enriched by bioconversion processes, with potential application of these enriched residue in ruminant diet formulations (Revello, et al., 2020). We have applied this fermentation process to other agroindustry residue substrates with excellent results (Faria et al., 2022).

The use of microorganisms to bioconvert agricultural residue into products with high nutritional content, mainly by increasing proteins, vitamins and digestibility (Tanruean et al., 2021), is an excel-lent option to add value to these substrates that, after cultivation, present significantly improved characteristics compared to the original substrate (Sekan et al., 2019; Paiva, 2021).

Bioconversion is a process of transformation of a certain raw material involving living cells, such as in fermentation processes using microorganisms or portions of the cell, like enzymes, providing the reuse of organic matter with high lignin and cellulose levels (Carvalho et al., 2021). The objective of this study was to implement a biotechnological method involving solid state cultivation utilizing *Pleurotus sajor-caju* and *Pleurotus ostreatus*, with the ultimate goal of enhancing nutritional value and integrating macauba residues into animal feed formulations.

Material and methods

Obtainment of pure bran and its mixtures

The macauba tree fruits were collected in the Panambi region, a district of Dourados, Brazil. The fruits were removed from the bunches and washed to remove dirt adhering to the skin, and then stored in plastic bags and refrigerated (-5 °C). The removal of the shell (epicarp) and pulp were performed manually, as well as the breaking of the endocarp to remove the kernels.

The pulp and kernel were crushed and then subjected to drying at room temperature and stored in closed plastic bags under refrigeration (-5 °C) until use.

The pulp and kernel underwent an exhaustive oil extraction procedure with hexane in a Sohxlet extractor (Oliveira et al., 2017). The pulp and kernel bran were packed in closed plastic bags and stored under refrigeration (-5 °C), after drying in an oven at 60 °C.

The epicarp was manually extracted, dried in an oven and ground in a knife mill fitted with a 1 mm sieve at the outlet.

The mixtures used in this study were all prepared in a 1:1 ratio on a dry basis, as follows: pulp bran + kernel bran and pulp bran + epicarp.

Bioconversion by the fungi *Pleurotus* sp.

The 100% pulp and kernel bran (initial pH of 5.1) and mixtures 1:1 (initial pH of 5.22) were submitted to the mycelium process with the fungi *P. ostreatus* and *P. sajor-caju*. The samples myceliated were: whole pulp, pulp bran, pulp bran-epicarp (1:1), pulp bran-epicarp (2:1), pulp bran-kernel bran (1:1). Both fungi were grown in the dark, at a temperature of 25 °C and humidity of 60%, following the methodology cited by Paz et al. (2013) and Giunco et al. (2021).

The fungi inoculated were *P. sajor-caju* strain/batch 146/16 and *P. ostreatus* strain/batch 147/16, purchased commercially in the form of spawn from the Company Funghi e Flora, located in Valinhos, São Paulo (fungal inoculants produced in sterile media using cereal grains).

Sample preparation

In transparent polypropylene bags (20 x 30 cm) equal amounts of the samples that served as substrate were weighed and water was added to each one so that the humidity was as high as possible, since 60 to 75% of humidity favors growth and 85 – 97 % favors fruiting (Aghajani et al., 2018; Bellettini et al., 2019).

The bags were closed with cotton plugs, to allow gas exchange during the process, and sterilized in an autoclave at 121 °C for 25 minutes. After this time, the samples were left in the autoclave to cool down and then placed inside the laminar flow chamber (Marconi, Piracicaba, SP, Brazil), previously sterilized with 70% ethyl alcohol and ultraviolet light.

A small amount (approximately 0.3 g) of mushroom spawn was inoculated into each sample with the aid of flame-sterilized anatomical dissection forceps. Each bag was opened by removing the cotton plug, trying to keep the bag opening above the flame, as well as the bag containing the mother culture. The initial culture was introduced into the center of the bag and quickly closed with the cotton plug. This procedure was performed for each sample of the experiment.

For the mycelial colonization phase, the samples were incubated in a biochemical oxygen demand (BOD) incubator (Quimis, model Q315M25) at a temperature of 25 °C, being monitored daily.

Mushroom production

The cultivation of *P. ostreatus* was carried out in two samples: 100% kernel bran and pulp-kernel bran in a 1:1 ratio. Initially, they were submitted to the mushroom inoculation and incubation steps, observing the growth of the mushroom in the mycelium phase in BOD in the absence of light.

For fruiting, the substrate, completely colonized in a dark environment, was transferred to an environment with natural light and ventilation, as described by Cardoso et al. (2013), in a container with water at the bottom, as a way to stimulate the formation of fruiting bodies (Aghajani et al., 2018).

The polypropylene bags were removed when the first signs of fruiting were seen and supported on metal grids until the mushrooms were harvested, where they were cut at the base of the stalk, at the point of union with the substrate, and then weighed for calculation of biological efficiency (BE), using the following equation according to Silva et al. (2020):

$$BE\ (\%) = \frac{\text{fresh mushroom weight (g)}}{\text{dry substrate (g)}} \times 100$$

Where: fresh mushroom weight is the total weight of harvested mushrooms and dry substrate weight is the initial amount of dehydrated substrate.

A pH measurement was carried out by soaking the samples in distilled water, with stirring in a magnetic stirrer, for 4 hours. Afterwards, the sample was filtered and the pH was measured in a bench potentiometer (Quimis Q400AS) calibrated with buffer solutions pH 4.0 and 7.0. According to Bellettini et al. (2019), the ideal conditions in which the mycelium of *P. ostreatus* can grow is a pH variation between 5.5 and 6.5.

Analysis for initial characterization, after enrichment with fungi and production of mushrooms

The determination of physical and chemical properties on a wet basis were performed as described by Faria et al. (2022) as follows: dry matter (DM) (#934.01), ash content or total mineral content (TMC) (#924.05), crude protein (CP) (#920.87, Nx6,25), neutral detergent fiber (NDF) (Mertens, 2002), acid detergent fiber (ADF) (Van Soest and Robertson, 1985),

lignin (from the value of cellulose, the calculation was carried out to determine the content of lignin according to Faria et al., 2022), cellulose (Goering and Van Soest, 1970), ether extract (EE) (#920.39), crude fiber (CF) (#962.09) and in vitro digestibility of all samples, before and after enrichment with fungi, was performed in triplicate, at the Animal Nutrition Laboratory/College of Agricultural Sciences at the Universidade Federal da Grande Dourados (UFGD).

Statistical analysis

The computer program R (R Core Team 2014) was used to analyze the data obtained in the experimental tests. The data were submitted to exploratory analyzes to eliminate the existence of outliers and the bases of analysis of variance (linearity, homocessance and error normality). After the preliminary analysis, analyzes of variance ($p > 0.005$) and Tukey´s test were performed following the statistical model $Yij = \mu+Ti+eij$, where: Yi = observed variable i; μ = overall mean; Ti = effect of in mixtures of pulp bran with kernel bran; eij = experimental error.

Results and discussion

Initial characterization of samples and substrate mixtures

The results of the preliminary evaluation of the pulp, including the initial amount of oil, pulp bran and pith bran, epicarp, macauba bunch and mixtures of pulp bran with pulp bran, macauba bunch and epicarp in a 1:1 ratio are showed in Table 1.

Table 1 - Results of bromatological analyzes and in vitro digestibility carried out on samples and substrate mixtures from the extraction of macauba oil (*Acrocomia aculeata*) on a dry basis

Analyzes (%)	Samples							
	MP	PB	NB	Epicarp	Bunch	PB + NB	PB + bunch	PB + Epicarp
Dry matter	93.13 ± 0.15	91.77 ± 0.17	93.85 ± 0.11	90.95 ± 0.06	93.56 ± 0.11	91.43 ± 0.02	90.86 ± 0.06	90.16 ± 0.24
TMC (ash content)	4.29 ± 0.10	6.64 ± 0.14	4.34 ± 0.09	4.05 ± 0.07	6.52 ± 0.12	5.40 ± 0.17	6.32 ± 0.07	5.78 ± 0.20
Crude protein	5.10 ± 0.32	7.83 ± 0.33	27.81 ± 0.52	2.69 ± 0.25	1.76 ± 0.41	16.63 ± 0.26	5.47 ± 0.17	4.73 ± 0.15
NDF	51.57 ± 0.37	56.95 ± 0.07	50.38 ± 0.73	79.41 ± 0.89	83.96 ± 0.35	49.04 ± 0.92	58.49 ± 1.05	51.89 ± 0.79
ADF	27.88 ± 0.29	23.33 ± 0.88	34.71 ± 0.46	55.66 ± 0.70	59.31 ± 0.50	26.29 ± 0.90	36.38 ± 0.81	30.70 ± 0.65
Lignin	7.64 ± 0.42	4.00 ± 0.43	16.01 ± 0.31	9.41 ± 0.99	16.79 ± 0.15	9.19 ± 0.15	10.06 ± 0.35	5.09 ± 0.26
Cellulose	8.82 ± 0.60	13.69 ± 0.52	12.35 ± 1.78	45.56 ± 0.98	35.60 ± 1.11	17.09 ± 0.20	25.85 ± 0.63	26.01 ± 0.85
Hemicellulose theoretical*	79.45± 0.00	80.28± 0.00	85.09 ± 0.00	135.07± 0.00	143.27± 0.00	75.33± 0.00	94.87 ± 0.00	82.59 ± 0.00
Organic mater theoretical**	95.71± 0.00	93.36± 0.00	95.66± 0.00	95.95± 0.00	93.48± 0.00	94.60± 0.00	93.68 ± 0.00	94.22 ± 0.00
Crude fiber	23.91 ± 0.63	17.04 ± 0.51	29.76 ± 0.35	55.47 ± 1.07	56.59 ± 1.42	21.45 ± 1.34	32.95 ± 3.84	26.22 ± 0.44
Ether extract	34.26 ± 0.24	4.00 ± 0.02	20.59 ± 1.28	2.12 ± 0.45	not done	10.93 ± 0.05	1.86 ± 0.06	3.23 ± 0.08
Digestibility	70.91 ± 3.50	72.91 ± 4.95	67.97 ± 0.69	22.26 ± 0.50	19.58 ± 1.40	63.64 ± 1.21	51.73 ± 0.50	59.73 ± 1.85

Note: Data presented as average of triplicates ± standart deviation. MP = macauba pulp; PB = pulp bran; NB = nut bran; TMC = total mineral concentration; NDF = neutral detergent fiber; ADF = acid detergent fiber. *%NDF + %ADF; **100 – ash conent.

The pulp of macauba was analyzed with its total oil content (34.26%). After the process of extracting the oil from the pulp, a minimum amount of it (4%) was obtained. The pulp bran obtained after oil extraction showed a higher proportion shared among ash, crude protein, NDF and cellulose.

Ash or mineral matter results reflect the inorganic portion of the food, indicating how rich the sample is in mineral elements. A higher index can be verified in the pulp bran followed by the peduncle, kernel bran and, lastly, the epicarp.

In the analysis of crude protein, the highlight was the kernel bran with much higher levels than the other analyzed samples. This protein value of kernel bran signals its potential as an animal feed ingredient.

The epicarp, as expected, showed higher values for NDF and ADF, since the main components of these fibers are cellulose, hemicellulose, and lignin. High NDF values are indicative of limiting its use in large quantities, as they cause the gastrointestinal tract to fill up, making digestion by food enzymes in the animal organism and absorption in the intestine difficult (Revello et al., 2020). The analysis of the cellulose content, as well as the NDF and ADF already mentioned, showed very high values for the epicarp (45.56%). The rates for pulp bran (13.69%) and kernel bran (12.35%) were much lower.

Through the digestibility analysis, it was possible to prove the consistency of the values mentioned above, where the samples with the highest levels of NDF, ADF, lignin and cellulose are precisely those that presented the lowest digestibility, in line with the statement by Rodrigues (2010): "negatively correlated with digestibility, higher values indicate lower digestibility". The epicarp resulted in 22.26% of digestibility, while the pulp and kernel bran presented values above 65%.

Of all the analyzed samples, only the pulp bran could be classified as energy (CP < 20% in dry matter) concentrate (CF < 18% in dry matter). The other samples, according to the fiber result, were classified as bulky (CF > 18% in dry matter) dry (moisture < 12%) (Gonçalves et al., 2009; Salman et al., 2010).

The values present in all blends correspond to the average of each constituent. The addition of almond bran to the mixture (pulp bran + almond bran) improved the protein content, while combinations containing epicarp resulted in a marginal reduction in digestibility compared to pulp bran alone (72.91%).

Bioconversion and enrichment by mycelium growth

Humidity variation and mycelial growth

In this study several imbibition water proportions were tested envisioning to obtain the highest humidity content for each material and mixture tested, resulting in the maximum water adsorption for each sample. The humidity obtained for each sample were: up to 60% humidity for the pulp bran and up to 48% for the bran with fat, showing that the fat prevents the entry of water. In this composition there was no growth of any of the fungi tested.

In the pulp bran with kernel in a proportion of 1:1 the humidity obtained was up to 56%, where there was growth of *P. ostreatus* with 51% humidity and growth of *P. sajor-caju* with 56%. In the mixture of pulp bran with epicarp also in the proportion 1:1, there was a maximum humidity of 68% with growth of *P. ostreatus* at 68% and *P. sajor-caju* at 65%, showing that the composition is very important in the relation humidity/aeration .

Each substrate has a different water retention capacity and it is important to consider the maximum water addition for the final consistency of the sample, which must present a friable aspect to allow good aeration. A pasty mass hinders the porosity of the material and directly influences the fungal growth process (Bellettini et al., 2019). The ideal humidity recommended in the substrate for the development of fungi is between 50 and 80% depending not only on the species of fungi being cultivated, but also on the substrate used. That percentage varies from author to author, and higher percentages can be found.

Oxygen percolation through the sample proved to be the most important criterion, which was facilitated by the use of the epicarp.

It is noteworthy that the sample of the mixture of pulp bran and epicarp performed by weighing both in a 1:1 ratio showed favorable characteristics for growth. Again, this test proved the efficiency of using the epicarp as a humectant.

*Characterization of post-*mycelium *samples*

The results obtained, before and after inoculation with fungi, for the samples that showed mycelium growth, are presented in Figure 1 (data presented in Tables 2 and 3).

Comparing the fungi *P. ostreatus* and *P. sajor-caju* in the different samples, it can be seen that for the ash analyses all *P. ostreatus* values were higher and all lignin values for this same fungus were lower values, meaning they are better in terms of animal nutrition. As for protein, only in the kernel + pulp samples did the PO fungus show the best value. For pulp + bunch and pulp + epicarp, the best protein result was with *P. sajor-caju*.

In general, for the mixture of pulp bran + kernel bran, the best fungus was *P. ostreatus*, while the mixtures of pulp bran + bunch and pulp bran + epicarp showed a relatively greater potential with the fungus *P. sajor-caju*. However, none of them excelled in all aspects. They alternate best values between one and another sample.

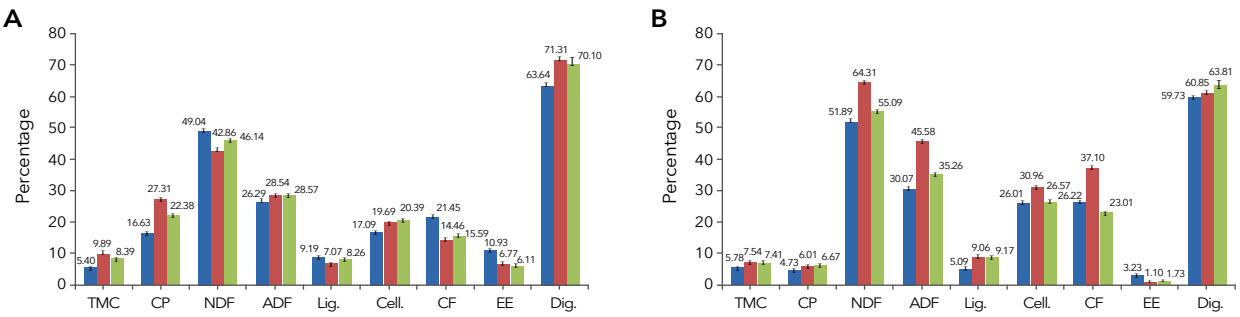

Figure 1 - Substrate enrichment by mycelium growth: (A) macauba's pulp and kernel bran, (B) macauba's pulp bran and epicarp.

Note: TMC = total mineral content; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Lig. = ligning; Cell. = cellulose; CF = crude fiber; EE = ether extract; Dig. = digestibility. Blue bars = control sample; red bars = with *Pleurotus ostreatus*; green bars = with *Pleurotus sajor-caju*.

Table 2 - Bromatological composition and in vitro digestibility of dry matter, of mixtures of pulp bran with macauba almond bran, in a 1:1 ratio, non-myceliated and myceliated with the fungi *Pleurotus ostreatus* (PO) and *Pleurotus sajor-caju* (PS)

Analyzes performed (%)	**Samples**		
	PB + NB standard	**PB + NB with PO**	**PB + NB with PS**
Dry matter	91.43^{b} ± 0.02	88.78^{c} ± 0.06	94.79^{a} ± 0.21
Total mineral content (ash content)	5.40^{c} ± 0.17	9.89^{a} ± 0.86	8.39^{b} ± 0.10
Crude protein	16.63^{c} ± 0.26	27.31^{a} ± 0.18	22.38^{b} ± 0.27
Neutral detergent fiber	49.04^{a} ± 0.92	42.86^{b} ± 0.94	46.14^{a} ± 0.33
Acid detergent fiber	26.29^{b} ± 0.90	28.54^{a} ± 0.43	28.57^{a} ± 0.35
Lignin	9.19^{a} ± 0.15	7.07^{b} ± 0.04	8.26^{a} ± 0.50
Cellulose	17.09^{b} ± 0.20	19.69^{a} ± 0.66	20.39^{a} ± 0.38
Hemicellulose theoretical*	75.33 ± 0.00	71.40 ± 0.00	74.71 ± 0.00
Organic mater theoretical**	94.60 ± 0.00	90.11 ± 0.00	91.61 ± 0.00
Crude fiber	21.45^{a} ± 1.34	14.46^{b} ± 0.80	15.59^{b} ± 0.33
Ether extract	10.93^{a} ± 0.05	6.77^{b} ± 0.22	6.45^{b} ± 0.07
Digestibility	63.64^{b} ± 1.21	71.31^{a} ± 1.29	70.10^{ab} ± 2.58

Note: Data presented as average of triplicates ± standart deviation. On the line, means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 5% error probability. PB = pulp bran; NB = nut bran. *%NDF + %ADF; **100 – ash content.

Table 3 - Compilation of the results of bromatological and digestibility analyzes, on a dry basis, carried out on mixtures of pulp bran with macauba epicarp, in a 1:1 ratio, non-myceliated and myceliated with the fungi *Pleurotus ostreatus* (PO) and *Pleurotus sajor-caju* (PS) applying the Tukey test

Analyzes performed (%)	Samples		
	PB + Ep standard	PB + Ep with PO	PB + Ep with PS
Dry matter	90.16^{b} ± 0.24	89.18^{c} ± 0.12	96.42^{a} ± 0.08
Total mineral content (ash content)	5.78^{b} ± 0.20	7.54^{a} ± 0.17	7.41^{a} ± 0.15
Crude protein	4.73^{c} ± 0.15	6.01^{b} ± 0.17	6.67^{a} ± 0.29
Neutral detergent fiber	51.89^{c} ± 0.79	64.31^{a} ± 1.02	55.09^{b} ± 1.52
Acid detergent fiber	30.70^{c} ± 0.65	45.58^{a} ± 0.32	35.26^{b} ± 0.37
Lignin	5.09^{b} ± 0.26	9.06^{a} ± 0.71	9.17^{a} ± 0.48
Cellulose	26.01^{b} ± 0.85	30.96^{a} ± 0.84	26.57^{b} ± 0.42
Hemicellulose theoretical*	82.59 ± 0.00	109.89 ± 0.00	90.35 ± 0.00
Organic mater theoretical**	94.22 ± 0.00	92.46 ± 0.00	92.59 ± 0.00
Crude fiber	26.22^{b} ± 0.44	37.10^{a} ± 0.94	23.01^{c} ± 0.22
Ether extract	3.23^{a} ± 0.08	1.10^{c} ± 0.12	1.73^{b} ± 0.10
Digestibility	59.73^{a} ± 1.85	60.85^{a} ± 1.79	63.81^{a} ± 2.09

Note: Data presented as average of triplicates ± standart deviation. On the line, means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 5% error probability. PB = pulp bran; Ep = epicarp. *%NDF + %ADF; **100 – ash content.

Only the mixture of pulp bran with kernel, after enrichment with both fungi, went from roughage (CF > 18% in dry matter) to concentrate (CF < 18% in dry matter) and classified as protein (crude protein - CP > 20% in dry matter), this characteristic being of great value because it gives the food a high energy content per unit volume.

Enrichment of substrates by mycelium growth

Analyzing the data referring to the same sample (mixture of pulp bran with kernel bran) for the ash analysis, the treatments with *P. ostreatus* and *P. sajor-caju* presented similar performances among themselves and superior to the treatment without fungus. The same occurred for ADF, cellulose and digestibility (Figure 1).

It is worth emphasizing that the mixture of pulp and kernel bran had an increase of 10.68% with *P. ostreatus* and 5.35% with *P. sajor-caju* for crude protein. The protein increase occurs due to an increase in the amount of protein-rich mycelium whose protein content is found between vegetables and meat, thus increasing the proportion of proteins when it permeates the substrate (Fayssal et al., 2021). It is noteworthy that there exists a substantial disparity in the total yields and biological efficiency of two mushroom species. Furthermore, this discrepancy persists even when the same species is cultivated on distinct substrates, indicating metabolic dissimilarities between them, including variations in their enzymatic machinery (Kurt and Buyukalaca, 2010). This difference in growth rate (mycelialation) clearly interferes with the composition of the mycelial substrates as a result of the density of the mycelial mass that permeates it.

The findings indicated that the blend of kernel bran and pulp bran was myceliated with *P. ostreatus*, exhibiting significant variances in all evaluations compared to the control sample, particularly in terms of NDF, lignin, and digestibility parameters. Nonetheless, as intended, the mycelial specimen displayed lower levels of NDF and lignin while presenting marginally higher values for digestibility. Such outcomes suggest a propensity towards enrichment.

Comparing the two mycelial samples with different types of fungi, it can be seen that there was no relevant difference between both fungi for the parameters: ADF, cellulose, crude fiber, ether extract and digestibility. None of the fungi stood out in all aspects. They alternated better values between one and another analysis.

Furthermore, for the mixture of pulp bran with kernel bran, analyzing now the pulp bran mixed with bunch for the analysis of ash, crude protein, ADF, cellulose and digestibility, the treatments with *P. ostreatus* and *P. sajor-caju* presented performances similar to each other and superior to the control sample.

Also, as desired, the reduction of values for both fungi, in relation to the control sample, for the parameters NDF, crude fiber and ether extract, can be seen. Only lignin remained practically similar in all treatments. Lignin, being a recalcitrant material, presents very slow degradation, so there was no time for its degradation. Lignin degradation strongly depends on the enzymatic activity of laccases, mainly multicopper oxidases. These crucial enzymes facilitate the ligninolytic mechanism by catalyzing the degradation of aromatic compounds through oxygen-dependent reactions (Durán-Aranguren et al., 2021). Moreover, laccases contain four copper ions, which are required primarily for the synthesis of the enzyme (Akpinar and Urek, 2012).

With the exception of dry matter, ash, and cellulose, there was no statistical difference between the two fungi tested in most parameters.

In the case of the mixture of pulp bran and macauba epicarp (Table 3), the fungus *P. ostreatus* does not statistically differ from the control sample in relation to in vitro digestibility, although it shows a positive trend towards an increase in the result. As for the fungus *P. sajor-caju*, in addition to digestibility, the cellulose result did not show statistical difference.

When the comparison is performed on the same sample (pulp/epicarp) between the two types of fungi, it is once again verified that they alternated better values between one and another. For the parameters: ash, lignin, and digestibility, there was no significant difference between both fungi.

Comparing the three tests (Figure 1), the first aspect that draws attention positively is the significant increase in ash, protein and digestibility for all samples in relation to the initial characterization, that is, without fungus. The ash value was higher for both treatments with fungi, being slightly higher for *P. ostreatus*. This increase in ash is related to the constant use of organic matter by the fungus, releasing minerals into the substrate, thus improving its nutritional quality through the bioavailability of these minerals (Mbassi et al., 2018; Araújo et al., 2020; Fayssal et al., 2021). The increase in ash content was also observed on various substrates (Bonatti et al., 2004; Fonseca et al., 2009; Araújo et al., 2020; Fozia et al., 2022).

The increase in protein content is observed in several studies with *Pleurotus* sp. (Fiorin, 2016; Vasconcelos et al., 2019; Faria et al., 2022). Unlike ashes, protein obtained better results with *P. sajor-caju*. This difference in protein content between *P. sajor-caju* and *P. ostreaus* is an inherent characteristic of the species, with the former being marginally more protein-rich than the latter. Additionally, there exists a dissimilarity in the density of mycelia that permeates throughout the substrate. This condition was observed by other authors (Bonatti et al., 2004).

This same relationship did not apply to digestibility, as two samples had better results with the *P. ostreaus* fungus and only pulp bran + epicarp showed a better value with *P. sajor-caju*. The digestibility of fungi is contingent upon the specific fungal species and substrate utilized. This variation in digestibility is intricately connected to the enzymatic complexity of each unique fungus as well as its media composition (Mendes et al., 2005; Araújo et al., 2020).

According to Rodrigues (2010), NDF levels in corn silage vary greatly, but around 50% is considered a good level. For Signoretti (2011), the minimum fiber content in diets for cows in early lactation should vary between 27 and 30% of NDF and 18 and 20% of ADF. The pulp bran + kernel bran samples showed NDF results below 50% in all treatments. The mixtures of pulp bran + epicarp had values around 51 to 64%, due to the contribution of epicarp fibers in the elevation of these values. The ADF results ranged from 26 to 45% among all samples.

The lignin and cellulose content in the samples did not show considerable variation after treatment with the fungi. The lignin of the pulp + kernel bran mixture had a slight decrease. For the pulp + epicarp sample, a considerable increase (from 5 to 9%). Cellulose, after mycelium growth, had an increase in values for all samples.

Availability, competitive prices and nutritional value are the main factors that influence the demand for protein foods in animal feeds. It is expected that, with the growth in the supply of other oilseeds destined for the production of biodiesel (canola, crambe, sunflower, macauba, castor bean, palm, jatropha, turnip, etc.), the dependence of soybean

meal in feed for animals is reduced, decreasing competition with human food and increasing options for producers (Oliveira et al., 2012).

Cultivation of *Pleurotus ostreatus*

The total colonization of the pure kernel bran and the mixture with the pulp bran took place in 30 days, and the induction of fruiting by light in 68 days. There was only fruitification for the mixture of pulp bran and kernel bran (1:1) with 75 days of testing; the treatment with pure kernel showed dehydration characteristics (yellowish and dry appearance) and was discarded. The fruiting bodies matured within five days. Each phase can be seen in Figure 2.

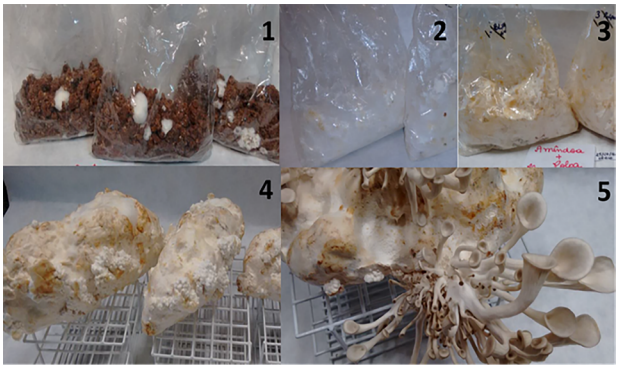

Figure 2 - Growth of the *Pleurotus ostreatus* fungus: (1) beginning of mycelium; (2) complete mycelium growth with 30 days; (3) day 68; (4) beginning of fructification with 75 days; and (5) fructification bodies.

The fruiting bodies were harvested at 80 days old, weighed and dried in an oven, together with the enriched substrates, at 40 °C for 24h. The biological efficiency obtained was 34.42%. In their work with a mixture of sugarcane and macauba bagasse, Cardoso et al. (2013) reported a biological efficiency value of 30.81% for a compound of 70% of sugarcane bagasse and 30 % of bocaiuva bagasse from a juice industry, material similar to the pulp used in this work, and 37.51% for a compound of 50% of sugarcane bagasse and 50% of bocaiuva bagasse.

Characterization of mushroom cultivation samples

The analysis carried out on the mushroom produced, on the substrate from which the mushroom was removed – which consists of a mixture of pulp bran + bocaiuva kernel bran in a 1:1 ratio – and on the mixture of both – substrate plus mushroom – are shown in Figure 3 and Table 4.

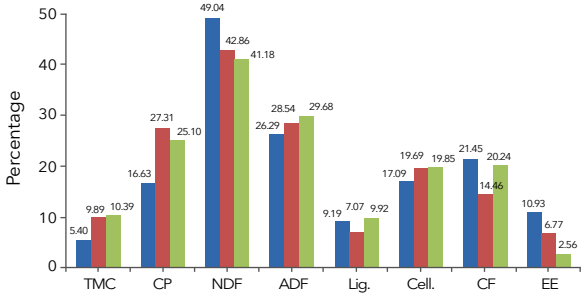

Figure 3 - Results of the parameters analyzed for the mixture of macauba pulp and nut bran.

Note: TMC = total mineral content; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Lig. = ligning; Cell. = cellulose; CF = crude fiber; EE = ether extract; blue bars = without fungus; red bars = *Pleurotus ostreatus* 30 days; green bars = *Pleurotus ostreatus* 80 days.

It presents a comparison with the mixture of bran from the pulp and kernel (1:1) with the characterization of the samples without treatment with fungi, with those left to mycelium for a period of 30 days and, finally, with those that remained for 80 days in the mycelium process, evaluating whether the time increase would make a difference in the nutritional values.

The use of *P. ostreatus* in human food has the advantage of being a food supplement, since it contains around 60% carbohydrates, 26% protein, 12% fiber, 0.9% to 1.8% fat, based on its dry weight; it also has vitamins such as niacin, thiamine (B1), vitamin B12, vitamin C, and minerals such as potassium, calcium and phosphorus have been detected (Mbassi et al., 2018; Fayssal et al., 2021). The high value of crude protein of the mushroom produced in this experiment (33.11%) stands out, and the result of crude fiber (11.75%) is consistent with Vasconcelos et al. (2019) and Faria et al. (2022) (12%).

With the exception of the crude fiber of the fungus-free mixture compared with the same mycelial sample for 80 days, all other parameters show differences between the control sample and with *P. ostreatus* fungus.

Table 4 - Results of bromatological and digestibility analyzes carried out on samples of pure mushroom, pure substrate and mixture of substrate plus mushroom - on a dry basis. Compilation of the results of bromatological and digestibility analyzes, on a dry basis, carried out on mixtures of pulp bran with macauba nut bran, in a 1:1 ratio, non-myceliated and myceliated with the fungi *Pleurotus ostreatus* (PO) for a period of 80 days (d), applying Tukey test

Analyzes (%)	Samples					
	Pure mushroom	Pure substrate	Substrate + mushroom	PB + NB no myceliation	PB + NB with with PO 30d	PB + NB with with PO 80d
Dry matter	90.71 ± 0.01	93.29 ± 0.17	93.26 ± 0.19	91.43^{b} ± 0.02	88.78^{c} ± 0.06	93.29^{a} ± 0.17
TMC	7.40 ± 0.22	10.39 ± 0.08	7.45 ± 0.16	5.40^{b} ± 0.17	9.89^{a} ± 0.86	10.39^{a} ± 0.08
Crude protein	33.11 ± 0.11	25.10 ± 0.39	23.97 ± 0.71	16.63^{c} ± 0.26	27.31^{a} ± 0.18	25.10^{b} ± 0.39
NDF	40.23 ± 0.45	41.18 ± 0.11	44.89 ± 0.91	49.04^{a} ± 0.92	42.86^{b} ± 0.94	41.18^{b} ± 0.11
ADF	20.53 ± 0.68	29.68 ± 0.23	29.50 ± 1.12	26.29^{b} ± 0.90	28.54^{a} ± 0.43	29.68^{a} ± 0.23
Lignin	5.03 ± 0.52	9.92 ± 0.38	8.24 ± 0.53	9.19^{b} ± 0.15	7.07^{c} ± 0.04	9.92^{a} ± 0.38
Cellulose	17.85 ± 1.23	19.85 ± 0.27	21.94 ± 0.64	17.09^{b} ± 0.20	19.69^{a} ± 0.66	19.85^{a} ± 0.27
Crude fiber	11.75 ± 0.66	20.24 ± 0.00	23.25 ± 0.89	21.45^{a} ± 1.34	14.46^{b} ± 0.80	20.24^{a} ± 0.00
Ether extract	not done	2.56 ± 0.37	4.35 ± 0.21	10.93^{a} ± 0.05	6.77^{b} ± 0.22	2.56^{c} ± 0.37
Digestibility	84.16 ± 0.44	not done	75.87 ± 0.24	not done	not done	not done

Note: Data presented as average of triplicates ± standart deviation. On the line, means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 5% error probability. PB = pulp bran; NB = nut bran; TMC = total mineral content (ash content); NDF = neutral detergent fiber; ADF = acid detergent fiber.

Comparing the myceliated samples for 30 and 80 days with each other, it appears that ash, NDF, ADF and cellulose present averages that do not differ from each other. The other parameters point to differences with negative trends for the longer incubation time. The protein had a small reduction (27.31% with 30 days to 25.10% with 80 days) remaining even, with a very expressive value in relation to the same sample without fungus (16.63%). The lignin and crude fiber content were higher after 80 days of mycelium growth, presenting values close to the samples without treat-ment with fungi. The ether extract had a significant reduction – from approximately 11% without fungi to 6.77% with 30 days of mycelium growth and, finally, 2.56% after 80 days of cultivation with *P. ostreatus* fungus, showing itself to be an excellent decomposer of the fat present in the substrate.

In view of this, it is possible to state that the increase of time, in days, that exceeds what is necessary for the fungus to cover the entire substrate (on average 30 days), is not necessary to obtain good results in the enrichment of the samples. This time of more than one month of colonization (in this case, 50 days more) does not justify better nutritional results; the same is only feasible if the objective is the production of mushrooms.

Conclusion

In view of the concern with new alternatives for enriched substrates and the cultivation of mushrooms in Brazil, giving priority to waste produced in the region itself, taking advantage of the particular characteristics of each one and collaborating to avoid waste during the production chain, it can be seen that *P. ostreatus* and *P. sajor-caju* resulted in great potential in mycelial samples, adding a considerable increase in ash, protein and digestibility in relation to the initial characterization, that is, without fungi. On the other hand, the mixture of pulp bran with kernel changed from bulky (CF > 18% in dry matter) to concentrate (CF < 18% in dry matter) and classified as protein (CP > 20% in dry matter), this characteristic being of great value for attributing to the food a high energy content per unit of volume. The cultivation of *P. ostreatus* with the sample of the mixture of pulp and kernel

bran resulted in a biological efficiency of 34.42% and the high value of crude protein of the produced mushroom (33.11%) was highlighted.

This way, the use of residue from macauba processing, from the points of view of clean technology and socio-environmental and economic issues, proved feasible. The results obtained in this study show the enrichment of the nutritional and digestive parameters of these residues, showing as their main benefit the possibility of their use in animal feed and human food, as is the case with mushroom production.

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