



# Nutritional and antioxidant potential of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn produced on agronomic wastes banana leaves and sugarcane bagasse substrates

Rossana Lucena de Medeiros<sup>1</sup> · Giuliane Moura Andrade<sup>1</sup> · Renata Barros Crispim<sup>2</sup> · Niellyson Nardan dos Santos Silva<sup>2</sup> · Sabrina Alves da Silva<sup>3</sup> · Háimyk Andressa Nóbrega de Souza<sup>3</sup> · Jhonatan Rafael Zárate-Salazar<sup>3</sup> · Francinalva Dantas de Medeiros<sup>4</sup> · Carlos Eduardo Alves Dantas<sup>5</sup> · Vanessa Bordin Viera<sup>5</sup> · Anauara Lima e Silva<sup>6</sup> · Josean Fechine Tavares<sup>6</sup> · Fillipe de Oliveira Pereira<sup>2</sup>

Received: 30 September 2023 / Accepted: 3 April 2024 / Published online: 22 April 2024 © The Author(s) under exclusive licence to Sociedade Brasileira de Microbiologia 2024

# Abstract

Global food production faces challenges concerning access to nutritious and sustainably produced food. *Pleurotus djamor*, however, is an edible mushroom that can be cultivated on agricultural waste. Considering that nutritional and functional potential of mushrooms can change based on cultivation conditions, we examined the influence of substrates with different compositions of banana leaf and sugarcane bagasse on the nutritional, mycochemical, and antioxidant properties of *P. djamor*. The mushrooms were grown for 120 days and dried in a circulating air oven at 45 °C for three days. We conducted bromatological analyses and mycochemical characterization (<sup>1</sup>H-NMR, total phenolics, and flavonoids) of the mushrooms and assayed the antioxidant activity of extracts from the dried mushrooms using an ethanol/water solution (70:30 v/v). In general, the substrates produced mushrooms with high protein (18.77 ± 0.24% to 17.80 ± 0.34%) and dietary fiber content (18.02 ± 0.05% to 19.32 ± 0.39%), and with low lipid (0.28 + 0.08% to 0.4 + 0.6%), and caloric content (maximum value: 258.42 + 8.49), with no significant differences between the groups ( $p \ge 0.05$ ). The mushrooms also exhibited high levels of total phenolics and flavonoids. The mushrooms cultivated on sugarcane bagasse substrates presented the highest values (p < 0.05). Analysis of the <sup>1</sup>H-NMR spectra indicates an abundant presence of heteropolysaccharides,  $\beta$ -glucans,  $\alpha$ -glucans, and oligosaccharides, and all the mushroom extracts exhibited high antioxidant activity. In conclusion, our study demonstrates that agricultural residues permit sustainable production of edible mushrooms while maintaining nutritional and functional properties.

Keywords Edible mushrooms · Fungi · NMR · Antioxidants · Mycochemical

Responsible Editor: Gisele Monteiro.

Fillipe de Oliveira Pereira fillipe.oliveira@professor.ufcg.edu.br

- <sup>1</sup> Postgraduate Program in Nutrition Sciences, Health Sciences Center, Federal University of Paraíba, João Pessoa, Brazil
- <sup>2</sup> Fungi Research Group, Academic Unit of Health, Education and Health Center, Federal University of Campina Grande, Cuité, Brazil
- <sup>3</sup> Grupo de Pesquisa & Produção de Cogumelos Comestíveis, Department of Soils and Rural Engineering, Center of Agricultural Sciences, Federal University of Paraíba, Areia, Brazil
- <sup>4</sup> Laboratory of Pharmacognosy, Academic Unit of Health, Education and Health Center, Federal University of Campina Grande, Cuité, Brazil
- <sup>5</sup> Laboratory of Bromatology, Academic Unit of Health, Education and Health Center, Federal University of Campina Grande, Cuité, Brazil
- <sup>6</sup> Postgraduate Program in Natural and Synthetic Bioactive Products, Health Sciences Center, Federal University of Paraíba, João Pessoa, Brazil

# Introduction

Mushrooms have been consumed as food and for their nutritional and medicinal properties for thousands of years. These fungi are considered a significant source of bioactive ingredients and present high contents of polysaccharides, proteins, dietary fibers, proteoglycans, vitamins like riboflavin and thiamine, and minerals such as potassium, phosphorus, magnesium, iron, and copper, as well as antioxidants, terpenoids, lectins, phenolics/polyphenolics, polysaccharides, and ergosterols [1]. Consumption of mushrooms is associated not only with nutritional benefits and health but also with sensory attributes [2].

Currently, there are around 2,000 edible mushroom species worldwide. The button mushroom (*Agaricus bisporus*), the shiitake (*Lentinula edodes*), and the oyster mushroom (*Pleurotus* spp.) are among the most cultivated and consumed species in Brazil and the world (Wan Mahari et al., 3). Global mushroom consumption is about 12.74 million tons and is projected to increase to 20.84 million tons by 2026 [4]. Due to ease of cultivation and high biological efficiency of *Pleurotus* spp, they account for about 25% of the global production of mushrooms, [5].

Species of the *Pleurotus* genus are classified as saprophytic. Some of the most common species in this genus are *Pleurotus ostreatus* ("oyster mushroom"), *P. djamor* ("pink oyster"), *P. citrinopileatus* ("golden oyster"), and *P. eryngii* ("king oyster"), among others. Thanks to an extensive enzymatic system capable of degrading and utilizing organic compounds [6], these species can utilize lignocellulosic substrates to develop, including byproducts from the food industry such as tea residues and spent beer grains [2].

The edible mushroom *Pleurotus djamor* Rumph. ex Fr. Boedijn (Basidiomycota) is naturally found in Brazil, in the north, the south, the northeast, and Paraíba state. *P. djamor* can be cultivated on various substrates and presents a shorter fruiting period than other commercial species of the genus. *P. djamor* is rich in proteins, dietary fibers, essential amino acids, carbohydrates, water-soluble vitamins and minerals, and abundant in bioactive molecules that can influence health [5]. The indigenous Yanomami people of Brazil have historically consumed *P. djamor*, especially when hunting was scarce. They mention that *P. djamor* is found on *embauba* tree trunks and, to a lesser extent, in the forests [7].

Cultivating edible mushrooms may help address the challenge of producing and consuming foods with fewer negative impacts on the environment yet offer beneficial biochemical components to human nutrition and health. Such cultivation follows the 17 Sustainable Development Goals (SDGs) of the United Nations' 2030 Agenda for social, economic, and environmentally sustainable development [8]. Mushroom cultivation represents a crucial biotechnological alternative for obtaining high-value nutritional and medicinal foods. It also enhances agricultural sustainability by recycling agroindustrial products. Moreover, when well managed within efficient systems (e.g., circular chain), mushroom production can increase crop profitability since the waste generated during production can also serve as both animal feed and soil improvement agents [9, 10].

Banana production and sugarcane cultivation hold significant social and economic importance for Northeastern Brazil. The "Brejo" microregion of Paraíba (Brazil) is a major banana producer at around 100,000 tons yearly. Sugarcane production in Brazil has witnessed a growth of 56.7% (231 million tons) between 2006 and 2017 [11]. Inadequate management of wastes generated from these activities could lead to serious environmental problems, including greenhouse gas emissions, pest proliferation, and nitrogen immobilization in the soil (Zárate-Salazar et al., 2020).

In *Pleurotus* spp. cultivation, substrate plays a crucial role due to its direct influence on productivity, chemical composition, functional attributes, nutritional content, and sensory characteristics [13]. Studies indicate that substrate composition variations affect mushroom yield and bromatological quality. Selecting an appropriate residual material as a substrate is essential for *P. djamor* cultivation since it will serve as a source of nutrition and lignocellulosic material to support growth, development, and fruiting [6]. In this context, the present study aims to analyze the influence of substrates using banana leaves and sugarcane bagasse (in differing percentages) on the nutritional potential, mycochemical attributes, and antioxidant properties of *P. djamor*.

## **Materials and methods**

## **Production of edible mushrooms**

#### Inoculation

The biological material for inoculum preparation was the strain *P. djamor* PDJR2/UFPB, provided by the Edible Mushroom Research & Production Group at the Federal University of Paraíba. Preparation of the inoculum followed the methodology outlined by Moreaux [14] and Zárate-Salazar et al. (2020) with few modifications. Initially, canary grass seeds (*Phalaris canariensis* L.) were cooked for 20 min. After air-drying, 3 g of calcium carbonate (CaCO<sub>3</sub>) and 13 g of calcium sulfate (CaSO<sub>4</sub>) per kilogram of the cooked grain were added. The material was sterilized in an autoclave at 121 °C and 103.4 kPa for 30 min. Then, 5 disks of 1 cm<sup>2</sup>, containing potato dextrose agar with *P. djamor* mycelium were aseptically transferred to the grains.

The mixed material was incubated in darkness for 15 days at  $28 \pm 2^{\circ}$ C until complete mycelial proliferation and subsequently stored in a refrigerator at 10 °C for use in the inoculation stage.

#### Substrate preparation

Two types of substrates were used to cultivate the *P. djamor* mushrooms: banana leaves (*Musa* spp.) and sugarcane bagasse (*Saccharum* spp.), combined in 5 formulations as presented in Table 1. The substrates were obtained from local producers (Areia, Brazil) of bananas, rapadura (unrefined sugar), and cachaça (sugarcane spirit). The materials were individually air-dried in a greenhouse, then crushed to 0.5–1 cm particle sizes and sifted through a 2 mm metal screen. The homogeneous mixture was transferred to polypropylene bags (25 cm × 30 cm), and distilled water was added until achieving an average moisture content of  $66.70 \pm 5.21\%$ . The substrates were then sterilized in an autoclave at 120 °C and 103.4 kPa for 60 min [6].

#### **Mushroom cultivation**

The bags containing the substrates were inoculated at a ratio of 4 g of P. djamor per kilogram of sterilized substrate. The material was kept in the absence of light at  $24 \pm 2$  °C and  $85 \pm 1\%$  relative humidity for 15 days until complete mycelial colonization. Two vertical cuts of 5 cm each were made on either side of each bag to stimulate fungal growth. The bags were then transferred to a fruiting area and maintained at  $28 \pm 2$  °C, relative humidity of  $92 \pm 1\%$ , and a light intensity of 200 lx (8.5 volts). Automated daily spraying of 2 L of water was conducted throughout the production process to maintain the optimal humidity range in the cultivation room. The total time from the inoculation to harvesting was 120 days. The harvested mushrooms were dried in a circulating air oven at 45 °C for three days. The dried mushrooms were then ground into a powder using a knife mill with a 10-mesh sieve. The resulting powder was stored in dry containers for subsequent analyses [12].

 Table 1
 Various substrate formulations used to grow Pleurotus djamor

Substrates	Banana leaves	Sugarcane bagasse
100%BL	100%	0%
75%BL+25%SB	75%	25%
50%BL + 50%SB	50%	50%
25%BL+75%SB	25%	75%
100%SB	0%	100%

BL: banana leaves; SB: sugarcane bagasse

#### **Bromatological analysis of mushrooms**

Bromatological analysis of the dried mushrooms was performed in accordance with the Association of Official Analytical Chemists – AOAC (2016) standards [15]. Moisture content was determined by drying in an oven (Med clave - Model 4) set at 105 °C for 24 h. Water activity was determined by directly reading the sample using a water activity measuring device (Aqua lab - Model Dew point 4). Ash was analyzed gravimetrically after sample incineration in a muffle furnace (Jung - Model 0612) at 550 °C for eight hours. Protein content was quantified using the Kjedahl method, adopting a conversion factor of 4.38 (in place of 6.25) for total nitrogen - crude protein, due to the high proportions of non-proteic N in mushrooms [16]. Total fat was determined using prior cold extraction with chloroform and methanol in a 2:1 ratio and subsequent gravimetric quantification, according to the method of Folch et al. [17]. Crude fiber content was analyzed gravimetrically after sequential digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH. The total carbohydrate content was estimated by difference, according to Eq. 1 [9]:

The energy value was determined according to Eq. 2 [9]:

Energy (kcal/100 g) = 
$$(4 \times g_{Carbohydrate})$$
  
+  $(4 \times g_{Protein}) + (9 \times g_{Fat})$  (2)

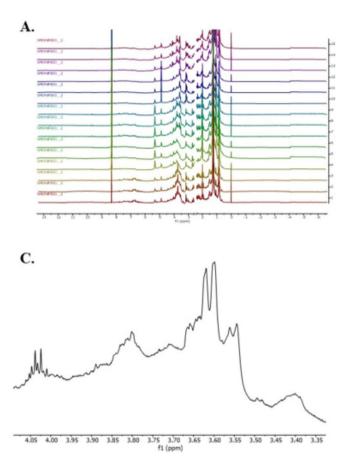
#### Preparation of mushroom extracts

To prepare the extracts, 2 g of dried *P. djamor* mushrooms were extracted by maceration with 50 mL of ethanol/water solution (70:30 v/v) at room temperature and protected from light for five days. The extracts were then filtered with a 0.45  $\mu$ m nylon syringe filter, stored under refrigeration, and protected from light [18].

#### Mycochemical characterization of the extracts

## <sup>1</sup>H nuclear magnetic resonance spectrometry (<sup>1</sup>H NMR)

To obtain the <sup>1</sup>H NMR spectra, we used dimethyl sulfoxide (DMSO-d6, 99.96 atom % D, containing 0.03% (v/v) TMS) solvent purchased from Sigma (Sigma Co., St Louis, MO). For analysis of the *P. djamor* hydroalcoholic extracts, we used the method proposed by Refaie et al. (2009), with some modifications. Three 30 mg aliquots of each extract were weighed and solubilized in 550  $\mu$ L of DMSO. The

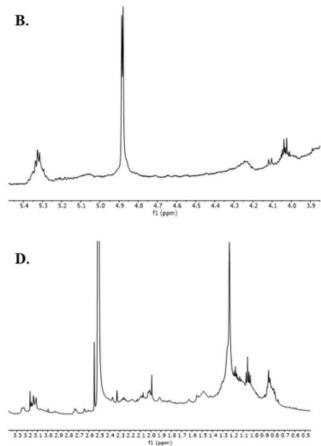


**Fig. 1** 1 H-NMR spectra (500 MHz, DMSO-d6) of *Pleurotus djamor*. B. Region between 4.1–5.4 corresponds to anomeric proton signals. C. Region between 3.4-4.0 corresponds to other protons belonging to

 Table 2
 Acquisition parameters for the <sup>1</sup>H-NMR spectra of *Pleurotus djamor*

Parameters	Dada
Number of Scans	8
Gain	101.0
Relaxation Delay	4.6100
Pulse Rate	8.0000
Pre-saturation frequency	3.36150
Acquisition time	3.2768

resulting solution was transferred to an NMR tube (inner diameter: 5 mm, length: 7 inches) for <sup>1</sup>H NMR spectral analysis (Fig. 1). The <sup>1</sup>H NMR spectra were obtained on a Bruker ASCEND 500 MHz spectrometer (Bruker, Coventry, UK), and followed the parameters shown in Table 2. Chemical shifts for all samples were referenced using the DMSO-d6 solvent signal at 2.50 ppm. The spectra were processed using MestReNova® (MNova) software - version 14.2.0. The <sup>1</sup>H NMR spectra were subjected to preprocessing based on referencing procedures for the solvent signal, baseline correction, phase correction, alignment, normalization of the total spectrum area, removal of the solvent



osidic units. D. Region between 0.55–3.3 indicates aliphatic hydrogens or methyl groups linked to glycosylated chain hydrogens

signal, and finally, a binning spectrum was obtained with a range of 0 to 11 ppm and a range of 0.040 ppm.

We applied two methods of exploratory data analysis, namely Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), using PAST software (version 4.03). The data were derived from the matrix generated from binning spectroscopy, and prior normalization was performed. PCA was executed using the cross-validation method. The binning spectrum, generated in MestReNova®, led to a data table (matrix) processed in Microsoft Excel® and exported in.csv format to PAST (version 4.03).

## **Total phenolic content**

The total phenolic content of the extracts was determined using Folin-Ciocalteu reagent with gallic acid as the standard phenolic compound [20]. For the assay, we mixed 200  $\mu$ L of mushroom extract or gallic acid, 40  $\mu$ L of Folin-Ciocalteu reagent, and 1640  $\mu$ L of distilled water and then homogenized by stirring for 1 min. We then added 120  $\mu$ L of a 15% sodium carbonate solution. The mixture was stirred and stood for 2 h, protected from light. After the reaction period, absorbance was detected at 760 nm. The total phenolic content was expressed as  $\mu$ g gallic acid equivalents per mL of extract ( $\mu$ gGAE/mL), using an equation obtained from the calibration curve of the gallic acid standard (20 to 100  $\mu$ g/mL).

#### **Total flavonoid content**

Total flavonoid content was determined using the method of Tambe & Bhambar [21], with quercetin as the flavonoid standard. We mixed 200  $\mu$ L of mushroom extract or quercetin and 1000  $\mu$ L of a 5% aluminum chloride (AlCl3) solution in methanol for the assay. The volume was completed with distilled water to 2000  $\mu$ L. After 10 min, the absorbance was measured in a spectrophotometer at 425 nm. The total flavonoid content was expressed as  $\mu$ g of quercetin equivalents per mL of extract ( $\mu$ gQE/mL), using the equation obtained from the calibration curve of the gallic acid standard (10 to 50  $\mu$ g/mL).

## **Antioxidant activity**

#### **ABTS** assay

The 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity assay was performed according to the methodology of Re et al. [22]. The ABTS radical cation was prepared by reacting a 7 mM aqueous solution of ABTS (2.5 mL) with 140 mM of potassium persulfate (44  $\mu$ L). The mixture was kept in the dark for 16 h. Before the assay, the ABTS reagent was diluted with ethanol to obtain an absorbance of  $0.70 \pm 0.05$  at 734 nm. We then mixed appropriate aliquots of the extracts with 1800 µL of the ABTS radical solution to obtain final concentrations of 12.5  $\mu$ L/mL to 100  $\mu$ L/mL. The solutions were kept without light for 6 min before reading absorbance at 734 nm in a spectrophotometer using an ethanol blank. We used Trolox (2  $\mu$ M to 16  $\mu$ M), a water-soluble vitamin E analog for a positive control. The percentage of scavenging activity (% SA) was calculated according to Eq. 3, described below:

$$%SA = (100 \times (A_{control} - A_{sample})) / (A_{control})$$
(3)

Where  $A_{control}$  is the absorbance of the control, containing only the ethanolic ABTS solution, and  $A_{sample}$  is the absorbance of the radical in the presence of the extract or Trolox. The results were expressed as  $\mu M$  trolox equivalents per mL of the extract ( $\mu M$  TE/mL).

#### **DPPH** assay

The antiradical activity was determined by DPPH radical (purple coloration) methodology - hydrogen donation, which reduces to form a yellow-colored DPPH-H (hydrazine), according to Rufino et al. [23]. Initially, we prepared a DPPH solution (1.18 mg in 50 mL of ethanol). Appropriate aliquots of the samples were then mixed with ethanol to obtain final concentrations ranging from 12.5  $\mu$ L/mL to 100 µL/mL. After 30 min of incubation in the dark, we read the absorbance at 515 nm in a spectrophotometer. We used ethanol as a blank and ascorbic acid (3µM to 23 µM) as the positive control. The percentage of sequestering activity (%SA) was calculated using Eq. 3. A<sub>control</sub> is the absorbance of the control, containing only the ethanolic solution of DPPH, and A<sub>sample</sub> is the absorbance of the radical in the presence of the extract or the standard ascorbic acid. The results were expressed as uM ascorbic acid equivalents per mL of the extract ( $\mu$ M AAE/mL).

## **Statistical analysis**

All experiments involving physicochemical analysis, total phenolic and flavonoid content, and antioxidant activity were performed in triplicate, and the results were expressed as mean  $\pm$  SD (standard deviation). Normal distribution of the data was assessed with the Shapiro-Wilk normality test, and homogeneity of variance was assessed using Bartlett's test. We performed a one-way ANOVA analysis of variance to determine significant differences (p < 0.05) between treatments, and when significant, we applied Tukey's test. All analyses were performed using R software Version 4.1.0 in the RStudio interface [24].

## Results

The data for the physicochemical analysis of *P. djamor* mushrooms cultivated in different substrate compositions are presented in Table 3. Overall, due to the nature of the cultivation substrates, the data reveal non-significant variations in values ( $p \ge 0.05$ ). The dried mushrooms maintained an average moisture content ranging from  $16.09 \pm 1.25\%$  to  $12.06 \pm 2.88\%$ , while water activity varied from  $0.50 \pm 0.21$  to  $0.67 \pm 0.05$ . Ash content ranged from  $4.51 \pm 0.23\%$  to  $5.05 \pm 0.04\%$ . The protein content was uniform among the mushrooms, ranging from  $17.80 \pm 0.34\%$  to  $18.77 \pm 0.24\%$ . The lipid content in the 25%BL+75%SB and 100%SB composition was higher than in mushrooms cultivated in the other substrates (p < 0.05). The crude fiber content of the mushrooms varied from  $18.02 \pm 0.05\%$ 

Table 3 Effects of different banana leaves and sugarcane bagasse	different banans	a leaves and sugar	cane bagasse su	ubstrates on the	physicochemical	characterization	substrates on the physicochemical characterization of Pleurotus djamor mushrooms	or mushrooms		
Substrates	Moisture (%)	Moisture (%) Water activity Ash (%)		Protein (%)	Carbohydrates (%)	Lipids (%)	Protein (%) Carbohydrates Lipids (%) Crude fiber (%) Energy (%) (%)	Energy (Kcal/100 g)	Total polyphenolsTotal flavonoids(μgGAE/mL)(μgQE/mL)	Total flavonoids (μgQE/mL)
100%BL	$16.09 \pm 1.25^{a}$ $0.67 \pm 0.05^{a}$	$0.67 \pm 0.05^{a}$	$4.71 \pm 0.86^{a}$	$18.77 \pm 0.24^{a}$ $42.13 \pm 1.85^{a}$	$42.13 \pm 1.85^{a}$	$0.28 \pm 0.08^{a}$	$0.28 \pm 0.08^{a}$ $18.02 \pm 0.05^{a}$	$246.14 \pm 8.49^{a}$	$246.14 \pm 8.49^{a}$ $272.27 \pm 54.45^{a}$	$82.14 \pm 12.97^{a}$
75%BL $\pm$ 25%SB 15.43 $\pm$ 3.20 <sup>a</sup> 0.65 $\pm$ 0.10 <sup>a</sup>	$15.43 \pm 3.20^{a}$	$0.65 \pm 0.10^{a}$	$4.82 \pm 0.34^{a}$	$17.80 \pm 0.34^{a}$	$17.80 \pm 0.34^{a}$ $43.09 \pm 4.28^{a}$	$0.24 \pm 0.06^{a}$	$0.24 \pm 0.06^{a}$ $18.62 \pm 0.66^{a}$	$245.71 \pm 16.03^{a}$	$245.71 \pm 16.03^{a}$ $204.52 \pm 61.13^{a}$	$72.24 \pm 19.41^{a}$
$50\%$ BL $\pm 50\%$ SB 14.79 $\pm 3.41^{a}$ 0.60 $\pm 0.13$	$14.79 \pm 3.41^{a}$	$0.60 \pm 0.13$ <sup>a</sup>	$4.77 \pm 0.62^{a}$	$18.37 \pm 0.18^{a}$	$18.37 \pm 0.18^{a}$ $43.73 \pm 3.19^{a}$	$0.26 \pm 0.03^{a}$	$0.26 \pm 0.03^{a}$ 18.08 $\pm 2.55^{a}$	$250.77 \pm 13.20^{a}$	$250.77 \pm 13.20^{a}$ 123.65 $\pm 13.58^{a}$	$117.24 \pm 13.37^{b}$
$25\%BL \pm 75\%SB$ 13.22 $\pm 3.44^{a}$ 0.50 $\pm 0.21^{a}$	$13.22 \pm 3.44^{a}$	$0.50 \pm 0.21^{a}$	$4.51 \pm 0.23^{a}$	$18.36 \pm 0.81^{a}$	$18.36 \pm 0.81^{a}$ 44.16 ± 3.89 <sup>a</sup>	$0.43 \pm 0.17^{\rm b}$	$0.43 \pm 0.17^{\rm b}$ $19.32 \pm 0.39^{\rm a}$	$253.95 \pm 15.30^{a}$	$253.95 \pm 15.30^{a}$ $346.36 \pm 80.52^{b}$	$113.86 \pm 9.60^{b}$
100%SB	$12.06 \pm 2.88^{a}$ $0.55 \pm 0.15^{a}$	$0.55 \pm 0.15^{a}$	$5.05 \pm 0.04^{a}$	$18.23 \pm 1.36^{a}$	$18.23 \pm 1.36^{a}$ $46.30 \pm 3.39^{a}$	$0.40 \pm 0.06^{\rm b}$	$0.40 \pm 0.06^{b}$ 18.32 $\pm 0.65^{a}$	$258.42 \pm 8.49^{a}$	$258.42 \pm 8.49^{a}$ $480.17 \pm 98.78^{b}$	$113.21 \pm 21.08^{b}$
Values are express	ied as means ±	SD. Different lett	ers within a co	lumn indicate:	significant differen	nces between s	ubstrates $(p < 0.05)$	). BL: banana leav	Values are expressed as means $\pm$ SD. Different letters within a column indicate significant differences between substrates ( $p < 0.05$ ). BL: banana leaves; SB: sugarcane bagasse; GAE: gallic	gasse; GAE: gallic
acid equivalent · OF· quercetin equivalent	VE: amercetin ea	mivalent								

from the various compounds present in the mixture. Visually comparing the spectra before and after data grouping revealed no changes. No alterations were detected in the behavior of the scores when compared to the preliminary PCA, indicating that the principal information was not modified by data reduction. Unused regions of the spectra were removed to avoid analysis interference. No distortion of spectral signals was observed after aligning the spectra in the new dataset, demonstrating that the peak shifts were satisfactorily corrected. The analysis of <sup>1</sup>H-NMR spectra indicated an abundant presence of polysaccharides. The spectra exhibited characteristic signals of anomeric protons in 4.1-5.4 (Fig. 1A) and other signals related to protons from osidic units between 3.4 and 4.0 (Fig. 1B). There were also intense signals at 0.55-3.3, indicating the presence of aliphatic hydrogens or methyl groups linked to hydrogen in glycosylated chains (Fig. 1C). Fourteen principal components (PC) were necessary to construct the PCA model, and 69.166% of the explained variance was accumulated in the first two components

(PC1 and PC2) (Fig. 2). Figure 2A displays the scores plot of the first two PCs and reveals that the mushrooms from the 25%BL+75%SB, 50%BL+50%SB, 75%BL+25%SB, and 100%BL treatments tend to cluster with low dispersion in the PCA model. The treatments were grouped according to the presence of hydrogen signals, with relative shifts to anomeric protons and protons belonging to osidic units, as observed in the loadings plot (Fig. 2B), suggesting little difference in their metabolic profiles. However, the 100% SB treatment and its corresponding replicas clustered on the opposing side. These signals also presented shifts in the sugar protons region, with lower shifts between 0.8 and 2.9 related to methyl groups or sugars. Two distinct groups emerged in the analysis, with no sample lying outside the Hotelling Ellipse (95%). Similar results were also observed in the cluster analysis using the UPGMA method, adopting the Euclidean distance with a cophenetic correlation coefficient of 0.9 and significant bootstrapping.

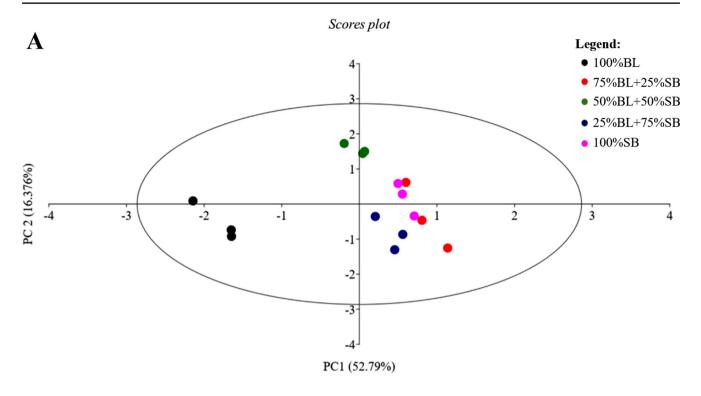
The total polyphenol and flavonoid contents in *P. djamor* mushrooms are presented in Table 3. Polyphenol content was higher in mushrooms cultivated on substrates with higher proportions of sugarcane bagasse (25%BL+75%SB, and 100%SB), with a significant difference compared to the other groups (p < 0.05). The mushrooms exhibited total polyphenol values ranging from 123.65±13.58 to 480.17±98.78 µg GAE/mL. Increased sugarcane bagasse

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to  $19.32 \pm 0.39\%$ , and carbohydrate content was around  $42.13 \pm 1.85\%$  to  $46.30 \pm 3.39\%$ . The highest average energy value was found for the 75%BL+25%SB treatment at 258.42 kcal/100 g. However, the results were similar to

The <sup>1</sup>H-NMR spectroscopic data revealed a set of signals

the other groups ( $p \ge 0.05$ ).



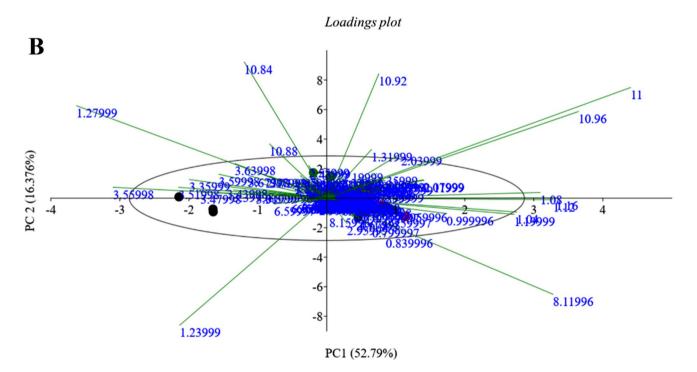


Fig. 2 PCA analysis of 1 H-NMR data from hydroalcoholic extracts of *Pleurotus djamor*. (A) Score plot with 87.94% of the variance explained in the first two components (PC1 and PC2). (B) Loadings graph of PC1 and PC2

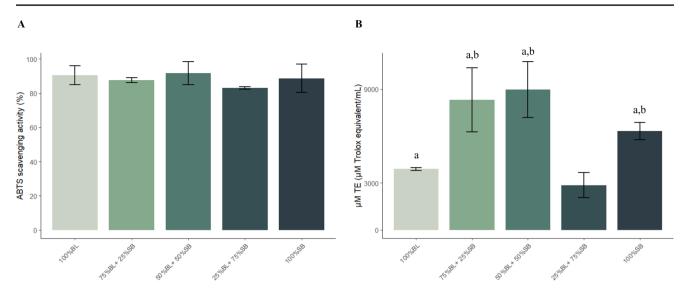


Fig. 3 Effect of different substrate formulations on the antioxidant capacity of *Pleurotus djamor* mushrooms. Values are expressed as means  $\pm$  SD. a: significant difference compared to 25%BL + 75%SB. b, : significant difference compared to 100%BL. FB: banana leaves; BC: sugarcane bagasse

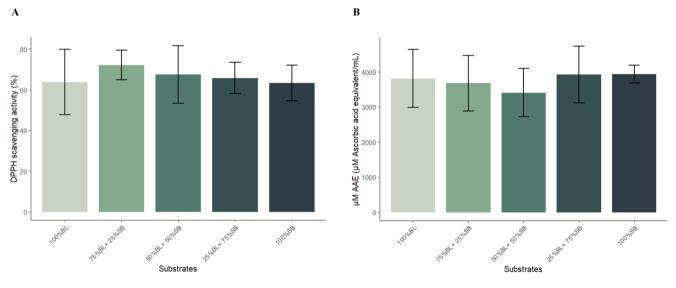


Fig. 4 Effect of different substrate formulations on the antioxidant capacity of *Pleurotus djamor* mushrooms. Values are expressed as means  $\pm$  SD. a: significant difference compared to 25%BL+75%SB. b: significant difference compared to 100%BL. FB: banana leaves; BC: sugarcane bagasse

content in the substrates also led to a significant rise in flavonoids. The groups 50%BL+50%SB, 25%BL+75%SB, and 100%SB presented higher total flavonoid values (µg QE/mL) compared to the other groups (p < 0.05).

Considering the polyphenol and flavonoid contents, we analyzed their antioxidant capacity using the DPPH (Fig. 3A) and the ABTS (Fig. 4A) methods. At 100  $\mu$ L/mL, all extracts exhibited high antioxidant activity in both tests, with radical scavenging activity variations between 83.01% and 91.6%. No significant differences were detected between mushrooms cultivated on either substrate ( $p \ge 0.05$ ). We calculated the trolox equivalents ( $\mu$ M TE/mL) (Fig. 3B) and ascorbic acid equivalents ( $\mu$ M AAE/mL) (Fig. 4B) per mL for all extracts. The mushrooms cultivated

on 25%BL+75%SB presented lower  $\mu$ M TE/mL than the others (p < 0.05). No differences were observed between mushrooms regarding  $\mu$ M AAE/mL ( $p \ge 0.05$ ).

## Discussion

Culturing *P. djamor* mushrooms on the substrates formulated in this study were suitable for producing mushrooms with high protein, dietary fiber, phenolic, and low lipid content. Lignocellulosic materials from local agronomic wastes support sustainable food production, being readily available and primarily underutilized. Fresh mushrooms are composed mainly of water, making them highly perishable. To extend their shelf life, they are often sold in dried form [25, 26]. In the present study, the mushrooms also underwent a drying process, and their moisture values after drying (Table 3), were much lower than the moisture content of fresh *P. eryngii* mushrooms (85.10%) cultivated on sugarcane bagasse [6]. Yet the values were similar to those reported for *P. sajor* caju (13.14%) and *P. djamor* (11.54%) subjected to cabinet drying oven at 60 °C [27].

Water activity is an important quality parameter, especially in dehydrated foods, since it refers to the water available for microbial growth, affecting product stability during storage [28, 29]. Our results (Table 3) align with those observed in dried *P. ostreatus* at temperatures of 45 to 65 °C, with values ranging from 0.556 to 0.597 [30]. However, they were above the range of 0.236 to 0.436 observed for *P. djamor* powders obtained through drying combined with blanching and sulfiting [29]. The variations may well be attributed to the different drying techniques and equipment used in the experiments. Although no significant differences were found between treatments (Table 3), lower water activity values were generally observed in the samples with lower moisture content. This was expected, as water activity is influenced by water removal [28].

Ash content is essential for understanding the mineral content in food [31]. Compared with previous *P. djamor* studies where ash contents ranged from 5.24 to 6.98% and 5.91% [27, 32] our ash concentrations (Table 3) were slightly lower. Under the conditions of the present study, no statistical difference was observed in the ash content due to different substrates. Similar behavior was reported by Valenzuela-Cobos et al. [33] for *P. djamor* cultivated from wheat straw and peat moss in various proportions; they found no significant influence of substrate on ash content. Yet when cultivated from wheat bran supplemented with sugarcane bagasse (20%), *P. djamor* presented the effects of supplementation, and ash concentrations reached up to 9.51% [34].

The literature contains several studies with similar results regarding *P. djamor* mushroom nutritional composition. Nayak et al. [35] and Das et al. [36] investigated the physicochemical properties of dried *P. djamor* mushroom powder and also found high amounts of protein and fiber, with low energy values. In a study conducted by [9], *P. djamor* was cultivated in three different substrates composed of rice straw, corncobs, and coffee pulp. Unlike our study, they detected a significant effect of the different substrates on the protein content of the samples, with a range of 21.61–27.09%. These examples highlight the influence of substrate type on the nutritional composition of *P. djamor* mushrooms.

To achieve more sustainable and healthy dietary patterns in the future, consumption of animal products should be limited, and alternative sources should be sought. The consumption of red meat has been associated with a range of cardiometabolic issues [37] and its production significantly contributes to greenhouse gas emissions [38]. Fungi-based proteins are thus gaining popularity as alternatives to animal proteins. Edible mushrooms have been recognized as good sources of high-quality protein, and they can be used by individuals who do not consume animal protein [39]. In the context of a diverse diet, *P. djamor* cultivated under the conditions adopted in this study may serve as a sustainable alternative source of proteins.

The results suggest that mushrooms are a good source of carbohydrates as a major constituent (Table 3). The averages are similar to those reported by [9] and slightly lower than those reported by [27], respectively, with values of 41.45-51.06% and 48.36-52.37%. Lipid content was as low as expected (Table 3); mushrooms generally present low-fat contents, making them low-calorie foods [32]. Fat was the only parameter that showed significant differences among the macronutrients, with mushrooms cultivated in the substrate composed of 25%BL+75%SB and 100%SB presenting the highest fat percentages (Table 3). Hasan et al. (2015) found that the lipid content of P. djamor decreased as the substrate base was supplemented with sugarcane bagasse, with values ranging from 3.58 to 6.34%. Another study has reported lipid percentages in P. diamor ranging from 1.63 to 2.09% [40]. These variations in data may be attributed to the species, the growth medium, or the analytical methods used [41].

Energy values ranged from 245.71 to 258.42 kcal/100 g (Table 3), with no significant differences between treatments. The results fall within the 240.55 to 295.36 kcal/100 g range previously reported for *P. djamor* var. roseus by [40]. The World Health Organization [42] recommends reducing consumption of energy-dense, high-fat, and high-sugar foods to prevent noncommunicable diseases such as diabetes and heart disease. In alignment with WHO recommendations, the Dietary Guidelines for the Brazilian Population [43] advises consuming minimally processed or unprocessed foods for a healthy diet. *P. djamor* (in line with both dietary guidelines) cultivated under the conditions of the present study proves to be strategic when consumed in minimally processed forms, offering a balanced nutritional value.

Mycochemical components provide edible mushrooms with functional biological properties for human nutrition and health. Bromatological analysis and <sup>1</sup>H-NMR spectra of *P. djamor* mushrooms indicated a rich presence of polysaccharides, which are common in extracts of edible mushrooms of the *Pleurotus* genus and are the most studied metabolites in this species [44]. They are the predominant components in mushroom extracts, as the major fraction corresponds to carbohydrates and a small fraction to soluble proteins [19]. When using substrates with varying agricultural residue compositions, certain variations in the resulting mix of compounds present in the fungal extracts are expected due to the differences. The signals obtained from the spectra may correspond to a mixture of polysaccharides commonly found in the species, such as heteropolysaccharides,  $\beta$ -glucans,  $\alpha$ -glucans, and oligosaccharides, as is reported in the literature for extracts of the species [44–48] These fiber-like components, phenolic compounds, and flavonoids are relevant since they directly relate to antioxidant and prebiotic activity.

We applied two exploratory data analysis methods (PCA and HCA) to identify patterns, similarities, or differences in the hydroalcoholic extracts of P. djamor. The mushroom extracts are complex mixtures comprising various components (metabolites, proteins, carbohydrates, etc.), causing extensive overlap of chemical shifts and signal amplitudes. Due to this characteristic and the volume of generated data, we employed statistical analyses to uncover hidden relationships among the samples through unsupervised exploration [49]. The <sup>1</sup>H-NMR technique is a fundamental tool in carbohydrate analysis; however, assigning resonance signals from specific sugar residues can be challenging with unidimensional experiments due to signal overlap. Bidimensional correlation NMR experiments can assist in resolving interpretation issues. However, bidimensional experiments were unnecessary because our primary goal was to understand the chemical profile of the extracts and identify potential differences among them through visual graphical representation. We can conclude that groups exhibit signals in the sugar region indicative of polysaccharides. However, there is a slight difference in the chemical profile of the group formed by 100%SB compared to the mushrooms that grew with banana leaf substrates. Future studies may investigate the components responsible for this difference.

Fiber-like polysaccharide compounds influence lipid metabolism by reducing fats and sugars' absorption and increasing fecal matter volume [50] Further, they interact with the human intestinal microbiome, producing a prebiotic effect [51, 52]. These polysaccharides confer antioxidant activity to extracts without cytotoxic effects, enhancing protection against  $H_2O_2$ -induced oxidative stress in cellular models [45]. Studies with other *Pleurotus* species, such as *P. ostreatus* and *P. eryngii* have confirmed this immunomodulatory potential [53], antitumor [54], and improved cardiometabolic parameters [55].

Previous studies have demonstrated the presence of mycochemical components in *P. djamor* mushrooms, which confer antioxidant activity [56–59]. Total phenolic compounds and flavonoid content are generally related to antioxidant activity. Phenolic acids are very commonly found in edible mushrooms. Tannic acid, gallic acid, protocatechuic acid, p-coumaric acid, ferulic acid, 5-O-Caffeoylquinic acid, syringic acid, gentisic acid, cinnamic acid, and vanillic acid are examples of phenolic compounds commonly found in *P. djamor* mushrooms [57, 60–63].

Just as the nutritional profile can be modified according to the substrate in which mushrooms are cultivated, the content of mycochemical components and antioxidant activity can also vary. We used five different compositions of sugarcane bagasse and banana leaves and detected significant changes in mycochemical characterization without affecting the antioxidant activity of the mushrooms. We used two controls to cross-validate the results to increase the reliability of the antioxidant activity analyses. In this sense, we used trolox and ascorbic acid, which are antioxidant compounds with different chemical structures and activity profiles. Agroindustrial residues such as agave bagasse and barley straw have also been used to cultivate *P. djamor* mushrooms [58]. The researchers found that the cultivation substrate influences the concentration of bioactive molecules in P. djamor fruiting bodies. Similarly, rice straw, corncobs, and coffee pulp have also been shown to modify the mycochemical profile and antioxidant activity of *P. djamor* [9].

# Conclusion

This study showed that using agronomic waste such as banana leaves and sugarcane bagasse allowed the production of *P. djamor* mushrooms with a high content of nutrients and antioxidant mycochemicals. We highlight the presence of proteins, dietary fibers, polysaccharides, and antioxidant compounds related to the bioactivity attributed to *P. djamor* mushrooms. Few studies have been conducted with *P. djamor* to assess its prebiotic activity and resulting beneficial effects on human health. However, due to its interesting fiber content and based on our results, future investigations into its in *vitro/in vivo* prebiotic potential are warranted. Similarly, using this mushroom to produce other food products (either in flour or dehydrated powder form) promises enhanced nutritional value.

**Acknowledgements** The authors thankfully acknowledge teacher Jonathan for reviewing the English and our local farmers for providing the agronomic wastes.

## Declarations

Competing interests The authors declare no competing interests.

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