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Bacuri and macaxeira waste: physical-chemical characterization and production of coconut bioaroma by solid-state fermentation

Resíduos de bacuri e macaxeira: caracterização físico-química e produção de bioaroma de coco por fermentação em estado sólido

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ABSTRACT

Agro-industrial waste is considered a global concern. Many of these residues are composed of considerable amounts of lipids and starch that can potentially be applied in bioprocesses, as is the case with residues from the bacuri fruit *(Platonia insignis Mart.)* and sweet cassava *(Manihot esculenta Crantz),* practically unexploited in the bioproduction of aromas. This work aimed to characterize these residues and evaluate the bioproduction of coconut aroma 6-pentyl-α-pyrone from solid-state fermentation using the fungi *Trichoderma harzianum.* The waste underwent characterization. Fermentations were conducted under different humidification conditions (water, nutrient solution without additives, and nutrient solutions with glucose or sucrose) for nine days. Aromatic compounds were extracted by solid-phase microextraction and subsequently quantified by gas chromatography. Analyses with bacuri residue revealed the presence of some compounds with nutritional potential for the fungus. Still, the inhibition halo detected for *Trichoderma* proved others, such as resinous derivatives that were probably responsible for the lack of growth and bioproduction. In sweet cassava, the compounds detected were not growth inhibitors and had low aroma production, not exceeding 7 ppm (weight/weight). Strategically, these residues were mixed and, in the presence of a nutrient solution with sucrose, a maximum production of 202.46±1.30 ppm (w/w) of 6-pentyl-α-pyrone was achieved, proving an excellent alternative. Cassava probably served as an environment for easy germination of fungal spores and bacuri, as an important source for bioexploitation of nutrients, especially lipids, resulting in increased production of 6-pentyl-α-pyrone.

Keywords: agro-industrial waste; 6-pentyl-α-pyrone; solid-state fermentation; *Platonia insignis Mart*; sweet cassava.

RESUMO

Os resíduos agroindustriais são considerados uma preocupação global. Muitos desses resíduos são compostos por quantidades consideráveis de lipídios e amido que podem potencialmente ser aplicados em bioprocessos, como os resíduos do fruto do bacuri (*Platonia insignis Mart*.) e da mandioca-doce (*Manihot esculenta Crantz*), praticamente inexplorados na bioprodução de aromas. Este trabalho teve como objetivo caracterizar esses resíduos e avaliar a bioprodução do aroma de coco 6-pentil-α-pirona proveniente da fermentação em estado sólido utilizando o fungo *Trichoderma harzianum*. Os resíduos passaram por caracterização. As fermentações foram conduzidas sob diferentes condições de umidificação (água, solução nutritiva sem aditivos e soluções nutritivas com glicose ou sacarose) durante n dias. Os compostos aromáticos foram extraídos por microextração em fase sólida e posteriormente quantificados por cromatografia gasosa. Análises do resíduo de bacuri revelaram a presença de alguns compostos com potencial nutricional para o fungo. Ainda assim, o halo de inibição para o *Trichoderma*, comprovou que outros como derivados resinosos foram provavelmente responsáveis pela ausência de crescimento e bioprodução. Na mandioca-doce, os compostos detectados não foram inibidores de crescimento e apresentaram baixa produção de aroma, não ultrapassando 7 ppm (peso/peso). Estrategicamente, esses resíduos foram misturados e, na presença de solução nutritiva com sacarose, foi alcançada uma produção máxima de 202,46±1,30 ppm (m/m) de 6-pentil-α-pirona, mostrando-se uma excelente alternativa. A mandioca provavelmente serviu como ambiente para fácil germinação de esporos de fungos e bacuri, como importante fonte para bioexploração de nutrientes, principalmente lipídios, resultando no aumento da produção de 6-pentil-α-pirona.

Palavras-chave: resíduos agroindustriais; 6-pentil-α-pirona; fermentação em estado sólido; *Platonia insignis Mart.*; mandioca-doce.

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Introduction

Agro-industrial plant residues, found in abundance in the environment, are heterogeneous materials formed mainly by lignocellulosic components (hemicellulose, lignin, and cellulose) (Ventura et al., 2022), that is, polymeric substances with high molecular weight (Lindsay et al., 2022) that present several properties and may be related to structural function.

They can also be rich in starch (cassava bagasse, corn straw, and rice), free sugars (sugar cane bagasse and fruit), lipids (babassu cakes, coconut, peanuts, and chestnuts), fibers (coconut shell, corn cobs, and plums), and minerals (açaí pomace, cotton, and coffee grounds), among others. These residues' characteristics have wide application in the biotechnological context, mainly in fermentation processes. Ho ever, bioproduction's success depends not only on the type of residue to be explored but also on other factors such as humidity, porosity, temperature, aeration, and even the type of microorganisms.

The most extensive application of agro-industrial waste in fermentation processes is in solid-state fermentation (SSF) systems. This is because they allow the acquisition of high value-added products (antibiotics, enzymes of industrial interest, dyes, metabolites, among others) and, often, in higher concentrations, when compared to submerged fermentation, in addition to positively influencing the reduction of environmental impacts (Dulf et al., 2016). Solid-state fermentation comprises a medium that resembles the natural microbial habitat (Zaier et al., 2021) where microorganisms grow on the surface and inside solid support (inert or substrate) (El-Sayed et al., 2020a) in the absence or near absence of free water (Afiqah et al., 2021). These are non-toxic carbon sources, capable of encouraging resistance to catabolic repression (El-Sayed et al., 2020b) and the production of metabolites at higher concentrations (El-Sayed et al. 2020); therefore, they are seen as essential substrates and excellent supports for the development of microorganisms (Wang et al., 2020).

Within the context that relates SSF and microorganisms, fungi are considered the most adapted because the growth of their hyphae can occur on the particle surfaces and, at the same time, penetrate the interparticle spaces, colonizing the entire solid substrate (Zaier et al., 2021). Therefore, the chosen substrate must meet the nutritional needs of microorganisms and be supported with favorable physical properties that facilitate water availability and can allow the initial anchoring of conidia, heat, mass transfer, and mycelial elongation in the solid medium within a given time (Hamrouni et al., 2019).

Otherwise, adjusting the solid medium to better adapt it to the microorganism is interesting. According to Buffi et al. (2023), modifying solid media tends to allow the combination of nutritional conditions. From an environmental point of view, conditions can also influence the production of metabolites, including 6-PP, where research reveals that the most outstanding production of this metabolite occurs in total darkness (Missbach et al., 2023).

However, it is worth mentioning that, in some contexts, some components may be harmful due to disobedience caused by the lignin-cellulose complex, the crystalline structure of cellulose itself (Budenkova et al., 2021), or even the presence of inhibitory components, responsible for the natural defense of plants. In cases of microbial inhibition, for the best exploitation of waste components, it is necessary to carry out pre-treatments as bioproduction depends, in particular, on the acquiescence of the microorganism about the cultivation medium. Reducing particle sizes, cutting, scraping, adjusting pH and humidity, removing contaminants by heat treatment, and even physical, chemical, or enzymatic (biological) hydrolysis (Mattedi et al., 2023) are methods constantly used in SSF.

It is essential to highlight that SSF has disadvantages, including mismatches in the control of parameters such as temperature, humidity, pH, accumulation of gases, and aeration of the medium. Another critical factor is the very heterogeneous composition of the substrate and the difficulty in increasing the production scale (Naeimi et al., 2020).

Despite this, obtaining valuable bioproducts makes the process advantageous, such as the acquisition of secondary metabolites with specific properties essential to human health, such as 6-pentyl-α-pyrone (6-PP), the primary secondary metabolite of fungi of the genus *Trichoderma* spp. It is a non-toxic natural flavoring with antibiotic activity, approved (Flores et al., 2019) by the Joint Committee of Experts on Food Additives (Fadel et al., 2015). The 6-PP is an unsaturated lactone with a characteristic coconut smell (Ramos et al., 2008). It also has antifungal properties capable of inhibiting other fungi growth (Lim et al., 2023).

In the production of this lactone, *Trichoderma* has been widely used because it is considered to excrete a complex of enzymes, such as chitinases, gluconases, and proteases (Baiyee et al., 2019), organic acids, biomass, and bioactive products (Hamrouni et al., 2020), and also for its vast capacity to explore lignocellulosic materials.

Although Brazil is one of the largest producers of agro-industrial waste in the world, little is explored of these materials. The wastes result primarily from the natural production of fruits like bacuri (rich in fatty acids and pectin) and roots like sweet cassava (with high starch content), from biomes such as Caatinga, Cerrado, Amazon Forest, and Atlantic Forest.

Bacuri (BA) is a fruit known by the scientific name *Platonia insignis Mart.*, belonging to the class of angiosperms of the *Clusiaceae* (or *Guttiferae)* family. It is a native species found in a part of the Amazon rainforest (Lima et al., 2022). It has high production, where 50 BA trees produce around 9.5 tons of fruit per hectare—a single tree can produce 2,000 fruits per year (Silva, 2016). Of this total, 1 ton is equivalent to pulp, 2.5 tons are seeds, and 6 tons are equivalent to resin peels. Its fruit comprises three parts: skin, pulp, and stone, which weigh 250 g on average and are oval. Its skin has thicknesses ranging from 0.6 to

1.7 cm representing 70% of the fruit's total weight. From BA, we can take advantage of the fruit, the wood, and its energy potential (Silva, 2016). It is a product exploited by extractivism. However, according to Lima et al. (2022), this production already exceeds 2,250 tons per year on average, with the expansion of the Amazon region. Even with large percentages of carbohydrates, lipids, pectin, fatty acids, and other micronutrients, BA bark is still considered of low value due to the need for more in-depth studies on antioxidant and anti-inflammatory properties, among others (Yamaguchi et al., 2021).

On the other hand, sweet cassava (SC) residue, whose scientific name is *Manihot esculenta Crantz*, is widely explored but is generally included as cassava residue. Nevertheless, this research used this species of cassava to produce flour, starch, etc. It has a low cyanide content, with a concentration <50 $mg.kg^{-1}$, therefore requiring no treatments (such as eliminating the cyanide content) to be used in fermentative processes. As it is rich in starch, representing 30% of the wet weight of the root (Alrefai et al., 2020), it is considered an excellent source of carbon for microorganisms. As sweet or wild cassava, it is one of the largest subsistence food sources in the world, representing the third largest source of food in tropical regions, behind only rice and corn (Carvalho et al., 2018).

Data from the Brazilian National Supply Council (*Companhia Nacional de Abastecimento* — Conab) from January 2021 showed that cassava production in Brazil in 2020 was 18.96 million tons. During its processing, the shell is the pre-cleaning residue, and it contains around 75% starch (m/m), with 21.70 and 62.51% of amylopectin and amylose (Morgan, 2016) wasted, respectively. The use of these residues can be advantageous in bioproduction since starch can be hydrolyzed by fungi into monosaccharides and used as a precursor in the biosynthesis of biomolecules (Wang et al., 2020).

Based on the advantages presented by SSF and the high amount of disposal of BA and SC residues, mainly for their nutritional contributions, the objective of this work was to carry out the physical-chemical characterization aiming at the possibility of application in the production of coconut bioaroma.

Materials and Methods

The biotechnological production of aroma using agro-industrial residues (BA and SC), still little explored for this purpose, motivated the completion of important steps:

- Characterization of materials — carried out to understand the properties of the residues that served as support and carbon sources for fungal growth in the fermentation process.
- Fermentation — stage conducted with residues (BA and SC) separately. As a strategy, a support made up of 50% of each residue was used; that is, the residues were mixed to provide conditions for the germination of fungal spores and, consequently increase aroma production.
- Aroma compounds — extracted by solid-phase microextraction (SPME), followed by the identification and quantification of the aroma by gas chromatography.

Obtaining and preparing waste

SC and BA peels were acquired in the state of Maranhão (MA), Brazil. In the case of BA, the residue was obtained in artisanal cellulose production houses and commerce in the city of Viana (MA). The SC peels came from flour mills in several regions from São João Batista (MA). Around 10 kg of waste were stored in Styrofoam boxes until pre-treatment procedures began, followed by characterization and fermentation processes. The waste was thawed, cut into small pieces, and placed in an oven to dry at 60°C for 48 hours. Then, it was crushed in a domestic blender and stored in plastic containers. The fractions used in this research were based on the maximum water absorption capacity defined as 212 mm for the SC shell and 450 mm for the BA shell.

Physicochemical characterization of wastes

The determination of pH, lipids, and ash was made according to Ramo et al. (2024), and protein as per Langyan et al. (2022). Total carbohydrates were determined based on the difference between the previous ones. Humidity was calculated by water loss during sample drying in an oven (IAL, 2005). The effective moisture content of the solid medium was acquired using the moisture balance (model Shimadzu MOC63V), weighing 1 g of the residue and determining the humidity for the four samples in two stages: initial (dry) and post-inoculation.

Lignocellulosic and reducing sugar determination

The composition of lignocellulosic materials was defined by the method proposed by van Soest (1994), which initially consists of the separation between the cellular contents (proteins, pectin, lipids, among others) and the components of the cell wall through digestion in a neutral detergent, obtaining the neutral detergent fiber (NDF) fraction, composed of cellulose, hemicellulose, and lignin. This fraction was subjected to digestion with acid detergent fiber (ADF), where cellulose and hemicellulose were completely solubilized and separated from lignin by filtration. The cellulose contained in the ADF was taken to the muffle furnace, and, by difference, the percentage of cellulose was calculated. Hemicellulose was determined by the difference between NDF and ADF (Madzingira et al., 2021). The percentage of starch was analyzed by titration with Fehling solutions, as described in the Analytical Standards of the Adolfo Lutz Institute (IAL, 2005).

The quantification of reducing sugars followed the protocol described by Miller (1959) and Zhao et al. (2021) using dinitro salicylic acid (DNS).

Metals quantification

To define metal content, the samples were digested in an acid/oxidant mixture using nitric acid (HNO_3) reagents and hydrogen peroxide (H_2O_2). Then, they were centrifuged, filtered, and collected in validities for analysis of the determination of the elements through Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). The analyses occurred in triplicates and were carried out at the Laboratory of Environmental and Mineral Analysis from the Institute of Chemistry of the Federal University of Rio de Janeiro (LAM/IQ/UFRJ).

Antimicrobial activity determination

To evaluate the antimicrobial activity of BA and SC residues, an ethanolic extract (50 mL) was produced with 1 g of the residue. Microbial strains of the American Type Culture Collection (ATCC) and Oswaldo Cruz Institute (IOC) origins were used to investigate the antimicrobial activity: *Proteus mirabilis* ATCC7002; *Staphylococcus aureus* ATCC25923; *Pseudomonas aeruginosa* ATCC27853; *Salmonella* ATCC14028; *Staphylococcus saprophyticus* ATCC19701; *Escherichia coli* ATCC25922; and *Trichoderma harzianum* IOC4042, previously grown in nutrient agar culture medium. The antimicrobial analysis of the residues was developed (Clinical and Laboratory Standard Institute, 2014) using the agar drilling technique. It was carried out at the Food Microbiology Laboratory of the Department of Pharmacy in the Federal University of Maranhão, Brazil. The sizes of the inhibition haloes were measured in millimeters through a standardized ruler. At the time, positive and negative controls were defined. Chloramphenicol and nystatin were used as positive controls for bacteria and fungi, and ethanolic solution was used as an injection for the negative control. The entire procedure was performed in triplicate.

An antimicrobial analysis was also carried out to determine whether the resin present in the BA residue was a potent inhibitor. The resin was collected by boiling 50 g of BA bark in 300 mL of water at a temperature of 110°C for 30 min. In this process, the emergence of the resin was observed, which was collected with a spatula and transferred to a glass flask, covered with aluminum foil and kept in a refrigerator until analysis.

The phytochemical prospecting of residues (BA and SC peels) followed the methodology of Toledo et al. (2022). The components investigated, regarding their absence or presence, were anthocyanins, flavonoids, anthroquinones, phenols, anthocanidins, leucoanthocyanidins, catechins, coumarins, tannins, flavonols, flavones, anthraquinones, anthrones, xanthones, and saponins.

Solid-state fermentation systems

Microorganism

The strain of *T. harzianum* (IOC4042) used in the inoculation process was acquired from the Department of Mycology of the Oswaldo Cruz Institute (Fiocruz). Its maintenance was performed with potato dextrose agar (PDA) medium composed of agar-agar (2% m/v), potato extract (20% m/v), and glucose (2% m/v), sterilized at 120°C for 20 min. The culture was incubated for seven days at 30°C, and the spores were subsequently resuspended in saline solution (0.9%) and counted in a Neubauer chamber to start the fermentations.

Conducting fermentations in SSF were carried out using hydration solutions to maintain humidity values between 75 and 80%. One of four distinct solutions could be added to the solid medium: a. distilled water; b. nutrient solution; c. nutrient solution with glucose; and d. nutrient solution with sucrose. All materials were autoclaved at 1 atm pressure for 20 min. The nutrient solution presented the following components per gram of residue: 0.0024 g of sodium nitrate ($NaNO_3$); 0.0018 g of ammonium sulfate ($(NH_4)_2SO_4$); and 0.001 g of monopotassium phosphate (KH_2PO_4). Solutions: glucose or sucrose at 0.03 g/g of residue.

In 20 mL vials (vails), 1g of residue was added as follows: either 1g of residue from BA or SC when the SSF was conducted only by a single residue, or 0.5 g of each residue when conducted through the mixture of bacuri and sweet cassava (BASC). Then, added to the residue, 2.0 mL of each nutrient solution or distilled water were autoclaved at 1 atm for 20 min. To allow air to enter the fermentation flasks, they were covered with a cloth, also previously sterilized, and then were inoculated with (0.5 mL) of 6.25×10^4 spores per gram of residue. Afterward, they were incubated at 30°C for nine days in a bacteriological oven.

Fermentation control was also performed using distilled water as a moisturizing solution without inoculum to prove the system's non-formation and/or existence of 6-PP. It was decided to carry out the SSF with only 1 g of the residues or their mixture in the analysis vial to avoid loss of volatiles. The bottles were covered with corks and replaced by the appropriate lid with a septum at the instant of extraction.

Extraction and quantification of aroma compounds

In the extraction step, 5 mL of 25% saline solution (m/v) was added to the 20 mL vail containing the fermented one. The addition of this solution allows the reduction of the surface tension of the medium, facilitating the release of aromatic compounds to the headspace and the direct contact with the fiber for SPME of composition: Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), with 100 µm thickness (SUPELCO), gray color. After adding the physiological solution, a magnetic bar was introduced into the vial to assist in the agitation of the system. The bottle was immediately closed with a crimping cap, magnetic bimetallic, with a 20 mm polytetrafluoroethylene (PTFE) silicone septum and a 10 mm hole. Next, the sample was heated in a water bath, under agitation, and kept at a temperature of 79°C. A balanced time of 2 min was required before the fiber was exposed, which was subsequently exposed for 30

min. At the end of the extraction time, the plunger was removed, and the fiber was directed to the gas chromatography (GC) to be exposed again for 4 min for thermal desorption, according to the procedure of Ramos et al. (2008).

The quantification and identification of aromatic compounds obtained in SSF was conducted by GC, using the GCMS-QP2010 Plus – Shimadzu. The chromatographic column used was the RESTEK RTX-5, with a 30 m length, internal diameter of 0.25 mm and 0.25 µm, with injector and flame ionization detector, both at 250°C, in splitless mode. The sample's temperature collected in the headspace by SPME occurred directly in the GC injector, using temperature gradients as specified below.

The initial column temperature was 100°C maintained for 4 min. After that, the heating was increased from 100 to 150°C at a rate of 10°C/min, remaining for another 4 min. The column was then heated from 150 to 200°C at a rate of 20°C/min for another 4 min. Finally, the temperature was increased from 200 to 230°C at a rate of 10°C/min for another 4 min. The carrier gas flow (N_2) was 1.2 mL/min. The total race time was 21 min.

From the stock solution, 2,000 ppm of the standard 6-PP (purity≥96% Sigma-Aldrich) dilutions were performed of 5; 10, 50, 100, and 200 ppm and 1 mL of each solution were added in 1 g of BA residues, then in 1 g of SC, and also in the BASC mixture in the ratio 1:1, thus obtaining the calibration curves for SC and BASC. The points were analyzed in triplicate.

Results and Discussion

Waste characterization

The granulometry (212 mm SC and 450 mm BA), the nutrient solution volume (2.5 mL), and the residue quantity/headspace ratio used in the fermentations were chosen to maintain an adequate moisture content (75–80%) sufficient to allow the process to be carried out for seven to nine days without significant loss of water in the process (Penha et al., 2012). This was proved to result in practically no moisture loss during fermentation in the absence of an inoculum.

Table 1 shows the composition analysis data. Both residues showed acidic characteristics, which does not appear to be an obstacle to the

Table 1 – Physical-chemical and phytochemical characterization of bacuri and sweet cassava waste.

Waste characteristics							
Characteristics		**BA**	**SC**	**Characteristics**		**BA**	**SC**
	pH	3.13 ±0.01	4.78±0.76	**Phytochemical prospecting**	Phenols	+	-
	GRAN (mm)	300	212		Tannins	-	+
Centesimal composition (%)	MT	6.00±0.00	8.30±0.06		Anthocyanins	+	-
	MC	3.50±0.07	2.80±0.07		Anthocanidins	+	-
	TCAR	86.36±0.12	91.90±0.21		Flavonoids	+	+
	EE	10.10±0.34	1.10±0.03		Leucoanthocyanidins	+	-
	CP	4.40±0.18	2.70±0.00		Catechins	+	-
	ADF	26.25±0.39	8.65±1.05		Flavonols/Flavones	-	-
	NDF	44.20±1.52	8.65±2.59		Anthraquinone	+	-
Lignocellulosic composition (%)	ST	11.14±0.01	49.50±0.3		Coumarin	+	-
	TRS/100g	3.49±0.17	16.39±0.12		Antrona	+	-
	CEL	23.65±0.30	5.10±0.4		Xanthones	+	-
	LIG	1.80±0.02	17.9±0.21		Anthroquinone	+	-
	HEM	2.20±0.81	24.50±1.5		Saponin	+	-
Metal composition (%)	Na	1.10±0.60	0.16±0.00	**EDS (%)**	C	60.00	49.00
	K	7.30±0.13	5.00±0.13		N	-	5.36
	Mg	0.47±0.00	0.70±0.02		O	39.00	45.55
	Ca	1.20±0.20	1.40±0.01		Zn	0.13	-
	Zn	0.013±0.00	0.011±0.00		-	-	-

BA: bacuri; SC: sweet cassava; pH: potential of hydrogen; GRAN: granulometry; MT: moisture; MC: mineral content; TCAR: total carbohydrates; EE: ethereal extract; CP: crude protein; ADF: acid digestive fiber; NDF: neutral digestive fiber; ST: starch; TRS: total reducing sugars; CEL: cellulose; HEM: hemicelluloses; LIG: lignin; Na: sodium; K: potassium; Mg: magnesium; Ca: calcium; Zn: zinc; +: presence of the compound; -: absence of the compound; C: carbon; N: nitrogen; O: oxygen; EDS: energy dispersive spectroscopy.

growth of *T. harzianum*. According to Mendoza et al. (2003), fungi of this species exhibit excellent growth in acidified medium, which justifies fungal behavior in modifying the environment to meet its growth needs (Conde-Ávila et al., 2023).

The centesimal composition shows the content of carbohydrates, fibers, proteins, and lipids that could be used to grow the filamentous fungus as nutritional sources in the presence of lignin, cellulose, hemicellulose, and total reducing sugars in both residues, with higher cellulose values in BA and higher levels of starch and free sugars in SC.

This composition favors the development of filamentous fungi since many of them colonize different ecological niches. For example, fungi of the genus *Trichoderma* can degrade lignocellulosic material through extracellular enzymes, absorbing compounds that are then made available for the growth of hyphae. Cellulases produced by *T. harzianum* are considered efficient systems for the complete hydrolysis of cellulosic substrates in glucose (Xia and Lin, 2022).

The SC peel, obtained during the processing of flour, starch, or other products, is composed of two regions: the outer with a thinner thickness brown color, which surrounds the entire root serving as a protective barrier, and the internal part, which is much denser, thicker, and white. For this study, the shell composed of two parts (internal and external) was used. The bark of the BA, in general, is oval, presenting a yellow color and quite aromatized. It is possible to observe externally, which is smooth and shiny. The inner part is thick and has a large cavity that holds the lumps (usually two, depending on the species), surrounded by white pulp, widely used in producing sweets, ice cream, etc. Another observed point detected in this bark was the presence of resin, an essential component for several vegetables, as it protects the plant.

The shell is saturated with resin, which will spill if there is any crack in the shell region. Despite the benefits offered by the resin for the plant, it also presents itself as an obstacle to using the bark of the BA, contributing to the high disposal of these materials in the environment. Regarding morphology, the dry bark has a spongy aspect with pores.

The highest mineral content was 3.50, standard deviation (±): 0.07% for BA and 2.80±0.07% for SC. Both residues presented high total carbohydrate contents with values above 85% for BA and exceeding 90% for SC. The percentage difference in lipid content is evident for BA, whose content was 10.10±0.34%, while SC was only 1.10±0.03%. The highest protein and fiber content were also found in the BA residue. The highest cellulose content was found in BA, 23.65±0.30%. The highest values of hemicellulose, lignin, and starch were present in SC. The amount of reducing sugar was also more expressive in SC, with a value eight times higher for each 100 g of residue than that of BA.

In comparison to SC, BA has higher contents of potassium (K) (7.30±%0.13), sodium (Na) (1.10±0.6%), and zinc (Zn) (0.013±0.00%). Magnesium (Mg) was not detected in SC, only in BA. The calcium (Ca) value was higher than other metals in SC.

However, for the consumption of carbon (C) sources, other nutrients such as minerals are also necessary to develop fungi, some essential for the activity of most different enzymes, with emphasis on Zn and copper (Cu), which are necessary for the proper development of living organisms (Wiewióra and Żurek, 2021). The concentrations of metals seen in the residues can be considered not harmful to the activities of *Trichoderma* (Kaur and Garg, 2018).

In the SC, the phytochemical test revealed only the presence of tannins and flavonoids, while in the BA, the following were identified: phenolics, anthocyanins, flavonoids, leucoanthocyanidins, catechins, anthraquinones, coumarins, xanthones, and saponins originating. Among the phytochemicals in the BA, the metabolites with antioxidant action (for example, carotenoids, phenolic acids, and flavonoids) stand out, which guarantee the reduction of oxidation rates of lipids present in the vegetables and can be better utilized by microorganisms during SSF.

Ribeiro et al. (2021) reported a compilation of some works with the composition of BA shell, indicating a selective extraction of a specific compound identified as morelloflavone. Low concentrations of oleic, linoleic, stearic, and palmitic acids are constituents of this matrix. The flavonoid class presents, in general, essential antioxidant properties due to the delocalization of the free radical formed. However, phenolic compounds can act as important antimicrobial agents, alkaloids, and terpenoids (Vaou et al., 2021).

Da Silva et al. (2020), studying the *Platonia insignis* hydroalcoholic extract (PiHE) for antifungal and antivirulence activities against vaginal *Candida* species, verified that its extract, rich in flavonoids, could act as a novel candidate for developing new therapeutic treatments against fungal infections. The BA residue, as well as the resin extracted from this residue, shows a significant growth reduction for *T. harzianum* and for the other microorganisms tested in comparison with the SC residue (Table 2).

The negative control shows the absence of growth inhibition, while the positive control indicates the inhibition effect, which is greater when the halo size is larger. Fluconazole and nystatin were used as a positive control, but they were not adequate for *T. harzianum*; there was no positive effect for the control (indicated with * in Table 2). Another product, amphotericin B, was used for this purpose but failed. In this case, another step with pesticides is suggested, as it is an environmental fungus.

Data show that both BA and BA resin presented antimicrobial activity for all microorganisms tested. However, the largest inhibition halo for almost everyone, including *T. harzianum* IOC4042, was the presence of the resin. On the other hand, the SC extract shows less inhibition for the tested microorganisms and no effect on *T. harzianum*. The most significant inhibition haloes were for *Staphylococcus saprophytic* ATCC19701 *and Escherichia coli* ATCC25922, with 10 mm each.

Solid-state fermentation

In this study, four conditions were chosen to verify the influence of supplementation since supplementation is necessary when the solid medium does not provide sufficient nutrients.

In the BA residue, no growth or aroma production was observed. Even after extracting the resin to quantify antimicrobial activity, this residue was used for an SSF and did not produce growth or aroma (Table 3). Fungal growth was already quite intense during fermentation with SC. No aroma production was observed from the fermentation performed with BA or heat-treated BA residue (data not presented), probably due to the inhibition caused by the compound of the residue and also by the loss of nutrients caused by thermal extraction. While in the fermentation with SC, there was a discreet production of aroma, but the amounts did not exceed 7 ppm (w/w).

Contrary to what happened with the fermentation using BA bark, the SC bark was a favorable support for the germination of the spores and the production of the coconut bioaroma. This residue has a starchy characteristic, with around 50% starch.

In general, the germination and growth of the fungus *T. harzianum* in SC waste was efficient in the four humidification conditions, including distilled water and nutrient solution, whose main source of carbon and energy was starch. Because it is a polysaccharide, starch requires additional digestion by fungi, tending to the occurrence of extracellular cleavage, releasing to the solid medium more bioavailable absorption units, such as mono-, di-, and oligosaccharides (Hamad et al., 2014). It was observed that, with the addition of the nutrient solution only, there was an increase in the productivity of the aroma (6.10±0.49 ppm [w/w]) about fermentation conducted only with distilled water (1.70±0.11 ppm [w/w]). However, despite the high exploitation of the nutrients in the SC residue by the fungi, production was still considered low.

Differently, from the two fermentations mentioned above, the one with the BASC mixture residues promoted a very expressive increase in the production of coconut bioaroma. However, the SSF in the presence of water and sucrose provided the highest concentrations, 142.46±2.46 and 202.46±1.03 ppm (w/w), respectively.

Due to the inhibition of fungus growth in the BA residue and the low aroma concentration under all humidification conditions in the SC residue, the mixture of residues had a positive effect. In all fermentation conditions, the fungus grew in the BASC mixture, and there was a considerable increase in the production of 6-PP. The concentrations obtained were 202.46±1.30 ppm (w/w), 142.46±2.46 ppm (w/w), 42.18±6.80 ppm (w/w), and 26.43±5.27 ppm (w/w) with sucrose

Table 2 – Determination of the antimicrobial activity of bacuri, bacuri resin, and sweet cassava.

Antimicrobial activity					
Microorganisms	Size of inhibition haloes (mm)				
	SC	BA	BA resin	C+	C-
Proteus mirabilis ATCC7002	9	10	28	27	0
Staphylococcus aureus ATCC25923	0	15	15	40	0
Pseudomonas aeruginosa ATCC27853	0	7	20	15	0
Salmonellas ATCC14028	9	10	15	43	0
Staphylococcus saprophyticus ATCC19701	10	15	8	35	0
Escherichia coli ATCC25922	10	14	7	45	0
Trichoderma harzianum IOC4042	0	10	14	*	0

BA: bacuri; SC: sweet cassava; C+: positive control (use of antibiotics); C-: negative control (absence of antibiotics); *Haloes not detected.

Table 3 – Bioproduction of coconut aroma (6-pentyl-α-pyrone) in distinct solutions added to waste.

	Waste					
	6-pentyl-α-pyrone concentration (expressed as dry mass)					
	ppm			mg/g		
Solutions	BA	SC	BASC	BA	SC	BASC
Water	Nd	1.70±0.11	142.46±2.46	Nd	0.00170	0.14246
Nutrient solutions	Nd	6.18±0.43	42.18±0.10	Nd	0.00618	0.04218
Nutrient+glucose	Nd	1.48±0.09	26.43±0.70	Nd	0.00140	0.00264
Nutrient+sucrose	Nd	2.15±0.10	202.46±1.03	Nd	0.00215	0.20246

Nd: not detected; BA: bacuri; SC: sweet cassava; BASC: bacuri+sweet cassava.

nutrient solution, water, nutrient solution, and nutrient solution with glucose, respectively. A lower aroma concentration was detected in the presence of medium containing glucose, both in BA and BASC, possibly due to the use of this sugar preferentially only for growth or maintenance. According to Abrol et al. (2022), the influence of nutritional composition on producing spores or biocompounds is hampered by solid media's indefinite or semi-defined nature. As reported by Garbin et al. (2021), inhibition can occur when the inhibitor and substrate compete for the same binding site, that is, the inhibitor binds to another region of the enzyme.

This may be related to the action of cellulases and amylases that saccharify cellulose and starch, releasing glucose into the medium (Ventura et al., 2022). However, it is worth highlighting that the composition and quantity of hydrolyzed sugars vary depending on the composition, in this case, of the agro-industrial residue, processing (Budenkova et al., 2021), and even the type of treatment.

The strategy of mixing SC residues with BA residues possibly enabled the accommodation of SC starch grains in the pores of the BA bark. This may have allowed good water absorption (Hamrouni et al., 2020) and oxygen transfer, in addition to being an important compartment for storing substances that improve spore production. Residues with accessible cavities favor the mass production of spores, especially in the presence of starchy substrates, which are considered the best for producing spores of filamentous fungi. This mixture may have facilitated the expansion of fungal hyphae throughout the waste, consequently allowing better use of available nutrients for the enzymatic activities necessary to obtain the products of interest.

Hamrouni et al. (2020) carried out an SSF using the metabolism mixture of vine, potato, jatropha, olive pomace, and olive oil as substrate *Trichoderma asperellum* TV104 for the production and 6-PP, emphasizing the starch and lipid content without solid medium, associated with aeration system. The compound production was 3.06±0.15 mg g DM^{-1}, a very high bioaroma value, with this waste mix subjected to aeration.

The mixture also provided an increase in lipid and starch content. According to Fadel et al. (2015), lipids and sugars are recommended to increase the production of 6-PP. Lipids have another essential function in SSF, acting as a detoxifying agent and combating the toxicity of the environment, as the concentration of coconut bioaroma harms the existence of fungi. This fact may be related to the ability of 6-PP to be absorbed by the hydrophobic cell membrane (Bonnarme, 1997). In this case, a possible manipulation of 6-PP, representing a defense mechanism related to toxic effects on other microorganisms, and the fungus itself, occurs when a certain concentration is reached (Liu et al., 2022), as it is a natural fungicide.

Authors state that the presence of glucose in high concentrations can inhibit the growth and/or sporulation of some fungi, such as *Beauveria bassiana, Metarhizium anisopliae, and Paecilomyces farinosus* (Ooijkaas et al., 1998). However, there is always a greater preference of microorganisms for simpler sugars. It is important to remember that fungi cannot sporulate in a medium saturated with available nutrients, and dormancy breakdown requires water and glucose (Baiyee et al., 2019). As for glucose, it is rapidly absorbed, and most of this carbon source may be directed to fungal growth, which is why high microbial growth and low concentration of the secondary metabolite (6-PP) were observed in SC. The availability of glucose in the medium suggests greater fungal growth. According to Chung and Kim (2018), the germination rate of the fungus evaluated in the presence of glucose was 98%, followed by sucrose (81%) and starch (70%).

They believe that producing these metabolites is possible when there is the accumulation of large amounts of precursors of primary metabolites (amino acids, acetate, pyruvate, among others). In this case, in a certain way, supplementation with glucose and sucrose may have contributed to the reduction of the enzymatic activity of the fungi. However, the worst yield of 6-PP resulted from the presence of glucose.

According to the model proposed by Nopharatana et al. (1998), the increase in height in surface fungal growth occurs as tip diffusivity increases or as the Monod saturation constant for glucose decreases. The lower saturation constant for glucose means that longer duration of the exponential phase would facilitate the diffusion of the hyphae, possibly towards the interior of the support, and increased use of the nutrients in producing the aroma.

The addition of only nutrient solution, with the reduction of sugar coming only from the residues, should maintain a good condition for hyphal diffusion and the production of precursors for subsequent synthesis of the bioaroma. Sucrose improves this condition a little since the previous hydrolysis necessary for this substrate can reduce glucose saturation and increase bioproduction. However, using readily soluble glucose could allow a less stressful environment for the production of interest, which agrees with the study by Moreno-Ruiz et al. (2020), which showed that 6-PP production is increased. The growth of *Trichoderma atroviride* is decreased when challenged by the presence of mycelia or metabolites of some pathogenic fungi.

Some works are reported in the literature on the production of different bioaromas using different agricultural residues, almost all adding nutrient solutions with glucose, and obtaining different levels. Some of them had similar contents to those obtained in this study (0.202 mg/g and 202.46 ppm) using sugarcane bagasse and the fungus of the genus *Trichoderma* in the production of 6-PP. We can mention Hamrouni et al. (2019) with 0.085 mg/g, Ladeira et al. (2010) with 0.254 mg/g, and Penha et al. (2012) with 0.093 mg/g. Other studies showed higher levels, such as Fadel et al. (2015), obtaining 3.62 mg/g of sugarcane bagasse with slightly higher carbohydrate content, and Ramos et al. (2008), who used green coconut residue with 0.8 mg/g of 6-PP.

According to reports by Lima et al. (2022), there has been a significant increase in the number of patents deposited in the last ten years,

with the prospect that with the advancement of studies on their properties, results for application in the most diverse areas will prove increasingly viable and promising. Studies related to agronomic aspects of BA are still predominant, such as cultivation and reproduction strategies, and recently, those related to species maintenance and biodiversity conservation stand out. Registrations of patent applications and/or patents granted with products developed from BA are primarily focused on the cosmetic, pharmaceutical, and phytotherapeutic areas, based mainly on leaf and seed extracts and, to a lesser extent, on the bark. Another area of emphasis has been the search to elucidate the chemical compounds responsible for the reported biological effects on the body or to elucidate compounds that have not yet been identified and their mechanisms of action, which opens the way for further studies.

The market looks very promising in different areas, which suggests that the amount of BA waste will increase. However, work so far has yet to address the use of these residues in applying SSF, and fungal growth was not possible even with natural residues. Yet, the mixture with SC and the addition of sucrose, or granulated sugar that can be bought in local markets, and with some nutrients also common in low concentrations, allowed an initial production of 0.202 mg/g.

Such production can be further amplified if some subsequent optimization is carried out, as was the case with Ramos et al. (2008), who managed to increase the production of 6-PP from coconut residue from 0.8 to 5.0 mg/g using an experimental design. This can also be proven in the work of Hamrouni et al. (2019), who found an increase in 6-PP production from 0.085 mg/g to 3.06±0.15 mg/g after selecting different species of *T. asperellum* and substrates, when a mixture of vine shoots, four potatoes, jatropha, olive pomace, and olive oil was used under aeration in SSF conducted with *T. asperellum* TV104 (Hamrouni et al., 2020).

As no work using these residues in the production of bioaroma has been found in the literature so far, this could be a promising biotechnological process to be further optimized.

Conclusion

The SSF performed with the bark of BA to produce coconut bioaroma was unsuccessful. This was due to inhibitory compounds, as previously pointed out, preventing the germination of spores of the fungus *T. harzianum*, even in the presence of sugars such as glucose and sucrose. On the other hand, the residue of the SC bark favored microbial growth, generating, however, a low aroma. By mixing SC and BA residues, it was possible to produce 202.46±1.30 ppm (w/w) of 6-PP with the addition of nutritional and sucrose solution. Possibly, the growth of the fungus provided by the SC facilitated the dispersion of the hyphae along the BA waste and, consequently, the enzymatic activities necessary for the conversion of the nutrients into the aroma of interest.

Authors' contributions

nASCIMENTO, A.S: project administration; investigation; writing – review & editing. LEITE, S.G.F.: conceptualization; supervision; validation; data curation; writing – review & editing. NASCIMENTO, U.M.: investigation; methodology; project administration; resources. MUCHAVE, G.J.: investigation; methodology; software. SILVA, A.Z.: resources; conceptualization. RIBEIRO, J.B.: methodology; conceptualization. PENHA, M.S.C.: methodology; conceptualization. RIBEIRO, E.C.: methodology; writing – original draft. BORGES, C.P.: methodology.

References

Abrol, V.; Kushwaha, M.; Mallubhotla, S.; Jaglan, S., 2022. Chemical mutagenesis and high throughput media optimization in *Tolypocladium inflatum* MTCC-3538 leads to enhanced production of cyclosporine A. 3 Biotech, v. 12, (8), 158. https://doi.org/10.1007/s13205-022-03219-x

Afiqah Razali, S.; Rasit, N.; Kuan Ooi, C., 2021. Statistical analysis of xylanase production from solid state fermentation of rice husk associated fungus A*spergillus niger*. Materials Today: Proceedings, v. 39, 1082-1087. https://doi.org/10.1016/j.matpr.2020.06.366

Alrefai, A.M.; Alrefai, R.; Benyounis, K.Y.; Stokes, J., 2020. Impact of starch from cassava peel on biogas produced through the anaerobic digestion process. Energies, v. 13, (11), 2713. https://doi.org/10.3390/en13112713

Baiyee, B.; Ito, S.; Sunpapao, A., 2019. *Trichoderma asperellum* T1 mediated antifungal activity and induced defense response against leaf spot fungi in lettuce (Lactuca sativa L.). Physiological and Molecular Plant Pathology, v. 106, 96-101. https://doi.org/10.1016/j.pmpp.2018.12.009

Bonnarme, P.; Djian, A.; Latrasse, A.; Féron, G.; Giniès, C.; Durand, A.; Le Queré, J.L., 1997. Production of 6-pentyl-α-pyrone by Trichoderma sp. from vegetable oils. Journal of Biotechnology, v. 56, (2), 143-150. https://doi.org/10.1016/S0168-1656(97)00108-9

Budenkova, E.; Sukhikh, S.; Ivanova, S.; Babich, O.; Dolganyuk, V.; Michaud, P.; Kriger, O., 2021. Improvement of enzymatic saccharification of cellulose-containing raw materials using *Aspergillus niger*. Processes, v. 9, (8), 1360. https://doi.org/10.3390/pr9081360

Buffi, M.; Cailleau, G.; Kuhn, T.; Li Richter, X.-Y.; Stanley, C.E.; Wick, L.Y.; Chain, P.S.; Bindschedler, S.; Junier, P., 2023. Fungal drops: a novel approach for macro- and microscopic analyses of fungal mycelial growth. MicroLife, v. 4. https://doi.org/10.1093/femsml/uqad042

Carvalho, J.C.; Borghetti, I.A.; Cartas, L.C.; Woiciechowski, A.L.; Soccol, V.T.; Soccol, C.R., 2018. Biorefinery integration of microalgae production into cassava processing industry: Potential and perspectives. Bioresource Technology, v. 247, 1165-1172. https://doi.org/10.1016/j.biortech.2017.09.213

Chung, K.H.; Kim, J.W., 2018. Role of sugars in early stage of spore germination in filamentous fungi, *Aspergillus nidulans*. Korean Journal of Mycology, v. 46, (4), 511-518. https://doi.org/10.4489/KJM.20180056

Clinical and Laboratory Standard Institute, 2014. M39-A4: analysis and presentation of cumulative antimicrobial susceptibility test data: approved guideline. Wayne, PA, USA. ISBN: 610.688.0700.

Conde-Ávila, V.; Loera-Corral, O.; Díaz, R.; Sánchez, C., 2023. Cutinolytic esterases are induced by the growth of the fungus *Trichoderma harzianum* on glyceryl monostearate in solid-state fermentation. BioResources, v. 18, (4), 8515-8527. https://doi.org/10.15376/biores.18.4.8515-8527

Companhia Nacional de Abastecimento (Conab), 2021. Informações Agropecuárias. Análises do Mercado Agropecuário e Extrativista. Análises do Mercado. Histórico Mensal de Mandioca. Mandioca – Análise Mensal. Novembro 2021 (Accessed April 04, 2023) at:. https://www.conab.gov.br/info-agro/analises-do-mercado-agropecuario-e-extrativista/analises-do-mercado/historico-mensal-de-mandioca/item/17525-mandioca-analise-mensal-novembro-2021

Da Silva, A.F.; da Rocha, C.Q.; da Silva, L.C.N.; Carvalho Júnior, A.R.; Mendes, I.N.F.V.; de Araruna, A.B.; Motta, E.P.; Silva, R.S.; Campos, C.D.L.; Farias, J.R.; Oliveira, A.S.; Silva, D.H.S.; Nascimento, F.R.F.; Guerra, R.N.M.; Monteiro, C.A., 2020. Antifungal and antivirulence activities of hydroalcoholic extract and fractions of *Platonia insignis* leaves against vaginal isolates of *Candida* species. Pathogens, v. 9, (2), 84. https://doi.org/10.3390/pathogens9020084

Dulf, F.V.; Vodnar, D.C.; Socaciu, C., 2016. Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities, and lipid fractions of plum fruit (*Prunus domestica L.*) by-products. Food Chemistry, v. 209, 27-36. https://doi.org/10.1016/j.foodchem.2016.04.016

El-Sayed, E.R.; Ahmed, A.S.; Al-Hagar, O.E.A., 2020a. Agro-industrial wastes for production of paclitaxel by irradiated *Aspergillus fumigatus* under solid-state fermentation. Journal of Applied Microbiology, v. 128, (5), 1427-1439. https://doi.org/10.1111/jam.14574

El-Sayed, E.R.; Ahmed, A.S.; Al-Hagar, O.E.A., 2020b. Agro-industrial wastes for production of paclitaxel by irradiated *Aspergillus fumigatus* under solid-state fermentation. Journal of Applied Microbiology, v. 128, (5), 1427-1439. https://doi.org/10.1111/jam.14574

Fadel, H.H.M.; Mahmoud, M.G.; Asker, M.M.S.; Lotfy, S.N., 2015. Characterization and evaluation of coconut aroma produced by *Trichoderma viride* EMCC-107 in solid state fermentation on sugarcane bagasse. Electronic Journal of Biotechnology, v. 18, (1), 5-9. https://doi.org/10.1016/j.ejbt.2014.10.006

Flores, C.; Nieto, M.; Millán-Gómez, D.V.; Caro, M.; Galindo, E.; Serrano-Carreón, L., 2019. Elicitation and biotransformation of *6-pentyl-α-pyrone* in *Trichoderma atroviride* cultures. Process Biochemistry, v. 82, 68-74. https://doi.org/10.1016/j.procbio.2019.04.019

Garbin, A.P.; Garcia Nayara F.L.; Cavalheiro, G.F.; Silvestre, M.A.; Rodrigues, A.; Paz, M.F.; Fonseca, G.G.; Leite, R.S.R., 2021. β-glucosidase from thermophilic fungus *Thermoascus crustaceus*: production and industrial potential. Anais da Academia Brasileira de Ciências, v. 93, (1), e20191349. https://doi.org/10.1590/0001-3765202120191349

Hamad, H.O.; Alma, M.H.; Ismael, H.M.; Goceri, A., 2014. the effect of some sugars on the growth of *Aspergillus niger.* Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, v. 17, (4). https://doi.org/10.18016/KSUJNS.28479

Hamrouni, R.; Molinet, J.; Dupuy, N.; Taieb, N.; Carboue, Q.; Masmoudi, A.; Roussos, S., 2020. The effect of aeration for *6-pentyl-alpha-pyrone,* conidia and lytic enzymes production by *Trichoderma asperellum* strains grown in solid-state fermentation. Waste and Biomass Valorization, v. 11, (11), 5711-5720. https://doi.org/10.1007/s12649-019-00809-4

Hamrouni, R.; Molinet, J.; Miché, L.; Carboué, Q.; Dupuy, N.; Masmoudi, A.; Roussos, S., 2019. Production of coconut aroma in solid-state cultivation: Screening and identification of *Trichoderma strains* for *6-pentyl-alpha-pyrone* and conidia production. Journal of Chemistry, 2019. https://doi.org/10.1155/2019/8562384

Instituto Adolfo Lutz (IAL), 2005. Physical-chemical methods for food analysis. IAL, São Paulo.

Kaur, H.; Garg, N., 2018. Recent perspectives on cross talk between cadmium, zinc, and arbuscular mycorrhizal fungi in plants. Journal of Plant Growth Regulation, v. 37, (2), 680-693. https://doi.org/10.1007/s00344-017-9750-2

Ladeira, N.; Peixoto, V.; Penha, M.; Barros, E.L.S., 2010. Optimization of *6-pentyl-alpha-pyrne* production by solid state fermentation using sugarcane bagasse as residue. BioResources, v. 5, (4), 2297-2306. https://doi.org/10.15376/biores.5.4.2297-2306

Langyan, S.; Bhardwaj, R.; Radhamani, J.; Yadav, R.; Gautam, R.K.; Kalia, S.; Kumar, A., 2022. A quick analysis method for protein quantification in oilseed crops: a comparison with standard protocol. Frontiers in Nutrition, v. 9. https://doi.org/10.3389/fnut.2022.892695

Lim, J.S.; Hong, J.-H.; Lee, D.Y.; Li, X.; Lee, D.E.; Choi, J.U.; Lee, K.Y.; Kim, K.H.; Cho, Y.-C., 2023. *6-Pentyl-α-Pyrone* from *Trichoderma gamsii* Exert Antioxidant and Anti-Inflammatory Properties in Lipopolysaccharide-Stimulated Mouse Macrophages. *Antioxidants*, v. 12, (12), 2028. https://doi.org/10.3390/antiox12122028

Lima, S.K.R.; Coêlho, A.G.; Lucarini, M.; Durazzo, A.; Arcanjo, D.D.R., 2022. The *Platonia insignis Mart*. as the promising Brazilian 'Amazon Gold': the state-of-the-art and prospects. Agriculture, v. 12, (11), 1827. https://doi.org/10.3390/agriculture12111827

Lindsay, M.A.; Granucci, N.; Greenwood, D.R.; Villas-Boas, S.G., 2022. Identification of new natural sources of flavour and aroma metabolites from solid-state fermentation of agro-industrial by-products. Metabolites, v. 12, (2), 157. https://doi.org/10.3390/metabo12020157

Liu, Y.; He, P.; He, P.; Munir, S.; Ahmed, A.; Wu, Y.; Yang, Y.; Lu, J.; Wang, J.; Yang, J.; Pan, X.; Tian, Y.; He, Y., 2022. Potential biocontrol efficiency of *Trichoderma* species against oomycete pathogens. Frontiers in Microbiology, v. 13, 974024. https://doi.org/10.3389/fmicb.2022.974024

Madzingira, O.; Hepute, V.; Mwenda, E.N.; Kandiwa, E.; Mushonga, B.; Mupangwa, J.F., 2021. Nutritional assessment of three baled rice straw varieties intended for use as ruminant feed in Namibia. Cogent Food & Agriculture, v. 7, (1). https://doi.org/10.1080/23311932.2021.1950402

Mattedi, A.; Sabbi, E.; Farda, B.; Djebaili, R.; Mitra, D.; Ercole, C.; Cacchio, P.; Del Gallo, M.; Pellegrini, M., 2023. Solid-state fermentation: applications and future perspectives for biostimulant and biopesticides production. Microorganisms, v. 11, (6), 1408. https://doi.org/10.3390/microorganisms11061408

Mendoza G.R.A.; Martijn ten Hoopen, G.; Kass, D.C.J.; Sánchez, G.V.A.; Krauss, U., 2003. Evaluation of mycoparasites as biocontrol agents of *Rosellinia root* rot in cocoa. Biological Control, v. 27, (2), 210-227. https://doi.org/10.1016/S1049-9644(03)00014-8

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, v. 31, (3), 426-428. https://doi.org/10.1021/ac60147a030

Missbach, K.; Flatschacher, D.; Bueschl, C.; Samson, J. M.; Leibetseder, S.; Marchetti-Deschmann, M.; Zeilinger, S.; Schuhmacher, R., 2023. Light-induced changes in secondary metabolite production of *Trichoderma atroviride*. Journal of Fungi, v. 9, (8), 785. https://doi.org/10.3390/jof9080785

Moreno-Ruiz, D.; Lichius, A.; Turrà, D.; Di Pietro, A.; Zeilinger, S., 2020. Chemotropism assays for plant symbiosis and mycoparasitism related compound screening in *Trichoderma atroviride*. Frontiers in Microbiology, v. 11, 601251. https://doi.org/10.3389/fmicb.2020.601251

Morgan, N.C.M., 2016. Cassava: nutrient composition and nutritive value in poultry diets. Animation Nourish, v. 2, 253-261. https://doi.org/10.1016/j.aninu.2016.08.010

Naeimi, S.; Khosravi, V.; Varga, A.; Vágvölgyi, C.; Kredics, L., 2020. Screening of organic substrates for solid-state fermentation, viability and bioefficacy of *Trichoderma harzianum* AS12-2, a biocontrol strain against rice sheath blight disease. Agronomy, v. 10, (9), 1258. https://doi.org/10.3390/agronomy10091258

Nopharatana, M.; Howes. T.; Mitchell. D., 1998. Modelling fungal growth on surfaces. Biotechnology Techniques, v. 12, 313-318. https://doi.org/10.1023/A:1008810500243

Ooijkaas, L.P.; Ifoeng, C.J.; Tramper, J.; Buitelaar, R.M., 1998. Spore production of *Coniothyrium minitans* during solid-state fermentation on different nitrogen sources with glucose or starch as carbon source. Biotechnology Letters, v. 20, (8), 785-788. https://doi.org/10.1023/B:BILE.0000015923.64200.15

Penha, M.P.; Rocha-Leão, M.; Leite. S.G.F., 2012. Sugarcane bagasse as support for the production of coconut aroma by solid state fermentation (SSF). BioResources, v. 7, (2), 2366-2375.

Ramo, L.B.; Nobrega, R.O.; Fernandes, D.D.S.; Lyra, W.S.; Diniz, P.H.G.D.; Araujo, M.C.U., 2024. Determination of moisture and total protein and phosphorus contents in powdered chicken egg samples using digital images, NIR spectra, data fusion, and multivariate calibration. Journal of Food Composition and Analysis, v. 127, 105940. https://doi.org/10.1016/j.jfca.2023.105940

Ramos, A.S.; Fiaux, S.B.; Leite, S.G.F., 2008. Production of *6-pentyl-α-pyrone* by *Trichoderma harzianum* in solid-state fermentation. Brazilian Journal of Microbiology, v. 39, (4), 712-717. https://doi.org/10.1590/S1517-83822008000400022

Ribeiro, D.; Russo, H.; Fraige, K.; Zeraik, M.; Nogueira, C.; da Silva, P.; Codo, A.; Calixto, G.; de Medeiros, A.; Chorilli, M.; Bolzani, V., 2021. Bioactive bioflavonoids from *Platonia insignis (Bacuri)* residues as added value compounds. Journal of the Brazilian Chemical Society, v. 32, (4), 786-799. https://doi.org/10.21577/0103-5053.20200230

Silva, L.; Freitas, K.; Rocha, J.; Cunha, E., 2016. Study of bacuri bark (Platonia insignis mart.) as a source of a new sustainable agglomerated material. In: Brazilian Chemistry Congress. Chemistry: Technology, Challenges and Perspectives in the Amazon, 56., 2016, Belém, PA (Accessed November 04, 2023) at:. https://www.abq.org.br/cbq/2016/trabalhos/12/9810-23132.html

Toledo, H.; Sánchez, C. I.; Marín, L.; Amich, J.; Calera, J.A., 2022. Regulation of zinc homeostatic genes by environmental pH in the filamentous fungus *Aspergillus fumigatus*. Environmental Microbiology, v. 24, (2), 643-666. https://doi.org/10.1111/1462-2920.15452

van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2 nd ed. v. 476. Cornell University Press, Ithaca. 476 p. https://doi.org/10.4236/ojapps.2015.510061

Vaou, N.; Stavropoulou, E.; Voidarou, C.; Tsigalou, C.; Bezirtzoglou, E., 2021. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. Microorganisms, v. 9, (10), 2041. https://doi.org/10.3390/microorganisms9102041

Ventura, M.; Puyol, D.; Melero, J.A., 2022. The synergy of catalysis and biotechnology as a tool to modulate the composition of biopolymers (polyhydroxyalkanoates) with lignocellulosic wastes. Catalysis Today, v. 397-399, 220-231. https://doi.org/10.1016/j.cattod.2021.09.032

Wang, B.-T.; Hu, S.; Yu, X.-Y.; Jin, L.; Zhu, Y.-J.; Jin, F.-J., 2020. Studies of Cellulose and Starch Utilization and the Regulatory Mechanisms of Related Enzymes in Fungi. Polymers, v. 12, (3), 530. https://doi.org/10.3390/polym12030530

Wiewióra, B.; Żurek, G., 2021. the response of the associations of grass and Epichloë endophytes to the increased content of heavy metals in the soil. Plants, v. 10, (3), 429. https://doi.org/10.3390/plants10030429

Xia, Y.; Lin, X., 2022. Efficient biodegradation of straw and persistent organic pollutants by a novel strategy using recombinant *Trichoderma reesei*. BioResources and Bioprocessing, v. 9, (1), 91. https://doi.org/10.1186/s40643-022-00581-9

Yamaguchi, K.K.L.; Dias, D.S.; Lamarão, C.V.; Castelo, K.F.A.; Lima, M.S.; Antonio, A.S.; Converti, A.; Lima, E.S.; Veiga-Junior, V.F., 2021. Amazonian *Bacuri (Platonia insignis Mart.)* fruit waste valorisation using response surface methodology. Biomolecules, v. 11, (12), 1767. https://doi.org/10.3390/biom11121767

Zaier, H.; Maktouf, S.; Roussos, S.; Rhouma, A., 2021. Filamentous fungi isolated from Tunisian olive mill wastes: use of solid-state fermentation for enzyme production. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, v. 49, (1), 12125. https://doi.org/10.15835/nbha49112125

Zhao, B.; Al Rasheed, H.; Ali, I.; Hu, S., 2021. Efficient enzymatic saccharification of alkaline and ionic liquid-pretreated bamboo by highly active extremozymes produced by the co-culture of two halophilic fungi. Bioresource Technology, v. 319, 124115. https://doi.org/10.1016/j.biortech.2020.124115