

Characterization and Evaluation of Agro-industrial Residues of Bacuri (*Platonia Insignis* Mart.) and Sweet Cassava (*Manihot Esculenta* Crantz) in the Production of 6pentyl- α -pyrone by Solid State Fermentation

ADRIANA SILVA DO NASCIMENTO (✉ adriana_nascimento08@yahoo.com.br)

Federal University of Rio de Janeiro: Universidade Federal do Rio de Janeiro <https://orcid.org/0000-0003-2959-7069>

Research Article

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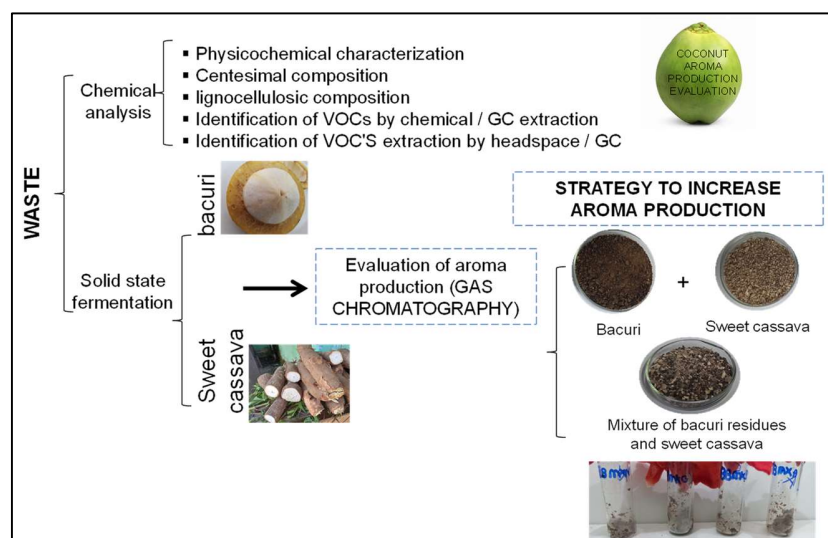
CHARACTERIZATION AND EVALUATION OF AGRO-INDUSTRIAL RESIDUES OF BACURI (*Platonia insignis* Mart.) AND SWEET CASSAVA (*Manihot esculenta* Crantz) IN THE PRODUCTION OF 6PENTYL- α -PYRONE BY SOLID STATE FERMENTATION.

NASCIMENTO, AS¹; LEITE, SGF¹; BORGES, CP¹; NASCIMENTO, UM²; SILVA, AZ²; MUCHAVE, GJ¹; RIBEIRO, JB¹; PENHA, MSC²; MARQUES, GEC³.

SUMMARY

The 6 pentyl- α -pyrone (6-PP) is a natural aroma, with a coconut smell, obtained by fermentation processes with fungi of the genus *Trichoderma*. The objective of this research was to evaluate the nutritional potential of bacuri bark and sweet cassava peels in the production of coconut bioaroma through solid state fermentation (SSF). To this end, analyses of physicochemical characterization, antimicrobial, volatile, surface, and composition activities were performed, including lignocellulosic profile, among others. The fermentations were conducted under humidification conditions with water, nutrient solution without additive and nutrient solutions with glucose or sucrose during 9 days. The quantification of aroma compounds was performed by gas chromatography, aided by solid phase microextraction. The results showed that there was inhibition of fungal growth in the bacuri residue motivated by the presence of inhibitory compounds, especially resin. The residues of sweet cassava, with a high percentage of starch, were favorable to microbial growth, however, the quantification of the bioaroma presented values below 7.0 ppm. Therefore, strategies to increase this production were necessary and the use of sweet cassava bark as a solid supplement, mixed with bacuri residue, was satisfactory reaching concentrations of 26.43 ± 5.72 ppm in the presence of nutrient solution with glucose; 42 ± 6.8 ppm with nutrient solution only; 142 ± 2.46 ppm only with distilled water and 202.46 ± 1.30 ppm with sucrose nutrient solution, showing the efficiency of the mixture for fungal development conditions and also for aroma production.

Graphical abstract



Keywords: Agro-industrial waste; Waste recovery; Solid-state fermentation; bacuri; sweet cassava.

¹Federal University of Rio de Janeiro

²Federal University of Maranhão

³Federal Institute of Education Science and Technology of Maranhão

Statement of Novelty

The production of aroma (6 pentyl- α -pyrone) with residues isolated from bacuri and sweet cassava showed different results. In the case of fermentations that took place in bacuri bark, there was inhibition of fungal growth (*Trichoderma harzianum*) by the presence of interfering constituents of the bark, especially resin, under humidification conditions (with water, with nutrient solution, nutrient solution with glucose and nutrient with sucrose). Fermentations with sweet cassava peels had low production under the same humidification conditions. As a strategy to increase the production of coconut aroma, it was decided to mix the residues (bacuri and sweet cassava) which, in turn, allowed a favorable environment for the germination of fungal spores and greater action of enzymatic activity, providing greater exploitation. constituents present in the waste mix. The production of aroma jumped from 7ppm, just with sweet cassava residue, to just over 200ppm in the mixture of residues, supplemented with a nutrient solution with glucose. This solid supplementation with sweet cassava residues was also efficient, even in humidification only with distilled water whose concentrations reached around 142ppm, proving the efficiency of the strategy.

1. INTRODUCTION

The nutritional evaluation of agro-industrial residues has favored the identification of large amounts of carbon sources, which can be exploited by microorganisms to obtain bio compounds. Bioproduction by Solid State Fermentation processes (SSF), or even submerged (SF), has achieved good results, however, better yields have been achieved through SSF, which sees in the feasible use of agro-industrial residues advantages, by owning substrates with different sources of carbon, simulating the natural habitat and rapid growth of aerobic microorganisms; in addition to requiring lower energy expenditure and sterilization[1], [2] resulting in processes at low cost and high concentrations of the product of interest

With this, several researches have seen in the bioconversion of agro-industrial residues, advantages in acquiring bioproducts of greater industrial interest, through SSF: [3], has succeeded in the use of potato pomace as a resource for the production of natural pigments (red, yellow and orange), using filamentous fungi, *Manacus spp.* [4] achieved high concentrations of pectinases, using rice husks and hay, with *Aspergillus niger ATCC 9642* as the fermenting agent. Sugarcane bagasse and green coconut shell powder were the residues used to obtain 6 pentyl- α - pyrone (6PP), (coconut bioaroma) using fungi of the genus *Trichoderma harzianum*. The concentrations of the metabolite found were in the range of 2.8 to 3.62 ppm as revealed by the studies of [5], [6],[7], [8]

The 6PP is an unsaturated lactone with a characteristic coconut smell [6]. Although it presents antibiotic activity it is also a non-toxic natural flavoring [9], [10]approved by the Joint Expert

Committee on Food Additives [8], with the highest concentrations of this bio compound acquired by SSF.

In SSF, microbial growth happens in non-soluble material or solid substrates in the absence or near absence of free water [11]. Based on the broad ability to explore lignocellulosic materials and the possibility of obtaining different bioproducts, fungi of the genus *Trichoderma spp.*, have been widely used for this function because they are considered excretory of a complex of enzymes, such as: chitinases, gluconases and proteases [12], [13]; organic acids, biomass, and other bioactive products [14], [15] important producers of primary and secondary metabolites, in particular, 6PP. Given this exploration capacity, other residues were used to achieve higher concentrations of 6PP. In this study, the sweet cassava (SC) bark and othe bacuri bark were used as a support and carbon source in solid state fermentation.

The scientific name of SC is *Manihot esculenta Crantz*, a type of sweet cassava, also called *mansa*, very used in the production of flours, starches, etc., as well as *wild* cassava. It has a low cyanide content and it is rich in starch [16], representing 30% of the wet weight of the root [16], [17]. Being *sweet* or *wild cassava*, it is one of the largest sources food of subsistence in the world, representing the third largest source of food in tropical regions, only behind of rice and corn [18], [19]

Data from the National Supply Council in Brazil [20] from January 2021 showed that cassava production in Brazil in 2020 was 18.96 million tons. During its processing the bark is the residue of the pre-cleaning [21] and this contains about 75% starch (w / w), with and 21.70% and 62.51% of amylopectin amylose [22], [23] wasted. The use of these residues can be advantageous in the biotechnological route, since starch can be hydrolyzed by fungi into monosaccharides and used as precursors in the biosynthesis of biomolecules [24]. For this research we used sweet cassava with cyanide concentration ($< 50 \text{ mg.kg}^{-1}$), which qualifies it as non-toxic for human consumption.

Bacuri (BA) is a fruit known by the scientific name *Platonia insignis Mart.* belonging to the class of angiosperms, of the family *Clusiaceae* (or *Guttiferae*). It is a native species, found in the rainfall part of the Amazon rainforest (Homma, 2018). It has high production, where 50 bacuri trees produce about 9.5 tons of fruits per hectare and a single tree can produce 2,000 fruits per year [26]. Of this total, 1 ton is equivalent to pulp, 2.5 tons are seeds, and 6 tons are equivalent to resin shells. Its fruit is constituted of three parts: bark, pulp, and the stone, which weighs on average 250g and has an oval shape. Its peel has thicknesses ranging from 0.6 to 1.7cm, which represents 70% of the total weight of the fruit. From the bacuri tree, we can take advantage of the fruit, the wood, and its energy potential [25]. It is a product exploited by extractivism.

The bark of BA, even with large percentages of carbohydrates, lipids, pectin and other micronutrients, still has its utilization considered low, due to the lack of more in-depth studies on the toxicological and carcinogenic potential of its components. In addition, its resin is a complicating factor in the use of this material [27] which favors large accumulations of these materials in the environment.

In view of the above, this research aimed to evaluate, through the physicochemical characterization, composition and lignocellulosic the potential of the residues of sweet cassava and bacuri in the production of coconut bioaroma (6PP), by solid state fermentation. The process was preceded by the characterization of the residue (nutritional, physical and chemical) followed by production, extraction (by solid phase microextraction - SPME) and quantification by gas chromatography (GC). Other strategies were necessary to acquire higher concentrations of 6PP in the residues, with emphasis on the accumulation of additional sources of carbon (glucose and sucrose), nutrients and even the mixture between the residues.

2. MATERIALS AND METHODS

2.1 Waste production and pre-treatment

The peels sweet cassava (SC) and bacuri bark (BA) were found in countryside cities in the State of Maranhão. In the case of bacuri, they were acquired in artisanal pulp production houses and in the commerce of the city of Viana – MA, in the period from December 2018 to January 2019. The peels of sweet cassava come from the flour houses located in several regions in São João Batista – MA. The harvesting was made in November 2018.

About 10 kg of the waste was stored in styrofoam boxes and transported to the city of São Luís-MA, to be frozen at -18°C in a freezer and then transported again (for 3:30h) to the Industrial Microbiology Laboratory - Block E - of the Technology Center - CT- of the Federal University of Rio de Janeiro - RJ, where the pre-treatment procedures, characterizations and fermentation processes began. The residues were defrosted, cut into smaller pieces, and placed in an oven for drying at 60°C for 48 hours, then crushed in a domestic blender and stored in plastic containers.

2.2 Physicochemical characterizations of matrices

The characterization of the samples consisted of the evaluation of pH, humidity, granulometry, composition analysis: centesimal, lignocellulosic, metallic, and volatile nutrients (extraction by headspace and dichloromethane). In addition, the amount of reducing sugars and

antimicrobial activity were identified. Associated with these evaluations, the superficial evaluation of the materials by scanning electron microscopy (SEM) was also performed.

The pH was determined by the official method [28]. The determination of lipids was possible by the [29]. With the aid of the Kjeldahl method [30] the percentage of proteins of the samples was obtained. The acquisition of ashes followed the [30] and the total carbohydrates were acquired by difference in relation to the previous ones. Humidity was determined by water loss during drying of the sample in an oven [31]. The effective moisture content of the solid medium was acquired using the moisture balance (model Shimadzu MOC63V), weighing 1g of the residue and determining the humidity for the 4 samples in the two stages: initial (dry) and post-inoculation.

The composition of lignocellulosic materials was defined by the method proposed by [32], 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 neutral detergent, obtaining the neutral detergent fiber fraction (NDF), composed of cellulose, hemicellulose and lignin. This fraction was subjected to digestion with acid detergent (ADF), where cellulose and hemicellulose are 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 is obtained. Hemicellulose was obtained by the difference between NDF and ADF [33]. 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 sugar followed the protocol described by [34]. Initially consisting of hydrolysis, where 1g of residue was placed in contact with 5mL of sodium acetate 50mM, pH 4. After filtration, the supernatant was centrifuged to separate the solids. Then, 0.1mL of the supernatant + 0.3mL of DNS (dinitrosalicylic acid) was added in an *eppendorf*, boiling for 5 min. The solution plus 1mL of distilled water was added for later reading in a spectrophotometer at 540nm. In this step, a white was produced with 0.1mL H₂O + 0.3mL of DNS. Quantification was performed with the aid of a standard curve ($y = 0.3694x + 0.0466$ $R^2 = 0.9987$).

To define the metal content, 0.5 g of sample was weighed in a Shimadzu analytical balance with precision of the tenth of mg (0.0001 g). The samples were digested in an acid/oxidant mixture, using the reagents nitric acid (HNO₃) and hydrogen peroxide (H₂O₂). Digestion was performed and the samples were increased to 25 mL. Then, centrifuged and filtered, collected in vials for analysis of 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 - LAM/IQ/UFRJ.

The identification of the volatile composition of the residues was performed in two ways, by extraction directly in the headspace and by the evaluation of the extract acquired by dichloromethane. For the extraction of the headspace, the residue samples were macerated, weighed 0.5g of each residue and placed in 10mL vats for subsequent analysis of the volatiles in a Gas Chromatograph (CG-2010) coupled to the Mass Spectrometer (CG-EM QP2010 Plus), both from Shimadzu, using a DB-5MS capillary column (30m x 0.25mm x 0.25 μ m). Carrier gas flow, Helium, at a linear velocity of 30 cm/s and column flow of 1.0 mL/min. The furnace temperature was 35°C for 3 min with a heating ramp of 10°C/min at 240°C, remaining for 5 min. The temperature of the injector and the ion source is 250°C and 200°C respectively. Split injection mode with 1:30 aspect ratio. Duration 28.50 min. MS Data: Acquisition mode: Full scan – m/z from 35 to 500. Identification was performed by normalizing peak areas and identification using the NIST (National Institute of Standards and Technology) Equipment Library (AOC-5000).

The liquid-liquid extraction with dichloromethane was initiated with the addition of 10mL of 95% ethanol to the vials containing 2g of residue, which were subsequently shaken for 30min at 30°C. The material was centrifuged for 15 min and the supernatant was collected in a separation funnel. Then, 10mL of dichloromethane ($\geq 99\%$) were added to proceed to the liquid-liquid extraction, from which the fraction of interest was obtained. It was necessary to dehydrate this extraction by adding anhydrous Na₂SO₄ PA.

After this step, the solution was filtered with paper and then the filtrate was collected in vats of 2mL in duplicate and quantified by gas chromatography model: CGMS-QP2010 Plus - SHIMADZU, DB5 MS. Thermal desorption in the 1:100 split injector occurred for a period of 36 minutes on all occasions. The initial temperature of the column was 40°C for 2 minutes, then it varied: 20°C/min up to 120°C contained for 2 minutes and then 10°C/minute up to 210°C. Injector and carrier gas detector 250°C 1.2mL/min.

The identifications were performed by the normalization of the areas of the peaks and identification through the Equipment Library of NIST (National Institute of Standards and Technology). Automatic injector (AOC-5000). It is worth remembering that this same methodology was used to determine the chemical composition of the resin contained in the bark of the bacuri, previously obtained by boiling 50g of bark in 150mL of distilled water for 20min, at a temperature of 90°C.

Through the analysis of Scanning Electron Microscopy (SEM) the surface of the residues was characterized, observing mainly the morphology and porosity. The equipment used was the *TM3030 plus* from *Hitachi* located in the Surface Analysis Laboratory of CETEM (Mineral Technology Center) – UFRJ.

To evaluate the antimicrobial activity of bacuri and sweet cassava residues, an ethanoic extract (50mL) was produced with 1g of the residue. The extract was filtered and stored. Microbial strains of *American Type Culture Collection* (ATCC) origin were used to investigate the antimicrobial activity: *Proteus mirabilis* ATCC7002; *Staphylococcus aureus* ATCC25923; *Pseudomonas aeruginosa* ATCC27853; *Salmonellas* ATCC14028; *Staphylococcus saprophyticus* ATCC19701; *Escherichia coli* 25922; *Trichoderma harzianum* 4042, previously grown in nutrient agar culture medium.

The antimicrobial analysis of the residues was developed with the aid of the agar drilling technique and was carried out at the Food Microbiology Laboratory of the Department of Pharmacy (DEFAR) of the Federal University of Maranhão. After microbial suspension in 0.9% saline solution following the McFarland scale at 0.5 as a clouding pattern, with the aid of a swab they were inoculated in the Mueller-Hinton agar plate (Difco) in three directions: horizontal, vertical and diagonal, to avoid the growth of isolated colonies forming a microbial mat.

To standardize the diameter of the perforation, the sources were drilled with cylinders of 6mm equidistant diameter with a sterile tip; in the wells were added 50µL of the extracts, sufficient for their filling. The Petri dishes were kept at room temperature for 2h for pre-diffusion of the substances, and then incubated at 37°C for 24h for the bacteria and 48h at 34°C for the fungi.

Subsequently, the inspection was performed in relation to the growth and inhibition regions. The sizes of the inhibition halos were measured in millimeters using a standardized ruler. At the time, positive and negative control was defined. Chloramphenicol and Nystatin were used as positive control for bacteria and fungi, and distilled water was used as injection for the negative control. The entire procedure was performed in triplicate.

On the possibility that the resin present in the bacuri residue is a potent inhibitor, the antimicrobial analysis was also carried out. Resin was collected by boiling 50g of bacuri 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 the aid of a spatula and transferred to a glass flask, which was covered with aluminum foil and kept in a refrigerator.

2.3 Solid State Fermentation Systems

Microorganism - The strain of *Trichoderma harzianum* (IOC 4042) used in the inoculation process was acquired at the Department of Mycology of the Oswaldo Cruz Institute (FIOCRUZ). Its maintenance was performed with PDA medium (*potato dextrose agar*) composed of agar-agar (2% m/v); potato extract (20% m/v); glucose (2% m/v), sterilized at 120°C for 20min. The culture was

incubated for 7 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.

Conduction of solid state fermentations Conducting fermentations in solid state - Fermentations were carried out using hydration solutions to maintain humidity values between 75 and 80%, and one of four distinct solutions can be added to the solid medium: a) distilled water; b) nutrient solution; c) nutrient solution with glucose; d) nutrient solution with sucrose. All material was autoclaved at 1atm pressure for 20min. The nutrient solution presented the following components per gram of residue NaNO_3 (0.0024g); $(\text{NH}_4)_2\text{SO}_4$ (0.0018g) e (KH_2PO_4) 0.001g. Solutions: glucose or sucrose at a concentration of 0.03g/g of residue.

In 20mL vials (vails) 1g of residue was added as follows: 1g of residue from BA or SC, when the FES was conducted only by a single residue, but when conducted through the mixture (BASC), 0.5g of each residue was used. Then added to the residue 2.0mL of each nutrient solution or distilled were autoclaved at 1 atm for 20 minutes and were inoculated with (0.5mL) of 6.25×10^4 spores per gram of residue. Then, they were incubated at a temperature of 30°C for 9 days in a bacteriological oven. Fermentation control was also performed using distilled water as a moisturizing solution without inoculum.

Extraction and quantification of aroma compounds – In the extraction step, 5mL of 25% saline solution (m/v) was added to the 20mL 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 the addition of 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 20mm PTFE silicone septum and a 10mm hole. Then, the sample was heated in a water bath, under agitation and kept at a temperature of 79°C, according to [6], with adaptations. The fiber used in SPME, once exposed, is in contact with the gas phase in the *headspace*, remaining for an adequate period for the adsorption of the compound of interest. A balance time of 2 minutes was required before the fiber was exposed, which was subsequently exposed for 30 minutes. At the end of the extraction time, the plunger was removed, and the fiber was directed to the CG to be exposed again for 4 minutes for thermal desorption, according to the procedure of [35]

To quantify the aromatic compounds obtained in solid state fermentations, the model equipment used was: CGMS-QP2010 Plus – SHIMADZU. The chromatographic column used was the RESTEK RTX -5, length: 30m, 0.25mm internal diameter and 0.25 μm . The initial temperature of

the column was 100°C for 4 minutes, then it varied: 10°C/min to 150°C maintained for 4 minutes and then 20°C/minute until 200°C. And 10°C to 230°C also for 4min. Injector and detector 260. °C carrier gas (N₂) 1.2mL/min, totaling 25min. each sample. The concentrations of 6PP were determined from the equations of lines obtained through the calibration curves.

From the stock solution 2,000 ppm of the standard 6pentyl- α -pyrone (purity <96% Sigma-Aldrich) dilutions of 5 were performed; 10; 50; 100 and 200 ppm and 1mL of each solution was added in 1g of bacuri residues (BA), then in 1g of sweet cassava (SC) and also in the mixture of bacuri and cassava (BA+SC) in the ratio 1:1, thus obtaining the calibration curves for SC and BASC were $y = 109014x - 148828$ ($R^2 = 0.9715$) and $y = 50615x - 29538$ ($R^2 = 0.987$), respectively. The points were analyzed in triplicate. An important to emphasize that is the curved relation of bacuri, which was not constructed because it is a residue that presented inhibition in fungal growth and, therefore, there was no production of aroma.

3. RESULTS

3.1 Characterization of matrices

The shell of sweet cassava, obtained during the processing of flour, starch or other products, is composed of two regions: the outer with 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, as can be seen in Figure 1. For this study, the shell composed of the two parts (internal and external) was used.

The bark of the bacuri, 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 2, depending on the species), surrounded by white pulp, which in turn, is widely used in the production of sweets, ice cream, etc.

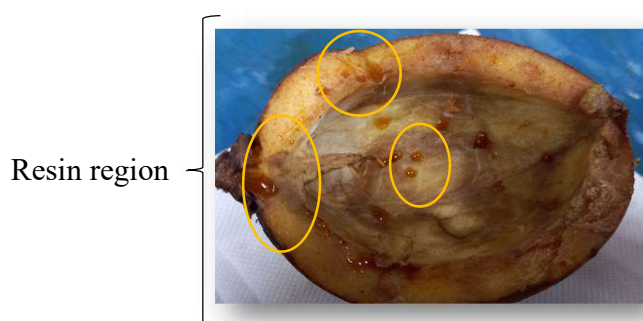
The visual characteristics can also be seen in Figure 1 b. Another observed point detected in this bark was the presence of resin, which is a very important component for several vegetables, as they act in the protection of the plant. In the case of the bacuri fruit, the resin (Figure 2) helps in the resistance to strong impacts, which justifies the conservation of the fruit when falling from great heights[26].

Fig.1: General characteristics of the bark (a) sweet cassava and (b) bacuri



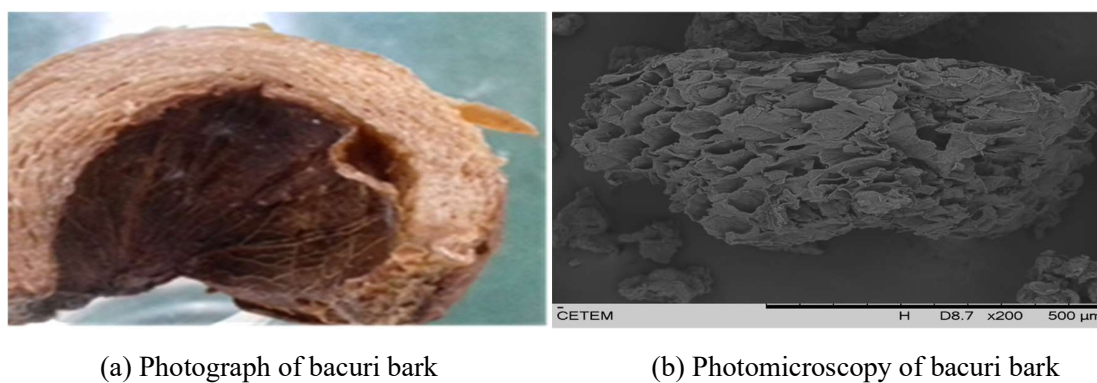
The shell is saturated with resin, and any crack in the shell region, the resin will spill. Despite the benefits offered by the resin for the plant, it also presents itself as an obstacle in the use of the bark of the bacuri contributing to the high disposal of these materials in the environment.

Fig. 2: Predominance of resin in bacuri bark



Regarding morphology, the dry bark has a spongy aspect, with the presence of pores, as observed in Figure 3a.

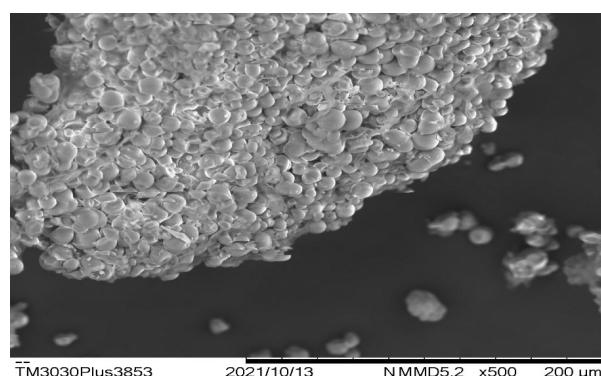
Fig. 3: Images of bacuri bark (a) by photography and (b) photomicroscopy by SEM



The SEM image showed that the entire bark of the bacuri is composed of vessels (Figure 3b), which serve for transport and storage of substances, including resin [19].

For the shells of the SC, the SEM (Figure 4) revealed agglomerated formations with rounded granules, characteristic of starch grains, with white coloration. The same characteristics of the cassava granules observed were also described by [36] who stated the presence of starch granules in different botanical sources, including cassava species.

Fig. 4: SEM Images of the granules found in the bark of the sweet cassava.



Other characteristics referring to the shells of SC and BA can be verified in tables 1- 6. Table 1 shows the data of the analysis of pH, humidity and centesimal composition. The BA presented a pH of 3.13 ± 4.01 , lower than those of the SC, which was 4.78 ± 0.76 . The moisture content of the residues was $6.0 \pm$ and 8.3 ± 0.06 for the SC and BA, respectively. The highest mineral content was BA with 3.5 ± 0.077 and 2.8 ± 0.07 for SC

Table 1: Physicochemical composition of the residues

WASTE	pH	MT	MC	CART	EE	CP	ADF	NDF
		%						
BA	3.13 ± 4.01	6.0 ± 0.006	3.5 ± 0.07	86.36 ± 0.12	10.1 ± 0.34	4.4 ± 0.18	26.25 ± 0.39	44.2 ± 1.52
SC	4.78 ± 0.76	8.3 ± 0.06	2.8 ± 0.07	91.9 ± 0.21	1.1 ± 0.03	2.7 ± 0.006	8.65 ± 1.05	8.65 ± 2.59

Caption: pH –hydrogen potential. CART- Total carbohydrates. MT – Moisture. MC -Mineral Content. EE - Ethereal extract. CP - Crude protein. ADF- Acid digestive fiber; NDF – neutral digestive fiber. BA – bacuri; SC – sweet cassava

Both residues presented high CART contents, with values above $85\pm\%$, but only the SC exceeded $90\pm\%$. Regarding the lipid content, the difference in percentages is very evident for the bark of the BA, whose content was $10.1\pm0.34\%$, while the SC was only $1.1\pm0.03\%$. The highest protein and fiber content were also found in the BA residue.

The lignocellulosic content is presented in Table 2. The highest cellulose content was found in BA bark, $23.65\pm0.3\%$. The highest values of hemicellulose, lignin and starch are present in the bark of the SC. The amount of reducing sugar was also more expressive in SC, with a value 8 times higher for each 100g of residue, compared to that of the bacuri bark.

Table 2: Lignocellulosic composition of residues

WASTE	CEL	HEM	LIG	ST	TRS	
	%				TRS/g Waste (p/p)	TRS/100g Waste
BA	23.65 ± 0.3	17.9 ± 0.21	1.8 ± 0.02	11.14 ± 0.01	0.03 ± 0.001	3.49 ± 0.17
SC	5.1 ± 0.4	24.5 ± 1.5	2.2 ± 0.81	49.5 ± 0.3	0.1639 ± 0.0012	16.39 ± 0.12

Caption: CEL-cellulose. HEM-hemicelluloses. LIG-lignin. ST-starch. TRS: Total Reducing Sugars.

BA – bacuri; SC – sweet cassava

The presence of nutrients is essential for the development of microorganisms. Some metallic nutrients were evaluated in this research and are available in Table 3.

Table 3: Composition of metallic nutrients

WASTE	METALS (%) p/p				
	Na	K	Mg	Ca	Zn
BA	1.1 ± 0.6	7.3 ± 0.13	0.47 ± 0.006	1.2 ± 0.2	0.013 ± 0.002
SC	0.16 ± 0.006	5.00 ± 0.138	0.7 ± 0.02	1.4 ± 0.013	0.011 ± 0.002

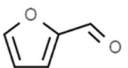
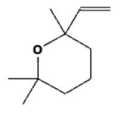
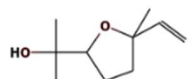
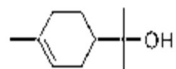
Caption: Na – Sodium. K – Potassium. Mg – Magnesium. Ca – Calcium. Zn – Zinc. BA – bacuri; SC – sweet cassava

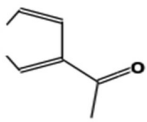
The bark of BA, in comparison to those of SC, has higher content of K (7.3 ± 0.13), Na ($1.1 \pm 0.6\%$) and Zn ($0.013 \pm 0.002\%$). Mg was not detected in the SC shells and was therefore present only in the BA peels. Only the Ca value was higher in relation to the other metals for SC.

Another parameter evaluated was the characterization of the volatile composition of the shells. The process was carried out in two ways: with direct analysis of volatile compounds in the headspace system and with liquid extraction using dichloromethane as solvent. The results are shown in Tables 4 and 5. The purpose of these analyses was to prove the absence of 6PP in the volatile constituents of the residues and to identify compounds with bioactive characteristics.

In the analysis of volatiles by *headspace* (Table 4), it was observed in the bark of bacuri, the presence of furfural, a toxic furan aldehyde, considered immediately dangerous (IDLH) for life or health with a concentration in the air of 100.00ppm [37]. The presence of furfural and derivatives add greater value to the bark of BA[38].

Table 4: List the components detected by the *headspace*, as well as the general characteristics of the compounds.

COMPOUNDS	MOLECULAR STRUCTURE	CHARACTERISTICS	RESIDUES
Furfural		Molecular formula: $C_5H_4O_2$ Molecular Weight: 96g/mol Boiling temperature: 161-162°C	BA
2H-Pyran,2-ethenyl tetrahydro-2,6,6-trimethyl		Molecular formula: $C_{10}H_{18}O_2$ Molecular weight: 154.2493 g/mol Boiling temperature: 223-224 °C	BA
2-furanmethanol,5-ethenyltetrahydro-alpha, alpha,5-trimethyl-cis-		Molecular formula: $C_{10}H_{18}O_2$ Molecular weight: 170.2487 g/mol Boiling temperature: 188°C Colorless to slightly yellow liquid; Powerful sweet-woody penetrating aroma with floral nuances.	BA
3-Cyclohexene-1-methanol, α,α -4-trimethyl-		Molecular formula: $C_{10}H_{18}O$ Molecular weight: 154.249g/mol Boiling temperature: 212 °C	BA

		Warm-peppery, slightly earthy and woody odor	
Ethanone,1-(3-ethyloxiranyl)		Molecular formula: C ₆ H ₈ O Molecular weight: 126,176 g/mol Boiling temperature: 190.5°C	SC

Source: NIST- National Institute of Standards and Technology - NIST. [39] BA – bacuri; SC – sweet cassava

In the shells of SC was found, as a volatile compound, only Ethanone, 1-(3-ethyloxiranyl). Other compounds were identified from the extracts obtained with dichloromethane and analyzed by gas chromatography coupled to a detector the masses. The compounds are available in Table 5 below.

Table 5: Compounds present in the residues, detected by GC/MS in analysis after extraction with dichloromethane

WASTE	COMPOUNDS
BA	3- furancetic acid; 4-hexyl – 2,5- dihydro-2,5-dioxo; Tridecanoic ethyl ester; bicyclo [3,3,1] nonan-9-one; 1,5,9 undecatriene; 2,6,10 dodecatrien-1-ol (farnesol); 5-hepten-3yn-2-ol, 6 metyl-5 (1-methylethyl); [3,3 –bi-1H, 1,2,4 – triazole] – 5,5dianamine, acid tetradecanoic (myristic); decanoic acid (capric acid); oleic acid (ácido oléico)
SC	Octane/ n-Octane/ 4-Methyl-8-propyl-1,5-diazabicyc/2-Pentene, 4-methyl-, (E)- p-Xylene/ Hexane, 3-ethyl

Caption: BA – bacuri; SC – sweet cassava

In the bacuri extracts, it was possible to verify the presence of different fatty acids, such as: tetradecanoic or myristic acid; Decanoic acid or capric acid and oleic acid. The presence of fatty acids in the shells of sweet cassava was not observed. Despite the existence of n-octane, being considered a toxic compound, according to [37], [40], the profile found did not cause toxic effect for the fungi *T. harzianum*, because the spores germinated well in SC.

Within this perspective, the evaluation of antimicrobial activity was also necessary. The extract of the bark of BA showed higher antimicrobial activities against the bacteria as observed in Table 6. The potential inhibition effect of BA bark extract was against *Proteus mirabilis* ATCC7002, *Salmonellas* ATCC14028, *Staphylococcus aureus* ATCC25923, *Staphylococcus saprophyticus* ATCC19701 and *Escherichia coli* 25922 with inhibition haloes of 10, 10, 15, 15 and 14 mm,

respectively. The isolated resin extract (pure) showed activities much superior to those of the BA bark, with haloes of 15, 20 and even 28mm, where the greatest inhibition was demonstrated for *Proteus mirabilis* ATCC7002, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Salmonellas* ATCC14028.

Table 6: Determination of antimicrobial activity of bacuri bark (BA) and sweet cassava (SC)

ANTIMICROBIAL ACTIVITY					
MICROORGANISMS	SIZE OF INHIBITION HALOES (mm)				
	SC	BA	RESIN	C+	C-
<i>Proteus mirabilis</i> ATCC7002	9	10	28	27	0
<i>Staphylococcus aureus</i> ATCC25923	-	15	15	40	0
<i>Pseudomonas aeruginosa</i> ATCC27853	-	7	20	15	0
<i>Salmonellas</i> ATCC14028	9	10	15	43	0
<i>Staphylococcus Saprophyticus</i> ATCC19701	10	15	8	35	0
<i>Escherichia coli</i> 25922	10	14	7	45	0
<i>Trichoderma harzianum</i> 4042	-	10	14	-	0

Caption: RESIN - plant material secreted by bacuri bark. BA – bacuri; SC – sweet cassava

For *Trichoderma harzianum* 4042 fungi, the extracts showed interesting antimicrobial activity, the resin and the BA extract. However, the largest inhibition haloes were acquired in the presence of the resin for the microorganisms. It showed no antimicrobial activity for *Trichoderma harzianum* 4042. The bacuri resin showed inhibition haloes of 14mm for *T. harzianum* 4042, revealing to have an important antimicrobial activity on the fungus. Regarding the extract of the cassava bark, this presented many inferiors haloes to those provided by the extract of the bacuri and the resin. The largest inhibition haloes were for *Staphylococcus saprophytic* ATCC19701 and *Escherichia coli* 25922, with 10mm each.

As a positive control, fluconazole and nystantin were used, but it was not effective for *T. harzianum*, that is, there was no positive effect for the control. Another product was used for this purpose, amphotericin B, but without success. Therefore, another step with pesticides is suggested, as it is an environmental fungus.

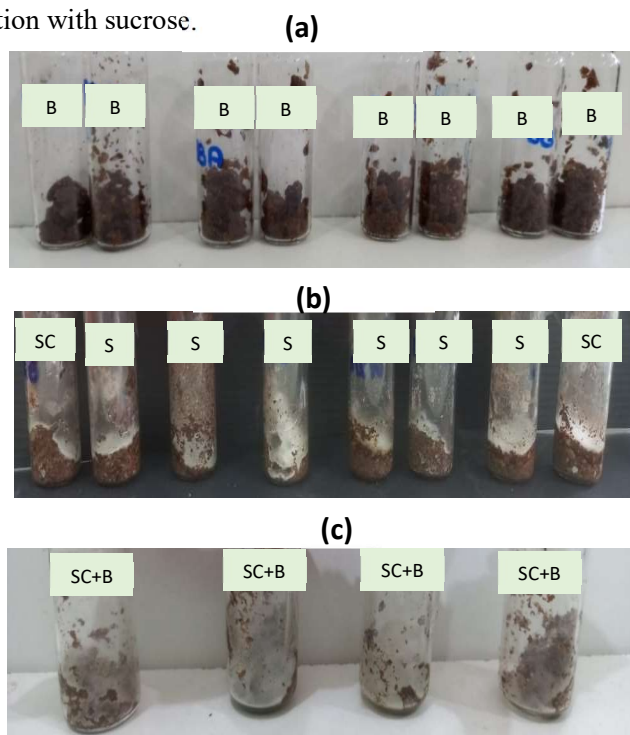
3.2 Solid State Fermentation

The solid-state fermentation step conducted in the presence of distilled water, nutrient solution, nutrient solution with glucose and nutrient solution with sucrose allowed us to understand the behavior of the fungus in relation to the exploitation of the nutrients contained in the residues of bacuri, sweet cassava, also the mixture between them (ratio 1:1). The illustrative fermentations can be seen in Figure 5 (a, b and c).

Figure 5a shows the fermentation using the BA residue in all humidification conditions (water, nutritive with sucrose, nutritive with glucose and nutritive) where total inhibition of germination of spores of the fungus *Trichoderma harzianum* was observed in all four conditions.

Fungal growth was already quite intense in the fermentation with SC. Also, intense growth was verified in Figure 5c, where the fermentation was conducted by mixing the residues (BA and SC). This conduct allowed us to understand that the strategy of mixing the matrices was favorable for the development of the spores and for the increase of the production of 6PP.

Fig. 5: Illustrative images of the fermentations, with residues (a) of BA, (b) SC and (c) associated condition BA and SC (1:1), conducted with water, nutrient solution, nutrient solution with glucose and nutrient solution with sucrose.

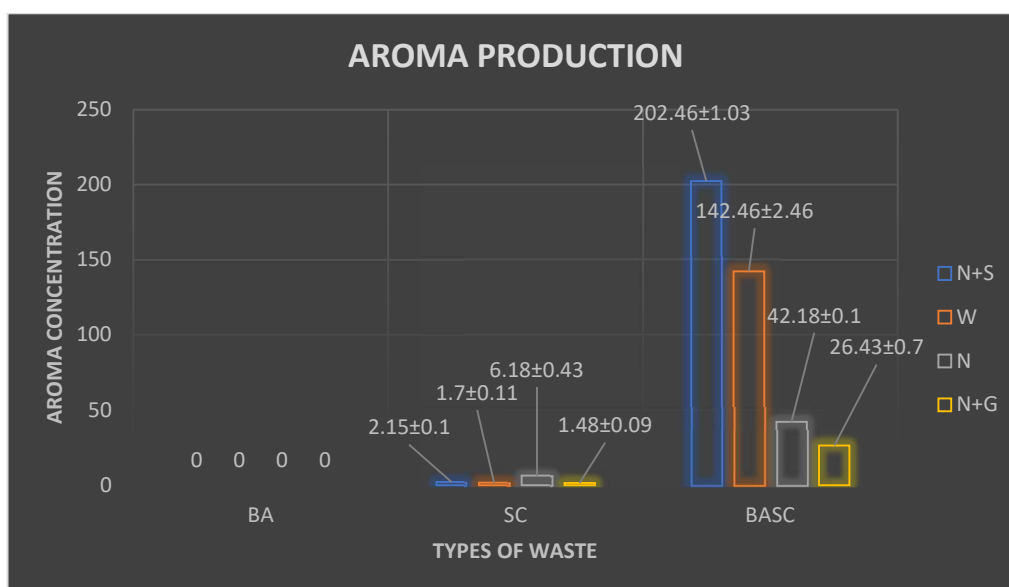


Caption: G: nutrient solution with glucose; S: nutrient solution with sucrose; N: nutrient solution; W: water. Caption: BA – bacuri; SC– sweet cassava

The results of fungal growth and quantification of the coconut aroma produced in each medium can be seen in Graph 1. As observed in the figure above, no aroma production was observed from the fermentation performed with BA residue, probably due to the inhibition caused by compounds existing in the residue. While in the fermentation with sweet cassava there was a discreet production of aroma, but the amounts did not exceed 7 ppm. Differently, from the two fermentations mentioned above, the fermentation with the mixture of SC and BA residues promoted a very expressive increase in the production of coconut bioaroma, however the SSF in the presence of water and sucrose were the ones that provided the highest concentrations, 142.46±2.46 and 202.46± 1.03 ppm, respectively.

The characteristic peaks can be seen in figures 6 (a, b and c). Figure 6a shows the peak of 6PP acquired through the standard solution of 6PP, at a concentration of 200 ppm, revealing the retention time of 16.6 and peak intensity.

Graph 1: Graph of the quantification, in (ppm), of the production of coconut aroma



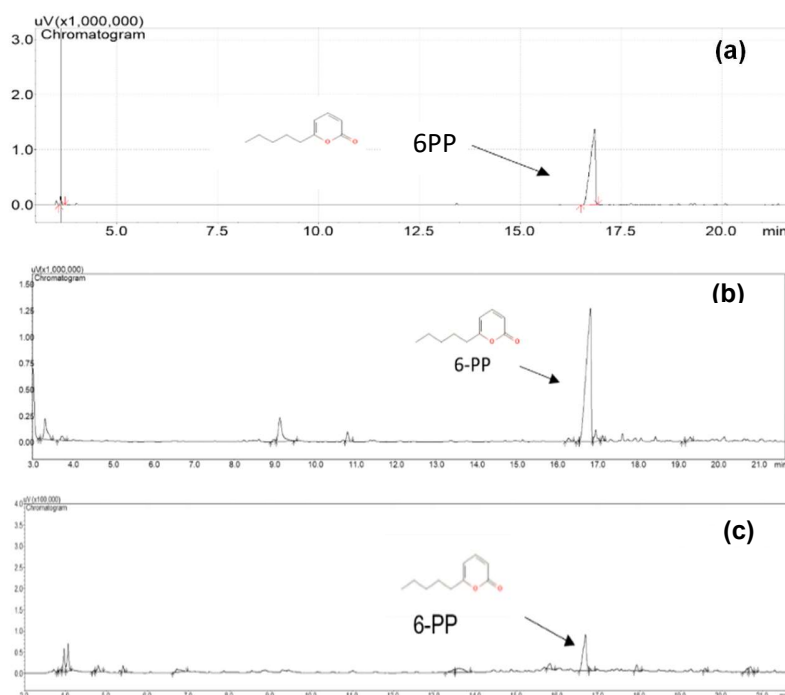
Caption: BA – bacuri; SC– sweet cassava; BA+SC – mixture of bacuri and sweet cassava residues. W – water.

Figures 6b and 6c show characteristic peaks of 6PP obtained by SSF, obtained through the fermentations of the mixture (SC+BA) and the sweet cassava, confirming the production of the aroma by the microorganisms in these matrices.

The assimilation of the sample as a carbon source was visually perceived through scanning electron microscopy images, which in addition to allowing the characterization of the surface of the residue, it was possible to observe the development of the fungus with the end of fermentation. Figure

7 (a and b) and 8 (a and b) shows the SEM images of the cassava residue and the BASC mixture. The 7th shows the residues of the unfermented SC, with the presence of white granules and agglomerates.

Fig. 6: Illustrative chromatogram of the 6PP peak, obtained: (a) mixture BA + SC + standard 200 ppm ; (b) BA+SC fermentation + nutrient solution with sucrose and (c) SC fermentation + nutrient solution with sucrose.



In 7b, the fermented residue is observed, with intense growth of fungal hyphae. The sweet cassava favored the germination of spores and the intense growth during the SSF.

Fig. 7: Comparison between the non-fermented and fermented phases of the SC residue

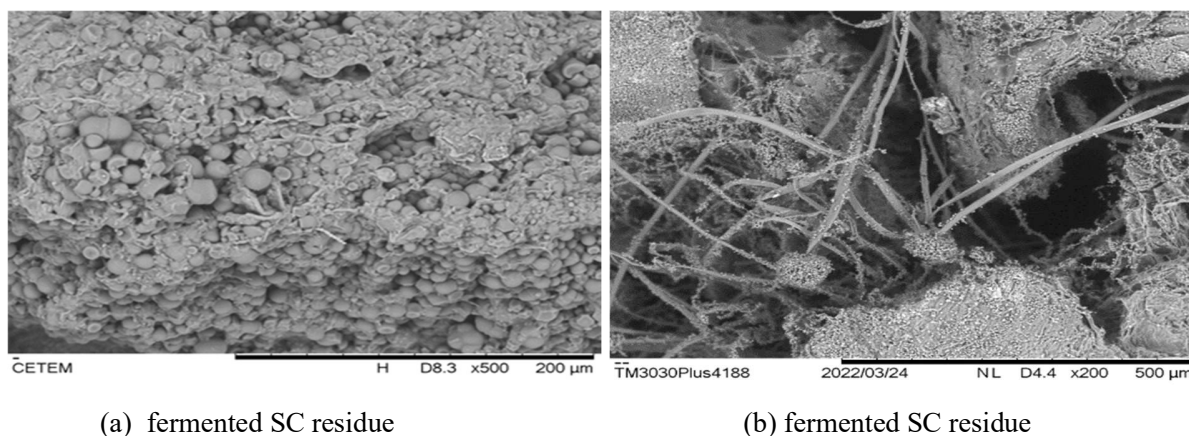
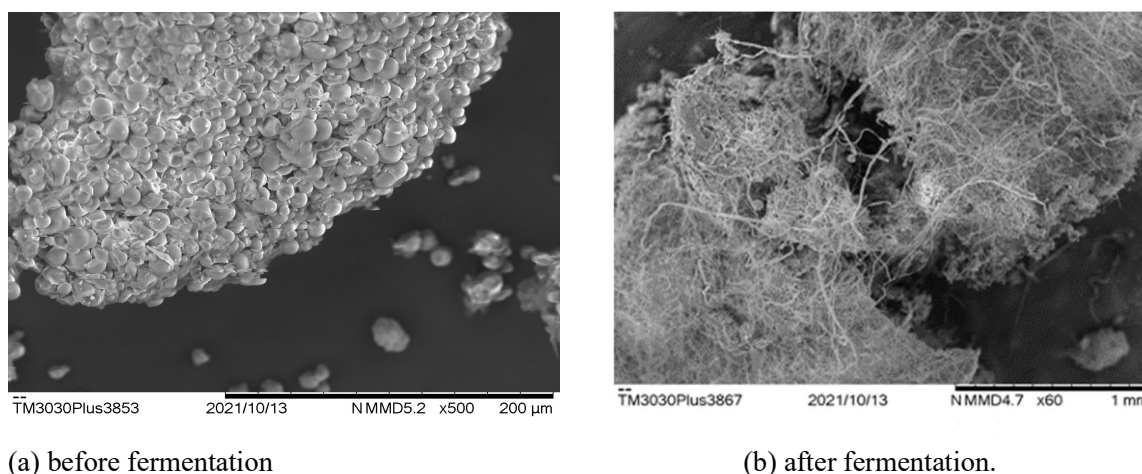


Figure 8 shows the mixture of residues before and after fermentation. Figure 8a shows the packaging of the cavities of the bacuri bark with the mixture of the granules from the cassava bark before fermentation. This packaging was facilitated by the difference in particle size and by the morphology of the residues used in this research.

Fig. 8: Comparison between the non-fermented and fermented phases of the BA+ SC residue



After fermentation, the presence of interlaced hyphae in the residues was verified, probably due to the favoring of the solid medium in the fungal development and due to the ample consumption of the nutrients present in the SC+BA mixture. Unlike what happened in fermentation, only with BA such development was not possible.

4. DISCUSSION

4.1 General Aspects of Residues

In this research the residues of bacuri and sweet cassava presented acidic characteristics. This characteristic was not an obstacle to fungal growth in SSF using fungi of the genus *Trichoderma harzianum*. According to [41] fungi of the genus have optimal growth in acidified medium, having, therefore, the ability to grow in all pH ranges. Another factor evaluated was the granulometry. Humidity is a factor that needs to be adjusted in SSF.

The initial humidity of the residues of the SC and BA were, respectively, $8.3\pm\%$ and $6.0\pm\%$ and the adjustment was based on the maximum capacity of water retention, aided by the moisture test, thus obtaining adequate final humidity. The fractions used in this research were based on the maximum water absorption capacity, defined as 212 mm for the SC bark and 450 mm for the BA

bark, with the addition of 2.5mL of water raising the final humidity to $79.91 \pm 0.06\%$ for cassava and $78.36 \pm 0.006\%$ for bacuri, thus trying to guarantee moisture until the end of fermentation, as observed by Penha et al. (2012) slight decrease in humidity in SSF conducted under the same conditions as this work. These values are, therefore, in line with what is cited by the studies by [42] and Clique ou toque aqui para inserir o texto. who assign SSF values around 70% and 74%, respectively. Regarding the centesimal composition (Table 1), it was observed that the shells of the SC exhibited a higher percentage of carbohydrates ($91.9 \pm 0.21\%$), in addition to free sugars and proteins. The other parameters such as ether extract, fibers (ADF and NDF) and mineral content were lower than those found in the BA residues.

The bark of the bacuri showed a lipid content of $10.17 \pm 0.34\%$. Values were much higher than those found by [27] with 3.84%, probably because they are different species. The total carbohydrate content was $83.787 \pm 0.12\%$; $3.5 \pm 0.07\%$ mineral content and $4.4 \pm 0.18\%$ crude protein. [44] also performed the composition analysis of the bacuri bark, obtaining lipid content equal to 4.12%; crude protein 2.16%; ash 2.17%; crude fiber 23.07%, on average. Based on the centesimal compositions of each residue, large contributions of carbohydrates, fibers and other components were observed, since carbohydrates are considered the main food sources, producers of energy. The conversion of these nutrients by microorganisms during SSF makes it possible to obtain commercial products or even raw materials for other processes.

Table 2 showed the lignocellulosic composition. Several types of fungi have excellent ability to adapt and colonize different ecological niches due to the great synthesis of compounds that allow them to increase their fitness. Those of the genus *Trichoderma*, for example, have an extremely important characteristic, which is their ability to degrade lignocellulosic materials (Andlar et al., 2018). With the release of extracellular enzymes, and subsequent enzymatic action, the fungi begin to feed, through their hyphae, absorbing the bioavailable nutrients. The lignocellulosic contributions of the evaluated residues are shown in Table 2.

The results showed that the bacuri residues presented low lignin content ($1.8 \pm 0.02\%$) and much higher values of cellulose ($23.65 \pm 0.34\%$) and hemicellulose ($17.9 \pm 0.21\%$) than those of sweet cassava, $13.8 \pm 0.17\%$ and $7.2 \pm 0.23\%$, respectively.

Species of the type *Trichoderma harzianum*, for example, in addition to acting as biopesticides and biofertilizers [46] are excretory of enzymes such as chitinases, glucanases and proteases [47]; [13] The performance of these microorganisms as decomposers is made efficient by the presence of cellulases. Cellulases produced by *T. harzianum* are considered as efficient systems for the complete hydrolysis of cellulosic substrates into glucose [48]

According to [49] the dehydrated cassava bark has 58.1% starch, 28.6% neutral detergent fiber and only 3.4% crude protein. In this research the percentages of starch obtained were 49.5 ± 0.17 for the peels of sweet cassava. Regarding the bark of the bacuri, only $11.4 \pm 0.1\%$ were found.

The amount of total reducing sugars in the BA residues was $2.97 \pm 0.2\%$ and for SC, 16.39 ± 0.12 ART/100g of residue. Thus, it can be understood that the greatest contribution of ART from starch comes from cassava that, by the action of hydrolases, are able to cleave the O-glycosidic bonds of polymers contained in cellulosic materials, releasing sugars, such as glucose, for example [50]. However, after high sources of carbon, essential minerals are unavailable in microbial nutrition, as they aid in the bioconversion of other nutrients.

Minerals are another group of nutrients present in plants performing various functions. [12] state that plants need to absorb macro and micronutrients, Na, P, K, Mg, Ca, etc. and, Fe, Mn, B, Zn, Mo, Cu, respectively, available in the soil. With emphasis on Zn and Cu, necessary for the proper development of living organisms [51], [52]. According to the largest amount of nutrients, present in vegetables, is concentrated in the peels of the fruits and not in the pulp, so the peels can be considered sources of nutrients, which are most often discarded. The evaluation of the mineral contributions from the bark of bacuri and sweet cassava is shown in Table 3.

Among the samples evaluated, the bacuri peels presented the highest concentrations of sodium (Na). The residue of bacuri and sweet cassava has a high concentration of K. Mg was found only in the bacuri residue, with $0.47 \pm \%$. The values of Ca and Zn were very close in both residuals. Although some minerals (metallic or non-metallic) are essential for the metabolism of fungi (copper, iron, manganese, molybdenum, zinc and nickel [53], in excess they are considered toxic [54], [55]

The action of filamentous fungi through the exploitation of these minerals is satisfactory, since they are microorganisms with high ability to exploit the most diverse sources of energy by excreting a variety of extracellular enzymes and metabolites interacting with organic and inorganic substrates (metals and minerals) in natural and synthetic environments, modifying their physical and chemical properties [56]

In the case of filamentous fungi, they can solubilize insoluble metals and minerals through their exploratory lifestyle, in which the filamentous branching allows excreting organic acids, such as oxalic and citric acid, which not only reduce the pH, but also complex metals present increasing their solubility [56], [57]

The action of microorganisms allows an increase in bioavailable metal and also potential toxicity, while actions of others immobilize and reduce bioavailability [58], [59]. For this, there are mechanisms that allow fungi to deal, for example, with metals, among them stand out; extracellular metal sequestration and precipitation; metal binding to the cell walls of the fungus; intracellular

sequestration and complexation or volatilization[53], [58]. Fungal mycelium provides the main binding sites for toxic metals. These regions are composed of chitin and melanin, for example, [58], [60]. Similarly, the cell wall is also shown to be an excellent metal ligand [55], [61].

In the case of fungi of the genus *Trichoderma*, organic acids such as gluconic acid, fumaric acid and citric acid can reduce the pH of the medium, allowing the dissolution of phosphate, and even macro and micronutrients present in vegetables. With the acidification of the medium increases the mobility of metals, including zinc. These fungi can concentrate several ions such as Pb, Cd, Cu, Zn and Ni[62]. The use of agro-industrial residues is strongly influenced by the presence of minerals. The fungal strains of the genus *Trichoderma* can tolerate more than one type of metal [63], [64]

Volatile composition obtained by both forms (headspace and dichloromethane) proved the non-existence of the compound 6pentyl- α -pyrone in the matrices. The compound Ethanone, 1-(3-ethyloxiranyl) present in SC, according to [65] may have antimicrobial activity, but did not cause inhibition for the fungus *Trichoderma harzianum*.

4.2 Production of 6pentyl- α -pyrone

The production of 6PP using residues from BA and SC showed results with very different realities, especially in the germination phase of the spores, a very important question, because according to [66] the origin of this metabolite is in the spores [66], [67] states that spores are metabolically dormant, needing to be activated or induced by enzymes that must be synthesized before the fungus begins to use the substrate for its own growth and these fungal structures act as biocatalysts in bioconversion reactions, as well as its own mycelium.

Although the use of agro-industrial residues is an excellent alternative in the production of bio compounds it is not always possible to obtain satisfactory results, because they are very heterogeneous media, rich in compounds that perform different functions in plants, including defense, inhibiting microbial development. Thus, alternatives have been used to minimize the inhibitory effects. The supplementation of the solid medium with additional sources of carbon (glucose, sucrose, starch, fructose, among others) and heat treatments are some examples.

The SSF performed with the bark of bacuri (BA) to the production of coconut bioaroma was unsuccessful, due to the high rate of inhibitory compounds (including resin), preventing the germination of spores of the fungus *T. harzianum*, even in the presence of sugars such as glucose and sucrose. According to [68] the influence of nutritional composition to produce spores or bio compounds are hampered by the indefinite or semi-defined nature of solid media.

This inhibitory action from the bacuri residue was confirmed by the analysis of the antimicrobial activity performed from the extract of the fruit peel and from the resin, where this action was effective not only for this fungus, but for other microorganisms analyzed, as observed in Table 6. Another factor that may also have influenced the inhibition of the germination of spores in this medium was the existence of furfural (Table 4) and fatty acids, such as: tetradecanoic or myristic acid; decanoic or capric and oleic acid (Table 5). Authors state that fatty acids present in various vegetables have great antibacterial and antifungal potential [69], [70].

Contrary to what happened with the fermentation using the bark of the bacuri, the bark of the sweet cassava was a very favorable support both for the germination of the spores and to the production of the coconut bioaroma. This residue has a starchy characteristic, with a percentage around 50% of starch. Carbon sources such as glucose, sucrose, lactose, starch, among others, facilitate the germination of fungi, playing an important role in the induction of enzymatic secretion, in the affinity of transport and digestion of simple sugars [71]

In general, the germination and growth of the fungus *T. harzianum* in this medium was efficient in the four humidification conditions, including the presence of distilled water and nutrient solution, whose main source of carbon and energy was obtained from 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 [71]–[73]. Thus, with different humidification conditions, values of 6PP between 1.5 and 6.2 ppm were also achieved. The highest concentration of the metabolite was acquired in the hydration condition only with nutrient solution, that is, without the addition of carbon sources (glucose or sucrose).

In a correlation between the demand for carbon sources and the production of aroma, the idea was to obtain much higher concentrations of 6PP, considering the presence of minerals and the high concentration of fermentable sugars from starch (n-glucose), and from supplementation with glucose or sucrose at concentrations of 0.03g/mL per gram of residue.

[74] evaluated the role of sugars in the initial stage of germination of the filamentous fungus, *Aspergillus nidulans*, revealing the presence of receptors on the surface of the spores, which transmit signals to their inner part, with consequent activation of proteins responsible for germination, when the spores are exclusively in the presence of sugars such as glucose, sucrose, fruits, galactose and starch. According to the authors, the germination rate of the fungus evaluated in the presence of glucose was 98%, followed by sucrose (81%) and starch (70%), which obtained percentages perceiving a reduction in germination rates in the presence of starch and sucrose in relation to glucose.

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* [75], [76], although there is always a greater preference of microorganisms for simpler sugars.

In the solid medium where there is presence of glucose and starch or sucrose and starch, for example, microorganisms give needs to the hydrolyze more complex molecules, transforming them into monomeric ones, and then being absorbed. In the case of 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 (6PP) were observed in cassava tame. The availability of glucose in the medium suggested greater fungal growth.

[77] reinforce that when microbial growth is in the stationary phase it is that secondary metabolites (they are often bioactive and low molecular weight) are synthesized. They believe that the production of these metabolites is possible when there is 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 6PP was with the presence of glucose.

The existence of minerals was also a factor observed in relation to the natural production of coconut aroma. It was observed that, with the addition of the nutrient solution only, there was a significant increase in the productivity of the aroma (27.5%), in relation to fermentation conducted only with distilled water (1.7 ± 0.11 ppm). However, despite the high exploitation of the nutrients present in the sweet cassava residue by the fungi, the production was still considered low.

Due to the inhibition of fungus growth in the bacuri residue and the low aroma concentration under all humidification conditions in the sweet cassava residues, the mixture of residues was performed strategically, as observed in Figure 9.

The initial idea was to promote a favorable environment for the germination of the spores, thus increasing the enzymatic activity in the medium and, consequently, the exploitation of the carbon sources also existing in the bark of the bacuri, including lipids.

The mixture of cassava and bacuri residues in the same ratio (1:1) facilitated germination conditions for the spores, considering that starch acted as a potential inducer on the germination of *Trichoderma harzianum*. Another important point was the increase in the nutritional load of the solid medium, especially that of minerals, in addition, the embedding of cassava granules in the cavities of the bacuri bark (Figure 8a), promoted a favorable support, especially with respect to oxygenation and humidity above 75%.

In all fermentation conditions it was observed a very high growth of the fungus in the BA+SC mixture and a considerable increase in the production of 6PP. The concentrations obtained were: 202.46 ± 1.03 ppm; 142.46 ± 2.46 ppm; 42.18 ± 0.1 ppm and 26.43 ± 0.7 ppm with sucrose nutrient solution; water; nutrient solution and nutrient solution with glucose, respectively. A similar behavior was observed in relation to fermentation with glucose, with the lowest production rate, as well as in fermentation with SC.

On the contrary, the sweet cassava, in the fermentations containing nutrient solution only, the aroma production was less than 50 ppm. In this case, the excess of salts in the medium may have influenced the low production. It is important to remember that fungi cannot sporulate in the medium saturated with available nutrients and, the breakdown of dormancy requires the presence of water and glucose, mainly[12]

Supplementation may not always have a positive effect on a particular fermentation system. This can be proven when compared with SSF in the presence of distilled water alone. The absence of salts tripled the production of aroma. This increase may also be related to the excellent conversion of starch into simpler sugars and a less complex medium. However, the highest concentration obtained was in the presence of the nutrient solution with sucrose. In this case, sucrose left the system conducive to the development of spores, as well as starch, a monosaccharide that allows good assimilation by filamentous fungi [71], in addition to acting as excellent inducers of enzymatic activity with greater exploitation of nutrients, enabling a production of 202.46 ± 1.03 ppm, that is an increase of 94.16% in relation to the production of sweet cassava with sucrose.

The fermentation time was a favorable factor, considering that in previous tests, much lower values were achieved in 9 days. The adjusted moisture rate for 2.0mL of humidifying liquids and 0.5mL of spore solution also showed higher yields for the mixture. In addition, the fermentation carried out in the aroma extraction bottle itself provided quantities closer to reality. It is worth mentioning that the non-immediate targeting of glucose only for fungal growth may be interesting to the production of secondary metabolites, which is why the production of aroma was much more effective in the presence of sucrose and in the presence of nutrient solution than in the presence of glucose.

5. CONCLUSION

The extensive disposal of materials has wasted tons of nutrients that can be used in the acquisition of new bioproducts, especially through fermentation processes. In the case of BA (bacuri) and SC (Cassava) waste, the disposal volume is also considered high. They are rich in nutrients such

as K, Zn, Ca among others, BA holds 3.5% of these nutrients, while SC has 2.8%. In addition, BA exhibits the highest levels of lipids, fibers, and proteins. For SC, the highest content was hemicellulose and starch.

The presence of inhibitory compounds such as resin, fatty and furfural acids, identified by gas chromatography analyses coupled to the masses, may have had an inhibitory effect on the development of spores during BA FES. With SC residues, there was no such inhibition since the presence of its components had no inhibitory effect.

To the purpose of producing coconut bioaroma, the SC residue showed the best result in the presence of nutrient solution, followed by the nutrient solution with sucrose. Low concentrations were obtained in the presence of water and nutrient solution with glucose. The addition of nutritive salts in SSF with SC was a decisive factor for a considerable increase in the production of 6PP, in relation to the production in the presence of distilled water. A better result was obtained in the presence of sucrose compared to the presence of glucose for SSF with this residue.

The non-germination of *T. harzianum* spores in BA residues also implied in the non-production of aroma. To minimize the effects of inhibitors and increase aroma production, we strategically opted for the mixture of BA+SC residues. This mixture promoted with the increase of nutrients and increase of the lignocellulosic composition, the germination of the spores of *T. harzianum* and the action of enzymes capable of exploiting less bioavailable sources, reaching maximum 6-PP production (200 ppm).

The structure and performance of the enzymatic complex produced by the fungi, favored by starch, allowed the degradation of these compounds even with antimicrobial properties, serving as an important nutritional source for the fungi. Factors such as bioavailability of fermentable nutrients, presence of minerals, minimizing the toxic effects of the support, may have positively influenced the bioconversion of nutrients to the production of 6PP in the BA+SC mixture.

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Author Contributions

NAS: Performed all the practical stages of the research and wrote the manuscript;

PMSC: Assisted in the antimicrobial analyzes and interpreted the equivalent of the findings in the analysis.

MGJ: Assisted in the chromatographic analysis and identification of compounds present in the residues and interpretation of the results;

SAZ: Assisted in the botanical analyzes of the residues and in the antimicrobial interpretations;

RJB: Assisted in the solid state fermentation and microbial cultivation steps and in the interpretation of the results;

MGEC: Assisted in the physical-chemical analysis of the residues and also in the interpretation of the results.

BCP: Supervised all stages of analysis.

MUN: Supervised the chromatographic analyses, interpreted the results and revised the manuscript

LSGF: Supervised all stages of analysis, interpretation of results and writing of the manuscript; final revision of the manuscript.

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ORCID ID

A. S. do Nascimento <https://orcid.org/0000-0003-2959-7069>

J. B. Ribeiro <https://orcid.org/000000018049>

S. G. F. Leite <https://orcid.org/0000000295476014>

G.EC. Marques <https://orcid.org/0000-0001-7801-3117>

U.M Nascimento <https://orcid.org/0000-0003-0710-4745>

M.S.C Penha <https://orcid.org/0000-0002-7677-1827>

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