AIMS Bioengineering, 11(4): 561–573.
DOI: 10.3934/bioeng.2024025
Received: 22 July 2024
Revised: 22 Novembe 2024
Accepted: 26 November 2024
Published: 09 December 2024

https://www.aimspress.com/journal/Bioengineering

*Research article*

# Optimization of laccase production by *Pleurotus ostreatus (Jacq.)* P. Kumm. using agro-industrial residues: a comparative study on peels of tucumã (*Astrocaryum aculeatum* G. Mey.) and pupunha (*Bactris gasipaes* Kunth) fruits

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**Abstract:** This work focuses on optimizing laccase production from *Pleurotus ostreatus* (Jacq.) P. Kumm. using tucumã (*Astrocaryum aculeatum* G. Mey.) and pupunha (*Bactris gasipaes* Kunth) fruit peels as substrates and assessing the influence of environmental parameters such as moisture content % (w/w) and wheat bran supplementation % (w/w). Both substrates demonstrated potential for substantial laccase activity, with tucumã peels reaching optimal production levels at 902 IU/kg under conditions of 70% moisture and 5% wheat bran supplementation, whereas pupunha peels exhibited a maximum

yield of 1486 IU/kg with 70% moisture and 15% bran supplementation. This study exemplifies the integration of Amazonian agro-industrial residues in biotechnological applications, highlighting the potential of these substrates in laccase production. In the context of the bioeconomy, this work underscores the importance of utilizing local biomass residues as sources of valuable enzymes, which play a critical role in biotechnological solutions, including pollution remediation and sustainable manufacturing processes.

**Keywords:** tucumã peel; pupunha peel; agro-industrial residues; enzyme optimization and bioconversion, Amazonian agro-industrial residues

## 1. Introduction

Laccase enzymes belong to the multicopper oxidase family and have an important role in the bioremediation of industrial effluents and the biotransformation of lignocellulosic waste into valuable bioproducts [1–3]. The economic production of laccase is a significant challenge in the biotechnology sector, given the enzyme's wide-ranging applications in biofuel production, the textile industry, and in environmental cleaning [1, 4–6]. Recent advances have focused on optimizing microbial sources for laccase production, and there is a keen interest in reducing production costs through the utilization of cost-effective substrates. The ability of laccase to oxidize a wide spectrum of phenolic and non-phenolic compounds, coupled with its minimal requirement for cofactors, positions it as a valuable enzyme in green chemistry and sustainable industrial processes [7–9].

The quest for economical and sustainable methods of laccase production has led to the exploration of agro-industrial residues as potential substrates. Scientific investigations have increasingly centered on the use of filamentous fungi, particularly from the genus *Pleurotus*, due to their robust laccase-producing capabilities and adaptability to various substrates [10–15]. These studies have revealed the impact of substrate composition, moisture content, and supplementation with additional nutrients on enzyme yield [16,17].

Despite the well-documented potential of agricultural residues in bioprocesses, there is a noticeable lack of regional research on the utilization of the fruit peels of tucumã (*Astrocaryum aculeatum* G. Mey.) and pupunha (*Bactris gasipaes* Kunth), two abundant resources in the Amazon region. From the total production of 1032 tons of tucumã and 5084 tons of pupunha, approximately 918–1223 tons of peel are generated annually [18,19]. These residues, commonly found in street markets in Brazil, present a significant challenge in terms of disposal due to their volume [20,21]. However, the literature suggests that they possess the necessary components to serve as effective substrates for enzyme production, particularly laccase [22]. The absence of studies that explore these specific residues in laccase production represents a critical regional knowledge gap, especially considering their potential to reduce the costs associated with bioprocesses.

This study aimed to optimize laccase production from *Pleurotus ostreatus* (Jacq.) P. Kumm. using tucumã (*Astrocaryum aculeatum* G. Mey.) and pupunha (*Bactris gasipaes* Kunth) fruit peels as substrates and evaluated the influence of parameters such as moisture content and wheat bran supplementation. The quantitative goals included achieving a significant increase in laccase production compared to existing benchmarks, with the aim of establishing a sustainable and cost-effective method for enzyme production. This research not only contributes to the broader field of enzyme

biotechnology but also offers practical solutions for the valorization of regional agro-industrial residues.

## 2. Materials and methods

### *2.1. Pleurotus ostreatus (Jacq.) P. Kumm.*

A commercial strain of *Pleurotus ostreatus* (Jacq.) P. Kumm identified as Gumelos 1 from Gumelos®, Brazil (http://shopee.com.br/gumelos/), acquired in July 2020, was utilized for this study. This strain has been preserved in the Collection of Medically Relevant Microorganisms at INPA. Cultivation involved placing mycelium fragments on Petri dishes containing yeast extract-peptone-dextrose agar (YPD) medium and incubating at 25 °C for 7 days in an incubator (Panasonic® MIR-154, Osaka, Japan).

### *2.2. Peel of Astrocaryum aculeatum G. Meyer and Bactris gasipaes Kunth fruits*

Collections of peels of *A. aculeatum* and B. *gasipaes* fruits were obtained at local markets in Manaus, immediately followed by transportation to the Mycology Laboratory at INPA for processing. A meticulous selection process preceded a series of asepsis treatments, including immersion in a 0.03% chlorine solution for 15 min, ensuring the removal of potential microbial contaminants. A subsequent drying phase at 70 °C, lasting from 48 to 72 hours, was applied to achieve optimal moisture content, crucial for fungal growth. Mechanical grinding, employing a high-speed stainless steel industrial blender (Skymsen® LI2, Brusque, Brazil), facilitated the reduction of the peel to a standardized particle size (1–5 mm), ensuring a uniform substrate composition for mycelial colonization.

### *2.3. Wheat seeds and wheat bran (substrate supplement)*

The wheat seeds used to prepare the inoculum were sourced from Bunge Alimentos S.A. (Bunge Alimentos S.A., São Paulo, Brazil), while the wheat bran used to supplement the substrate was sourced from Moinho Globo® (Moinho Globo, Sertanópolis, Brazil). This wheat bran was standardized to a granularity of 1–5 mm. The proximate composition of the bran was as follows: moisture content 13.5%, crude protein 15.5%, crude fiber 11.0%, crude fat 4.0%, and ash 5.5%. Wheat bran is used as a supplement in the production of laccase mainly due to its nutritional content, affordable cost, and ability to stimulate enzyme production and mushroom growth.

### *2.4. Proximate composition of peels from A. aculeatum and B. gasipaes*

A comprehensive proximate analysis of the peels of *A. aculeatum* and *B. gasipaes* was conducted in accordance with the rigorous methodologies outlined by the Adolfo Lutz Institute [23]. This analytical phase was instrumental in quantifying key compositional elements such as protein, lipids, ash, moisture, and carbohydrate content, thereby providing invaluable insights into the nutritional profiles of the substrates and their implications for fungal growth and laccase enzyme production. Details of these analyses are further described in section 2.6.

### *2.5. Optimal moisture and wheat bran supplementation percentages for maximizing laccase (IU/g) production*

#### 2.5.1. Inoculum preparation

In the initial phase of the experiment, wheat grains designated for spawn preparation were subjected to thorough washing and soaking in filtered water for a period of 12 h. Once dried, 30 g of these grains was placed into 125 mL Erlenmeyer flasks, which were then sealed with cotton plugs and sterilized at 121 °C for 0.75 h under 1.1 atm pressure. Subsequently, for mycelial inoculation, six 1 cm diameter mycelium plugs, pre-cultivated on Petri dishes, were transferred aseptically into the flasks under a laminar flow hood AC2-4S3 (Esco Lifesciences, Singapura, Singapura). These inoculated flasks were then incubated in an incubator (Panasonic®, MIR-154, São Paulo, Brazil) at 25 °C for 14 days to promote mycelial proliferation.

#### 2.5.2. Bioprocess description

The substrates were collected, the moisture content was adjusted, and then they were enhanced with a calculated supplementation, adhering to a carefully designed factorial experimental framework, prior to being sealed in autoclavable HDPE (width 21 cm × length 30 cm, 0.8 kg bags) (Embrasil Embalagens Plásticas Ltda., São Paulo, Brazil) for sterilization and subsequent cultivation.

Following the preparation of the substrate, a $2^2$ experimental central composite design with axial points and central replication was employed to optimize moisture content and wheat bran supplementation to maximize the biological efficiency (BE). Axial points were set at 1.414, and the central point was triplicated. The experimental factors were moisture content and wheat bran supplementation, with BE as the response variable [24]. Details of the experimental setup are presented in Table 2. Once data were collected, analysis of variance (ANOVA) was used to determine which factors had significant effects on BE, either individually or through interactions. ANOVA helped identify both main effects—how each factor influenced the outcome on its own—and interaction effects, which show how the combination of factors impacted the results. A regression model was then developed, providing a predictive equation for the BE under different conditions. Finally, the results were visualized using a response surface plot, which offered a clear representation of the optimal conditions for maximizing BE.

#### 2.5.3. Incubation process

Following sterilization, the bags of substrate were inoculated with 30 g of the prepared mycelium spawn under sterile conditions under a laminar flow hood. The inoculated substrates were then transferred to a BOD chamber (Q315M25, Quimis®, Brazil, Diadema) set at a controlled temperature of 25 °C. This controlled environment facilitated optimal conditions for the growth and metabolic activity of the fungi. The incubation period lasted for 25 days. Upon completion of the 25-day incubation period, the laccase enzyme was harvested from the inoculated substrates.

### *2.6. Analytical methods*

#### 2.6.1. Composition of substrates

The chemical composition of the substrates and mushrooms was analyzed using standard protocols from the Physicochemical Methods for Food Analysis by the Adolfo Lutz Institute [18]. Moisture content was determined through gravimetric drying in a precision oven (Memmert GmbH + Co. KG, Schwabach, Germany) at 105 ± 2 °C until constant weight. Ash content was assessed by incinerating 2 g of dried sample at 550 °C for 6 h in a muffle furnace (Thermo Fisher Scientific, Waltham, MA, USA) to completely remove any organic material. Protein content was measured via Kjeldahl distillation (Buchi Labortechnik AG, Flawil, Switzerland), applying a nitrogen-to-protein conversion factor of 6.25 for the mushrooms and 5.71 for the substrates, following digestion with concentrated sulfuric acid (≥ 95%, Sigma-Aldrich, St. Louis, MO, USA). Lipid content was determined via Soxhlet extraction (Gerhardt GmbH & Co., Königswinter, Germany) using petroleum ether (Sigma-Aldrich, purity ≥ 99%) with 8 h reflux time. The total carbohydrate content was calculated by the difference, adding the values of moisture, ash, protein, and lipids, and subtracting the total from 100%. Each assay was performed in triplicate (n = 3), with the results reported as mean ± standard deviation to ensure precision and reproducibility.

#### 2.6.2. Laccase activity

Laccase activity (E.C. 1.10.3.2; benzenediol oxidoreductase) was quantitatively assessed using a spectrophotometric method on a basic spectrophotometer (Eppendorf, Hamburg, Germany) with o-dianisidine (Sigma-Aldrich, St. Louis, USA) as the substrate. The enzymatic reaction was performed in a 1.5 mL final reaction volume within a 100 mM sodium tartrate buffer at pH 4.5, set at room temperature (22–25 °C) to optimize enzyme stability. The initial substrate concentration was maintained at 5 mM. The reaction mixture was prepared in a 1 cm path-length quartz cuvette, with absorbance monitored at 460 nm, where o-dianisidine has a molar extinction coefficient of $1.16 \times 10^4$ $M^{-1}cm^{-1}$. Enzyme activity was calculated from the rate of the increase in absorbance, with one unit (U) defined as the amount of enzyme that oxidizes 1 μmol of o-dianisidine per minute. All the reagents, including sodium tartrate and o-dianisidine, were prepared with deionized water filtered through a 0.22 μm membrane (Millipore, Burlington, USA) to ensure reproducibility [25].

### *2.7. Statistical analysis*

For proximate composition analyses of fruit peels from *A. aculeatum* and *B. gasipaes*, Microsoft Excel® was utilized to calculate means and standard deviations and conduct parametric t-tests. All experiments were conducted in triplicate to ensure reliability and reproducibility.

For optimizing moisture content and wheat bran supplementation in laccase production, ANOVA with a 95% confidence interval was applied to identify significant main and interaction effects on biological efficiency (BE). Following the response surface methodology (RSM) guidelines of Barros Neto and Bruns (1995), a regression model was developed to predict BE under varying conditions, with results visualized through response surface plots, providing a clear representation of the optimal parameters for maximizing enzymatic activity. Statistical analyses were performed using

STATGRAPHICS® 9 (Statgraphics Technologies, Inc., The Plains, VA, USA) and Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA).

## 3. Results

### *3.1. Proximate analysis of tucumã and pupunha peels*

Employing standard analytical techniques for moisture, ash content, protein, lipids, and carbohydrates, we systematically quantified these components in both types of peels. Table 1 presents our findings, revealing a notably higher moisture content in the fresh peel of the fruit of *B. gasipaes* (61.68%) compared to that of *A. aculeatum* (36.9%), with the latter exhibiting a greater lipid content (23.92%).

**Table 1.** Comparative proximate analysis of peels of the fruit of *Astrocaryum aculeatum* G. Meyer and *Bactris gasipaes* Kunth used in laccase production by *Pleurotus ostreatus* (Jacq.) P. Kumm (Gumelos I).

| Parameter | Peel | |
|---|---|---|
| | *A. aculeatum* | *B. gasipaes* |
| Moisture (%) | $36.9 \pm 0.01^{a}$ | $61.68 \pm 0.01^{b}$ |
| Residual moisture (dry residue) (%) | $5.35 \pm 0.57^{a}$ | 4.09 ± 0.01b |
| Ash (dry residue) (%) | $2.61 \pm 0.01^{a}$ | 2.52 ± 0.01b |
| Protein (dry residue) [N × 6.25] (%) | $9.36 \pm 0.05^{a}$ | 8.52 ± 0.10b |
| Lipids (dry residue) (%) | $23.92 \pm 0.03^{b}$ | $38.04 \pm 0.12^{a}$ |
| Carbohydrates (dry residue) (%) | 58.76 | 46.83 |
| pH (dry residue in 10% distilled water solution) | $5.34 \pm 0.04^{a}$ | $4.04 \pm 0.04^{b}$ |

The values are expressed as mean ± standard deviation of the means, n = 3; the absence of matching letters on the same line indicates statistically significant differences (Student's t-test, $p < 0.05$) for mean comparisons among different substrates.

### *3.2. Influence of moisture and wheat bran supplementation on laccase production by P. ostreatus using peels of tucumã (A. aculeatum) and pupunha (B. gasipaes)*

This experimental design allowed for the systematic exploration of the interaction between moisture (w/w) % and wheat bran supplementation (w/w) %, with the aim of optimizing laccase production. Initial analyses (Table 2) indicated that both factors significantly affected laccase yields, highlighting the importance of nutritional conditions in enzyme expression. The highest laccase activity in tucumã peel was observed at a moisture content of 70% and 5% wheat bran supplementation, yielding 902.7 IU/kg, while pupunha peel demonstrated its peak production of 1,486.7 IU/kg under 70% moisture and 15% supplementation, suggesting substrate-specific responses to the experimental conditions (Table 2).

**Table 2.** Influence of moisture and wheat bran supplementation on laccase production by *Pleurotus ostreatus* (Gumelos I) using peels of the fruit of *Astrocaryum aculeatum* G. Meyer and *Bactris gasipaes* Kunth., employing a $2^2$ experimental design with axial points and central replication.

| Moisture (%) | Wheat bran supplementation (%) | Laccase produced in the peel of tucumã fruit (IU/kg) | Laccase produced in the peel of pupunha fruit (IU/kg) |
|---|---|---|---|
| 60 | 10 | 265.5 | 1327.4 |
| 70 | 15 | 212.4 | 1486.7 |
| 50 | 15 | 743.4 | 318.6 |
| 70 | 5 | 902.7 | 371.7 |
| 50 | 5 | 424.8 | 212.4 |
| 60 | 10 | 212.4 | 1221.2 |
| 60 | 10 | 265.5 | 1274.3 |
| 74.1421 | 10 | 902.7 | 1008.8 |
| 45.8579 | 10 | 371.7 | 106.2 |
| 60 | 17.0711 | 318.6 | 265.5 |
| 60 | 2.92893 | 159.3 | 477.9 |

### *3.3. Laccase production (IU/kg) by* Pleurotus ostreatus *in tucumã peels*

The results of the experiment highlighted a significant variation in laccase production (Ui/kg) by *P. ostreatus* in tucumã peels under different conditions of moisture and wheat bran supplementation. The highest laccase activity was recorded at 70% moisture and 5% wheat bran supplementation, reaching 903 IU/kg. Conversely, the lowest activity was observed at 60% moisture and 3% wheat bran supplementation, with a production of 159 IU/kg. After the data had been collected (Table 2), analysis of variance (ANOVA) was used to determine which factors had significant effects on laccase production using the peels of tucumã, either individually or through interactions (Table 3).

**Table 3.** Analysis of variance to determine the statistical significance of factors, interactions, and quadratic model for laccase production using the peels of tucumã (*Astrocaryum aculeatum* G. Meyer).

| Source | Sum of squares | DF | Mean square | F | P |
|---|---|---|---|---|---|
| A: Moisture | 60,867 | 1 | 60,867 | 64.77 | 0.0151 |
| B: Wheat bran | 2679 | 1 | 2679 | 2.85 | 0.2334 |
| AA | 297,617 | 1 | 297,617 | 316.69 | 0.0031 |
| AB | 254,444 | 1 | 254,444 | 270.75 | 0.0037 |
| Lack-of-fit | 165,260 | 4 | 41,315 | 43.96 | 0.0224 |
| Pure error | 1879 | 2 | 939 | | |
| Total (corr.) | 782,748 | 10 | | | |
| $R^2$ = 0.786472 (78.64%) | | | | | |

Equation 1: Laccase produced on tucumã peel = 5,053.36 − 214.30 × moisture + 276.00 × wheat_bran − 5.04 × (moisture × wheat_bran) + 2.28 × $moisture^2$+ 1.15 × $wheat\_bran^2$

This model (Equation 1) accurately represents the relationship between the variables and laccase production. The statistical analysis using ANOVA for the quadratic model confirmed its significance with a P-value < 0.05, indicating that the variations in laccase production could be attributed to changes in moisture and wheat bran supplementation levels. The model's lack-of-fit test was non-significant (P > 0.05), suggesting that the model fits the experimental data well. Furthermore, the coefficient of determination ($R^2$) was calculated to be 0.786, demonstrating that the model could explain 78.6% of the variability in laccase production. These results validate the efficacy of the $2^2$ experimental design with axial points and central replication in exploring the influence of moisture and wheat bran supplementation on laccase production in tucumã peels.

### 3.4. *Laccase production (IU/kg) by* Pleurotus ostreatus *in pupunha peel*

The analysis, based on an ANOVA (Table 4) of the data from Table 2 for laccase production by *P. ostreatus*, revealed that both factors, as well as their quadratic terms, have substantial significance in enhancing laccase yield when using pupunha peel.

**Table 4.** Analysis of variance to determine the statistical significance of factors and interactions and the quadratic model for laccase production using the peels of tucumã (*Astrocaryum aculeatum* G. Meyer).

| Source | Sum of squares | DF | Mean square | F | P |
|---|---|---|---|---|---|
| A: Moisture | 847,517 | 1 | 847,517 | 300.58 | 0.0033 |
| B: Bran | 105,991 | 1 | 105,991 | 37.59 | 0.0256 |
| AA | 597,252 | 1 | 597,252 | 211.82 | 0.0047 |
| AB | 254,419 | 1 | 254,419 | 90.23 | 0.0109 |
| BB | 987,212 | 1 | 987,212 | 350.12 | 0.0028 |
| Lack-of-fit | 324,966 | 3 | 108,322 | 38.42 | 0.0255 |
| Pure error | 5639 | 2 | 2819 | | |
| Total (corr.) | $2.77858 \times 10^6$ | 10 | | | |
| $R^2 = 0.8810$ (88.10%) | | | | | |

Equation 2: Laccase produced on pupunha peel = −1,260 + 372.3655 × moisture + 54.87 × wheat_bran − 3.2521 × $\text{moisture}^2$ − 16.7245 × $\text{wheat\_bran}^2$ + 5.044 × (moisture × wheat_bran)

Building on these findings, the research ventured into regression analysis, crafting a nuanced quadratic equation to model the laccase production process (Equation 2).

This model represents the relationships between the variables. The model's efficacy is further bolstered by an impressive R-squared value of 0.881, indicating that approximately 88.1% of the variability in laccase production is accounted for. This high degree of model fit, however, comes with an acknowledgment of the limitations presented by the absence of specific lack-of-fit test results, necessitating a cautious interpretation of the model's predictive capabilities within the scope of the experimental conditions.

In Figure 1, a detailed response surface is presented, showcasing laccase production by *Pleurotus ostreatus* when cultivated on the peels of tucumã (A) versus the peel of pupunha (B). This analysis highlights the effects of variations in moisture content and the addition of wheat bran on enzyme production.

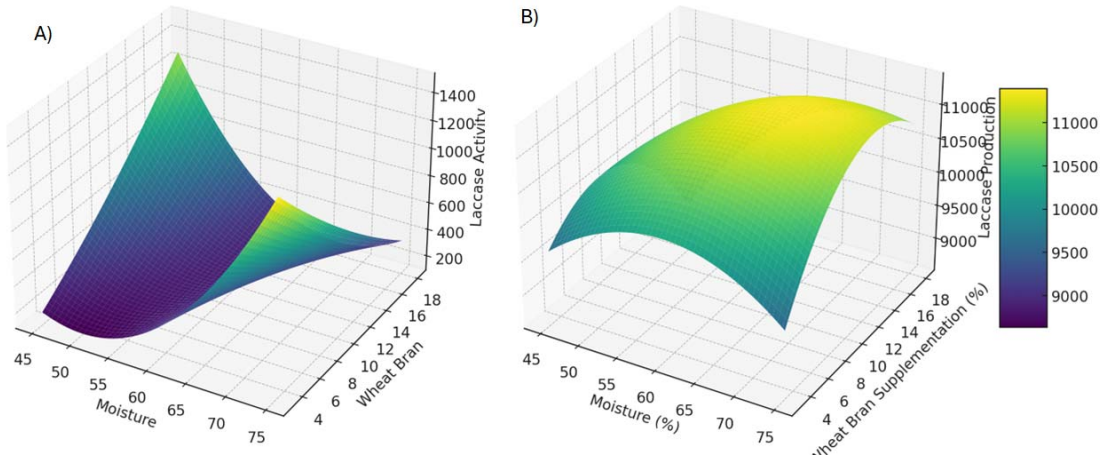


**Figure 1.** Response surface of laccase production by *Pleurotus ostreatus* (Gumelos I) cultivated on peels of *Astrocaryum aculeatum* G. Meyer and *Bactris gasipaes* Kunth.

The best predicted result for laccase production (Equation 1) using tucumã as substrate was 1510.21 units/kg, which was achieved at a moisture level of 75% and a wheat bran level of 3%. However, the best result in the model surface (Equation 2) indicates a maximum laccase production with pupunha of approximately 1,397.91 units. This optimal production occurs at a moisture level of approximately 66.21% and a wheat bran supplementation of approximately 11.64% (Figure 1).

## 4. Discussion

In this study, we successfully optimized laccase production from *Pleurotus ostreatus* using tucumã and pupunha peels. The results revealed that pupunha peels yielded a maximum laccase activity of 1486.7 IU/kg, significantly outperforming tucumã peels, which produced 902.7 IU/kg under optimal conditions. These findings demonstrate the potential of these Amazonian agro-industrial residues as low-cost substrates for sustainable laccase production, addressing both economic and environmental challenges. The analysis of the proximate composition of the tucumã and pupunha fruit peels showed differences in moisture and lipid percentages. Tucumã peel demonstrated a lower moisture and lipid percentage compared to the pupunha peel, but a higher percentage of carbohydrates [23–25]. This assertion is supported by several studies, including those conducted by [26–29], which similarly report on the proximate compositions of various fruit peels and underscore the relevance of such attributes in bioprocess assays.

Comparatively, Yamauchi et al. (2019) cultivated *P. ostreatus* on fermented bamboo sawdust, with 500 IU/Kg of laccase. Kumar et al. (2022) reported a high laccase yield of 12,500 IU/kg using cassava residues in solid-state fermentation (SSF), which aligns closely with our findings for pupunha. Similarly, An et al. (2023) achieved laccase activities of 1200 IU/kg and 1500 IU/kg with *Pleurotus eryngii* and *Lentinus edodes*, respectively, also under SSF with agro-industrial residues (sugar cane bagasse, wheat straw, and rice husk). Han et al. (2022) examined the influence of lignocellulosic biomass on laccase production by *Pleurotus* species, reporting activities between 800 and 1,600 IU/kg

in SSF, depending on the biomass used. However, direct comparisons with other studies are challenging due to the differences in substrates and analytical methods used for laccase quantification. Despite these challenges, the high laccase activity observed in this study, if compared with previous works, underscores the potential of these substrates in biotechnological applications [22,29,30].

Laccase production from the different substrates showed significant variability. The higher yield observed with pupunha peels may be attributed to (a) a greater presence of phenolic inducers in the substrate, thereby enhancing laccase activity, (b) the distinct nutritional contributions of each substrate, which may have influenced microbial growth and enzyme production, and (c) the potential interference of the substrate matrix in the enzyme extraction process, which affects laccase recovery. Little is known about the specific interactions between the fungal strains and these substrates, which could significantly influence enzyme production. Therefore, additional studies are necessary to explore the underlying mechanisms driving the variations in laccase yield.

The strategic use of these substrates not only promotes cost-effective enzyme production but also contributes to the valorization of agro-industrial waste, thereby supporting environmental sustainability and waste management. Our research encountered limitations related to the absence of fiber analysis, a limited range of factorial levels, and a singular approach to fungal cultivation. These constraints underscore the need for further studies to explore a broader spectrum of conditions and alternative cultivation methods. However, our findings significantly contribute to science and technology, especially in the Amazon region, by demonstrating the potential of agro-industrial waste valorization in the development of bioindustries, thereby supporting sustainable regional development.

The use of agro-industrial residues for enzyme production exemplifies sustainable development rooted in bioindustry, bioeconomy, and biotechnology. In the Amazon, local fungal biodiversity can be utilized to produce enzymes and food products of industrial value, fostering innovation and reducing waste. This approach aligns with circular economy principles while integrating regional value chains into broader bioeconomic networks. By transforming discarded materials into valuable resources, such initiatives promote environmental sustainability and economic growth. This reinforces the Amazon's potential as a hub for biotechnological advancements within sustainable industrial frameworks.

## 5. Conclusions

This study demonstrated the viability of using tucumã (*Astrocaryum aculeatum*) and pupunha (*Bactris gasipaes*) peels as effective substrates for laccase production by *Pleurotus ostreatus*. The optimization of moisture content and wheat bran supplementation yielded high enzymatic activity, with the pupunha peels achieving a maximum production of 1486 IU/kg. These findings suggest that agro-industrial residues, often regarded as waste, can be repurposed effectively, offering both an environmental solution and a cost-efficient alternative for enzyme production. By transforming these problematic residues into valuable resources for biotechnological applications, this approach provides a dual benefit: it addresses waste disposal issues and supports sustainable industrial processes.

## Use of AI tools declaration

The authors declare they have not used artificial intelligence (AI) tools in the creation of this article.

## Acknowledgments

We gratefully acknowledge the financial support from FAPEAM (Fundação de Amparo à Pesquisa do Estado do Amazonas), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), under process number 062.00898/2019. The project, titled "Production, Stability, and Applicability of Colorants Produced by Filamentous Fungi Isolated from Soil Samples of the Amazon Region", was funded by the Call No. 006/2019 - UNIVERSAL AMAZONAS. The project was coordinated by João Vicente Braga de Souza.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Authors contributions

All authors contributed to the article's conception and design. Author Contributions: João Vicente Braga de Souza, Kevyn Melo Lotas, and Érica Simplício de Souza conceived and designed the study. João Vicente Braga de Souza, Kevyn Melo Lotas, Raissa Sayumy Kataki Fonseca, Joice Camila Martins da Costa and Ana Claudia Alves Cortez performed the experiments and organized the database. João Vicente Braga de Souza, Érica Simplício de Souza, and Kevyn Melo Lotas wrote the first draft of the manuscript. João Vicente Braga de Souza, Érica Simplício de Souza, Kevyn Melo Lotas, Raissa Sayumi Kataki Fonseca, Joice Camila Martins da Costa, Ana Claudia Alves Cortez Francisca das Chagas do Amaral Souza, João Fernando Vieira Ennes, Márcio Barreto and Flávia da Silva Fernandes wrote sections of the manuscript. All authors contributed to the revision of the manuscript and read and approved the submitted version.

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