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Bioconversion of babassu mesocarp flour into single-cell protein in a controlled bioreactor system

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Abstract

This study demonstrated the application of the INFORS HT Multifors 2 bench-top bioreactor system for yeast production using babassu flour as substrate. Different process parameters were evaluated to maximize the conversion yield of sugars into cellular biomass, with the best condition achieving a yield of 0.4 g/g. The resulting product was successfully used in the formulation of plant-based burgers, demonstrating its functional applicability as a protein-rich ingredient.

Keywords

Saccharomyces cerevisiae, fermentation, SCP, babassu flour, plant-based ingredient, INFORS HT, Multifors 2.

Protein-rich ingredient and plant-based burger produced using fermented babassu flour.

Introduction

Babassu mesocarp flour is a byproduct derived from the inner part of the fruit of the babassu palm (Attalea speciosa), which is commonly found in various regions of Brazil, particularly within the Amazon and Cerrado biomes. From a nutritional stand-point, babassu mesocarp flour is composed predominantly of carbohydrates, accounting for approximately 80 % of its composition. Of this total, around 70 % is starch, while the protein content is relatively low, ranging between 1–2 %. Additionally, it contains a significant amount of phenolic compounds (1–2 %), which have inhibitory effects on microbial growth, including yeast.

Meat analog products made from alternative protein sources are rapidly gaining ground in the global food market. These products are developed to replicate the taste, texture, and nutritional value of conventional meat, using plant-based ingredients, microorganisms as single-cell protein (SCP), or even lab-grown proteins. Innovations in this field respond to a growing demand for healthy and sustainable food alternatives.

Objective

The objective of this study was to enhance the protein content of babassu flour by converting its sugars into yeast biomass, leveraging the naturally high protein content of yeast. This study aims to evaluate the best condition to propagate yeast as a source of single cell protein (SCP) using INFORS HT Multifors 2 bench-top bioreactor system using babassu hydrolysate as substrate.

To achieve this, babassu flour was hydrolyzed to release fermentable sugars, which were then metabolized by yeast in a cell propagation system for biomass production.
The resulting material was used as a protein-rich ingredient for the formulation of plant-based burger prototypes.
The yeast-enriched babassu flour proved to be a promising alternative for the development of meat analog products.

Methods and materials

Enzymatic hydrolysis

The babassu flour was acquired from Associação Agroextrativista Sementes da Floresta (AASFLOR), Pará, Brazil. Aiming to convert the starch and dextrin present in babassu flour into fermentable sugars (glucose, maltose, and maltotriose) for subsequent yeast metabolism, an enzymatic hydrolysis step was performed under controlled conditions using a Dubnoff water bath. The process parameters were as follows:

- Substrate concentration: 25 % (w/w, based on total mass)
- Temperature profile (3 hours of process): gradual decrease from 85 °C to 65 °C
- pH: adjusted and maintained at 5.0 throughout the process
- Enzyme dosage: 4 % of α-amylase and 1.2 % of glucoamylase, both relative to the dry weight of the flour.

Strain selection

Forty-four *Saccharomyces cerevisiae* strains from BIOINFOOD's yeast collection were evaluated in duplicate on solid medium containing 70 % babassu hydrolysate and incubated at 32 °C for 48 hours. Colonies were photographed, and colony size and cellular density were measured in pixels using ImageJ software. Growth data were normalized using the Z-score method. The strains showing the highest growth performance were selected for a second evaluation step.

These selected strains were then incubated in liquid medium containing 80 % babassu hydrolysate without nutrient supplementation. As control, one flask with a commercial yeast strain was cultivated in YPD medium (2 % glucose, 2 % peptone, 1 % yeast extract). Experiments were performed in duplicate using an INFORS HT Minitron incubator shaker with a 25 mm throw at 32 °C and agitation at 200 min^{-1} for 48 hours (Figure 1). Based on this assay, the strain with the highest biomass production and yield was selected for propagation in INFORS HT Multifors 2 bioreactor system.

Yeast propagation

Aiming to convert the sugars present in babassu hydrolysate into yeast biomass (SCP), fermentation experiments were conducted using the INFORS HT Multifors 2 bioreactor system. The inoculum of strain BFY195 was prepared in an INFORS HT Minitron incubator shaker with a 25 mm throw at 32 °C and agitation at 200 min^{-1}, to initiate fermentation using 4 g of dry cell weight (DCW).

Figure 1. Selection of *S. cerevisiae* strains for babassu hydrolysate fermentation. 11 pre-selected strains and one control flask.

The process was carried out at 32 °C, with agitation at 400 min^{-1}, aeration at 2 vvm, and operated in both fed-batch and batch modes. The process started with 400 mL of water and the bioreactors were programmed to feed 350 mL of babassu hydrolysate (with varying sugar concentrations) over 24 hours, followed by an additional 16 hours without feeding, totaling 40 hours of fermentation.

For Experiment F, the feeding was performed continuously over 40 hours. The pH was automatically controlled and maintained close to 4.0 for most experiments, except for Experiment F, where it was maintained at 5.2, using phosphoric acid and ammonium hydroxide as control agents. Nutrient supplementation was performed using urea and B-complex vitamins.

Extrusion and production of the protein ingredient

After drying the fermented material in a convection oven, it was blended in different ratios with non-hydrolyzed babassu flour and soy protein concentrate (SPC) to generate ingredient prototypes containing 50 % protein. Following tests for solubility, gel formation capacity, and water and oil retention, one formulation was selected based on its performance against the evaluated criterias. This selected formulation was then extruded using a twin-screw extruder (Process™ 16, Thermo Scientific) under varying process conditions — including feed rate, temperature, screw speed, and moisture content — in order to obtain a product with optimal techno-functional properties for application in plant-based burger production.

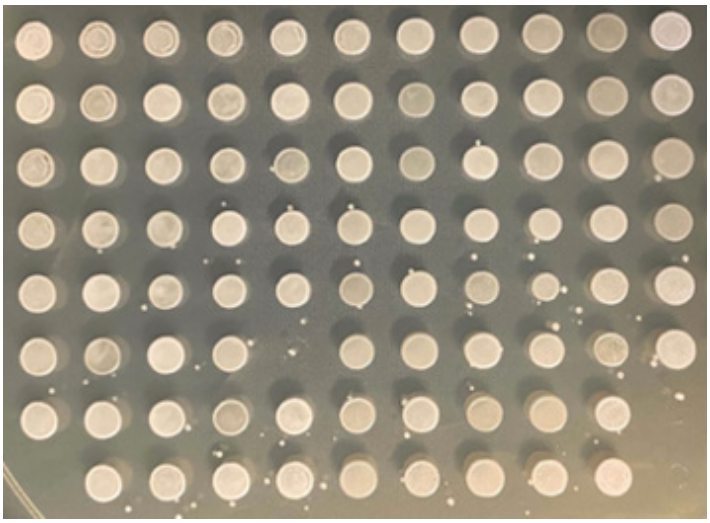
Results

Enzymatic hydrolysis

The enzymatic hydrolysis of babassu flour was performed with over 90 % efficiency, resulting in a hydrolysate containing 180 g/L of fermentable sugars (maltotriose, maltose, and glucose). This material was used in both the strain selection phase and the SCP production process.

Strain selection

For the selection of the 44 yeast strains, cultures were photographed and cell growth was quantified in pixels using ImageJ software. The normalized data using the Z-score method are presented in Figure 2. At this stage, 11 strains with Z-score values above 0.021 were selected and then incubated in liquid medium containing babassu hydrolysate using the INFORS HT Minitron incubator shaker (Figure 1). From this assay, strain BFY195 was selected based on its superior performance in biomass production and sugar consumption yield among the evaluated strains.



BFY001	0.028	BFY058	0.124	BFY074	0.019	BFY196	-0.063
BFY002	-0.009	BFY060	-0.039	BFY083	0.120	BFY205	0.012
BFY003	0.002	BFY061	-0.081	BFY086	0.030	BFY206	0.019
BFY010	-0.025	BFY063	-0.083	BFY088	0.041	BFY207	0.238
BFY011	0.016	BFY064	-0.154	BFY090	-0.037	A	0.140
BFY012	0.051	BFY065	0.003	BFY177	0.125	B	0.015
BFY019	-0.063	BFY066	0.012	BFY191	-0.028	C	-0.084
BFY021	-0.010	BFY067	-0.043	BFY192	-0.051	D	-0.037
BFY027	-0.122	BFY068	-0.088	BFY193	0.112	E	-0.130
BFY033	-0.124	BFY072	-0.108	BFY194	-0.143	F	-0.128
BFY047	-0.046	BFY073	-0.156	BFY195	0.022	G	-0.097

Figure 2. Spotting assays for the selection of strains best suited for the fermentation process of babassu flour hydrolysate. Colonies grow after 48 hours of cultivation, and the heatmap analysis shows normalized growth values (Z-score) for two replicates of each tested strain. Red and blue indicate higher and lower Z-score values, respectively.

Yeast propagation

SCP production was carried out using the INFORS HT Multifors 2 bioreactor system (Figure 3). Different process parameters were applied, and the corresponding data are presented in Table 1. Yield values were calculated based on the ratio between sugar consumed and biomass produced.

The experimental results indicate that nitrogen and vitamin supplementation are important for increasing both biomass yield and cell production. It was also observed that sugar concentration influences total biomass production but does not significantly affect yield (as seen in Trials D and E). Finally, continuous feeding of babassu hydrolysate throughout the entire 40-hour process (Trial F) resulted in the best performance in terms of both yield and biomass production.

Trials	Nutrients			Results	
	Initial sugars (g)	Nitrogen source	Vitamin source	DCW produced (g)	Y (x/s)
A	26.1	—	—	5.9	0.28
B	27.7	+	—	6.3	0.29
C	35.4	+	—	8.9	0.32
D	41.2	+	+	9.4	0.33
E	35.4	+	+	8.8	0.34
F	36.4	+	+	14.5	0.40

Table 1. Process parameters and results for biomass production and yield. — : without nutrient supplementation; +: with nutrient supplementation.

Figure 3. Propagation of yeast from babassu hydrolysate.

Plant-based burger production

To produce the protein ingredient, sequential fermentations were carried out using the protocol from Trial F. This process gen-erated a product composed of yeast and babassu flour with a protein content five times higher than the original flour. The resulting product was then blended with soy protein concentrate (SPC) and non-hydrolyzed babassu flour to achieve a final protein concentration of 50 % (Figure 4). The ingredient was extruded, and seasonings and water were incorporated to formu-late the plant-based burger (Figure 4). The final burger underwent microbiological testing, chemical and nutritional characteri-zation, and sensory evaluation, all of which showed positive results in their respective aspects.

Figure 4. Protein-rich ingredient and plant-based burger produced using fermented babassu flour.

Conclusion

The INFORS HT Multifors 2 bench-top bioreactor system delivered critical advantages for this study. The results demonstrated that the programming system of the INFORS HT Multifors 2 bioreactor, combined with optimized process parameter adjustments, was effective for SCP (single-cell protein) production. A continuous feeding profile (40 hours) was seamlessly implemented using the integrated eve® bioprocess platform software, ensuring consistent substrate delivery, enabling optimal yeast metabolism. Integrated sensors and automated feedback loops maintained ±2% deviation from setpoints (temperature, aeration, agitation). These capabilities directly contributed to overcoming babassu's phenolic inhibition challenges, achieving:

- 50% higher biomass production (14.5g DCW vs 9.4g in batch mode)
- 15% yield improvement (0.40 g/g vs 0.34 g/g)

This technological advantage was instrumental in transforming a low-value byproduct into a commercially viable protein in-gredient within a single experimental campaign.

Acknowledgments

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