EVALUATION OF THE ENZYMATIC HYDROLYSIS PROCESS OF BABASSU MESOCARP FLOUR TO OBTAIN FERMENTABLE SUGARS

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ABSTRACT

The aim of this study was to investigate the efficiency of enzymatic hydrolysis to convert starch present in babassu flour into fermentable sugars. In this context, different pH values ranging from 4.0 to 6.0 were evaluated. Babassu mesocarp flour hydrolysate was obtained through enzymatic hydrolysis using α-amylase and glucoamylase. In suspensions with 20 % (w v^{-1}) babassu mesocarp flour, α-amylase (0.3 %) and glucoamylase (0.3 %) were simultaneously added and maintained under constant agitation at 200 rpm for 3 hours, with a temperature ramp varying from 65 to 85 ºC. Results obtained in assay 2 (pH 5.0) indicated that the enzymatic hydrolysis process was 73.6 % efficient (9.65 g L^{-1} of maltose and 92.35 g L^{-1} of glucose). These findings demonstrate the significant potential for the enzymatic conversion of starch in babassu mesocarp flour into fermentable sugars.

Keywords: Sustainable. Starch. Hydrolysate. Efficiency. Bioprocess.

1 INTRODUCTION

The babassu palm (*Orbignya phalerata*) is of great importance in the Amazon region and northeastern Brazil, renowned for its versatility and the various products derived from its fruits. The babassu fruit comprises four main parts: the epicarp (outer shell), mesocarp (intermediate layer), endocarp (hard inner shell), and almonds (seeds). Each of these parts has distinct applications, including the production of oil, charcoal, food, animal feed, and even handicrafts.[1] The sustainable exploitation of babassu significantly impacts the local economy, fostering the socioeconomic development of riverine and extractive communities.[2]

The mesocarp of babassu is particularly valuable due to its high starch content. Traditionally used in food, mesocarp flour has garnered increasing interest as a raw material for various industrial applications.[3] This byproduct, obtained after the removal of the almonds, is rich in carbohydrates, especially starch, making it ideal for biotechnological transformation processes. In addition to its use in food, mesocarp flour can be converted into fermentable sugars through enzymatic hydrolysis, which may have wide applications in bioprocesses.[4]

In this scenario, the enzymatic hydrolysis, a process that employs enzymes such as amylases and glucoamylases to convert starch into smaller sugars, is an efficient and environmentally friendly approach.[5] Thus, the babassu mesocarp flour hydrolysate presents significant potential as substrate to microorganisms, particularly yeasts. These fermentable sugars may be utilized by yeasts such as *Saccharomyces cerevisiae*, which are widely employed in industrial fermentation processes.[6] The use of this hydrolysate not only provides a sustainable and renewable carbon source for microbial growth but also contributes to reducing dependence on conventional substrates such as molasses and corn.[4]

In this context, the utilization of babassu mesocarp flour to produce fermentable sugars represents a significant innovation in the field of bioprocesses. By integrating biotechnology and the utilization of natural resources, this study will contribute to the advancement of sustainable technologies and the valorization of products derived from Amazonian biodiversity. Thus, the aim of this study was to investigate the efficiency of enzymatic hydrolysis to convert starch present in babassu flour into fermentable sugars to be used as a carbon source for single-cell protein (SCP) production.

2 MATERIAL & METHODS

Babassu mesocarp flour was sourced from the Cantinas da Terra do Meio (Vem do Xingu), located in the city of Altamira, PA, Brazil. The enzymes α-amylase (LiqueStar) and glucoamylase (Fermentase HX) were provided by Suntaq International Limited, Brazil.

Babassu mesocarp flour hydrolysate was obtained through enzymatic hydrolysis using α-amylase and glucoamylase. In suspensions of 20 % (w v^{-1}) of babassu mesocarp flour, α-amylase (0.3 %) and glucoamylase (0.3 %) were simultaneously added to the process with 50 uL of $CaCl_2$ (60 ppm). To enhance the efficiency of the process, different pH values ranging from 4.0 to 6.0 were evaluated. The system was heated using a temperature ramp (65-85 ºC) and maintained under constant agitation at 200 rpm for 3 h.

Total reducing sugars (TRS) were determined in hydrolysis samples every hour using a 3,5-dinitrosalicylic acid (DNS) colorimetric method. A glucose standard curve was used, and absorbance was measured at 546 nm, as described by Miller[7]. At the end of the hydrolysis process, the fermentable sugars were determined. Chromatographic analysis conducted on a HPLC (Alliance HT, Waters, USA), Compounds separation occurred in a Bio-Rad HPX-87H column at 35 °C, using 5 mM sulfuric acid as mobile phase, at 0.6 mL min^{-1}. A refractive index detector at 35 °C was used and sugar concentrations were analyzed by comparing retention time and peak areas with those of a standard curve of HPLC grade sugars (maltose and glucose).[8]

3 RESULTS & DISCUSSION

During the hydrolysis process, it was observed that pH significantly influences the efficiency due to the optimal pH required for each enzyme's activity. Therefore, evaluating the pH value that results in the highest conversion of starch into fermentable sugars is crucial for improving process efficiency. The results are summarized in Table 1.

Table 1 Results of improvement of enzimatic hydrolysis of babassu mesocarp flour.

		TRS (g L^{-1})				Fermentable sugars (g L^{-1})	
N	**pH**	**0 h**	**1 h**	**2 h**	**3 h**	**Maltose**	**Glucose**
1	4.0	7.80	75.33	88.44	92.52	6.44	61.65
2	5.0	7.82	88.44	110.55	138.57	9.65	92.35
3	6.0	7.85	74.68	81.88	87.69	6.10	58.44

As presented in Table 1, monitoring the conversion of starch in babassu mesocarp flour into total reducing sugars (TRS) revealed that pH 5.0 yielded the best results over 3 hours. Specifically, TRS levels increased from 7.82 g L^{-1} at the beginning of the process to 138.57 g L^{-1}, representing a 177.2% increase. This performance was 49.8% and 58.1% higher compared to trials conducted at pH 4.0 and 6.0, respectively.

Regarding fermentable sugars, assay 2 exhibited the highest concentrations of maltose and glucose, indicating that the enzymatic hydrolysis process was 73.6 % efficient in converting starch into fermentable sugars. It is known that α-amylases break α-1,4 glycosidic bonds within the starch chain, forming dextrins and maltose, while glucoamylase hydrolyzes α-1,6 glycosidic bonds in starch and dextrins, releasing glucose as the final product.[5,9]

Thus, the results of this study are promising for the enzymatic conversion of starch in babassu mesocarp flour into fermentable sugars. However, further research is necessary to optimize the hydrolysis process to achieve higher efficiency. This optimization will enhance the sustainability and economic viability of utilizing babassu flour in industrial bioprocesses.

4 CONCLUSION

The findings of this study demonstrate significant potential for the enzymatic conversion of starch in babassu mesocarp flour into fermentable sugars. However, further research is necessary to refine the hydrolysis process to achieve greater efficiency. Such optimization will enhance the value of babassu byproducts and offer a sustainable alternative to carbohydrates derived from other agricultural crops.

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ACKNOWLEDGEMENTS

This study received financial support from The Good Food Institute (GFI), Brazil, in the research incentive program InovAmazônia: Ingredients for the Vegetable Food Market. Project: “Desenvolvimento de farinha a base de subprodutos do processamento de babaçu obtida a partir de hidrólise e fermentação para aplicação em produtos cárneos análogos”.