RESIDUAL FRACTIONS FROM DEFATTED RICE BRAN PROTEIN EXTRACTION AS SOURCE OF CARBOHYDRATES IN BIOPROCESS

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ABSTRACT

The study investigated the feasibility of using residual fractions from the extraction of proteins from Defatted Rice Bran (DRB) as a source of carbohydrates in bioprocesses. Enzyme activity tests indicated that a temperature of 70°C was ideal for simultaneous application of α-amylase and amyloglucosidase (AMG). The biomass was subjected to protein extraction in an alkaline medium assisted by ultrasound. The residual fractions (precipitate resulting from the extraction process (P) and supernatant from protein precipitation (S)) were combined and autoclaved to liquefy the starch. A Full Factorial Design (FFD) 2^2 was applied to study enzymatic hydrolysis (variables: concentrations of α-amylase and amyloglucosidase in the range 120 to 180 mL/m^3, and the substrate concentration (P/S ratio) in the range of 30 to 70 g/L), resulting in a maximum of 17 g/L of reducing sugars. Fermentation experiments showed that the hydrolysate of the residual fractions was efficient, yielding results comparable to control media, indicating potential for use in bioprocesses. These findings suggest a promising technological approach for valorizing rice bran waste in integrated biorefineries.

Keywords: Biorefinery. Biomass production. By-product. Fermentation. Enzymatic hydrolysis.

1 INTRODUCTION

Rice is a widely cultivated and globally consumed cereal, with a worldwide production of approximately 515 million tons in 2022/23. Brazil leads outside Asia, projecting an output of 7.5 million tons in 2023/24.[1] Besides its significance in food, rice by-products such as husks and bran offer valuable opportunities. These by-products can be underutilized as fodder, bedding in poultry farms, or fuel in boilers.[2]

The grain composition varies due to different cultivars, environmental conditions, and agricultural practices, impacting the fractions of rice processing. Rice bran, representing 6-12% of the grain, contains carbohydrates (68.65%), lipids (1.88%), proteins (16.75%), and ashes (12.72%).[3,4] After lipid extraction, DRB can be utilized for protein extraction. Although studies explore fermentative processes of rice bran biomass,[5,6] none have addressed the residual fractions from lipid and protein extraction processes for bioprocessing.

Considering the technological and nutritional importance of the protein concentrate, after applying a previously studied technological route for protein extraction from DRB, this study focused on the enzymatic hydrolysis of the residual fractions for application in fermentative processes. The integrated utilization of different fractions of rice bran in biorefineries can enhance the competitiveness of this resource and promote its valorization in the economic context.

2 MATERIAL & METHODS

The DRB was supplied by Indústria Riograndense de Óleos Vegetais (IRGOVEL – Pelotas/RS); α-amylase (LpHera®) and AMG (Saczyme Go 2X), both from Novozymes®, were used for the enzymatic hydrolysis. Cell growth was assessed using a locally acquired inoculum of *Saccharomyces cerevisiae* yeast (SAF-INSTANT®). The residual fractions used in this study were obtained following a method previously developed[4] to evaluate the extraction of proteins and carbohydrates from DRB to get a protein concentrate. Subsequently, the suspension underwent ultrasound treatment under previously defined conditions[7]. After centrifugation, the Precipitate (P1) was collected, dried in an oven for 16 h at 50 °C, ground (Basic Analytical Mill A 11 IKA), and stored for subsequent analysis. The Supernatant (S1) underwent protein precipitation for liquid phase separation. The pH was adjusted to 4.5 with 3.0 mol· L^{-1} HCl (isoelectric point of rice proteins[8]; the solution was left to rest overnight at 10 °C for precipitation, followed by centrifugation at 3000 rpm (25 °C) for 15 min (Hettich, 420 R, Germany). The Supernatant (S2) was stored for the subsequent hydrolysis. The Precipitate (P1) and the Supernatant (S2), derived from the protein extraction of DRB, served as the raw material for studying enzymatic hydrolysis of carbohydrates.

The enzymatic activity (EA) tests[9] of α-amylase were evaluated at 50, 70, and 90 °C, while AMG was tested at 50, 65, and 70 °C, with a constant pH of 5.0. EA was expressed in International Units per mL (U·mL^{-1}) (amount of enzyme capable of releasing 1 µmol of glucose per minute under conditions (pH, temperature) for each case.

For the enzymatic hydrolysis of residual fractions, an FFD 2^2 was applied. The variables were α-amylase and AMG concentrations (120 to 180 mL·m^3) and the substrate concentration (P1/S2 ratio) ranging from 30 to 70 g·L^{-1}. The experiments were conducted in 125 mL Erlenmeyer flasks (total volume of 50 mL), in an orbital shaker at 120 rpm. The pH of the substrates was adjusted to 5.0, and the autoclaving process at 121 °C/10 min was performed for starch gelatinization. The medium was cooled to the ideal temperature for the simultaneous addition of enzymes (α-amylase and AMG), as defined in the EA, and incubated for 12 h. The enzymatic hydrolysis progress was monitored by withdrawing 0.5 mL samples every 2 h. At the end of the reaction, 0.5 mL of 1 mol·L^{-1} NaOH was added, followed by 0.25 mL of Carrez I and II (potassium hexacyanoferrate and zinc acetate, respectively) to eliminate protein residues. Phase separation was achieved by centrifugation at 4500 rpm at 5 °C for 10 min, and the liquid phase was filtered through filter paper, followed by the determination of RS.[10,11]

The enzymatic hydrolysis of the combined residual fractions was conducted in 250 mL Erlenmeyer flasks, with a total volume of 200 mL, in an orbital shaking at 160 rpm. The ratio of the P1 to S2 was determined based on the results of the FFD. At the end of the hydrolysis, phase separation was carried out by centrifugation at 7000 rpm at 5 °C for 20 min. This process was repeated twice, and subsequently, the liquid phase was filtered through filter paper to ensure the removal of solid particles.

The fermentation assays (Table 1) were conducted in 500 mL Erlenmeyer flasks (total volume of 250 mL). The pH of the media was adjusted to 5.0 and autoclaved (121 °C, 1 atm, 10 min). After reaching room temperature, *S. cerevisiae* inoculum was added (10% (v·v^{-1}/10^8 CFU·mL^{-1})). Incubation was carried out at 30 °C under stationary conditions. Assays were performed in duplicate. Biomass concentration was determined by optical density at 600 nm[12] and the concentration of RS and total RS were determined[10,11]. Substrate-to-biomass conversion factors ($Y_{X/S}$), cellular productivity ($P_{biomass}$), and the maximum cellular growth rate (μmax) were determined.[13] Tukey's test was employed to assess differences between means at a significance level of 5% ($p < 0.05$) (ACTION STAT 3.7 software).

Table 1 Assays for evaluating the cellular growth of *S. cerevisiae* from the hydrolysate of residual fractions

Assays*/ Nutrients	RS (g·L^{-1})	Sucrose (g·L^{-1})
H	15	-
C1	-	15
HS	15	165
C2	-	180

* H – Hydrolysate pure, without added nutrients; C1 – Control medium 1, reduced in sugars in the same proportion as medium H.; HS – Hydrolysate supplemented with nutrients to achieve the same composition as C2; C2 – Control medium 2, simulating sugarcane bioethanol production. Except for assay H1, the other assays were supplemented with 5 g·L^{-1} of yeast extract, 3.5 g·L^{-1} of potassium phosphate, 0.75 g·L^{-1} of magnesium sulfate, and 1 g·L^{-1} of calcium chloride.

3 RESULTS & DISCUSSION

For α-amylase, the highest enzymatic activity (2.03 U·mL $_{enzyme}$ $^{-1}$) was achieved at 90 °C. This result differed significantly ($p < 0.05$) from the activities found at 50 °C and 70 °C (1.53 and 1.81 U·mL $_{enzyme}$$^{-1}$, respectively). The results for AMG were higher than those found for α-amylase and showed no significant difference ($p \geq 0.05$) at temperatures of 50 °C, 65 °C and 70 °C (6.60; 6.89; 6.41 U·mL $_{enzyme}$ $^{-1}$, respectively). Therefore, the temperature of 70ºC was chosen for the simultaneous application of enzymes in the reaction, minimizing losses due to evaporation and reducing energy costs associated with this process phase.

Figure 1 shows the results of RS released over time for the FFD hydrolysis assays. It is observed that experiments 1 and 2 resulted in lower concentrations of RS (7.94 and 7.68 g·L^{-1}, respectively), while experiments 3 and 4 exhibited higher release of RS (16.50 and 18.00 g·L^{-1}, respectively). The lower concentrations of RS in assays 1 and 2 can be attributed to the smaller amount of substrate used (30 g·L^{-1}), as the enzyme concentrations employed in these experiments (120 and 180 mL·m^{-3}, respectively) were the same as those used in assays 3 and 4, conducted with 70 g·L^{-1} of the substrate.

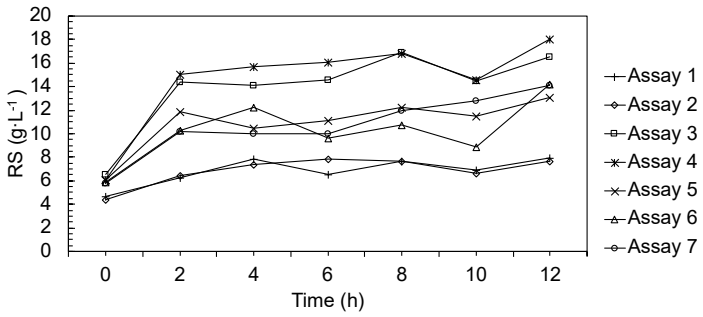

Figure 1 RS released overtime during the enzymatic hydrolysis of the FFD 2^2 assays

Through the analysis of effects, only variable x_2 (substrate concentration) showed a significant ($p < 0.05$) and positive effect on the studied response. This indicates that the highest hydrolysis responses were observed at higher substrate concentrations (P1/S2 ratio). Based on the results achieved in FDD assays, the conditions of assay 4 (180 mL·m^{-3} of enzymes and 70 g·L^{-1} of substrate) were chosen for obtaining the hydrolysate due to higher RS values at both 10 and 12 h (14.54 and 18.00 g·L^{-1}, respectively), indicating better hydrolysis efficiency.

Figure 2 shows the kinetic profiles of the fermentations conducted. The H and C1 experiments (Figures 2a and 2b) were concluded within 12 h of fermentation, a time shorter than the other experiments, due to the lower initial concentration of reducing sugars (RS). On the other hand, the fermentation processes of the HS and C2 experiments (Figure 2c and 2d) were interrupted at 36 h due to the stabilization of cell growth, with total RS concentrations of 18.12 and 61.08 g·L^{-1}, respectively. These results indicate no complete consumption of sugars in the HS and C2 experiments, suggesting the possibility of culture medium contamination or errors in result analysis.

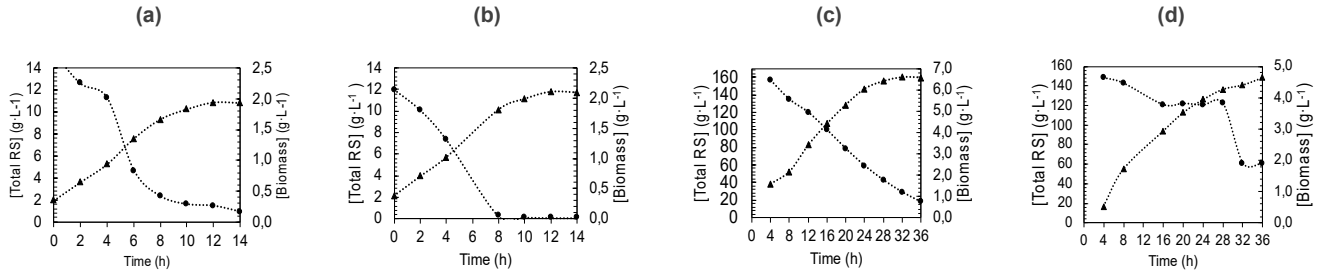

Figure 2 Kinetic profiles of the fermentation assays. (a) H; (b) C1; (c) HS C2; (d) C2.

The kinetic parameters of the fermentation experiments are presented in Table 3. Comparing the H and C1 experiments, as well as the HS and C2 experiments, in the analysis of the parameters $P_{biomass}$, $Y_{X/S}$, and μmax, the C1 experiment yielded superior results to H, and the experiments differed statistically ($p \leq 0.05$) from each other. Regarding the HS and C2 experiments, $P_{biomass}$ and μmax were higher in HS than in C2, differing statistically ($p \leq 0.05$) between them. Conversely, in the $Y_{X/S}$, the C2 and HS experiments did not differ statistically ($p \geq 0.05$) from each other. The hydrolysate proved to be an efficient medium, as it achieved comparable results with the control, which was supplemented with sucrose and other nutrients, even without the addition of nutrients. Similarly, the supplementation of the hydrolysate led to a significant increase in cellular productivity, indicating that the hydrolysate from residual fractions contained sufficient nutrients for yeast growth. In the alcoholic fermentation of hydrolyzed DRB with yeast stimulation by ultrasound, another study [6], achieved a maximum $Y_{X/S}$ of 0.21 $g_{biomass}\cdot(g_{RS})^{-1}$. Considering that in this study, residual fractions from the protein extraction process of DRB were used, although the results were lower, one can infer the potential for utilizing these residues in culture media for fermentative processes.

Table 2 Kinetics parameters from fermentation

Parameters	Assays			
	H	C1	HS	C2
$Y_{X/S}$ ($g_{biomass}\cdot(g_{RS})^{-1}$)	0.109 ± 0.0110^{b}	0.144 ± 0.0058^{a}	0.036 ± 0.0015^{c}	0.047 ± 0.0021^{c}
$P_{biomass}$ ($g_{biomass}\cdot L^{-1}\cdot h^{-1}$)	0.113 ± 0.0002^{c}	0.122 ± 0.0003^{b}	0.139 ± 0.0017^{a}	0.115 ± 0.0018^{c}
μmáx (h^{-1})	0.160 ± 0.0035^{b}	0.190 ± 0.0035^{a}	0.080 ± 0.0049^{c}	0.060 ± 0.0035^{d}

4 CONCLUSION

The results of this study indicate the feasibility of commercial amylolytic enzymes, α-amylase and AMG, in the enzymatic hydrolysis of residual fractions from the protein extraction of DRB. Achieving a maximum of 18 g·L^{-1}of RS in the hydrolysis stage, with a $Y_{X/S}$ of 0.109 $g_{biomass}\cdot(g_{RS})^{-1}$ and $P_{biomass}$ of 0.113 $g_{biomass}\cdot L^{-1}\cdot h^{-1}$ in the fermentative process using the hydrolysate of residual fractions, highlights the potential of these residues for utilization in bioprocesses. These findings pave the way for a new technological approach, where the initial extraction of lipids and proteins from DRB, both valuable components, can be followed by the utilization of residual fractions in fermentations. This approach may represent a promising alternative for integrated biorefineries, aiming to enhance the competitiveness of this resource against other matrices and promote its valorization.

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ACKNOWLEDGEMENTS

To Central Analítica Multiusuário de Medianeira (CEANMED) of UTFPR-Medianeira and CNPq (Financial assistance - Process 403786/2021-5).