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Valorization of Brewer's Spent Grain for the Obtention of Phenolic Compounds and Enzymes

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ABSTRACT

Brazil's agribusiness-centric economy is crucial part of the global agricultural product consumption and exportation. The Brazilian brewing industry exemplify this, contributing significantly to Brazil's GDP with substantial outputs of barley and beer, yet also generating substantial lignocellulosic waste, notably brewer's spent grain (BSG). Comprising cellulose, lignin, and hemicellulose, BSG represents a rich substrate for biotechnological conversion, yielding valuable products like phenolic compounds and enzymes. This study explores BSG valorization using Talaromyces stollii, focusing on phenolic compound production and industrial enzyme enhancement. Spectrophotometric methods measured total phenolics and protein content, revealing increasing trends over 7 days of fermentation with 7.85 ± 0.033 mg GAE/g and 96.42 ± 0.0047 µg/mL, respectively. Laccase activity peaked on day 4 with 29,444.44 U/L but declined thereafter. These findings exemplify BSG's biotechnological potential and highlight new options for a sustainable waste management and high-value product development in agro-industrial contexts.

Keywords: Biomass Valorization. Brewer's spent grain. *Talaromyces Stollii*. Phenolic Compounds; Laccase

1 INTRODUCTION

Brazil has an economy strongly centered on agribusiness, with an emphasis on agricultural products and their derivatives, both for domestic consumption and exportation[1]. In this context, this sector stands out as a target for optimization and innovation, especially regarding waste management, aiming to make this process more sustainable. A notable example is the brewing industry in the country, which plays a significant role in the national economy. With the increasing population and agricultural activity, especially in the context of products destined for the brewing industry, the waste generated by it tends to increase more and more. Beer production generates a considerable volume of lignocellulosic waste, estimated at 20 kg for every 100 L of beer produced, corresponding to up to 85% of the total waste from the brewing industry[2 3]. The Brewer's spent grain (BSG) is composed of three distinct organic polymer fractions: cellulose, lignin, and hemicellulose, making it a rich source of molecules and structures susceptible to biotechnological transformations[4]. These organic fractions can be converted into valuable products, such as biofuels or chemical intermediates. A relevant example of a chemical intermediate is phenolic compounds[5]. The valorization of BSG through the action of biological agents, such as the filamentous fungi *Talaromyces stollii*, represents a promising strategy for obtaining higher value-added products, such as phenolic compounds and enzymes of industrial interest.

2 MATERIAL & METHODS

The BSG degradation was carried out by submerged fermentation in triplicate using 250 mL Erlenmeyer flasks containing 50 mL of distilled water and 1 g of biomass (2% w/v). The inoculum load was $4 * 10^5$cells/mL. The control was conducted with only distilled water and biomass. The fermentation conditions were set at pH 5, 28°C, 200 rpm for 7 days. After the fermentation period, the contents of the Erlenmeyer flasks were centrifuged for 10 minutes at 3000 rpm, and the supernatant was collected. This supernatant is the fermentation extract used for the analyses in this study.

To measure the total phenolic content of the fermentative extracts the spectrophotometric method with Folin-Ciocalteau reagent was adopted. The reaction was carried out using 96-well plates. In the well were added 10 µL of the fermentative extract and 200 µL of Folin-Ciocalteau reagent diluted 1:10 in distilled water. After 3 minutes of incubation, the reaction was stopped using a 20% (w/v) calcium carbonate solution, and then the reading was taken in a microplate reader at a wavelength of 765 nm. The standard calibration curve was made using gallic acid (GAE) covering the range from 0 to 1 mg/mL[6].

The Bradford spectrophotometric method was used to determine the protein concentration in the fermentation extract. For the analysis, 10 µL of fermentation extract and 190 µL of Bradford reagent were pipetted, followed by a 5-minute incubation. The reading was then taken in a microplate reader at a wavelength of 595 nm[7].

Laccase was one of the enzymes evaluated and its activity was quantified through the oxidation of the ABTS substrate. The reaction took place in 1 mL cuvettes where 100 µL of 0.1 mM citrate / 0.2 mM phosphate buffer (pH 4.4), 800 µL of 0.4 mM ABTS, and 100 µL of fermentation extract were added. Absorbance readings were monitored from 0 min to 5 min at 30-sec intervals using a spectrophotometer at 420 nm. For the wavelength used, the molar extinction coefficient was 36,000 M^{-1} cm^{-1}. Enzyme activity was calculated using the equation below[8]:

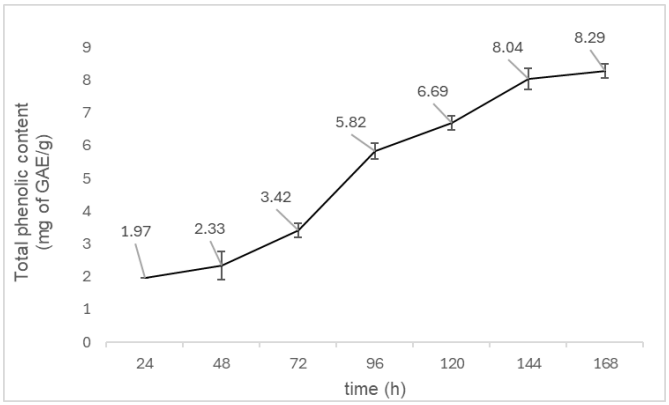
$$Enzyme\ activity\ \left(\frac{U}{L}\right) = \frac{\Delta abs\ .\ 10^6}{\varepsilon . v . t}$$

Where: Δabs is the difference in absorbance, ϵ is the molar extinction coefficient of ABTS; v is the volume of the enzyme extract (L) and "t" is the time (min).

3 RESULTS & DISCUSSION

The results of the total phenolic compounds analysis of the fermentation extracts from days 1 to 7 are presented below in the figure 1:

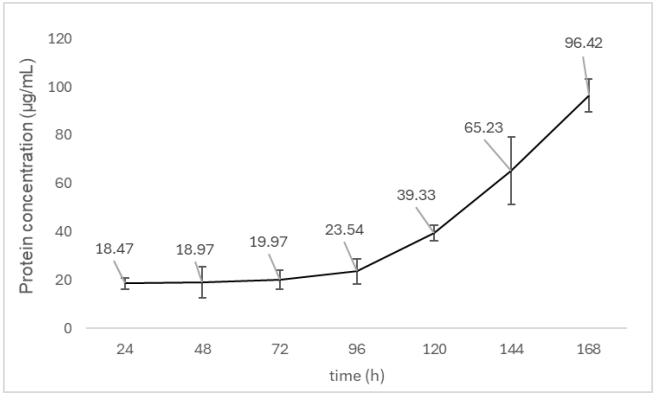
Figure 1: results of the total phenolic content quantification assay



By analyzing the obtained values, it is evident that there is a continuous increase in the concentration of total phenolic compounds in the fermentation extracts over the 7 days of fermentation. A significant increase can be seen between the third day, with a value of 3.42 ± 0.24 mg GAE/g, and the fourth day, with a value of 5.82 ± 0.22 mg GAE/g. The day with the highest production was the seventh day, with a value of 8.29 ± 1.12 mg GAE/g.

The results of the total protein content analysis of the fermentation extracts from days 1 to 7 are presented below in Figure 2:

Figure 2: results of the protein quantification assay

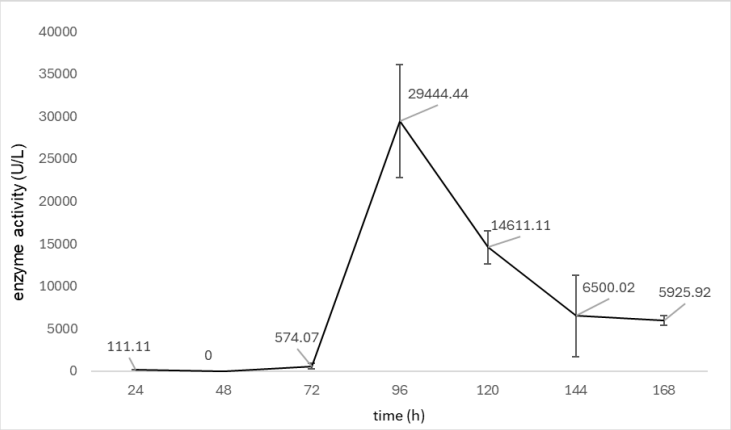


These values show an increase in the concentration of proteins in the fermentation extracts over the 7 days of fermentation. A peak in production can be observed starting from day 5, where a value of 39.33 ± 3.23 µg/mL was obtained, followed by day 6 with a value of 65.23 ± 13.90 µg/mL, and finally, the highest production on day 7 with a value of 96.42 ± 6,83 µg/mL.

The values obtained for the laccase enzyme activity are shown in figure 3:

The values indicate a peak in laccase enzyme activity on the fourth day of fermentation at 29,444.44 U/L, followed by a decline starting the following day with an activity of 14,611.11 U/L. Towards the final days of fermentation, there is a noticeable plateau in activity on days 6 and 7, with activities of 6,500 U/L and 5,925.92 U/L, respectively.

Figure 3: results of the laccase activity assay



The production of laccase using solid-state fermentation of malt bagasse by *Trametes versicolor* reached values of 10,108 U/g and 10,006.6 U/g using phenol and ethanol as inducers, respectively, after 12 days of fermentation[9]. As observed in other studies, the production of proteases can be achieved through other filamentous fungi such as *Aspergillus foetidus* (248 U/mg)[10] and *Rhizomucor miehei* (773.3 U/mg)[11]. Moreover, it was possible to express the gene of an acid protease from a microorganism of the same genus, *Talaromyces leycettanus*, in the yeast *Pichia pastoris*, obtaining an activity value of 1795.4 U/mg[12]. The capability of other filamentous fungi, including those from the genus *Talaromyces*, for protease production could indicate a possible degradation of proteins within the fermentative extract itself, perhaps explaining the decline in the laccase enzymatic activity obtained in the results. Further tests are still needed to support this hypothesis.

4 CONCLUSION

This study highlights the significant potential of brewer's spent grain (BSG) as a valuable substrate for biotechnological applications, specifically to produce phenolic compounds and industrial enzymes. The continuous increase in the concentration of total phenolic compounds and proteins over the 7-day fermentation period points to the potential of *Talaromyces stollii* in valorizing BSG. The observed peak in laccase enzyme activity on the fourth day of fermentation, followed by a decline, suggests the presence of regulatory mechanisms, potentially influenced by protease activity. These findings not only demonstrate the feasibility of using BSG for high-value product generation but also emphasize the need for further research the implementation of separation methods such as microfiltration, ultrafiltration, and even salting out steps to be used as a tool to help dissociate the protein fraction from the phenolic one and obtain purer extracts for characterizing the components being obtained in the fermentations. Ultimately, the bioconversion of BSG presents a promising strategy for sustainable waste management and the development of value-added products in the agro-industrial sector.

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