Effect of *Saccharomyces cerevisiae* and nitrogen compounds on the fermentation of banana pulp (*Musa* spp.)

Efecto de *Saccharomyces cerevisiae* y compuestos nitrogenados en la fermentación de la pulpa de banano (*Musa* spp.)

Efeito de *Saccharomyces cerevisiae* e compostos azotados na fermentação da polpa de banana (*Musa* spp.)

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Abstract

The use of agro-industrial by-products, such as banana pulp (*Musa* spp.), represents a sustainable alternative for animal production, reducing costs and improving resource utilization. The study aimed to evaluate the effect of *Saccharomyces cerevisiae*, urea, and ammonium sulfate on the nutritional value of banana pulp, seeking to optimize its bromatological properties to transform it into a nutritionally viable and sustainable input. A completely randomized experimental design with a factorial arrangement was employed, considering two treatment levels: 1 % *Saccharomyces cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate, and 1.5 % *Saccharomyces cerevisiae*, 1 % urea, and 0.2 % ammonium sulfate. The aerobic fermentation times studied were 2, 4, and 6 hours. The results showed that the best bromatological quality was achieved at 6 hours with 1 % *Saccharomyces cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate. However, the most economically efficient treatment was obtained with 1.5 % *Saccharomyces cerevisiae*, 1 % urea, and 0.2 % ammonium sulfate in 4 hours of fermentation, due to its lower energy consumption. These findings highlight the potential of banana pulp treated as a cost-effective and sustainable input, contributing to more efficient animal production systems.

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Resumen

El aprovechamiento de subproductos agroindustriales, como la pulpa de banano (*Musa* spp.), representa una alternativa sostenible para la producción animal, reduciendo costos y mejorando el uso de recursos. El estudio tuvo como objetivo evaluar el efecto de *Saccharomyces cerevisiae*, urea y sulfato de amonio sobre el valor nutricional de la pulpa de banano, buscando optimizar sus propiedades bromatológicas para convertirla en un insumo nutricionalmente viable y sostenible. Se empleó un diseño experimental completamente aleatorizado con un esquema factorial, considerando dos niveles de tratamiento: 1 % de *Saccharomyces cerevisiae*, 0,8 % de urea y 0,1 % de sulfato de amonio, y 1,5 % de *Saccharomyces cerevisiae*, 1 % de urea y 0,2 % de sulfato de amonio. Los tiempos de fermentación aeróbica estudiados fueron 2, 4 y 6 horas. Los resultados mostraron que la mejor calidad bromatológica se alcanzó a las 6 horas con 1 %de *Saccharomyces cerevisiae*, 0,8 % de urea y 0,1 % de sulfato de amonio. No obstante, el tratamiento más eficiente económicamente fue con 1,5 % de *Saccharomyces cerevisiae*, 1 % de urea y 0,2 % de sulfato de amonio en 4 horas de fermentación, debido a su menor consumo energético. Estos hallazgos destacan el potencial de la pulpa de banano tratada como un insumo rentable y sostenible, contribuyendo a sistemas de producción animal más eficientes.

Palabras clave: calidad bromatológica, banano, *Saccharomyces cerevisiae*, alimentación animal.

Resumo

A utilização de subprodutos agroindustriais, como a polpa de banana (*Musa* spp.), representa uma alternativa sustentável para a produção animal, reduzindo custos e melhorando a utilização dos recursos. O estudo teve como objetivo avaliar o efeito de Saccharomyces cerevisiae, ureia e sulfato de amónio no valor nutricional da polpa de banana, procurando otimizar as suas propriedades bromatológicas para a converter num input nutricionalmente viável e sustentável. Utilizou-se o delineamento experimental inteiramente casualizado, em esquema fatorial, considerando dois níveis de tratamento: 1 % de Saccharomyces cerevisiae, 0,8 % de ureia e 0,1 % de sulfato de amónio, e 1,5 % de Saccharomyces cerevisiae, 1 % de ureia e 0,2 % de sulfato de amónio. Os tempos de fermentação aeróbia estudados foram de 2, 4 e 6 horas. Os resultados mostraram que a melhor qualidade bromatológica foi atingida em 6 horas com 1% de *Saccharomyces cerevisiae*, 0,8 % de ureia e 0,1 % de sulfato de amónio. No entanto, o tratamento economicamente mais eficiente foi com 1,5 % de *Saccharomyces cerevisiae*, 1% de ureia e 0,2 % de sulfato de amónio em 4 horas de fermentação, devido ao seu menor consumo energético. Estas descobertas destacam o potencial da polpa de banana tratada como um insumo rentável e sustentável, contribuindo para sistemas de produção animal mais eficiente.

Palavras-chave: qualidade bromatológica, banana, *Saccharomyces cerevisiae*, ração animal.

Introduction

The development of efficient and nutritious diets is a central challenge in animal nutrition to optimize performance and health (Simeanu & Razvan, 2023), highlighting the importance of researching new food sources and applying biotechnology (Poel *et al*., 2020).

Banana pulp (*Musa* spp.) represents a potential energy source for animal feed in tropical areas (Mohd *et al*., 2022) however, its low protein content, the presence of antinutrients such as tannins, and its rapid degradation restrict its nutritional use (Vásquez *et al*., 2024).

According to Salazar-López *et al*. (2022), the addition of *Saccharomyces cerevisiae*, urea, and ammonium sulfate improves the nutritional value of agricultural by-products (Salazar *et al*., 2022). In this regard, Vera Chang *et al*. (2022) indicate that urea and ammonium sulfate promote the formation of microbial protein from the carbohydrates in banana pulp, increasing their nutritional value through ruminal action (Vásquez *et al*. , 2022). In this study, the effect of *Saccharomyces cerevisiae*, urea, and ammonium sulfate on the nutritional value of banana pulp was evaluated, seeking to optimize its bromatological properties to make it a nutritionally viable and sustainable input.

Materials and methods

Study location

The study was carried out at the Faculty of Agricultural Sciences of the Technical University of Babahoyo, located at kilometer 7.5 of the Babahoyo-Montalvo Road (UTM: X: 1.7723946; Y: 79.71025931). The area has a humid tropical climate, characterized by temperatures ranging from 24 to 26°C, 88 % relative humidity, 1,262 mm of annual precipitation, an altitude of 8 meters above sea level, and 990 hours of sunshine per year.

Population studied

The behavior of brewer's yeast under different fermentation times was analyzed to evaluate its performance under various experimental conditions.

Plant material

One hundred kilograms (100 kg) of banana pulp was used as a representative sample to evaluate treatments based on fermentation times and added ingredients.

Preparation and homogenization

The banana pulp samples were prepared in a single batch in order to ensure homogeneity in both quantity and quality of the material, which allowed maintaining consistent experimental conditions and ensuring the reliability of the results obtained.

Chemical ingredients

Addition of urea

In the experimental treatment, 0.8 % and 1 % urea were added to two mixtures of 100 kg of banana pulp, respectively. Urea facilitates the breakdown of proteins in the pulp into essential amino acids, enriching their nutritional profile. This process allows the protein and amino acid content to be significantly increased, thus improving the value of the pulp as an ingredient for animal feed (Rigueira *et al*., 2021).

Addition of ammonium sulfate

Zero-point one percent (0.1 %) ammonium sulfate was added to a mixture of 100 kg of banana pulp, and 0.2 % was added to another sample of equal mass. This compound, as a source of nitrogen, stimulates the growth and metabolism of brewer's yeast, enhancing its efficiency during fermentation and optimizing treatment results (Yang *et al*., 2021).

Determination of fermentation times

Three fermentation times (2, 4, and 6 hours) were considered in order to analyze the behavior of the yeast throughout the fermentation process, allowing a detailed comparison of its performance in each phase and an evaluation of its efficiency.

Grouping by fermentation time

The samples were fermented for 2, 4, and 6 hours, using mixtures with uniform ingredients to ensure that the observed variations are due exclusively to the fermentation time, thus guaranteeing the validity of the results.

Quality control

An exhaustive quality control was performed to ensure the homogeneity of the banana pulp before and after fermentation, evaluating key physical and chemical parameters. Samples that did not meet cleanliness and hygiene standards were discarded, guaranteeing the reliability of the experimental results.

Fermentation technique

Concentrations of *Saccharomyces cerevisiae* (1 % and 1.5 %) were incorporated into the banana pulp and stored in hermetically sealed, sterilized 200 mL tanks. The mixture was properly homogenized, and representative samples were taken for bromatological analysis.

Saccharification technique

An enzymatic saccharification process was applied to the previously treated banana pulp, following the methodology used by Gu *et al*., (2020) to evaluate the breakdown of structural carbohydrates, such as starch and cellulose, in banana pulp during processing.

The saccharification technique allows the evaluation of the breakdown of structural carbohydrates into reduced sugars, quantifying the simple sugars released, and providing key information about the efficiency of the process.

Treatments under study

Table 1 describes the treatments studied in the field.

Operationalization of variables

Dependent variables

Bromatological composition: protein, NDF, ADF, energy, fat, moisture, ash, dry matter, from experimental banana pulp treatments.

Independent variable

Incorporation levels of *Saccharomyces cerevisiae* yeast at 1 % and 1.5 %, urea at 0.8 % and 1 %, and ammonium sulfate at 0.1 % and 0.2 %. Aerobic fermentation times: 2, 4, and 6 hours.

Parameters to be evaluated

Bromatological parameters were evaluated using standard methods (AOAC, 2005): Kjeldahl, for crude protein (AOAC 984.13), Soxhlet, for ether extract (AOAC 920.39), bomb calorimetry, for gross energy (AOAC 983.23), oven drying, to determine moisture (AOAC 934.01), and calcination in a muffle oven, for total ash (AOAC 942.05).

Experimental design and statistical analysis

The study used a Completely Randomized Design (CRD) with six treatments and three replications, totaling 18 experimental units. Two main factors were evaluated: the concentration of *Saccharomyces cerevisiae* (1 % and 1.5 %) and the fermentation time (2, 4, and 6 hours). An analysis of variance (ANOVA) and Tukey's mean comparison tests ($p \leq 0.05$) were applied using IBM SPSS Statistics 24.0 software.

Results and discussion

Dry matter

Figure 1 presents the results of the dry matter content in fermented banana pulp with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

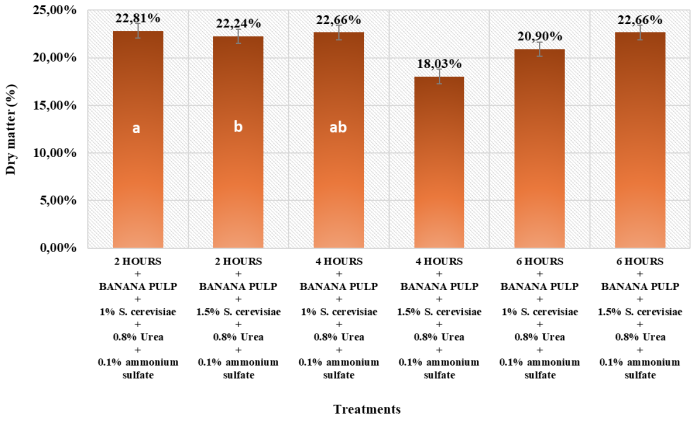

Figure 1. Dry matter content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

The statistical analysis showed a highly significant model ($p < 0.0001$), with relevant effects of the factors time, pulp, and their interaction (time × pulp) on this variable. The model fit was excellent ($R^2 = 0.99$; CV = 0.78 %), and Tukey's test showed significant differences ($p < 0.05$) between fermentation times. In the formulation with 1 % *S. cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate, the highest dry matter content was observed at 2 h (22.81 %), followed by a slight decrease at 4 h (22.66 %) and a more marked reduction at 6 h (20.90 %). These variations can be attributed to the enzymatic activity of the yeast, which temporarily alters the pulp's structure and its water-retention capacity, thus affecting the solids concentration. The significant interaction suggests that the proper combination of fermentation time and formulation can optimize the nutritional profile of the fermented product.

The dry matter content showed moderate variations depending on the fermentation time and the formulation. A higher content was observed at 2 h, a decrease at 4 h, and a new increase at 6 h. These changes are attributed to the enzymatic activity of *Saccharomyces cerevisiae*, which temporarily altered the pulp's structure and its water-retention capacity, thereby affecting the solids concentration. Suárez *et al*.(2016), described similar fluctuations during fermentation processes, attributing them to physical changes associated with contraction and expansion under microbial activity.

Protein

A significant effect ($p < 0.0001$) of time, formulation, and their interaction on protein content was evidenced (figure 2).

Table 1. Description of treatments.

Treatments	Description
T1	2 hours + Banana pulp + 1 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate
T2	2 hours + Banana pulp + 1.5 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate
T3	4 hours + Banana pulp + 1 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate
T4	4 hours + Banana pulp + 1.5 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate
T5	6 hours + Banana pulp + 1 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate
T6	6 hours + Banana pulp + 1.5 % *S. cerevisiae* + 0.8 % Urea + 0.1 % ammonium sulfate

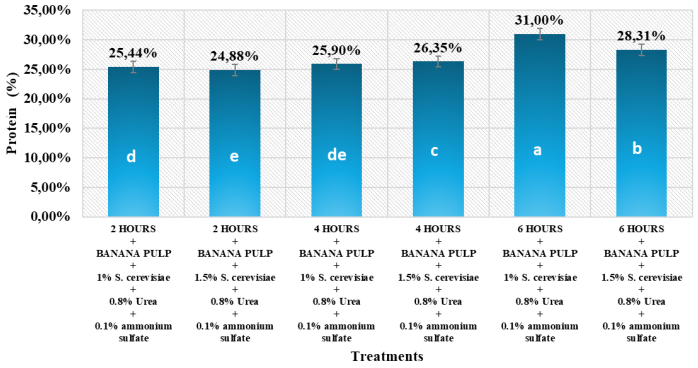

Figure 2. Protein content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

The highest concentration was obtained at 6 h with the formulation containing the lowest additive load, this being a value of 31 %, while the most concentrated registered the lowest value at 2 h, resulting in 24.88 % protein. These results underscore the importance of adjusting both factors to optimize protein synthesis. The increase in protein is related to the highest metabolic activity and nitrogen assimilation by *S. cerevisiae* in prolonged fermentations. Fernandez *et al*.(2021), reported similar findings in fruit matrices enriched with yeast and nitrogen, highlighting the potential of the process to valorize agro-industrial by-products and deepen the understanding of protein metabolism in tropical fruits.

Fat

Figure 3 shows the results obtained for the fat content (%) in banana pulp (*Musa* spp) fermented with different levels of *S cerevisiae*, urea, and ammonium sulfate.

Significant effects ($p < 0.05$) of the factors time, pulp, and their interaction on fat content (ether extract) were observed. Tukey's test indicated significant differences between 6 and 4 hours, but not between 4 and 2 hours. Regarding the pulp factor, the formulation with 1 % *Saccharomyces cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate had the highest fat content, being 2.84 % at 6 hours, while in the formulation of 1.5 %, the highest fat content was 5.88 % at 6 hours. These results confirm that both the fermentation time and the composition of the pulp significantly affect this parameter, highlighting this formulation as the most effective.

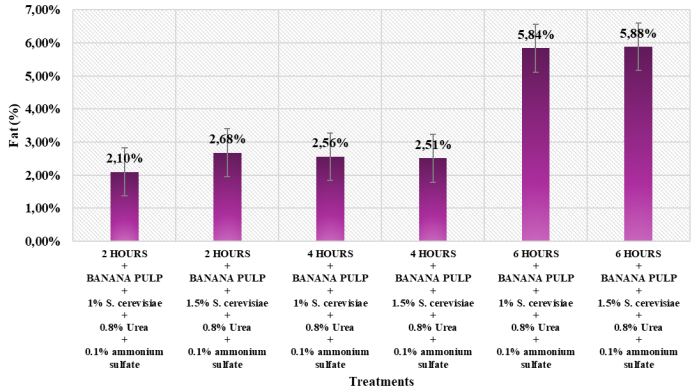

Figure 3. Fat content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

This behavior was consistent with the results obtained by Souza *et al*. (2025), who reported an increase in lipid content during the fermentation of agro-industrial by-products, with a maximum point at 6 hours. This pattern was attributed to the intensification of the lipolytic activity of *S. cerevisiae*, capable of mobilizing and transforming structural and residual lipids present in the plant matrix as the fermentation process progresses.

From a practical perspective, these results indicated that proper formulation selection and fermentation time could optimize fat content, an important attribute for the energy and functional value of the final product. At the theoretical level, the study provided evidence on the direct relationship between microbial lipid metabolism and fermentation conditions applied to fruit matrices.

Ashes

Figure 4 presents the results obtained from the ash content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

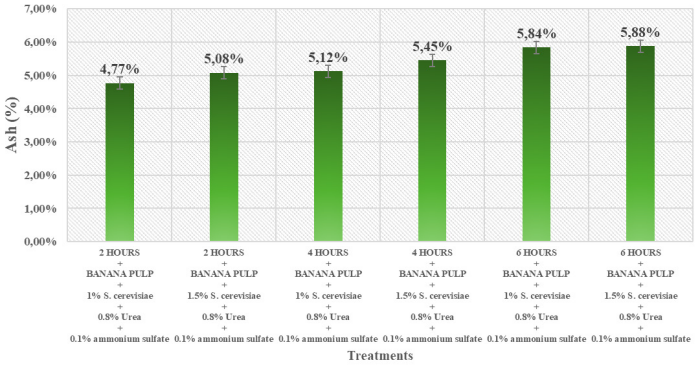

Figure 4. Ash content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

The analysis of variance indicated a significant model ($p < 0.0001$), with the effects of the factors time ($p < 0.0001$) and pulp ($p = 0.0333$) on ash content.

The time*pulp interaction was not significant ($p = 0.4021$), suggesting that the effect of the formulation does not vary with fermentation time. Tukey's test showed significant differences between the three times (2, 4, and 6 hours) and between the pulp formulations.

Basically, the ash content increased as a function of fermentation time, remaining within the acceptable ranges for animal feed (<10 % for poultry and <12 % for livestock), regardless of the composition used, in which the highest value was presented in the treatment 6 hours + banana pulp + 1.5 % *Saccharomyces cerevisiae* + 0.8 % urea + 0.1 % ammonium sulfate, with a value of 5.88 %, while the opposite occurred in the treatment 2 hours + banana pulp + 1 % *Saccharomyces cerevisiae* + 0.8 % urea + 0.1 % ammonium sulfate, in which a low value of 4.77 % was observed.

Ash content showed a significant increase with fermentation time ($p < 0.0001$), without significant interaction with the formulation ($p = 0.4021$), which indicated that the accumulation of minerals had an effect given by the fermentation time directly associated with time. This increase can be attributed to the degradation of organic matter by *Saccharomyces cerevisiae*, which concentrated the minerals present in the substrate. Similar results were reported in the study conducted by Kong *et al*. (2019), who observed increases in the mineral fraction of agro-industrial by-products after fermentation with yeasts.

Fiber

The results obtained from the fiber content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate are shown in figure 5.

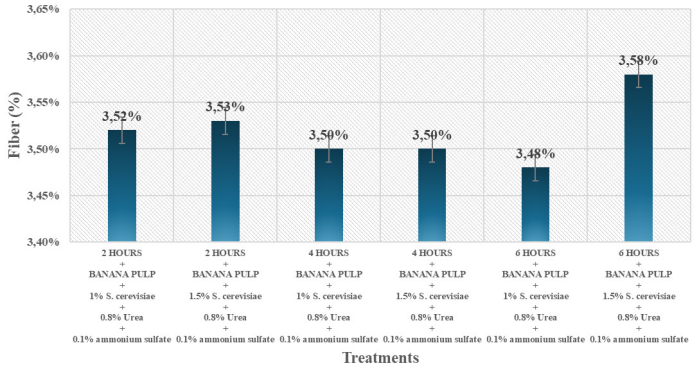

Figure 5. Fiber content (%) in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

The statistical analysis showed that the general model is not significant ($p = 0.9936$), indicating that the factors evaluated (time, pulp, and their interaction) do not have a relevant effect on fiber content. No significant differences were observed between time levels (2, 4, and 6 hours) or between pulp formulations. In addition, the time-pulp interaction also showed no significant effects. Although the data show low variability (CV = 5.84 %), the model lacks explanatory power regarding the response variable.

This result could be explained by the low degradability of the fibrous fraction under moderate fermentation conditions, especially when using yeasts such as *Saccharomyces cerevisiae*, which lack enzymes capable of hydrolyzing cellulose or hemicellulose. Instead, these yeasts preferentially metabolize simple sugars as monosaccharides and disaccharides. This phenomenon was also reported by Mutsokoti *et al*. (2017), who found little modification of crude fiber in residues fermented with non-cellulolytic yeasts.

Nitrogen-free extract

The results of the analysis of the nitrogen-free extract (NFE) presented in figure 6 showed an effect between the fermentation time and the composition of the pulp on the sugar and carbohydrate content ($p < 0.0001$).

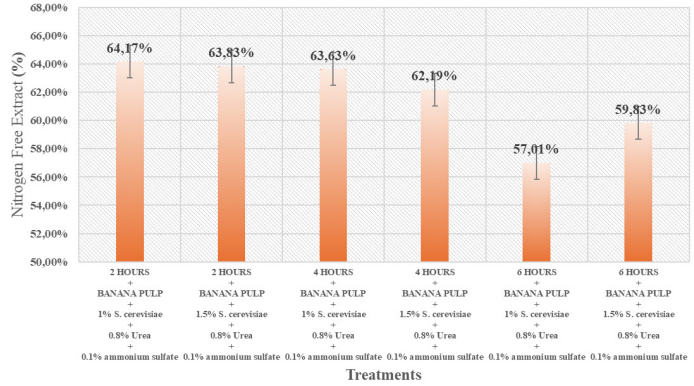

Figure 6. Nitrogen-free extract (%) content in banana pulp (*Musa* spp) fermented with different levels of *Saccharomyces cerevisiae*, urea, and ammonium sulfate.

Although a progressive decrease in NFE was observed with increasing time, no significant differences were found between the 2-, 4-, and 6-hour intervals. Regarding composition, the formulation with 1 % *Saccharomyces cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate presented significantly higher NFE values ($p = 0.0032$) compared to the formulation with higher additive concentrations, reaching 64.17 %. The time*pulp interaction was also significant, highlighting a higher retention of sugars in the first formulation, especially at 2 and 4 hours, although with an overall reduction at 6 hours.

Previous studies have shown that the decrease in carbohydrate content during fermentation is a recurrent phenomenon in fruit matrices, due to the use of sugars as a primary source of energy for microbial growth. (Briz *et al*., 2016), reported similar reductions in soluble sugars during fermentation of tropical fruits, explaining that this decline is related to the rapid glycolytic activity of *Saccharomyces cerevisiae*, which converts glucose and fructose into biomass, ethanol, and CO_2 under limited aerobic conditions.

Conclusions

Based on the objectives set and the results obtained in this research, it is concluded that the aerobic fermentation of banana pulp with the incorporation of *Saccharomyces cerevisiae*, urea, and ammonium sulfate produced significant improvements in its bromatological characteristics.

The treatment composed of 1 % *Saccharomyces cerevisiae*, 0.8 % urea, and 0.1 % ammonium sulfate at 6 hours of fermentation, proved to be optimal according to the bromatological and statistical analyses carried out. This treatment provided an adequate balance between nutritional quality and the efficiency of the fermentation process, clearly surpassing other formulations evaluated, where its use is recommended at animal production scale, highlighting the importance of using an agro-industrial banana by-product as a sustainable and profitable input.

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