CHARACTERIZATION OF RESIDUAL BREWER'S YEAST AND STUDY OF ITS POTENTIAL APPLICATION FOR HYALURONIC ACID PRODUCTION.

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ABSTRACT

The search for the reuse of waste is essential to promote sustainability and avoid environmental impacts. The brewing industry, responsible for producing billions of liters of beer, uses yeast as one of its main inputs. However, this yeast is discarded after a few cycles of use. This study characterized the yeast from beer production and investigated its potential for producing hyaluronic acid. Acidity, moisture, ash, lipids, proteins, sugars, and pH were analyzed. The residual yeast had a high protein content of 41.69% and was then used as a substitute for the conventional protein source to produce hyaluronic acid. The results indicated the feasibility of this approach. The residual yeast obtained the highest yield of hyaluronic acid, 0.37%, about the conventional yeast, 0.15%, an increase in the yield of 106%, demonstrating the potential of application in biotechnology, contributing to the valorization of this residue.

Keywords: Spent yeast. Fermentation Process, Beer Residue, Hyaluronic Acid, Industrial Waste Recovery.

1 INTRODUCTION

Beer stands out as one of the most consumed beverages worldwide. Large-scale by-products such as surplus malt grains, used yeasts, and leftover hops are generated during production. These wastes are rich in protein and energy sources and are often used as animal feed. However, these materials have great potential to be reused in biotechnological processes to produce compounds with higher added value. This includes using these by-products as a means for the cultivation of microorganisms, thus contributing to the reduction of waste disposal, valorization of materials, and mitigation of environmental impacts [1, 2, 3, 4]. Given this, companies are concerned about environmental responsibility since the brewing industry generates considerable waste throughout the process, including yeast residue discarded after use for certain cycles, with high polluting potential due to factors such as organic load and solids content [5].

In the brewing process, 1.5 to 3 kg of yeast waste is produced from the biomass of the microorganisms responsible for fermentation. These residues comprise proteins (35-60%), carbohydrates, minerals, vitamins, and amino acids[3]. The residual yeast extract from a craft brewery as a source of organic nitrogen in the submerged cultivation of *Bacillus sp*. was favorable to microbial biomass formation. The substrate's conversion factor into cells was favorable to the culture, and the cell yield of the cultures was supplemented with brewer's yeast extract[6].

Beer residues, such as residual yeast, are attractive for use as substrates for the cell growth of microorganisms with the potential for hyaluronic acid production since nitrogenous vitamins and nutrient complexes are essential for the cultivation of different classes of microorganisms, including *Streptococcus zooepidemicus* and the production of hyaluronic acid, a linear polysaccharide of high molecular weight, widely used in medicine, cosmetics, food and health, being considered the most influential biopolymer due to its properties of viscoelasticity, hygroscopicity, immunogenicity and biocompatibility[7].

For fermentation, *S. zooepidemicus* microorganisms submerged in a culture medium containing yeast extract, peptones, or casein hydrolysate is commonly used as a source of nitrogen and growth factors such as magnesium and phosphate [8, 9, 10, 11]. Given the above, this study aims to characterize the spent yeast of beer to evaluate its use as a source of protein in the fermentation process for the production of hyaluronic acid.

2 MATERIAL & METHODS

The residual yeast used for the gift was kindly provided from a brewery in the west of Santa Catarina, Brazil, after 6 cycles of the beer production process. An aliquot of residual yeast was dried in an oven for 24 hours at 60 ºC to determine lipids. A wet aliquot was used to determine acidity, moisture, ash, total nitrogen, proteins, and pH, and all determinations were performed in triplicate. The spent yeast was dehydrated in a lyophilizer LIOTOP L101 for the fermentation process, according to Figure 1.

The analysis of acidity, moisture, ash, lipids, and pH was performed according to the methodologies of the Adolfo Lutz Institute[12], the nitrogen and protein content by the Kjeldahl method[13], and the sugar content by the DNS method[14].

The microorganism used for microbial fermentation and hyaluronic acid production was *Streptococcus equi subsp. zooepidemicus* ATCC 39920 CCT FAT.

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Figure 1- a) Wet brewer's yeast, b) Freeze-dried brewer's yeast.

The *S. zooepidemicus* strain was cultured in a solid culture medium containing Soy Tryptone Agar (TSA) at 37 °C for 24 hours. The colonies were collected and suspended in sterile 0.9% saline solution until a standardized inoculum was obtained, with absorbance reading (optical density) between 0.08 and 0.1 (10^8 CFU/mL) at λ=600 nm, using a spectrophotometer. Fermentation was carried out in Erlenmeyer and kept under agitation at 150 rpm at 37 °C, pH 7.0 maintained for 12 hours.

The culture media for fermentation were prepared as shown in Table 1.

Table 1- Combinations of alternative and conventional culture media.

Substrate	Alternative	Conventional
Glicose (g/L)	40	40
Triptona (g/L)	-	20
Brewer's residual yeast (g/L)	20	-
Potassium phosphate KH_2PO_4 (g/L)	2,00	2,00
Sulfato de amônio $(NH_4)_2SO_4$ (g/L)	0,50	0,50
Magnesium sulfate $MgSO_4$ (g/L)	0,50	0,50

After fermentation, the fermented medium was centrifuged at 3300 rpm for 15 min, the cell-free supernatant was precipitated with ethanol in a 2:1 (v/v) ratio, and the supernatant ethanol was kept cool at 4 °C for 1 h for H.A. precipitation. It was then centrifuged again at 3300 rpm for 15 min; the hyaluronic acid separated from the ethanol was resuspended in distilled water and quantified by the CTAB method - Cetyltrimethylammonium Bromide[15].

3 RESULTS & DISCUSSION

The result of the characterization of the residual brewer's yeast can be seen in Table 2. The acidity found for the residual yeast was 6.53% ± 0.230. The humidity corresponds to the weight loss suffered by the product when heated in conditions in which water is removed; the quantification resulted in a value of 83.04%, ± 1.374, An ash content of 5.96% ± 0.001, lipid content of 0.64% ± 0.004, pH of 4.79 and sugar content of 2.35 g/L was obtained, The total nitrogen content was 6.67% and 41.69% total protein, a value within the range presented in the literature3 of 35-60%, indicating that it is a good protein source for several applications.

Table 2- Characterization of spent brewer's yeast

Parameter	Quantification
Acidity	6.53% ± 0.230
Humidity	83.04%, ± 1.374
Ash	5.96% ± 0.001
Lipid	0.64% ± 0.004
pH	4,79
Sugar	2.35 g/L
Nitrogen	6,67%
Protein	41,69%

After identifying the characteristics of the residual yeast from the brewing industry and verifying its considerable concentration of total nitrogen and proteins, its application in the fermentation process was proposed using the microorganism *S. zooepidemicus*. This approach proposes to replace the conventional nitrogen source with a sustainable alternative to the disposal of industrial waste.

The use of residual yeast as a nitrogen source in the fermentation process contributed significantly to the production of hyaluronic acid, Figure 2. For 20 g/L of residual yeast used as an alternative nitrogen source, 0.074 ± 1.034 g/L of hyaluronic acid was obtained, yielding 0.37%. 20 g/L of the conventional nitrogen source, 0.031 g/L was produced, ± 0.909 and 0.15% yield. Using the alternative nitrogen source increased the yield in hyaluronic acid production by 106% in the study, where the optimization of the composition of the fermentative medium composed of glucose and soy peptone as an alternative source of nitrogen increased the yield of hyaluronic acid produced by the fermentation of *S. zooepidermicus* by 65%[16].

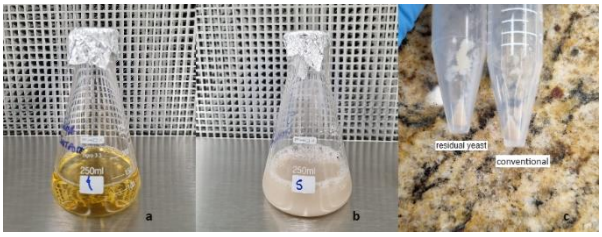

Figure 2- a) Fermentação convencional; b) Fermentation with residual brewer's yeast; c) Hyaluronic acid produced.

Studies indicate that the efficient production of hyaluronic acid by *S. zooepidermicus* requires a high concentration of glucose, moderate aeration, neutral pH, nitrogenous compounds, and vitamins[17]. The presence of B vitamins in the substrate is crucial for production and growth[11]. Carbon and nitrogen sources are vital for microbial growth and hyaluronic acid synthesis, and amino acids can increase production[18]. The results of this study corroborate these statements since the spent yeast residue used in fermentation, with high nitrogen and protein content, significantly increased the yield of hyaluronic acid compared to conventional methods due to the presence of carbohydrates, minerals, vitamins, and amino acids[3].

4 CONCLUSION

Analyses have shown that yeast is a promising protein source in the fermentation process with *S. zooepidemicus* and hyaluronic acid synthesis, resulting in a 106% increase in yield compared to conventional yeast. This increase is attributed to carbohydrates, minerals, vitamins, and amino acids, which favor microbial growth. The study highlights the biotechnological potential of residual brewer's yeast, contributing to the valorization of this waste and providing important parameters for its reuse.

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