KINETIC AND PHYSICO-CHEMICAL PROFILING OF A BEER PREPARED FROM SEQUENTIAL FERMENTATION WITH KEFIR AND *S. Cerevisiae* COLONIES

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ABSTRACT

The growing demand for premium beers signals a change in the country's beer consumption pattern, with consumers looking for better quality and more sensorial innovative beverages. In this context, this work evaluated and characterized the fermentation process of a beer produced using probiotic kefir colonies with a view to new possibilities for innovation. Fermentation was carried out sequentially, and during the fermentation process, the density, alcohol content, and dry biomass were evaluated to determine the specific cell growth rates, ethanol production, and kinetic parameters. In addition, the following physicochemical parameters were evaluated in the finished beer: apparent extract, real extract, primitive extract, alcohol by volume, and color. It was found that ethanol production was partially linked to cell growth and that the beer produced met the quality standards required by Brazilian regulations for classification as beer.

Keywords: Kefir. Beer. Fermentation.

1 INTRODUCTION

The pattern of beer consumption in Brazil has been directly impacted by the COVID-19 pandemic. It has continued over the following years, with consumers looking for drinks that deliver innovation and better quality. In 2023, Brazil produced 15.4 billion liters of beer, representing a more than 15% growth compared to previous research[1, 2]. This increase in consumption is due to the demand for premium beers, which differ from conventional beers in that they are produced from selected ingredients and offer a different sensory experience.

There are many ways to innovate in the brewing process. However, research has been carried out using non-traditional yeasts since it is during fermentation that an important part of the sensory profile of the drink is built. Thus, to meet these new market demands for products that offer quality combined with unique experiences, probiotic *kefir* colonies were applied to a brewing process with the traditional brewer's yeast *S. cerevisiae* in a sequential fermentation regime, aiming to evaluate and characterize the fermentation process kinetically and physico-chemically.

2 MATERIAL & METHODS

The brewing process begins with the production of the wort. For this, the Brew in a Bag (BIAB) technique was used, a method widely used by craft brewers, in which all the mashing is carried out in a single step with the aid of a grain bag made of non-toxic material, responsible for containing all the malt during the procedure, separating it from the wort in production and dispensing stages such as washing the grains with secondary water and recirculating the wort. Using a temperature ramp ranging from 62 °C to 76 °C for around 100 minutes, the wort was produced from 20 L of mineral water and 2.5 kg of previously milled Pilsen malt. The wort was then boiled for 60 minutes, and hops were added.

Once the mashing process was complete, the wort was cooled using a chiller and transferred to the fermentation tank to be inoculated with the desired strains in the appropriate order. Since fermentation occurred sequentially, the procedure began with inoculating probiotic kefir colonies (20 g. L^{-1}). Once the density reading had stabilized, the probiotic colonies were removed, and fermentation continued with the addition of *S. cerevisiae* (0.6 g. L^{-1}). The entire fermentation process was carried out at a temperature of 18 °C.

To study fermentation kinetics, daily and periodic samples were taken to monitor the evolution of dry biomass (g), using the gravimetric method, total dissolved solids (TSS, °P), measured using a digital refractometer, and alcohol by volume (ABV, %), determined using densimetry. Once these data were available, it was possible to determine the specific rate of cell growth (μ_X), substrate consumption (μ_S), and alcohol production (μ_P) according to the methodologies presented in the reference methodology[3, 4]. In addition, the yield factors from substrate to cell ($Y_{X/S}$), relationship between cell and product ($Y_{X/P}$), and yield factors from substrate to product ($Y_{P/S}$) were determined[3].

The physical-chemical characterization was carried out using methodologies 246/IV, 247/IV, 249/IV, 250/IV, and 251/IV, found in Instituto Adolfo Lutz (2008), to determine alcohol by volume and the apparent, real, and primitive extracts[5]. The color of the beer produced was determined using spectrophotometry[6].

3 RESULTS & DISCUSSION

The sequential fermentation was carried out in two separate stages. Initially, the beer wort was inoculated only with probiotic kefir colonies, and once the relative density reading had stabilized, the probiotic colonies were removed, and the commercial strain of *S. cerevisiae* was inoculated to finish attenuating the beer. The first stage lasted 120 hours, starting from a relative density of 1.052 g.cm^{-3} and reaching 1.018 g.cm^{-3}. In the final stage, which lasted 72 hours, the density varied from 1.018 g.cm^{-3} to 1.015 g.cm^{-3}. The whole fermentation lasted 192 hours, resulting in a beer with a final relative density of 1.015 g.cm^{-3}and an estimated alcohol content of 4.86%.

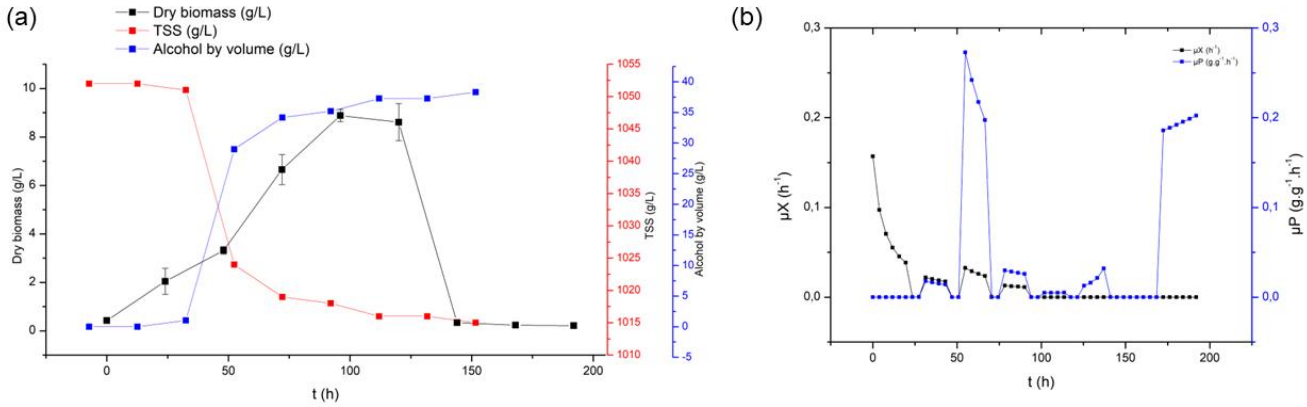

Figure 1 Kinetic curves (a), specific cell growth rate, and specific ethanol production rate (b).

The characterization of the fermentation process can also be divided into two stages. In the first phase, which extends from the start of fermentation to 120 hours later, it can be seen (Figure 1 (b)) that the maximum specific cell growth rate (0.16 h^{-1}) occurs at the start of fermentation, and the maximum ethanol production rate (0.27 g. g^{-1} h^{-1}) will only occur after approximately 54 hours of fermentation. In the second stage, which begins after the *kefir* colonies are removed and the *S. cerevisiae* is added, it can be seen (Figure 1 (b)) that there is no longer any cell growth but still ethanol production.

Regarding the kinetic parameters (Table 1), no studies were found in the literature that could be used for comparison purposes. Even so, it is important to highlight both negative values obtained for the substrate-to-cell ($Y_{X/S}$) and product-to-cell ($Y_{X/P}$) yield factors. These values can be explained by the method used to ferment the beverage: sequential fermentation. With the removal of the kefir colonies from the process after 120 hours of fermentation, there was a drastic reduction in the present biomass, as seen in Figure 1 (a). Since the calculation of the yield factors considered the fermentation process as a whole, without separating it into stages, at the end of the process, there was less biomass than at the beginning, thus justifying the negative values.

Table 1 Kinetic parameters

Kinetic parameters	P_X (g. L^{-1}.h^{-1})	P_P (g. L^{-1}.h^{-1})	$Y_{X/S}$ (g.g^{-1})	$Y_{X/P}$ (g.g^{-1})	$Y_{P/S}$ (g.g^{-1})
	0.04	0.20	0.24	0.23	1.04

Brazilian legislation, through Normative Instruction N° 65, published in 2019, establishes quality standards for beer. Starting with the sequential fermentation method used, the beer produced in this study, according to the third paragraph of the Normative Instruction, would be classified as a multiple-fermentation beer. As for the alcohol content, this legislation establishes that for the beverage produced to be classified as beer, its alcohol content must be higher than 2% by volume (v/v%). Therefore, the beverage resulting from this study, having obtained a percentage of alcohol by volume equal to 6.68 ± 0.47%, according to the legislation, can be classified as beer. As for extracts, a classification referring to the amount of solids dissolved in the beer wort, the legislation only states that the primitive extract must be greater than or equal to 5% by weight. Therefore, according to the information available in Table 2, it can be seen that the beer produced for this study meets the quality standards required by Brazilian legislation[7].

As for color, the legislation used to classify beers as light or dark, but this classification was revoked in 2019. Even so, it is required that the analytical methods of the European Brewers Convention (EBC) be applied to analyze beer color[6]. As a result, according to the value obtained in Table 2, compared to the EBC scale for traditional beer styles, the beer produced from kefir colonies is close to the color of Pilsen-type beers.

Table 2 Physico-chemical parameters

Parâmetros físico-químicos	
Alcohol by volume (%, v/v)	6.68 ± 0.47
Real extract (%, g/100 g)	4.49 ± 0.77

Apparent extract (%, g/100 g)	7.56 ± 0.07
Primitive extract (%, m/m)	14.79 ± 0.05
Color (EBC)	11.69 ± 0.33

4 CONCLUSION

This study presents an innovative methodology for producing beer, using sequential fermentation with probiotic kefir colonies and a commercial strain of *S. cerevisiae*, demonstrating the viability of the process. The resulting beer had an alcohol content of approximately 6.68% v/v, a primitive extract greater than 5% by weight, and the color of the beer was similar to Pilsen, meeting the quality standards set by Brazilian legislation. Sequential fermentation provided alcohol production partially related to cell growth in the first stage (*kefir*) and independent in the second (*S. cerevisiae*). The negative values of the $Y_{X/S}$ and $Y_{X/P}$ yield factors were attributed to the reduction in biomass during the process, which was influenced by the sequential methodology. As a result, the study demonstrated the potential of sequential fermentation with *kefir* and *S. cerevisiae* for producing high-quality beers, where the results are promising and open avenues for further research and development of differentiated beer products.

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