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FERMENTATIVE PROCESS EVALUATION OF MEAD USING KEFIR AND ENCAPSULATED YEASTS

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ABSTRACT:
Mead is an alcoholic beverage obtained from the fermentation of water and honey, traditionally carried out using pure cultures of unicellular yeasts of the genus Saccharomyces. Alternatively, the cell immobilization process can be used to encapsulate microorganisms in a support material. A similar system occurs in kefir grains. These grains are composed of a polysaccharide matrix that brings together a variety of microbiota with fermentative capacity. This study aimed to monitor mead fermentation using three treatments, encapsulated yeast, water kefir, and spontaneous fermentation (control), and to evaluate their kinetic and physicochemical parameters. Similar pH and total acidity values were found for all treatments. A significant difference was found for density and total soluble solids analyses, with greater emphasis on the use of yeast, followed by kefir and, finally, the control treatment. Regarding the kinetic parameters, the encapsulated yeast treatment showed the highest productivity (0.202 mL/100 mL h), fermentation yield (49.13%), and efficiency (96.15%), followed by kefir (0.064 mL/100 mL h, 44.22%, and 86.56%, respectively) and the control treatment (0.041 mL/100 mL h, 41.31%, and 80.84%, respectively). The final ethanol contents were 6.80% v/v for the encapsulated yeast treatment, 4.77% v/v for kefir, and 2.67% v/v for the control. The results demonstrated the feasibility of using encapsulated yeast and water kefir as fermentative agents for mead production, although further optimization of process conditions is required.

Keywords: Alcoholic fermentation; Cell immobilization; Fermentation kinetics.

AVALIAÇÃO DO PROCESSO FERMENTATIVO DE HIDROMEL UTILIZANDO KEFIR E LEVEDURAS ENCAPSULADAS

RESUMO:
O hidromel é uma bebida alcoólica obtida pela fermentação de água e mel, tradicionalmente realizada por cultivo puro de leveduras unicelulares do gênero *Saccharomyces*. Como alternativa, pode-se utilizar o processo de imobilização celular, no qual os microrganismos são envolvidos por um material suporte, formando um sistema semelhante aos grãos de kefir, compostos por uma matriz polissacarídica que reúne uma diversidade de microbiota com capacidade fermentativa. Com o objetivo de avaliar o comportamento dessas matrizes microbianas, este trabalho monitorou a fermentação do hidromel utilizando três diferentes tratamentos: leveduras encapsuladas, kefir e fermentação natural (controle), avaliando parâmetros cinéticos e físico-químicos do processo. Foram observados valores próximos de pH e acidez total para todos os tratamentos, porém com diferença significativa nas análises de densidade e sólidos solúveis totais, destacando-se o tratamento com leveduras encapsuladas, seguido pelo kefir e, por último, pelo tratamento controle. Em relação aos parâmetros cinéticos, o tratamento com leveduras encapsuladas apresentou a maior produtividade (0,202 mL/100 mL h), rendimento fermentativo (49,13%) e eficiência (96,15%), seguido pelo kefir (0,064

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mL/100 mL h, 44,22% e 86,56%, respectivamente) e pelo tratamento controle (0,041 mL/100 mL h, 41,31% e 80,84%, respectivamente). Os teores finais de etanol foram de 6,80% v/v para o tratamento com leveduras encapsuladas, 4,77% v/v para o kefir e 2,67% v/v para o controle. Os resultados demonstraram a viabilidade do uso de leveduras encapsuladas e do kefir de água na produção de hidromel, indicando possibilidades de aprimoramento das condições do processo e, consequentemente, da qualidade do produto obtido. Esses avanços poderão ser alcançados por meio de estudos mais aprofundados sobre as variáveis que interferem no processo de produção.

Palavras-chave: Fermentação alcoólica; Imobilização celular; Cinética fermentativa.

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INTRODUCTION

Dietary choices are crucial for ensuring balanced nutrition and preventing diseases through the incorporation of functional foods, which may represent a valuable strategy for maintaining long-term health (Altun *et al.*, 2021).

Functional foods are those that provide additional health benefits beyond their basic nutritional functions, thereby contributing to a reduced risk of chronic diseases (Granato *et al.*, 2017). Probiotics are live microorganisms that constitute functional components in foods. When consumed in adequate amounts, they can confer health benefits on the host through direct action or interactions with microorganisms in the intestine (Azizi *et al.*, 2021).

Examples of probiotics include kefir and bacteria belonging to the genera Lactobacillus and Bifidobacterium. The term kefir refers to a fermented beverage produced through the fermentation of kefir grains in milk- or water-based substrates (Azizi *et al.*, 2021). Kefir differs from other fermented products mainly because of its characteristic starter culture, known as kefir grains. These grains are approximately 1–4 cm in size and resemble small cauliflower florets because of their irregular, lobed morphology, with colors ranging from white to light yellow (Kesenkaş *et al.*, 2017). Studies indicate that microorganisms commonly found in these grains include lactic acid bacteria such as *Lactobacillus paracasei*, *L. nagelii*, and *L. hilgardii*, acetic acid bacteria belonging to the genus *Acetobacter*, and yeasts such as *S. cerevisiae* (Azizi *et al.*, 2021; Laureys and De Vuyst, 2014).

Given the fermentative potential of the microorganisms associated with kefir grains, their application can also be explored for the production of alcoholic beverages, including mead.

Mead is a beverage obtained through the alcoholic fermentation of a must prepared from honey, potable water, and, when necessary, nutrient salts**.** Yeasts belonging to the genus *Saccharomyces* are commonly used for mead fermentation. However, several factors can influence fermentation and impair the activity of free yeast cells in the must, potentially resulting in low ethanol production and the release of metabolites that may contribute to undesirable aromas, including esters, organic acids, and hydrogen sulfide. The use of free cells may also make downstream processes, such as cell separation, clarification, and filtration, more challenging.

Therefore, cell immobilization has been investigated as an alternative approach, particularly for yeast-based fermentations (Venturini Filho, 2016; Kovaleski, 2019). Cell immobilization consists of physically confining microbial cells within a support matrix while maintaining their metabolic activity. For yeast immobilization, calcium alginate can be used as a polymeric matrix to entrap cells and form beads that can subsequently be used in fermentation (Kovaleski, 2019).

Therefore, this study aimed to monitor mead fermentation and compare the kinetic and physicochemical parameters of three treatments: encapsulated yeast, water kefir, and spontaneous fermentation (control). The study also aimed to characterize the fermentative performance of these agents, assess the feasibility of using water kefir and encapsulated *Saccharomyces cerevisiae*, and evaluate the physicochemical characteristics of the final products.

MATERIAL AND METHODS

Activation of water kefir grains

The water kefir was purchased online, manufactured and shipped by the company Vida Probiótico in dehydrated form, with 1 gram per package. For activation, the water used in the process was filtered and boiled. The water kefir grains were activated according to the manufacturer's recommendations. The microbiological composition of the commercial water kefir grains was not provided by the manufacturer and is not characterized in the present study. Filtered and boiled water was used for the activation medium. For a volume of 200 mL, 8 g of brown sugar (4% w/v) was added. After homogenization and cooling to 25 °C, 1.1 g of sodium bicarbonate was added to the activation medium. Once the activation medium was ready, analyses were carried out following the methodology of the Adolfo Lutz Institute (IAL, 2008) for pH (pH meter Hanna Instruments, Italy) and total soluble solids (TSS) (Nova Instruments benchtop ABBE refractometer, Brazil) to verify suitable characteristics for kefir development.

Subsequently, the water kefir grains were transferred to sterilized glass jars containing the solution, homogenized, and incubated in a Shaker SL

222 (Solab Científica, Brazil) incubator at 25 ºC for two days. This activation process was performed in duplicate. After 48 h, the liquid was removed with the aid of a previously sanitized plastic sieve, and the kefir grains were washed with distilled water. The grains were then returned to the glass jar, and a new must prepared on the same day was added, following the same steps as the initial activation must. The withdrawn liquid was subjected to pH and TSS analyses again to evaluate the effective activation of the kefir.

The activation cycle was performed five times to ensure the recovery and adaptation of the dehydrated kefir grains to the activation medium and to obtain sufficient biomass for the subsequent fermentation assays. At the end of the activation period, the kefir grains were washed with distilled water and weighed, totaling approximately 4 g. The grains were then divided into three portions of 1.33 g for the experimental replicates.

Yeast encapsulation

Yeast encapsulation was performed following a modified version of the method described by Kovaleski (2019). One gram of sodium alginate was added to 30 mL of distilled water and allowed to stand for 5 min at room temperature to promote polymer hydration. The mixture was subsequently heated on a hot plate under continuous magnetic stirring until complete dissolution of the sodium alginate and gel formation. After cooling to room temperature, 1.33 g of active dry yeast *Saccharomyces cerevisiae* Lalvin K1-V1116 (Lallemand, Canada) was suspended in 10 mL of distilled water. The yeast suspension was then added to the cooled sodium alginate solution and homogenized under magnetic stirring until a uniform mixture was obtained. The initial cell concentration, cell viability, and viable cell count (CFU mL^{-1}) of the yeast inoculum were not experimentally determined in the present study.

The mixture was then placed into a previously sanitized syringe and suspended by a metal clamp attached to a universal stand. Extrusion took place in a 2% calcium chloride solution into which the spheres were dripped. This immobilization procedure was performed in triplicate, and the spheres remained immersed in the calcium chloride solution under refrigeration until their use.

Preparation and inoculation of the must

The must was prepared according to the adapted methodology described by Mendes-Ferreira *et al.* (2010), using boiled water and bee honey to obtain an initial concentration of approximately 14 °Brix. The mixture was homogenized under magnetic stirring until a homogeneous must was obtained and subsequently cooled to 25 °C. The pH, TSS, density, and total acidity of pure honey and the prepared must were determined in triplicate according to the methodology of the Instituto Adolfo Lutz (IAL, 2008). Density was determined using a hydrometer (HG Brasil, Brazil) while TSS, expressed as °Brix, was determined using a benchtop Abbe refractometer (Nova Instruments, Brazil).

Next, the total volume was divided into nine portions, each containing 1.140 L, and transferred to previously sanitized and labeled 2 L flasks. Three treatments were designated, each performed in triplicate: the first with water kefir (K1, K2, and K3), the second with encapsulated yeast (L1, L2, and L3), and the third, the control treatment, with natural fermentation (C1, C2, and C3).

For the kefir treatment, the pre-weighed and separated grains were added to the corresponding flasks. The encapsulated yeasts were removed from the calcium chloride solution, washed with distilled water, and then added to the respective musts. The control treatment consisted only of the must, without the addition of a starter culture.

Fermentation

Fermentation was carried out in an incubator at a controlled temperature of 25 °C, according to the conditions described by Borges *et al.* (2021), with adaptations. Throughout the fermentation, analyses of pH, total soluble solids (TSS), density, and total acidity were conducted at 0, 24, 48, 72, 96, 168, 216, 264, 336, 384, and 504 h (IAL, 2008). After fermentation, the meads were clarified by decantation to separate the beverage from the sedimented biomass and suspended solids, according to the post-fermentation procedures described by Simão *et al.* (2023), with adaptations. Subsequently, vacuum filtration was performed using a Büchner funnel connected to a vacuum filtration system with qualitative filter paper. The filtered meads were bottled in previously cleaned and sanitized glass bottles and stored under refrigeration at approximately 7 °C until subsequent analyses. The alcohol content (% v/v) was estimated from the original gravity (OG) and final gravity (FG) values according to Thesseling *et al.* (2019), using Equation 1:

$$ABV(\%) = (OG - FG) \times 131$$

(Equation 1)

Where: ABV is alcohol by volume (% v/v), OG is the original gravity before fermentation, and FG is the final gravity after fermentation.

Kinetic parameters

Fermentation yield and efficiency were calculated according to Aquarone *et al.* (2001). Fermentation yield ($Y_{s/p}$), fermentation efficiency (E, %), theoretical alcohol content (TA, %), productivity (mL/100 mL h), and fermentation rate (% v/v/h) were calculated according to Equations 2, 3, 4, 5, and 6, respectively.

$$Y_{S/P}(\%) = \frac{\text{Ethanol produced (\% v/v)}}{\text{Consumed sugars (\% v/v)}} \times 100$$

(Equation 2)

$$E(\%) = \frac{\text{Alcohol produced (\% v/v)}}{\text{Theoretical alcohol (\% v/v)}} \times 100$$

(Equation3)

$$TA(\%\, v/v) = \text{Consumed sugars (\% v/v)} \times 0.5111$$

(Equation 4)

$$P\left(\frac{mL}{100\,mL \cdot h}\right) = \frac{\text{Ethanol produced (\% v/v)}}{\text{Fermentation time (h)}}$$

(Equation 5)

$$V_s\left(\frac{\%\, v/v}{h}\right) = \frac{\text{Consumed sugars (\% v/v)}}{\text{Fermentation time (h)}}$$

(Equation 6)

Statistical analysis

The collected data were subjected to a single-factor analysis of variance (ANOVA) at a 95% confidence interval and the means were compared using the Tukey test at a 5% significance level ($p \leq 0.05$), with the assistance of Statistica 13.0 software (Statsoft Inc., USA).

RESULTS AND DISCUSSION

Obtaining fermentative agents

The activation process of the water kefir grains was considered successful, as the biomass increased from an initial mass of 1 g to approximately 4 g during the activation cycles. Previous studies have demonstrated that water kefir grain biomass can increase substantially during fermentation. Breselge *et al.* (2025) reported increases in grain mass of up to 236% after 48 h of fermentation. The authors also observed that fresh grains showed a mean biomass increase of 79.0%, whereas previously dried grains showed a mean increase of 18.2%, indicating that the physiological state of the grains can markedly influence their subsequent growth. These findings support the biomass development observed in the present study, particularly considering that the water kefir grains used herein were initially obtained in a dehydrated form. Further biomass development was also observed during mead fermentation, as some of the newly formed grains continued to increase in size.

During activation, the pH decreased from 9.08 ± 0.12 to 8.26 ± 0.06 after 48 h. This reduction is consistent with previous studies on water kefir fermentation, in which acidification is associated with microbial metabolism and the production of organic acids, particularly lactic and acetic acids. Lactic acid bacteria and acetic acid bacteria are mainly involved in the production of these acids, while yeasts contribute to the overall fermentative metabolism and the formation of ethanol and carbon dioxide (Laureys; De Vuyst, 2014).

Regarding total soluble solids, different behaviors have been reported. Esatbeyoglu *et al.* (2023) observed a significant reduction in °Brix during water kefir fermentation, from 10.4 ± 0.1 to 6.10 ± 0.28 °Brix in an aronia pomace-based substrate and from 11.8 ± 0.5 to 7.65 ± 0.92 °Brix in an aronia juice-based substrate after 72 h, which was attributed to sugar conversion into ethanol and CO_2. In contrast, Tireki (2025) reported an increase in total soluble solids after 72 h of water kefir fermentation, together with a decrease in pH, indicating that the behavior of this parameter may vary according to the physicochemical characteristics of the fermentation medium. Therefore, the slight increase in °Brix observed in the present study may be related to the specific composition of the activation medium and to soluble compounds formed or released during microbial metabolism.

The yeast encapsulation process was considered satisfactory, as the calcium alginate beads obtained in the present study had a diameter of approximately 0.2 cm (2.0 mm) and remained structurally stable, without visible fissures, throughout the fermentation process. This bead size is consistent with values reported in previous studies. Sousa-Dias *et al.* (2021) reported that alginate beads used for cell immobilization generally range from 0.2 to 2.0 mm in diameter, while a study with *Saccharomyces cerevisiae*-loaded calcium alginate beads reported a mean diameter of 1.62 ± 0.02 mm. Furthermore, they specifically evaluated immobilized *S. cerevisiae* for mead production and demonstrated the importance of maintaining the mechanical stability of alginate beads during fermentation. Therefore, the diameter and absence of visible fissures observed in the present study indicate adequate bead formation and structural stability under the evaluated fermentation conditions.

Physical-chemical parameters

Throughout fermentation, the microorganisms involved in the process produce organic acids, resulting in a decrease in pH and an increase in total acidity. This behavior has also been reported in previous mead studies and is associated with the formation of organic acids during microbial metabolism (Starowicz and Granvogl, 2020; Souza *et al.*, 2024). Among the treatments used in mead production, a more significant decrease in pH was observed during the first 24 h of fermentation with yeast, dropping from 4.43±0.06 to 3.98±0.05. In the initial days, yeast tends to consume nitrogen and produce H+ ions, along with acetic and succinic acids (Starowicz and Granvogl, 2020), resulting in a pronounced reduction in pH compared with the other two treatments throughout the process.

Kefir also influenced the pH and showed lower values compared to the naturally fermented must, with a more significant decrease after 48 h, reaching a pH of 3.94±0.02. This is associated with the diverse microbiota of the kefir grains, including yeasts and lactic acid bacteria like those of the *Leuconostoc* genus, which produce lactic and acetic acids as the main metabolites of glucose and fructose metabolism, components found in high amounts in honey (Monar *et al.*, 2014). In a study conducted by Barreto (2022) on the use of kefir for beer production, a more significant decrease was observed in the first 24 h after inoculation, ranging from an initial pH of 5.55±0.01 to 3.45±0.03. This decrease was greater than that observed in the present study. However, the temperature employed was 37 ºC, which falls within a more favorable range for the activity of lactic acid bacteria.

The temperature of 25 °C used in the present study was selected because it is commonly employed in mead fermentation and provides suitable conditions for yeast fermentative activity while also allowing the development of the microbial consortium associated with water kefir. Previous studies have successfully conducted mead fermentation at 25 °C using immobilized *Saccharomyces cerevisiae* and water kefir-associated microorganisms (Sousa-Dias *et al.*, 2021; Souza *et al.*, 2024). Temperature directly affects microbial growth and enzymatic activity. Lower temperatures generally reduce fermentation rates, whereas excessively high temperatures may affect microbial viability and metabolite production. Figure 1 presents the results of the pH analyses conducted.

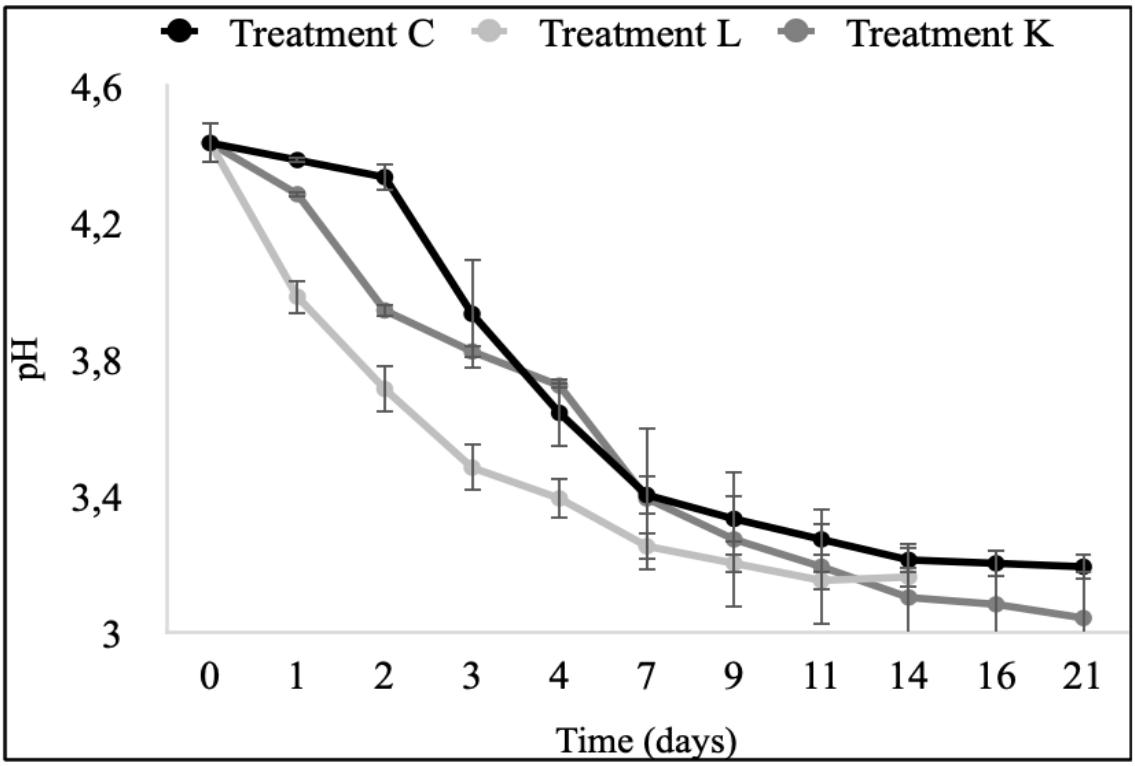


Figure 1. pH values during fermentation. Treatments: L = encapsulated yeast; K = water kefir; C = control (spontaneous fermentation).

Throughout the fermentation, the musts exhibited similar patterns of pH reduction, reaching close values in the final product: 3.19±0.04 for the control, 3.16±0.03 for the L treatment, and 3.04±0.14 for the K treatment. Although no significant differences in final pH were observed among treatments ($p > 0.05$), pH changed significantly over fermentation time in all treatments. This temporal variation is consistent with previous studies on mead fermentation, which have reported progressive acidification during fermentation as a consequence of organic acid formation by microbial metabolism. Souza *et al.* (2024), for example, observed a decrease in pH throughout mead fermentation with water kefir and *Saccharomyces boulardii*, accompanied by an increase in acidity, which was associated with the formation of organic acids during the fermentation process.

Total acidity increased earlier in the inoculated treatments. The increase became more pronounced after 24 h in the encapsulated yeast treatment and after 48 h in the kefir treatment, whereas the control showed a slower increase, with a more marked rise between the 3rd and 7th days of fermentation. This behavior is consistent with previous studies on mead fermentation. Sroka and Tuszyński (2007) reported that organic acid formation occurs mainly during the early stages of mead fermentation, particularly through the production of acetic and succinic acids, which contribute to the reduction in wort pH. Similarly, Souza *et al.* (2024) observed a progressive increase in total acidity and a decrease in pH during mead fermentation with water kefir and yeasts, with greater acidification in the treatment containing commercial *Saccharomyces cerevisiae*.

Therefore, the results of the present study are consistent with previous reports regarding the progressive acidification of mead and the greater acidification associated with inoculated yeast treatments. However, the present study showed a delayed increase in acidity in the spontaneous fermentation control, which may be related to the slower establishment of naturally occurring microorganisms in the absence of an added starter culture.

There were no significant differences among the treatments ($p > 0.05$). The acidity values were within the range of 50 to 130 meq L^{-1} established by Normative Instruction No. 34 of November 29, 2012 (Brasil, 2012). The values were 51.84±5.35, 54.08±1.47, and 64.32±6.00 meq L^{-1} for the control,

yeast, and the kefir treatments, respectively, as shown in Figure 2

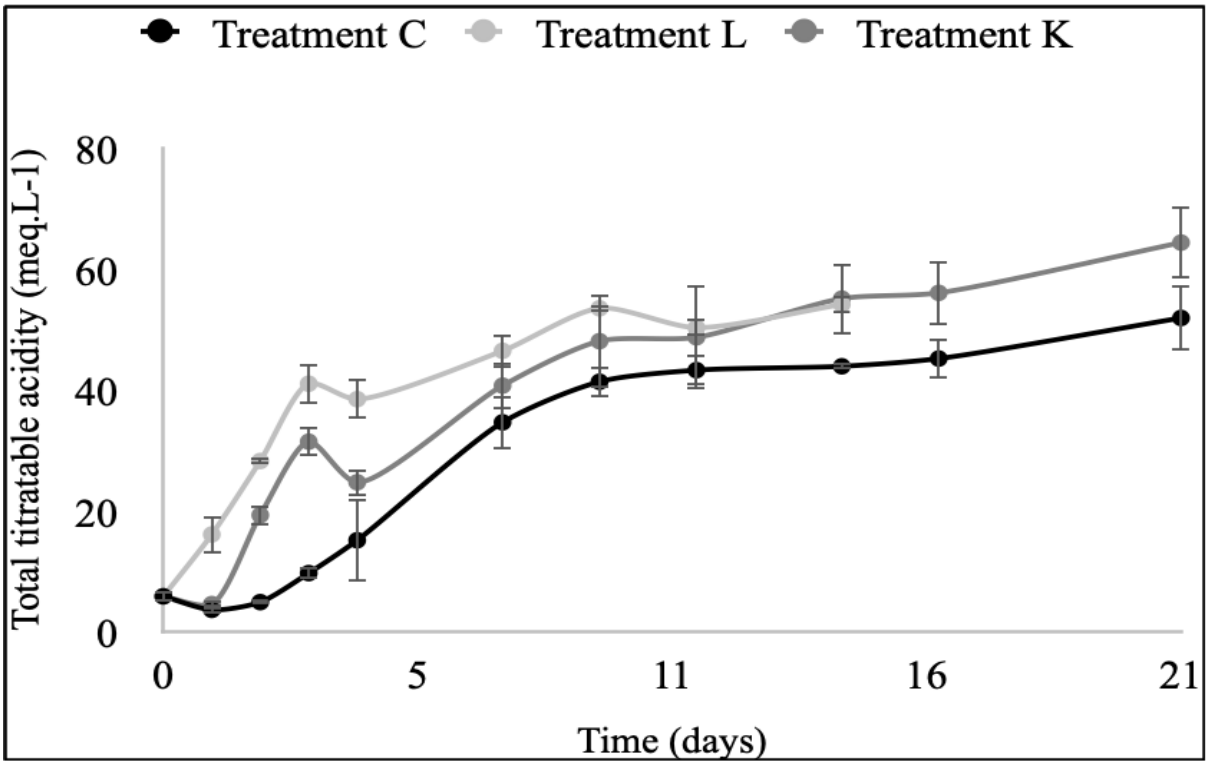


Figure 2. Total acidity obtained during fermentation. Treatments: L = encapsulated yeast; K = water kefir; C = control (spontaneous fermentation)

For mead produced using different Saccharomyces strains, Mileski (2016) found similar values, specifically 53.66±1.52 with the *Red Star champagne* strain and 65.00±1.00 with the Fleischmann yeast. Therefore, the results did not differ significantly from those obtained using the traditional fermentation method with free yeast.

The TSS analysis was used to monitor sugar consumption in the must, and the results are presented in Figure 3. The must fermented with encapsulated yeast exhibited the highest sugar consumption among the three treatments. TSS plummeted from the 4th day onward to a final value of 3.80 ± 0.06 °Brix. This reduction was approximately 10.92 °Brix, a value slightly lower than that reported by Matsuo and Steffen (2018), who observed a decrease of 11.40 °Brix in mead produced with encapsulated yeast.

The variation in soluble solids reduction across studies may be partially associated with differences in inoculum concentration. Pereira *et al.* (2013) evaluated different pitching rates of *Saccharomyces cerevisiae* in mead fermentation and demonstrated that inoculum size influenced fermentation kinetics. The authors showed that increasing the initial yeast concentration modified the fermentation profile and could accelerate the progression of the process, although the response also depended on the yeast strain. Therefore, variation in inoculum concentration between studies may partially explain the variation in substrate consumption observed in the present study.

Regarding the kefir treatment, a slower decrease in TSS was observed, indicating a slower substrate consumption rate than the one in the encapsulated yeast treatment. The mead reached a final value of 9.01 ± 0.29 °Brix after 21 days of fermentation. Slow and incomplete consumption of soluble solids has also been reported in meads fermented with kefir. Souza *et al.* (2024), for example, standardized the initial mead must at 25 °Brix. They reported final soluble solids values ranging from 16.10 to 17.95 °Brix after 9 days of fermentation at 25 °C, depending on the concentrations of water kefir grains and yeast used. Although the initial soluble solids concentration and fermentation time differed from those of the present

study, both studies indicate that residual substrate can remain in kefir-containing meads and that its consumption is influenced by fermentation time and inoculum concentration.

Thus, the residual sugars observed in the present study suggest that fermentation could potentially continue beyond 21 days. Also, adjustments in inoculum concentration could influence substrate consumption and fermentation kinetics. Similar findings were reported by Monar *et al.* (2014). The authors used water kefir from Ecuador to compare different sweeteners and observed greater consumption of the must sweetened with honey, with a final value of 5.57 °Brix. The control showed the smallest variation for this parameter, with a final ºBrix of 10.90±1.14, indicating that the absence of a starter culture influenced the maintenance of residual substrate in this process. The must fermented with encapsulated *Saccharomyces cerevisiae* showed a significant difference in TSS compared with the other two treatments ($p < 0.05$).

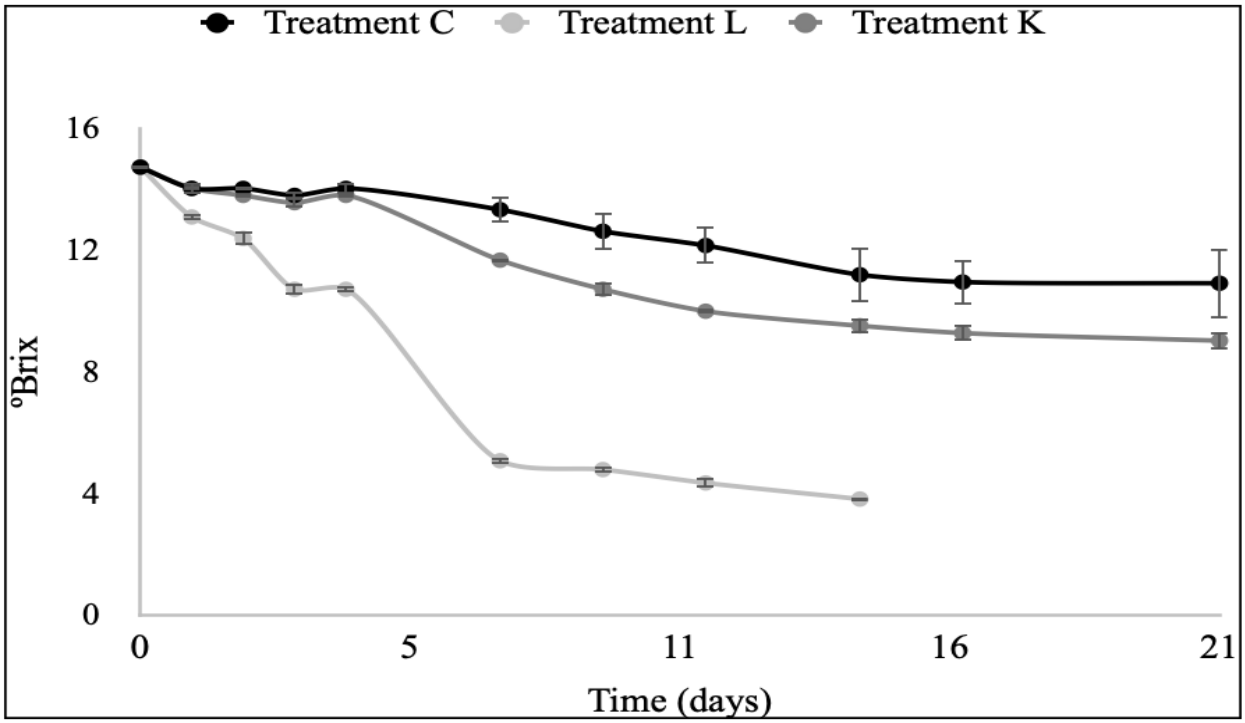


Figure 3. Brix degrees of the must during fermentation. Treatments: L = encapsulated yeast; K = water kefir; C = control (spontaneous fermentation).

In the analyses conducted, the encapsulated yeast treatment (Treatment L) showed the most pronounced decrease in density, particularly from the 4th day of fermentation onwards, which coincided with the greater reduction in total soluble solids (TSS). Treatment L reached a final density of 1.009 ± 0.00 g mL^{-1} after 14 days and differed significantly from the other treatments ($p < 0.05$). In comparison, the control reached a final density of 1.040 ± 0.01 g mL^{-1} after 21 days, whereas the kefir treatment reached 1.024 ± 0.00 g mL^{-1}.

The decrease in density observed during fermentation is consistent with the consumption of fermentable sugars and their conversion mainly into ethanol and CO_2. Thus, this behavior was expected as alcoholic fermentation progressed and was compatible with the simultaneous reduction in total soluble solids. The density plummeted in the encapsulated yeast treatment (Treatment L) suggests greater substrate consumption and fermentative activity compared with the other treatments. Similar behavior has been reported in studies on mead fermentation, in which the consumption of fermentable sugars was associated with the progression of fermentation and ethanol production (Pereira *et al.*, 2013; Souza *et al.*, 2024). Therefore, the density profile observed in Treatment L is consistent with the greater reduction in total soluble solids and the higher fermentative performance observed for this treatment. The density profiles of

the treatments throughout fermentation are presented in Figure 4.

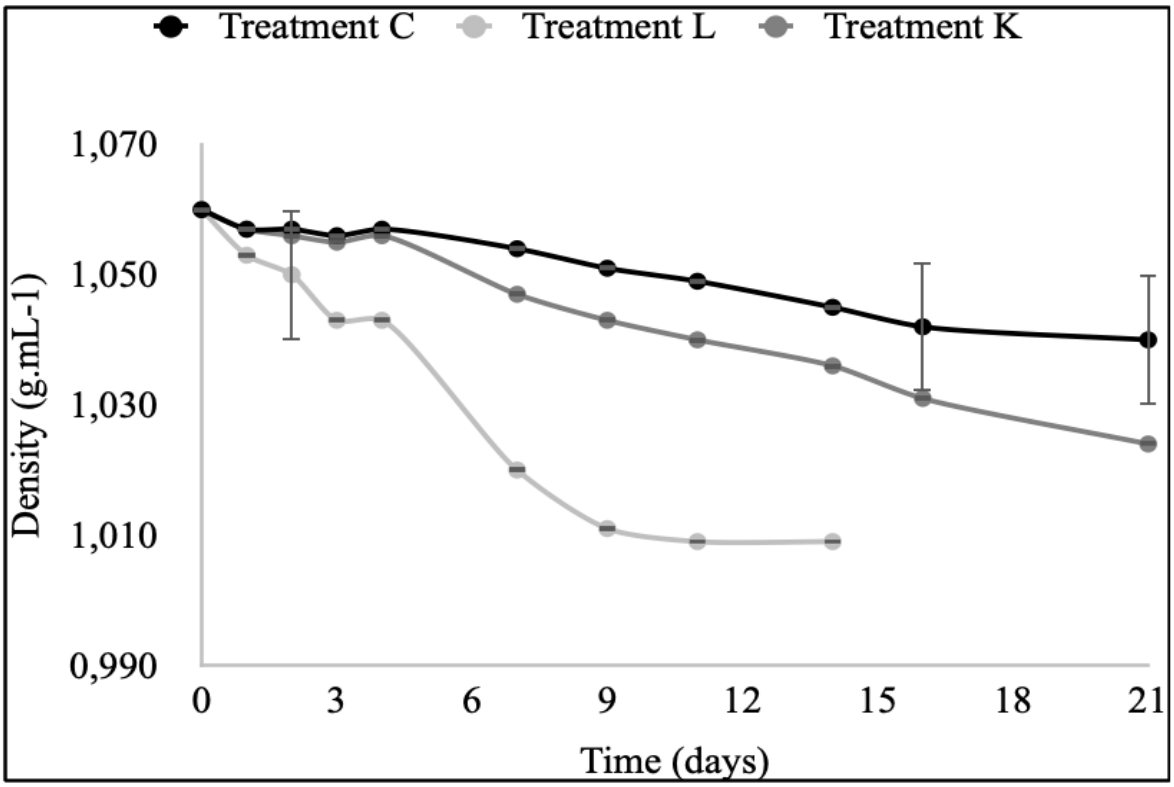


Figure 4. Density of the treatments during fermentation. Treatments: L = encapsulated yeast; K = water kefir; C = control (spontaneous fermentation).

Kinetic parameters

Based on the results of the analyses performed, the kinetic parameters of the treatments were calculated, as presented in Table 1.

Regarding product formation, the encapsulated yeast treatment showed a higher rate of substrate consumption, productivity, and a final alcohol content of the mead (6.8% v/v). This alcohol content is lower than that obtained in other studies in which immobilized yeasts were also used in mead fermentation, with reported values of 12.03 ± 0.12% (Fonseca, 2013) and 10.53 ± 0.12% (Borges, 2021), but close to those found by Matsuo and Steffen (2018) of 8.7% alcohol with an initial of 25 ºBrix. Thus, considering the initial sugar concentration in the must and its consumption, it is evident that the process was efficient, and immobilization did not hinder yeast activity, possibly helping to prevent stress caused by alcohol content, a condition reported by Mileski (2016) after the 11th day of fermentation in musts inoculated with free yeast cells.

The encapsulated yeast treatment (Treatment L) produced a mead with an alcohol content within the range established by Brazilian regulations (4–14% v/v) (Brasil, 2012). The mead fermented with kefir reached an alcohol content of 4.77% v/v after 21 days of fermentation. The presence of residual sugars indicates that additional substrate remained available for fermentation. Souza *et al.* (2024), studying mead fermented with water kefir and yeasts, also demonstrated that ethanol production and residual soluble solids are influenced by inoculum composition and fermentation conditions. Therefore, the residual sugars observed in the kefir treatment suggest that further fermentation could increase substrate consumption and ethanol production.

Although the kefir treatment (Treatment K) had a slower substrate consumption rate and ethanol production compared with the encapsulated yeast treatment, it also proved to be efficient. The control treatment showed the slowest fermentation and the lowest efficiency, as expected for spontaneous fermentation with a low initial population density of microorganisms (Venturini Filho, 2016). The encapsulated yeast treatment (Treatment L) showed a significantly higher alcohol content than the other

treatments ($p < 0.05$). This behavior was expected considering the greater substrate consumption and higher fermentative performance observed for this treatment. During alcoholic fermentation, the consumption of fermentable sugars is directly associated with ethanol formation.

Table 1. Kinetic parameters of the treatments

Treatment	Ethanol (%v/v)	VS (mL/100mL.)	Productivity (mL/100mL.)	YS/P (%)	E (%)
C	2.67	0.01	0.041	41.31	80.84
L	6.80	0.03	0.202	49.13	96.15
K	4.77	0.01	0.064	44.22	86.56

Treatments: C = control (spontaneous fermentation); L = encapsulated yeast; K = water kefir. Vs = substrate consumption rate; YS/P = substrate-to-product conversion yield; E = fermentation efficiency.

Previous studies on mead have reported a relationship between the reduction in soluble solids and the increase in alcohol content (Souza *et al.*, 2024). Pereira *et al.* (2013) also demonstrated that the fermentative performance of *Saccharomyces cerevisiae* in mead is influenced by the initial yeast population, with inoculum size affecting fermentation kinetics. Therefore, the higher alcohol content obtained in Treatment L is consistent with its greater consumption of soluble solids and higher fermentation productivity observed in the present study.

During fermentation, gas formation was visually observed, particularly in the treatment containing water kefir. This observation is consistent with CO_2 production resulting from microbial metabolism during the conversion of fermentable sugars. Similar fermentative activity has been reported in meads produced with water kefir and yeasts (Souza *et al.*, 2024). The meads obtained in the present study also showed a clear, light-yellow appearance without visible turbidity. Souza *et al.* (2024) reported yellowish coloration in meads produced with water kefir and yeasts, although color characteristics varied according to the microorganisms and fermentation conditions employed.

The observations in the present study were qualitative and were not subjected to instrumental or sensory evaluation. Therefore, no conclusions regarding aroma, flavor, or sensory acceptability were drawn.

CONCLUSION

Among the treatments evaluated, the encapsulated yeast treatment (Treatment L) showed the best overall fermentative performance, as evidenced by its faster substrate consumption, higher productivity and fermentation efficiency, and its highest ethanol production (6.80% v/v). These results indicate that yeast encapsulation did not impair the fermentative activity of *Saccharomyces cerevisiae* and may represent a suitable strategy for mead production. In contrast, spontaneous fermentation showed the lowest fermentative performance, with lower substrate consumption, ethanol production, productivity, and efficiency, highlighting the importance of using a defined inoculum to achieve a more efficient and controlled fermentation process.

Water kefir also demonstrated potential as a fermentative agent for mead production, although fermentation was slower than with encapsulated yeast. The water kefir treatment required 21 days to reach density values close to the expected range, but produced a final alcohol content of 4.77% (v/v), within the range established by Brazilian regulations. These findings demonstrate the potential application of water kefir as an alternative mixed microbial culture for mead fermentation, expanding the possibilities for the use of non-conventional fermentative agents in this beverage.

The main contribution of this study is the comparative evaluation of encapsulated *S. cerevisiae*, water kefir, and spontaneous fermentation under the same experimental conditions, providing information on their physicochemical behavior and fermentation kinetics. The results particularly highlight the

superior performance of encapsulated yeast while also demonstrating the feasibility of water kefir for mead production, although optimization of the fermentation conditions is still required.

The study has limitations that should be considered when interpreting these findings. The microbial composition of the commercial water kefir grains was not characterized. Also, the initial cell concentration, viability, and viable cell count of the yeast inoculum were not experimentally determined. Furthermore, sensory analysis was not performed, preventing conclusions regarding the sensory quality and consumer acceptance of the beverages. Future studies should therefore characterize the microbial composition of water kefir, standardize inoculum concentrations, and investigate the effects of fermentation variables such as temperature, time, pH, and initial soluble solids concentration. Sensory analysis and consumer acceptance tests should also be conducted to determine whether the physicochemical and kinetic advantages observed translate into desirable sensory characteristics.

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