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## Kinetic Modeling of Mead Production

Gustavo García<sup>a</sup>, Juan F. Moreno<sup>a</sup>, Tamara Bernal<sup>a</sup>, Fausto Posso<sup>b</sup> and Jorge Delgado-Noboa<sup>a</sup> 

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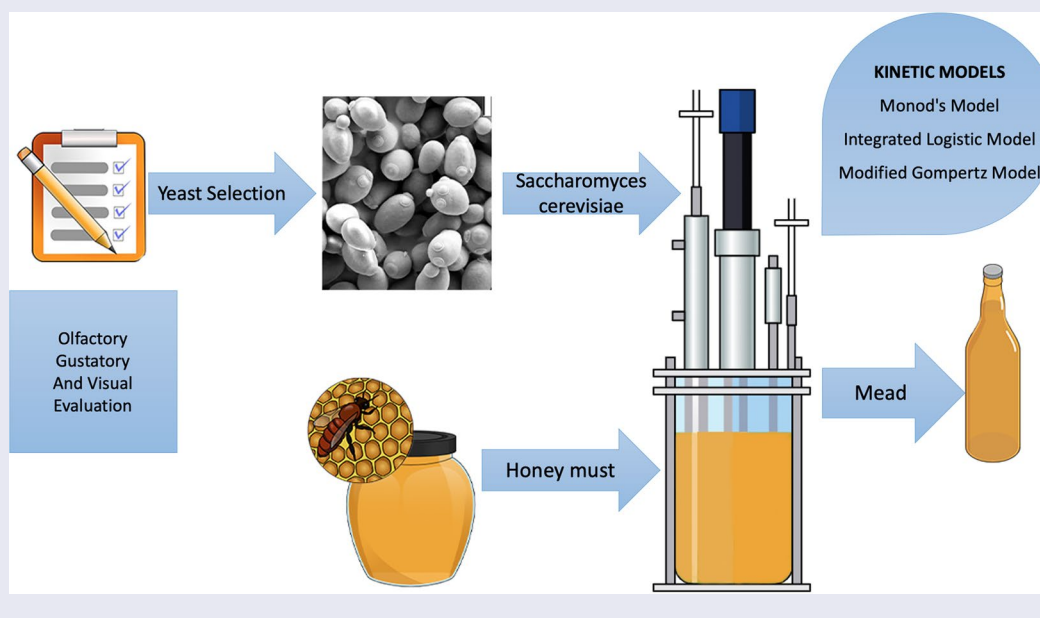
### ABSTRACT

This work studies the fermentation kinetics to produce mead using *Saccharomyces cerevisiae*, selected from three commercial yeasts to generate a product with better organoleptic characteristics and greater acceptance by a group of untrained tasters. The values of the kinetic parameters of the fermentation were obtained from a series of fermentations at laboratory scale, maintaining constant the initial concentration of biomass (1.5 g/L), the operating temperature (33°C) and the pH (4) and varying the initial soluble solids concentration in four values (10, 16, 22 and 25°Brix). Based on the experimental results, a mathematical modeling was developed to estimate the variables of interest. Thus, from the application of the *Monod* model, the saturation constant ( $K_s$ ) of 336.6 g/L was obtained, with a maximum specific growth rate ( $\mu_{max}$ ) of 0.071 h<sup>-1</sup>. Using the *integrated logistic* model, the experimental values were adjusted to obtain the average value of  $\mu_{max}$  of 0.0815 h<sup>-1</sup>. Finally, the maximum ethanol production rate ( $r_{pm}$ ) of 0.2621 g/L was obtained through the *modified Gompertz* model. Therefore, *Monod*, *integrated logistic* and *modified Gompertz* models were ideal mathematical tools to interpret the kinetic behavior of honey fermentations, predict and control this process, both on a laboratory scale and on a subsequent industrial scale. Thus, contributing to the knowledge of the dynamic behavior of mead production and its level of technological development.

### HIGHLIGHTS

- Fermentation for mead production with *Saccharomyces cerevisiae*.
- *Monod*, *integrated logistic* and *modified Gompertz* models checked for describing kinetics.
- Determination of key kinetic parameters for mead production in batch bioreactor.
- Influence of substrate concentration on mead production in a batch bioreactor.

### GRAPHICAL ABSTRACT



### KEYWORDS

Kinetic modeling; mead; *Saccharomyces cerevisiae*; sensory evaluation

## Introduction

Mead is an ancient alcoholic beverage made by fermenting honey and water.<sup>[1]</sup> It predates wine and beer and has been produced for around 5,000 years.<sup>[2]</sup> The term *mead* comes from the Greek word *hydromel*, the first batch of mead likely occurred when rainwater mixed with honey and wild yeasts fermented it.<sup>[2,3]</sup> Mead is also known by other names such as *metheglin*, *hydromel*, *medovukha*, and *ogol*.<sup>[4]</sup> It offers positive effects on human digestion and metabolism, reducing the risk of chronic diseases.<sup>[5]</sup> Mead has a high sugar content mainly fructose and glucose, making it ideal for fermentation under different conditions such as temperature, acidity (pH), fermentation time, and yeast concentration.<sup>[3,6,7]</sup> In recent years, mead production has experienced steady growth driven by the demand for new fermented products and the desire of honey producers to create high-value derivatives.<sup>[8]</sup> The global mead market has seen an estimated annual growth rate of 7% between 2018 and 2022.<sup>[9]</sup> The United States has been a significant contributor to this growth, with the number of mead producers increasing from 30 in 2003 to 520 in the subsequent years, representing a remarkable 73% growth between these years.<sup>[10]</sup> In Chile, mead is marketed as part of the wine and sparkling wine category, which has also seen an increase in value. Between August 2017 and July 2018, the wine industry grew by 2.1%, while the sparkling wine line grew by 8.1% as of 2021.<sup>[11]</sup> The global mead beverage market was valued at USD 487.9 million in 2020 and is projected to reach USD 1,621 million by 2028, with a compound annual growth rate (CAGR) of 18.71%.<sup>[12]</sup> Based on these results, it is important to identify the successful strategies for the Ecuadorian mead industry. With an average honey production of 10.2 kg per hive and 15,820 hives in the country, Ecuador has a potential market for mead production.<sup>[13,14]</sup>

Mead fermentation is a slow process that can take several months<sup>[14]</sup> and presents challenges such as interruptions, lack of product uniformity, and the development of off-flavors. These factors depend on honey variety, yeast strains, pH levels, and temperature. Secondary fermentation by bacteria can increase acidity and produce volatile esters.<sup>[2,15]</sup> The presence of nitrogen can create unpleasant aromatic compounds,<sup>[7]</sup> while maturation in glass containers contributes to organoleptic and physicochemical characteristics.<sup>[16]</sup> Additives like pollen and fruit juices can enhance mead quality and fermentation.<sup>[1]</sup> The variety of honey has the greatest impact on the final quality and sensory characteristics of mead.<sup>[17–19]</sup> The relevant sensory characteristics of beverages are evaluated by a panel of experts or consumers through smell, taste and, to a lesser extent, color and appearance.<sup>[20]</sup> Mead aroma is influenced by honey, yeast, and production processes.<sup>[2]</sup> Theoretical knowledge regarding mead production is limited, particularly in terms of honey fermentation. However, it is widely recognized that the quality of mead can be enhanced by developing formulations that incorporate additives like pollen or nitrogen sources to optimize fermentation conditions.<sup>[21,22]</sup> Research efforts have primarily concentrated on studying the composition, alcohol

concentration, volatile compounds, sensory analysis, and acceptability of honey in relation to mead production.<sup>[18,23]</sup>

Polynomial equations are applied to determine output variables such as the maximum specific growth rate ( $\mu_{\max}$ ), the *Monod* growth constant ( $K_s$ ), and the maximum ethanol production rate ( $r_{\text{pm}}$ ) based on input variables including biomass amounts, substrate concentration, and ethanol concentration.<sup>[1,24]</sup> In the field of biotechnology, various models have been developed to predict and control yeast fermentation and other fermentative systems.<sup>[25]</sup> This includes growth models for biological populations and models for product generation. Machine learning algorithms have also been employed to improve predictive control in complex biosystems due to their ability to handle nonlinear dynamics.<sup>[26]</sup> The *Monod* model, employing the *Lineweaver-Burk* linearization method, explains microbial growth by considering the effect of substrate concentration on the specific rate of cell growth.<sup>[27–30]</sup> The logistic function model, widely used in microbial growth studies, assumes that the growth rate is proportional to the current population and available resources in a confined environment.<sup>[31–34]</sup> To model ethanol generation, the *modified Gompertz* model, originally used to describe age distribution in human populations and whose modification allows modeling microbial growth, has been applied.<sup>[35]</sup> It predicts microbial growth, metabolite formation, and provides information on maximum product concentration, maximum production rate, and lag phase.<sup>[32]</sup> In mead production kinetics, the *Gompertz* and *logistic* models with three or four parameters have been utilized to describe substrate consumption, product generation, and determine the maximum yeast growth rate during the alcoholic fermentation of honey.<sup>[36]</sup> These mathematical models contribute to a better understanding of the fermentation process and can aid in optimizing mead production.

In this context, the aim of this research was to study the mead production process using a yeast of the *Saccharomyces cerevisiae* type, supported by an experimental stage and kinetic modeling, seeking to optimize its production. For this, the *Monod* model, and variations of the *logistic* and *Gompertz* models were applied, such as the *integrated logistic* model and the *modified Gompertz* model, respectively. Thus, it was intended to contribute to the knowledge of the dynamics of honey fermentation.

## Experimental

### Pilot fermentation

Honey is a natural complex product that contains at least 181 substances, mainly carbohydrates, as well as other minor substances.<sup>[14]</sup> Bee honey can be classified as monofloral when the nectar is collected mostly from a single flower species and on the other hand, multifloral honey is made from the nectar of many different flowers, plants or trees.<sup>[37]</sup> In this study, monofloral eucalyptus honey was used, this honey comes predominantly from the flowering of eucalyptus (at least 80% eucalyptus pollen) from SCHULLO S.A. produced in Quito (Ecuador). Additionally, this honey

complied with national and international quality standards (NTE INEN-CODEX, USDA). For each test, around 1.8 kg of honey was used, and the time elapsed from its harvest was 12 months.

In the pilot test, three different yeasts were used and the evolution of the soluble solids (°Brix) during fermentation was recorded. The yeasts used were freeze-dried yeast, *Levapan* brand; multipurpose alcoholic fermentation yeast, *Fermentis* brand (*Saf-ale T-58* type) and a specific yeast to produce mead, *Mangrove Jack's* brand (*Mead Yeast M05* type). The mead samples obtained in each case were subjected to a tasting test to assess the acceptance of the final product by consumers based on a sensory analysis, and thus select the yeast that allows obtaining the product with the best visual, olfactory and taste characteristics.

### Sensory evaluation

The sensory analysis was carried out in the city of *Azogues* located in the geographic area number 6 of Ecuador, selected for ease of recruitment of the respondents. A non-probabilistic convenience sampling was performed for a sample size of 30 participants, preferring individuals who regularly consume alcoholic beverages to resemble the results of a panel of trained judges. Once the panel of judges was selected, they were trained in topics of visual appreciation and tasting of the meads.

The sensory analysis carried out followed the techniques and specifications of wine tasting used by Blanco<sup>[28]</sup> and complemented with what is established in the Guide for the Training of Judges for the evaluation of meads of the *Certification Program for Brewers Judges*.<sup>[28]</sup> The visual, olfactory and taste parameters of the mead samples were evaluated to select the yeast that generates a product with the best organoleptic characteristics.

### Batch alcoholic fermentation

This process was carried out in a 2-liter *Biotron GX* batch reactor, using a maximum volume of 80%, with stirring between 150 and 250 rpm to ensure homogeneity in the reactive mass. The yeast used was the one selected after the experimental stage. The operating conditions in all cases were as follows: constant temperature of 33 °C, pH of 4 and initial biomass concentration of 1.5 g/L. The initial concentration of soluble solids (°Brix) took the values of 25, 22, 16 and 10, in each of the experiments. These experiments lasted between 25 and 46 h, depending on the concentration of the honey.

### Ethanol quantification

For the quantification of ethanol, the non-chromatographic microdiffusion method was used. The procedure uses a *Conway* chamber to determine substances susceptible to volatilization and fixation to the appropriate medium to be quantified.<sup>[38,39]</sup> The chamber is hermetically sealed and

consists of two compartments. In the first one is the alcohol that, due to its high vapor pressure and test temperature, volatilizes towards the second compartment, where the oxidation of ethanol to acetic acid occurs due to the presence of potassium dichromate dissolved in sulfuric acid. Excess unreacted dichromate is measured by its reaction with potassium iodide to form iodine, which is titrated with sodium thiosulfate in the presence of starch as an indicator.

### Sugar quantification

The method used was the phenol-sulfuric acid test, which allows the determination and quantification of certain sugars, such as polysaccharides, oligosaccharides, monosaccharides and their derivatives. This method consists in the formation of orange-yellow complexes due to the reaction with phenol in concentrated sulfuric acid. The intensity of the color is directly related to the concentration of carbohydrates and is measured by absorbance at wavelengths between 488 nm and 492 nm. The assay was performed in triplicate in a *Thermo Scientific UV-Visible Genesys 180* spectrophotometer at a wavelength of 490 nm, previously performing the calibration curves.<sup>[40]</sup>

### Yeast quantification

This quantification was carried out by freeze-drying. The samples were centrifuged at 4,000 rpm for 15 min to discard the supernatant and stored in liquid nitrogen at -190 °C to avoid degradation reactions. Once the experimental process was completed, the samples were freeze-dried in an *Armfield* brand *FT 33* freeze-dryer, for 48 h, of which the first 24 h were frozen and the final 24 h were dried for subsequent weighing.<sup>[41]</sup>

### Mathematical modeling

The experimental results were used to determine the main variables associated with the dynamics of honey fermentation in the presence of yeasts. Thus, the *Monod* equation (equation 1) was used to model the fermentation kinetics. This equation establishes that the specific growth rate of the active biomass is directly related to the concentration of the limiting substrate.<sup>[42]</sup> Thus, the maximum specific growth rate ( $\mu_{\max}$ ) and the *Monod* growth constant ( $K_s$ ) are determined in two stages. In the first, the inverse function of equation 1 (equation 2) is obtained and in the second, both variables are obtained from the graph of their linearized expression, resulting from a convenient redefinition of the variables involved (equation 3).

$$\mu = \frac{\mu_{\max} \cdot S}{K_s + S} \quad (1)$$

where  $S$  growth-limiting substrate concentration [g/L];  $\mu_{\max}$  maximum specific rate of growth [h<sup>-1</sup>];  $\mu$  specific rate of cell growth [h<sup>-1</sup>];  $K_s$  Monod's growth constant [g/L].

$$\frac{1}{\mu} = \frac{K_s}{\mu_{\max}} \cdot \frac{1}{S} + \frac{1}{\mu_{\max}} \quad (2)$$

$$y = mx + b \quad (3)$$

where

$$y = \frac{1}{\mu} [\text{h}]$$

$$m = \frac{K_s}{\mu_{\max}} \left[ \frac{(\text{g} \cdot \text{h})}{\text{L}} \right]$$

$$x = \frac{1}{S} [\text{L} / \text{g}]$$

$$b = \frac{1}{\mu_{\max}} [\text{h}]$$

The *integrated logistic* equation (equation 4) was used to model the fermentation process and determine the maximum specific growth rate ( $\mu_{\max}$ ). Thus, the experimental data of biomass concentration and fermentation time were fitted to the *integrated logistic* model using a *Levenberg-Marquardt* non-linear least squares method programmed in *Matlab*.<sup>[40]</sup>

$$X = \frac{X_0 \cdot \exp(\mu_{\max} \cdot t)}{1 - \left[ \left( \frac{X_0}{X_{\max}} \right) \cdot (1 - \exp(\mu_{\max} \cdot t)) \right]} \quad (4)$$

where  $X$  yeast concentration at time  $t$  [g/L];  $X_0$  initial yeast concentration [g/L];  $X_{\max}$  maximum yeast concentration [g/L];  $\mu_{\max}$  maximum specific growth rate [ $\text{h}^{-1}$ ];  $t$  fermentation time [h].

The maximum ethanol production rate ( $r_{pm}$ ) was obtained by applying the modified *Gompertz* equation (equation 5), by means of a previous adjustment of the experimental data of ethanol production and fermentation time using the same linearization procedure previously indicated.

$$P_E = P_{E_{\max}} \cdot \exp \left\{ - \exp \left[ \frac{r_{pm} \cdot \exp(1)}{P_{E_{\max}}} \cdot (t_l - t) + 1 \right] \right\} \quad (5)$$

where  $r_{pm}$  maximum ethanol production rate [g/(L·h)];  $P_E$  ethanol concentration at time  $t$  [g/L];  $P_{E_{\max}}$  maximum ethanol concentration [g/L];  $t$  fermentation time [h];  $t_l$  delay time [h].

## Results and discussion

### Pilot fermentation and sensory evaluation

The evolution of the soluble solids ( $^{\circ}\text{Brix}$ ) is showed in Figure 1, highlighting that the yeasts *Fermentis Saf-ale T58*

and *Magroove Jacks Mead Yeast M05* were more robust compared to the *Levapan* brand yeast, mainly due to a higher consumption of substrate during the pilot fermentation.

In the pilot mead fermentations, key process parameters such as temperature and the temporal evolution of  $^{\circ}\text{Brix}$  were controlled. The results showed that the best yeast was the *Fermentis Saf-ale T58*, producing a mead with limpidity with light-yellow color, and with an aroma profile similar to green apple, honey, beer, cider, vinegar apple and champagne, presenting medium sweetness, low acidity and a low alcohol intensity with a medium body.

Regarding the visual evaluation of the tasting test, the three mead samples presented identical characteristics, light-yellow coloration with light intensity and without turbidity. Regarding the olfactory and gustatory evaluation, the results obtained are shown in Figure 2, highlighting the mead fermented with *Fermentis Saf-ale T58*, with an intensity of smell and aroma with a quality grade 4, which indicates that the mead presents medium intensity, without defects and has aromatic notes similar to green apple, honey, beer, cider, apple cider vinegar and champagne with pleasant intensities.

The intensity of sweetness presented a quality grade 4, which translates into a medium sweetness. The acidity had a quality grade 1, which indicates the absence of this parameter. The alcohol intensity of this mead was the best, having a quality grade 3, which represents a low alcohol intensity. The intensity of the bitterness was grade 1, which means that it is not appreciable in this mead.

The results of the visual, olfactory and taste characteristics allowed selecting *Fermentis Saf-ale T58* yeast for the determination of the kinetics of mead fermentation. These results were compared to a similar study,<sup>[28]</sup> where the same survey was applied in the tasting test and a mead was obtained with a profile similar to that obtained in this study, with similarities in the olfactory and taste characteristics.

The fermentations with the selected yeast were made for variations in the soluble solids ( $^{\circ}\text{Brix}$ ) concentration and their temporal evolution shows the effect on the decrease in the concentration of sugars due to the consumption of the

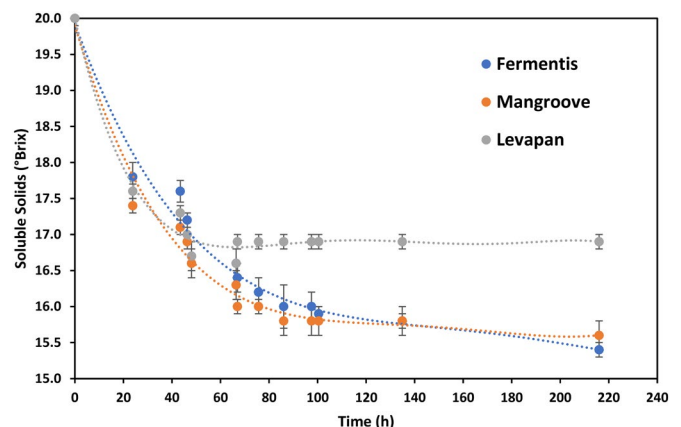
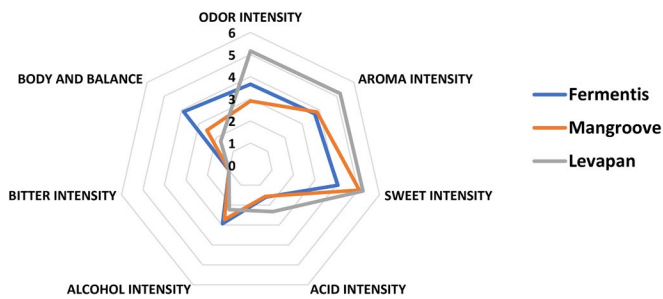


Figure 1. Evolution of soluble solids ( $^{\circ}\text{Brix}$ ) during pilot mead fermentations.



**Figure 2.** Comparison of olfactory and gustatory attributes of the meads obtained in the pilot fermentations.

substrate by the yeasts to produce ethanol and  $\text{CO}_2$  as metabolites.

### Laboratory fermentation

Once the best type of yeast for mead fermentation was selected, the effect of soluble solids ( $^{\circ}\text{Brix}$ ) on ethanol production was evaluated. For this, fermentations were carried out with different concentrations expressed in soluble solids ( $^{\circ}\text{Brix}$ ), these being 25, 22, 16 and 10, respectively. Figure 3 shows the evolution of the soluble solids ( $^{\circ}\text{Brix}$ ) during the different experiments.

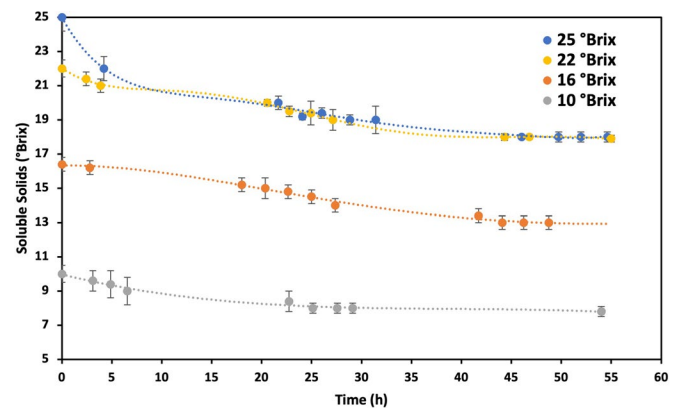
The initial substrate concentrations, expressed in soluble solids ( $^{\circ}\text{Brix}$ ), were taken in increments of soluble solids close to  $6^{\circ}\text{Brix}$ , to evaluate the behavior of the yeast with respect to the substrate concentration, since higher substrate concentrations can achieve higher ethanol concentrations. However, it must be considered that for concentrations of soluble solids higher than  $25^{\circ}\text{Brix}$  the ethanol fermentation process can be inhibited.<sup>[43]</sup> This fact, as the hypertonic environment, caused by excessive levels of substrate, could weaken the viability and fermentation capacity of the yeast. Therefore, if the substrate concentration is higher than a certain value, the product (ethanol) and yeast concentrations will not increase, thus wasting resources and energy.<sup>[44]</sup>

The evolution of the behavior of the  $^{\circ}\text{Brix}$  was comparable with a study of beer fermentation carried out by Grassi et al.<sup>[45]</sup> in fermentation conditions similar to those of this study, who reported that the total sugar content presented a rapid decrease in all the tests carried out in the three days of fermentation, with a decrease in soluble solids ( $^{\circ}\text{Brix}$ ) from  $10^{\circ}\text{Brix}$  to  $5^{\circ}\text{Brix}$ .

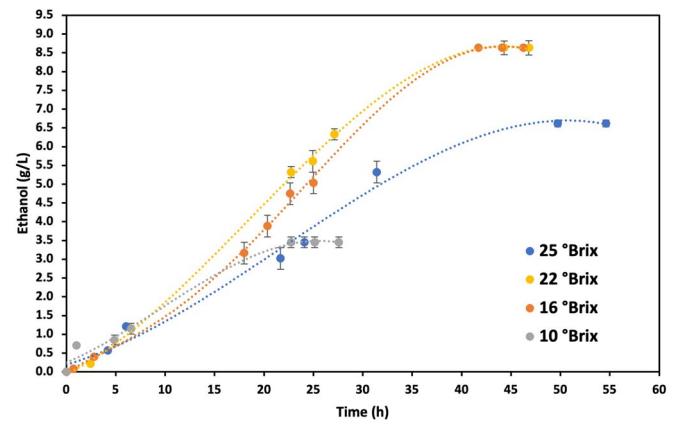
### Ethanol generation

Figure 4 shows the experimental results of ethanol generation in the fermentative processes for different  $^{\circ}\text{Brix}$  concentrations.

Regarding alcohol production, a maximum ethanol concentration of  $8.5\text{g/L}$  was obtained for the experiments with 16 and  $22^{\circ}\text{Brix}$ , after approximately 42h of fermentation. Ethanol generation was lower than that obtained in a mead production study, with a *Fleischmann* brand, *S. cerevisiae* yeast strain,<sup>[3]</sup> where ethanol concentrations of  $29.8\text{g/L}$  were obtained using  $2.5\text{g/L}$  yeast and  $92.4\text{g/L}$  of ethanol using



**Figure 3.** Temporal evolution of soluble solids ( $^{\circ}\text{Brix}$ ) during mead batch fermentations.



**Figure 4.** Time evolution of ethanol concentration during mead batch fermentations.

$5\text{g/L}$  of biomass for the experiments with the lowest and highest concentration of ethanol, respectively. The ethanol concentration obtained in this study was probably lower as a smaller amount of initial biomass was used, and the yeast strains were different. In addition, *Fleischmann* yeast is mainly used to produce bread, which explains why this strain is more robust compared to the *Fermentis Saf-ale T58* yeast, thus generating a product with a higher ethanol concentration.

### Biomass generation and substrate consumption

Table 1 shows the concentrations of yeast and substrate during the mead batch fermentations for different concentrations of soluble solids ( $^{\circ}\text{Brix}$ ), the data correspond to the average of the tests carried out in triplicate.

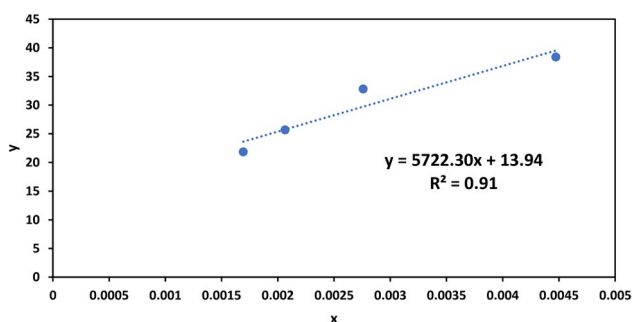
The increase in the yeast concentration indicates that this biomass is constantly generating new cells by mitosis, causing a higher concentration of cells throughout the experiment, until a moment in which the concentration remains constant (stationary phase).<sup>[46,47]</sup> Substrate consumption is due to the fact that yeasts use the medium substrate as food for their growth and reproduction, metabolizing the sugars in the medium, producing alcohol and carbon dioxide under anaerobic conditions.<sup>[48]</sup>

**Table 1.** Experimental results of yeast concentration and sugar concentration.

°Brix	Initial biomass concentration (g/L)	Final biomass concentration (g/L)	Initial sugar concentration (g/L)	Final sugar concentration (g/L)
25	1.5	3.80 ± 0.33	528.71 ± 29.68	389.75 ± 7.38
20	1.5	8.60 ± 0.22	484.22 ± 11.01	392.04 ± 13.05
15	1.5	5.80 ± 0.47	362.41 ± 1.22	284.87 ± 1.01
10	1.5	3.40 ± 0.32	223.58 ± 13.50	180.93 ± 17.61

**Table 2.** Parameters obtained for the *integrated logistic* equation.

<i>Integrated logistics model</i>		
°Brix	$\mu_{\max}$	$R^2$
25	0.051	0.96
22	0.087	0.98
16	0.071	0.98
10	0.086	0.95

**Figure 5.** *Lineweaver-Burk* linearization.

Regarding the generation of biomass, it is observed that the concentration of yeasts increases throughout the fermentation process and its final concentration is higher to the extent that the concentration of the substrate present in the medium is higher. However, this behavior occurs up to a limit value, after which the increase in the substrate concentration generates osmotic stress in the yeasts, preventing their proper development and delaying their growth.<sup>[44]</sup>

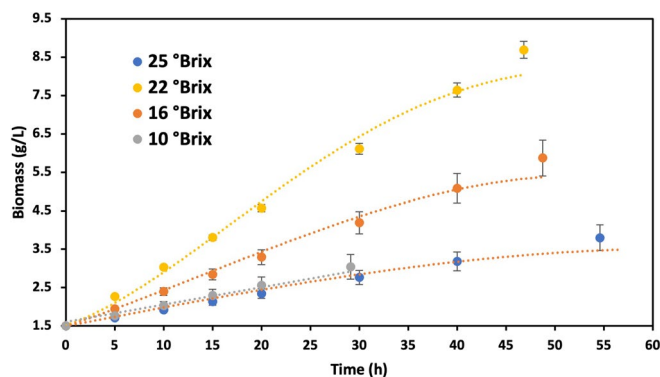
The amount of substrate decreased during the fermentations, similar to that described by Pereira et al.,<sup>[49]</sup> for a mead fermentation with an initial sugar concentration of 275 g/L, which decreased over a period of 48 h to a value of 112 g/L, these values are in ranges closer to the initial and final sugar concentrations presented in the 10°Brix experiment of this study, of 223.58 g/L and 180.93 g/L, respectively, in the same 48 h.

## Mathematical models

### Monod's kinetic model

Figure 5 shows the *Lineweaver-Burk* linearization method, with which the values of the kinetic constants,  $\mu_{\max}$  and  $K_s$  were determined. A correlation coefficient  $R^2 = 0.91$  was obtained, considered acceptable. The expression of the linear equation was  $y = 5722.30x + 13.94$ , whose slope represents the value of  $K_s/\mu_{\max}$  and the intercept with the  $y$  axis represents  $1/\mu_{\max}$ , obtaining values of  $K_s$  of 410.31 g/L and of  $\mu_{\max}$  of  $0.071 \text{ h}^{-1}$ .

The solution of the *Monod* kinetic model, supported by the *Lineweaver-Burk* method, allowed obtaining a maximum

**Figure 6.** Representation of the *integrated logistic* model. Dots indicate experimental data and lines indicate kinetic models adjustment.

specific growth rate ( $\mu_{\max}$ ) of  $0.071 \text{ h}^{-1}$  and a saturation constant ( $K_s$ ) of 410.31 g/L. These results have a variation with those obtained by Ahmad et al.<sup>[50]</sup> for the fermentation of a glucose solution, who reported a value of  $\mu_{\max}$  of  $0.084 \text{ h}^{-1}$  and a value of  $K_s$  of 213.60 g/L, indicating that the yeast had a greater affinity for glucose solution probably as it is a totally pure substrate.

### Integrated logistics model

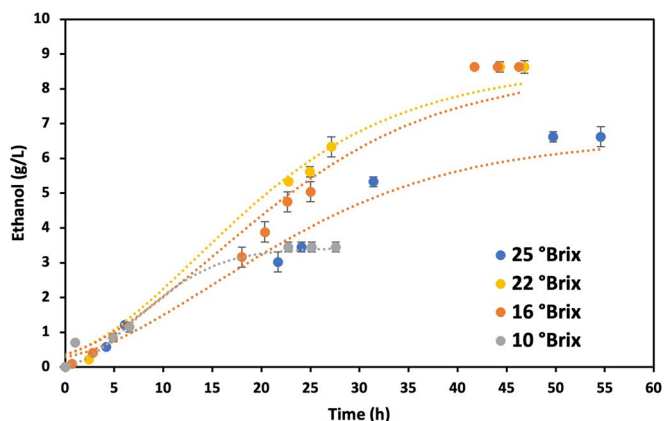
The *integrated logistic* equation allowed modeling the fermentation processes and determining the maximum specific growth rate ( $\mu_{\max}$ ) by fitting the experimental data. The experimental data were fitted to the *integrated logistic* model using a *Levenberg-Marquardt* non-linear least squares method programmed in *Matlab*®. Table 2 shows the parameters obtained for the *integrated logistic* equation of each of the experiments and Figure 6 shows the graphs obtained for the same equation.

When applying the *integrated logistic* model, an average maximum specific growth rate ( $\mu_{\max}$ ) of  $0.081 \text{ h}^{-1}$  was reached. This value was lower than that obtained by Dodić et al.,<sup>[51]</sup> who reported a  $\mu_{\max}$  value of  $0.19 \text{ h}^{-1}$ , for the fermentation of raw sugar beet juice carried out under conditions similar to those of this study. The value of the maximum specific growth rate ( $\mu_{\max}$ ) obtained indicates that mead is a fermentation must with less acceptance by yeasts than raw sugar beet juice; this difference may be due to factors such as substrate concentration, yeast strains used, inoculum size, changes or variations in nutrient composition, limiting substrate, and/or pH and temperature conditions.<sup>[29,44]</sup>

From the adjusted models, a  $\mu_{\max}$  of  $0.071 \text{ h}^{-1}$  and an average  $\mu_{\max}$  of  $0.081 \text{ h}^{-1}$  were obtained, for the *Monod* and *integrated logistic* model, respectively. These values are in ranges close to those reported in the literature for mead production

**Table 3.** Parameters obtained for the *modified Gompertz* equation.

Modified Gompertz model		
°Brix	$r_{pm}$	R <sup>2</sup>
25	0.18	0.97
22	0.27	0.98
16	0.24	0.96
10	0.26	0.97

**Figure 7.** Representation of the *modified Gompertz* model. Dots indicate experimental data and lines indicate kinetic models adjustment.

processes, highlighting a value of  $\mu_{max}$  of  $0.045\text{h}^{-1}$  from a study carried out by Mendes-Ferreira et al.,<sup>[52]</sup> and values of  $0.074\text{h}^{-1}$  and  $0.088\text{h}^{-1}$  in a study by Cuenca et al.<sup>[36]</sup>

### Modified Gompertz model

The *modified Gompertz* equation allowed modeling the fermentation processes and estimating the maximum ethanol production rate ( $r_{pm}$ ) supported by the adjustment of the experimental data of all cases. The results obtained were adjusted to the *modified Gompertz* model using a *Levenberg-Marquardt* non-linear least squares method programmed in *Matlab*®. Table 3 shows the results of the application of the *modified Gompertz* model and Figure 7 the corresponding graphs.

By applying the *modified Gompertz* model, a maximum ethanol production rate ( $r_{pm}$ ) of  $0.26\text{g}/(\text{L}\cdot\text{h})$  was obtained. This value is higher than those obtained by Srimachai et al.,<sup>[53]</sup> who reported an  $r_{pm}$  of  $0.24\text{g}/(\text{L}\cdot\text{h})$ . This indicates that mead is potentially a better fermentation medium than oil palm fronds. The variation in the value of the maximum speed of bioethanol production could be due to the types and compositions of the substrates used in the fermentation.

### Conclusions

In the present study, mead samples were prepared using three different yeasts, of which, through a tasting test, it was determined that the optimal yeast for mead fermentation was *Fermentis Saf-ale T58*. This yeast produced a mead with

superior organoleptic characteristics compared to the meads fermented with the other two commercial yeasts.

The *Monod* model, applied to the kinetic study of the mead fermentation process, allowed obtaining the value of the kinetic constants  $\mu_{max}$  and  $K_s$ , whose values were  $0.071\text{h}^{-1}$  and  $410.31\text{g}/\text{L}$ , respectively, with a correlation coefficient  $R^2 = 0.91$ .

The *integrated logistic* model allowed drawing the sigmoid curve, characteristic of population growth over time, for each experiment. They had a very high precision, since the correlation coefficient for all the experiments was greater than 0.9 and it was possible to determine that the optimal substrate concentration for mead fermentation was  $22^\circ\text{Brix}$ .

The *modified Gompertz* model allowed determining the value of the maximum ethanol production rate ( $r_{pm}$ ) with an optimal value of  $0.27\text{g}/(\text{L}\cdot\text{h})$  for the  $22^\circ\text{Brix}$  experiment with an  $R^2$  of 0.98.

The results show the suitability of the models used to determine the dynamic behavior of honey fermentation, contributing to the knowledge about mead production and its potential to emerge in the alcoholic beverages market.

Future research should be directed towards the application of these models considering three fundamental aspects such as the use of immobilized cells, different types of honey and the use of different additives to predict and compare bioethanol production results and later its industrial scaling.

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No potential conflict of interest was reported by the authors.

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