MEAD OF NATURAL FERMENTATION

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ABSTRACT

Mead is an alcoholic fermented obtained from the dilution of honey and water in different amounts, depending on the desired alcohol content. This study aimed to evaluate a natural alcoholic fermentation for mead process. Bee honey was used, also Tahiti lemon, Gala apple and black raisin in order to diversify beeking products and to evaluate the effect. The production of pure mead (A) was from 17.60º Brix, the production of lemon mead (B) was from 16.80º Brix, the production of mead raisin (C) was from 19.60º Brix, while mead with apple (D) was from 16.10º Brix, and all mead were produced from wild yeast present in the environment. The alcoholic fermentation occurred at room temperature for 56 days and obtained alcohol content (v/v) and volatile acidity (mEq/l) in A of 4.92% and 24.47, in B of 1.78%. and 8.71, in C of 6.47% and 11.26 and in D of 1.53% and 6.46, respectively. Moreover, after the 56 days of maturation of the mead were obtained the methanol (mg/l) and alcoholic (v/v) content, in this order, in A of 66.67 and 11.04%, in B of 1.000.00 and 6.71%, in C 200.00 and 13.28% and in D 833.33 and 5.06%. From the results obtained can be concluded that only C is within the legislation of the mead standard, but that A and C yeasts presents the highest fermentation potential. Thus, further studies on mead production and a reassessment of the quality and identity standard agreed by Normative Instruction no 34/2012 are required.

Keywords: wild yeast, beverage, Apis mellifera honey, honey beverage, beverage analysis

INTRODUCTION

The focus of the present study is natural fermentation. And the choice of mead by this process was due to the growing search for foods categorized as “comfort food”, which are foods that recall a pleasurable mental state, especially human diet for its therapeutic, nutritional and functional richness of its composition, including vitamins and minerals (Azeredo & Teixeira, 2018). Therefore, handcrafted fermented drinks are classic examples of this type of food. And it includes homemade fermentation obtained from the dilution of honey and water in different amounts, depending on the desired alcohol content. This study aimed to evaluate a natural alcoholic fermentation for mead process. Bee honey was used, also Tahiti lemon, Gala apple and black raisin in order to diversify beeking products and to evaluate the effect. The production of pure mead (A) was from 17.60º Brix, the production of lemon mead (B) was from 16.80º Brix, the production of mead raisin (C) was from 19.60º Brix, while mead with apple (D) was from 16.10º Brix, and all mead were produced from wild yeast present in the environment. The alcoholic fermentation occurred at room temperature for 56 days and obtained alcohol content (v/v) and volatile acidity (mEq/l) in A of 4.92% and 24.47, in B of 1.78%. and 8.71, in C of 6.47% and 11.26 and in D of 1.53% and 6.46, respectively. Moreover, after the 56 days of maturation of the mead were obtained the methanol (mg/l) and alcoholic (v/v) content, in this order, in A of 66.67 and 11.04%, in B of 1.000.00 and 6.71%, in C 200.00 and 13.28% and in D 833.33 and 5.06%. From the results obtained can be concluded that only C is within the legislation of the mead standard, but that A and C yeasts presents the highest fermentation potential. Thus, further studies on mead production and a reassessment of the quality and identity standard agreed by Normative Instruction no 34/2012 are required.

Keywords: wild yeast, beverage, Apis mellifera honey, honey beverage, beverage analysis
Mead is obtained through the preparation of the wort, preparation of vat feet (also called inoculum, which consists of a suspension of microorganisms of adequate concentration used for the fermentation of the must), inoculation of yeasts, fermentation, clarification and filling. The fermentation of the drink can take a few months and even years, depending on honey, yeast, nutrition and pH control (Mattietto, 2006; Milesik, 2016). According to Katz (2012), fermented beverages after storage continue to develop through several slow chemical reactions even after fermentation has stopped, it gets its name from aging, as they are matured over time in bottles. In the maturation of this fermented drink, the maturation and storage times are not universal. Generally, the maturation of alcoholic beverages can occur in glass containers that provide the organoleptic and physico-chemical characteristics of the traditionally manufactured product, which consists of a bittersweet flavor and a spicy aroma (EU, 2012; Milesí, 2016). However, in other containers, such as wooden, it is not recommended, because during the fermentation, the growth of microorganisms such as molds can occur in the pores of the wood, contaminating and altering the organoleptic values of the drink (Katz, 2012). During the production of the honey drink, several precautions must be used to avoid delay, the mixture does not result in the honey wine due to the stress of the yeast, such as nutrition, temperature, pH, and so that they do not meet the need for the microorganisms of interest and favor other microorganisms, such as bacteria. Because changing the conditions of the environment will consequently change the desired characteristics in the drink. As already seen, the fermentation for the manufacture of mead was natural, using only wild yeasts and in which fermentation is characterized by the microorganisms present spontaneously in food and environments. And so that fermentation is spontaneous, starting with existing microorganisms. And, therefore, the type of fermentation always depends on what was used. For example, if fermentation starts with a grape, yeasts start an alcoholic fermentation; however, if fermented from milk or vegetables, the lactic acid bacteria will dominate and start a lactic fermentation (Katz, 2012). Although natural fermentation has been widely used in the past, wild yeasts can harm alcoholic fermentation processes and, in contrast, some can also bring positive points proven through analysis. In the isolation and evaluation of some wild yeasts regarding their fermentative potential in ethanol production, it was found that the hegemony and rusticity of these strains are associated with an excellent fermentative performance (Ferrari et al., 1998; Parazzí & Oliveira, 1996; Adrïetta et al., 2007; Moreira et al., 2013). Likewise, wild yeasts are more resistant to adverse conditions, especially regarding pH and temperature (Cecato-Antonini & Parazzí, 1996; Moreira et al., 2013).

**MATERIAL AND METHODS**

The experiment was carried out at the Food, Chemistry, Microbiology and Chromatography Laboratories, at the Technology College of Piracicaba “Dep. Roque Trevisan”, in the city of Piracicaba, São Paulo State, Brazil, and the activities will be detailed.

**Reagents and materials**

Africanized bee honey, water, apple, lemon and raisin were purchased at the local market in the city of Piracicaba and immediately forwarded to the laboratories in order to be processed. The production of pure mead of honey (type A) was from 17.60º Brix, the production of lemon mead (type B) was from 16.80º Brix, the production of raisin mead (type C) was from 19.60º Brix, while mead with apple (type D) was from 16.10º Brix, and all mead were produced from wild yeast present in the environment.

The absolute ethyl alcohol (C₂H₅OH) used for the analysis in chromatography is of analytical standard grade (Neon Brand), while the methyl alcohol (CH₃OH) used for the analysis in chromatography is of standard chromatographic grade (Brand LiChrosolv® Merck), while the 0.01M sodium hydroxide solution and the methylene blue-sodium citrate solution were those that were available in the laboratory.

**Fermentation**

To carry out the natural fermentation process, honey, apple, lemon and raisin were left in contact with the atmosphere, so that there was contact with the microorganisms. After exposure, a 4 to 1 ratio of water and honey was added in a 2 l bottle, and 4 types of mead recipes were prepared to compare the alcohol content, with variations in accordance with the Table 02.

**Table 1 Mead shunts**

<table>
<thead>
<tr>
<th>Denomination</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mead</td>
<td>Water and honey fermented drink</td>
</tr>
<tr>
<td>Great mead</td>
<td>Aged mead</td>
</tr>
<tr>
<td>Melomel</td>
<td>Fruit-added mead (except grapes)</td>
</tr>
<tr>
<td>Pyment</td>
<td>Grape mead (preferably wine grapes)</td>
</tr>
<tr>
<td>Cyser</td>
<td>Apple mead</td>
</tr>
<tr>
<td>Metheglin</td>
<td>Mead with spice, hops and even rose petals</td>
</tr>
<tr>
<td>Bragot</td>
<td>Mead with added malt</td>
</tr>
<tr>
<td>Hipporras</td>
<td>Pepper mead</td>
</tr>
</tbody>
</table>

**Brandy**

Mead that after a fermentation step there is an addition of honey and honey brand obtained by distilling the mead.

Source: Adapted from Berry (2007); Iglesias et al. (2014); Brunelli (2015); Freitas et al. (2017).

**Table 2 Mead methods**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>type A</th>
<th>type B</th>
<th>type C</th>
<th>type D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey</td>
<td>200.00</td>
<td>200.00</td>
<td>200.00</td>
<td>200.00</td>
</tr>
<tr>
<td>Water</td>
<td>800.00</td>
<td>800.00</td>
<td>800.00</td>
<td>800.00</td>
</tr>
<tr>
<td>Lemon</td>
<td>-</td>
<td>92.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raisin</td>
<td>-</td>
<td>-</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>Gala apple</td>
<td>-</td>
<td>-</td>
<td></td>
<td>146.00</td>
</tr>
</tbody>
</table>

Source: Authors. * Values shown in grams

**Instrumental**

The alcohol content determinations were performed in a gas chromatograph PerkinElmer model Clarus 600, with flame ionization detector (flame ionization detector - FID). A capillary column PerkinElmer model Elite-WAX with dimensions of 30 m x 0.25 mm x 0.5 µm was used. The carrier gas used was nitrogen at a flow rate of 1.20 mL min⁻¹, of hydrogen was 45 mL min⁻¹ and the synthetic air was 450 mL min⁻¹, all with a high degree of purity (99.999%). The sample injection volume was 300 µL at a speed of 250 µL s⁻¹, using the “split” of 1:10. The oven temperature was 212º F per 5 minutes. The injector temperature was 302º F and the detector 572º F.

The gas chromatograph also has an automatic sampler of the Combipal brand, model CTC Analytics, Pal System, with the oven to Headspace.

**Preparation of standard solutions**

Standard solutions containing absolute ethyl alcohol, expressed in w/v (volume in mL of the analyte and 100 mL of solution), were prepared with the following concentrations: standard 1 (0.5% of C₂H₅OH), standard 2 (1.0% of C₂H₅OH), standard 3 (2.0% of C₂H₅OH), standard 4 (3.0% of C₂H₅OH), standard 5 (4.0% of C₂H₅OH), standard 6 (5.0% of C₂H₅OH), standard 7 (6.0% of C₂H₅OH), standard 8 (7.0% of C₂H₅OH), standard 9 (8.0% of C₂H₅OH), standard 10 (9.0% of C₂H₅OH), standard 11 (10.0% of C₂H₅OH), standard 12 (11.0% of C₂H₅OH), standard 13 (12.0% of C₂H₅OH) e standard 14 (14.0% of C₂H₅OH) presented by the equation y=(7186.637974)x+(950.097041) - 450.371921, of hydrogen was 45 mL min⁻¹, standard solutions containing methyl alcohol, expressed as w/v (weight in grams of the analyte and 100 mL of solution), were prepared with the following concentrations: standard 1 (0.0396% of CH₃OH), standard 2 (0.0792% of CH₃OH), standard 3 (0.1188% of CH₃OH), standard 4 (0.1584% of CH₃OH), standard 5 (0.1980% of CH₃OH), standard 6 (0.2376% of CH₃OH), standard 7 (0.2722% of CH₃OH) e standard 8 (0.3168% of CH₃OH). The analytical curve was constructed and the R² value of 0.999190 was obtained, presented by the equation y=-(12.271469)x+(7186.637974) x.

**Analysis of alcohol content and methanol**

In order to perform the analysis of alcohol content, 4 samples were collected every 7 days after the start of mead preparation and then another 7 days and so on until completing 56 days, totaling 36 samples, added after four more samples on the 57th day. To carry out maturation, the racking and filtration process was carried out, which consists of preventing the mead from being in contact with the sludge formed at the bottom of the container and with suspended materials (yeasts) (Mattietto, 2006; Brunelli, 2015). At the end of the maturation process, which took another 56 days after the помилиум collection and, therefore, after the 112th day of the beginning of the fermentation process, when the content of methanol and ethanol in these samples was then carried out, and thus decanting of yeasts that could still be in suspension after filtration. And all samples were then incubated in the oven of the automatic sampler to use the Headspace extraction, at 140º F during 5 min with a stirring of 500 rpm. After they were injected into the chromatograph, one by one.

**Physicochemical analysis**

Analyzes were carried out in triplicate of pH and soluble solids (* Brix). And for that we used the Quimis Q-400MTS bench pHmeter and the Homis VBR- 32T portable refractometer model with scale from 0 to 32% of soluble solids. Samples were taken after preparing the recipes, repeating this process every 7 days, for a period of 56 days, as well as on the 57th day.
Volatile acidity

For this analysis, it was used the Tecnal Volatile Acidity Determinator TE-0871, methodology recommended by the Adolfo Lutz Institute (2008) to determine the titratable volatile acidity of wines and other fermented beverages by volumetry, after steam distillation, following the formula:

\[ VA = \frac{( n \times f \times N \times 1000 )}{V} \]

In which:

- \( VA \) = Volatile acidity, in mEq/L
- \( n \) = Volume of sodium hydroxide solution spent on titration, in mL
- \( f \) = Sodium hydroxide solution correction factor
- \( N \) = Normality of sodium hydroxide solution
- \( V \) = Sample volume, in mL

Cell viability

The sodium methylene blue citrate solution was prepared as reported by Ceccato-Antonini (2010), weighing 0.01 g of methylene blue, dissolving it in a small amount of sterile distilled water, adding 2 g of sodium citrate, homogenizing the substance and completing the volume to 1000 mL with sterile distilled water. The slides (Neubauer chamber) were observed in an Olympus BX41 light-field optical microscope coupled to an Olympus DP72 camera, being digitally documented in the DP2-BSW software.

RESULTS AND DISCUSSION

Fermentation

The analysis of the evolution of the fermentation process was done by monitoring the pH, soluble solids and alcohol content over the months, as shown in Figures 1, 2, 3 and 4.

For Figure 1 there are showed the pH, tenor of soluble solids and tenor of alcohol results for sample Type A (pure mead of honey).

In Figure 3 we can observe the pH, tenor of soluble solids and tenor of alcohol results for sample Type C (mead raisin).

For Figure 4 there are showed the pH, tenor of soluble solids and tenor of alcohol results for sample Type D (mead with apple).

Through pH and soluble solids analyzes performed weekly, as well as visual analyzes of the four different types of meads, it was observed that meadows type B and D had their soluble solids content stabilizing as from the twenty-first day, thus indicating that yeasts were probably reaching their maximum capacity to tolerate the level of alcohol contained in each mead or until they were dying due to the below ideal pH, especially in the case of type B, in which the minimum pH was never reached, as indicated by Silva (2016). The pH of musts traditionally used in the manufacture of alcoholic beverages varies between 3.5 and 4.5. And according to Aquarone, Lima & Borzani (2001) and Oliveira et al. (2001), pH values between 3 and 4 do hinder bacterial contamination. Although the pH of type A mead is also below that indicated for fermentation, soluble solids and visual analyzes indicated that the fermentation process continued to occur. Therefore, sodium hydroxide (NaOH) was then added on the forty-sixth day only to mead types B and D, with the aim of increasing their pH and, consequently, their cell viability so that the fermentation process would continue to occur.

In 650 mL of mead type B with an initial pH of 3.38, 79.5 mL of NaOH reaching a final pH of 4.0, while in the type D mead it started from 850 mL and a pH of 3.41 adding 45.9 mL of NaOH reaching a final pH of 4.00, a value within the recommended for the alcoholic fermentation process.

Analysis of alcohol content and volatile acidity

The alcoholic content and volatile acidity in the mead (Table 3) are presented by Normative Instruction No 34/2012, with the maximum allowed amount of acetic acid of 20 mEq/L (milliequivalent-gram per liter) and ethyl alcohol is 4% up to 14%, in v/v at 68º F.
Table 3 Alcohol content on the 56th day

<table>
<thead>
<tr>
<th>Recipe</th>
<th>% of alcohol content (v/v)*</th>
<th>Volatile Acidity (mEq/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>4.92</td>
<td>24.47</td>
</tr>
<tr>
<td>Type B</td>
<td>1.78</td>
<td>8.71</td>
</tr>
<tr>
<td>Type C</td>
<td>6.47</td>
<td>11.26</td>
</tr>
<tr>
<td>Type D</td>
<td>1.53</td>
<td>6.46</td>
</tr>
</tbody>
</table>

*Medium values

Only mead C reached the standards of quality and identity that legally characterize them as such, as it had a content of ethyl alcohol and volatile acidity within the stipulated by Brazil (2012) for a fermented honey drink to be considered mead. However, types B and D meads had ethyl alcohol content below that determined by Brazil (2018), which strictly characterizes them only as a fermented honey drink, even with volatile acidity below the maximum limit. Mead A, on the other hand, cannot be characterized as mead according to the criteria of Brazil (2012), because despite having an alcohol content above the minimum limit, it also had volatile acidity above the maximum limit.

And, according to Gomes (2010) and according to Oliveira Neto (2013) yeasts, microorganisms responsible for the fermentation process, produce ethyl alcohol from the amount of total soluble solids (ºBrix), which can explain the different alcoholic levels obtained in analyzes of the different meads, since the mead type C, which had the highest initial Brix, which was 19.60º Brix, also had the highest final alcohol content, of 6.47%. And according Mileski (2016) which started from 27ºBrix with the addition of selected yeast (Saccharomyces cerevisiae), the alcohol content obtained was 15.69%, on average, presenting the possibility that wild yeasts may also have been responsible due to the low alcohol content of meads.

Each mead was produced in a different location. And in types B, C and D different substrates were added, thus indicating that the yeasts, despite being all wild and therefore adapted to the environment, as described by Vicente (2015), may be of different strains, which would also explain the difference in the final alcoholic content of meads, mainly types B and D, which had the lowest levels. Furthermore, according to Food Ingredients Brazil (2013), lemon has an antimicrobial effect thanks to citrus oil. Castro & Lima (2011) with according to Sarto & Zanussi junior (2014) reported his expressive antifungal potential, while Kosker, Feller & Esselen (1949) apud Ouvrèis (1997) reported yeast inhibition in apple cider.

It should also be borne in mind that the alcohol content of the four types of meads on the fifty-seventh day of the process without pasteurization was higher than on the fifty-sixth day of the process. And this shows that the pasteurization process was carried out correctly, which was proved by the analysis of the cell viability values, together with the pH and Brix analyzes, it was decided to add sodium hydroxide only in types B and mead D.

Table 4 Values of methanol and ethanol content after maturation

<table>
<thead>
<tr>
<th>Recipe</th>
<th>% of Alcohol content (v/v)*</th>
<th>% of Methanol content *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>11.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Type B</td>
<td>6.71</td>
<td>0.10</td>
</tr>
<tr>
<td>Type C</td>
<td>13.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Type D</td>
<td>5.06</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Average and approximate values

The alcohol content shown in Table 4 characterizes meads legally by this parameter determined by Brazil (2012). However, the values presented are very different from what is shown in Table 3. And the authors believe that the increase in alcohol content is due to the maturation process and the decantation of yeasts. And they understand that further studies on the matter are necessary since nothing was found in the literature to explain the cause of the increase in alcohol content.

Normative Instruction No. 14/2018 was used for the methanol content parameter, which establishes the quality and identity standard for wine and grape and wine derivatives, since mead is considered honey wine. Thus, the level of adequacy for this parameter was established, the maximum value of 400 mg/l of methyl alcohol present in the mead.

Thus, based on the information in Table 4, only Type C mead can be characterized as viable for consumption and commercialization, since the methanol content was 200 mg/l, while Types A, B and D, presented high levels above the stipulated, where in Type B and D it is expected due to the presence of pectin, which is a precursor to methanol, which comes from lemon and apple in their formulations. Where in lemon, as reported by Mendonça et al. (2006), it is rich in pectin, presenting 54.62% in dry matter, involving flavado, albedo and bagasse. And in the apple according to the works of Levigne, Ralet & Thibault (2002), Yapo et al. (2006) and Fertonani (2006) the content of pectin is between 25 and 30%. In the Type A mead, the high methanol value presented was not expected, since in its formulation no fruit was used to make the presence of pectin available, but as the volatile acidity also presented a high value, what can be proposed is the contamination by microorganisms that carry genes that encode methanol-producing enzymes.

Cell viability

Based on the evolution of the content of soluble solids during the fermentation process, the cell viability of the four types of mead was made, aiming at a more detailed knowledge about the microorganisms that were present in the yeasts, since the fermentation was done naturally.

The determination of yeast cell viability (Figures 5 and 6) was carried out according to Oliveira-Freguglia and Hori (1998), using a solution of methylene blue-sodium citrate as a dye. The samples were analyzed using a microscope and the result expressed as a percentage (%) of live cells determined using the following formula:

\[ V = CV/CT \times 100 \]

In which:

- \( V = \) Cell viability
- \( CV = \) Number of living cells
- \( CT = \) Number of total cells (live + dead)

Based on the cell viability formula and in Figure 5, it was possible to determine the cell viability of the four types of mead on the thirty-sixth day of the process, with cell viability of type A 38.14%, 19.10% of type B, 29.41% of type C and 10.20% that of type D. Based on the cell viability values, together with the pH and Brix analyzes, it was decided to add sodium hydroxide only in types B and mead D.
Figure 6 Yeasts of the four types of mead in the Neubauer chamber on the 57th day of the process. A) Mead of type A. B) Mead of type B. C) Mead of type C. D) Mead of type D.

The second analysis shows the improvement in viability in types B and D meads, indicating that the addition of sodium hydroxide had the expected effect, with type B viability increasing to 22.03% while type D increasing to 27.91%, however the viability of types A and C decreased, with type A reduced to 4.92%, while type C reduced to 22.39%, due to the high concentration of ethanol in the fermented medium, since According to the microbiologist, the main responsible for the decrease in cell viability in alcoholic fermentation is the product of the fermentation itself.

CONCLUSION

It is concluded that it is possible to make artisanal mead through natural fermentation, using wild yeasts. However, that during the fermentation process it is necessary to take extra care so that the process is carried out entirely in anaerobiosis so that there is no contamination with microorganisms that produce acetic acid and methanol, as this transforms the mead, which is the desired product, into vinegar of honey or unifl for human consumption. In spite of this, further assessments are necessary to verify whether the differences found in the four types of meads are repeated and whether the yeasts found in Type C mead are really more efficient, as well as whether the yeasts of the four meads will always differ each other.

In view of the results presented in this work, a reassessment of the quality and identity standards established by Normative Instruction No. 34/2012 for mead is considered appropriate, with the addition of a methanol content parameter in the fermented honey drink and by conducting a study more detailed to verify the real origin of methanol.

REFERENCES


