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Evaluation of Physicochemical and Sensory Aspects of Mead, Produced by Different Nitrogen Sources and Commercial Yeast

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Fermented products from honey are widely consumed around the world. However, the technological and scientific development on this is very low, compared to wine. Thus, the goal of this study was to evaluate the addition of commercial yeast and nitrogen sources upon the sensory and physicochemical aspects of mead. Honey was diluted with water to reach 24 °Brix. Every of the 12 treatments were supplemented with its respective nitrogen source (pollen, mix of pollen-ammonium di-hydrogen phosphate, pollen pretreated and yeast extract). Once the musts were ready, they were pasteurized at 60 °C during 15 minutes. At the same time, every commercial yeast culture (UVAFERM, LALVIN-QA23 and FERMIBLANC AROM) was activated at 37 °C during 30 minutes and then added. The media were incubated at 30 °C; the time consumed varied from 16 to 20 days. The concentration of glucose, fructose, ethanol, glycerol and organic acids, were measured throughout the fermentation process. A sensory analysis was carried out, using the methodology proposed by the University Of California, Davis, with the help of eight trained panelists. Treatments with yeast extract and UVAFERM, exhibited the higher fermentation rate. Moreover, not all the sugars were totally consumed at the end. The concentration of ethanol (12.7 – 13.6% v/v) and glycerol (5.0 – 10 g/L), reached the expected levels. The acids identified, were those produced by yeast (succinic, lactic and acetic). The obtained levels of acetic acid ranged from 0.2 to 1.1 g/L. On the other hand, mead with FERMIBLANC and the Mix, showed the highest score on the sensory analysis test. In conclusion, the treatment with the mix and FERMIBLANC, presented the best physicochemical and sensory characteristics; these parameters can be used to produce quality type meads.

1. Introduction

One of the factors affecting the production chain of honey in Colombia is product falsification. This has a direct impact on crystalized honey sales. This type of honey does not draw consumer attention, and sometimes it is considered second class, no matter its actual quality. Mead production is an important alternative giving this an added value, improving beekeepers revenues. Mead is an alcoholic beverage made from honey and water, in which *Saccharomyces cerevisiae* is used to conduct the fermentation process. The ethanol content in mead can vary from 8 to 18 % (v/v) (Gomes et al. 2013). Mead has been elaborated since ancient times. At the present, the global wine industry is in expansion. the market grew at 10% during 2005 and 2010 (Rivaldi et al. 2009). Due to its aroma, taste, and long history of production, there are certain niches made up demanding consumers for organic products, boosting even more the market dynamics (Rivaldi et al. 2009). Besides, mead has gained economic importance, because of the therapeutic and nutraceutical properties attributed to honey and by an increasing demand of gourmet products (Mendes-Ferreira et al. 2010). Homemade mead is becoming popular among local beekeepers, but there are certain problems related to this practice, such as a

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lack of uniformity of the final product, honey composition, re-fermentation by yeast or by acetic acid-producing bacteria and lactic acid-producing bacteria, that could abnormally increase, the production of ester or volatile acidity, affecting the sensory quality of the final product (Pereira et al. 2012). On the other hand, mead fermentation is a time-consuming process often taking several months to complete, and this depends on the type of honey used, yeast strain, and honey-must composition (Mendes-Ferreira et al. 2010). To overcome those aforementioned situations, honey/honey-wine isolated yeast cultures or commercial yeast starter cultures have been employed, but problems still persists (Mendes-Ferreira et al. 2010). This may be due to the use of inappropriate yeast strains not suitable for the specific fermentation composition/conditions and the limited nitrogen content presented in honey (Mendes-Ferreira et al. 2010). Although, the determination of the physicochemical parameters of mead is quite important, the sensory analysis is a tool that helps to establish if some samples have particular characteristics that would make them superior among others, as well as detecting faults (off-odours, off-flavours, and others) that would make their commercialization impossible. Several investigations have been carried out, in order to determine the effect of some fermentation conditions on the production of certain volatile compounds that are related to the gustatory and olfactory sensations, as well as the effect of certain nutritive supplements applied to the honey-must, on some sensory aspects (Rivaldi et al. 2009; Roldán et al. 2011; Vidrih and Hribar 2007). Therefore, the aim of the present investigation was to evaluate the physicochemical and sensory aspects of mead, produced with different nitrogen sources and commercial yeasts, to obtain a product with better quality characteristics.

2. Materials and Methods

2.1 Honey, yeasts and nitrogen sources

Crystalized honey (*Apis mellifera*) from beekeepers located in Boyacá (Colombia) was used. Honey was stored in refrigeration at 4 ± 2 °C, during the research. Three commercial starter cultures (*Saccharomyces cerevisiae)* were employed. The cultures (UVAFERM BC, FERBLANC AROM, and LALVIN QA23) were maintained in refrigeration. Four nitrogen sources were used (Pollen, Yeast Extract, a mix of pollen and Ammonium di-Hydrogen Phosphate of equal parts, and protease-pretreated pollen). Pollen was collected from CORPOICA´s apiary (Cundinamarca – Colombia) and added to the wort at 3.1 g/L. The Ammonium di-Hydrogen Phosphate (ADP - PANREAC) and pollen were supplemented at 0.575 g/L and 1.55 g/L, respectively. The protease-pretreated pollen was obtained from another investigation that brought under pollen to hydrolysis with Protamex (NOVOZYME). The later was applied at 2.5 g/L. All the nitrogen sources gave 140 mg Nitrogen/L in the honey-must (measured by Kjeldahl).

2.2 Fermentation, wort and inoculum preparation

The wort was elaborated diluting honey with spring-water (from the local market), to obtain a solution of 24 °Brix. Then, the solution was passed through a stainless steel sieve (Tyler standard screen scale 100 mesh) to remove any impurity. Every prepared solution was supplemented with one nitrogen source according to the experiment design, and pasteurized at 65 °C for 10 minutes. After that, they were cooled to reach room temperature. Once the musts were ready; they were inoculated with the commercial yeast. Every inoculum was made activating 0.450 g of the culture in 200 mL of wort, during 30 minutes at 37 °C. The inoculum (200 mL) was poured into the experimental unit to obtain a fermentation medium of 900 mL. All media (12 treatments with two replicates each) were fermented at 30 °C during 20 days. Throughout this time, samples were taken out to measure the concentration of sugars (glucose + fructose), glycerol, ethanol and acids (acetic, succinic and lactic). Once the fermentation (process) was finished, all the treatments were clarified with bentonite (2 g/L) to prepare them for the sensory evaluation.

2.3 Determination of glucose, fructose, glycerol, ethanol and acids (acetic, succinic and lactic) by HPLC, and sensory evaluation

Glucose, fructose, glycerol and ethanol were analysed using a Dionex Ultimate 3000 HPLC system, with a Shodex SC-1011 column, held at 80 °C, a refractive index detector (Shodex) at 40 °C, HPLC-grade water as mobile phase, and a working flow rate of 0.8 mL/min. The injection volume was 10 µL and the retention time 30 minutes (Tanimura et al. 2012). The acids (succinic. lactic and acetic) were analysed with the same system, an Aminex HPX-87H column, held at 65 °C, HPLC-grade water with sulphuric acid at 0.005 mol/L, and a working flow rate of 0.7 mL/min. The injection volume was 20 µL and the retention time 30 minutes (López & Gómez 1996). The sensory evaluation was performed by eight panellists, trained in the evaluation of meads. Samples were presented in glasses, covered to minimize evaporation. Every tester followed the methodology created by the University of California, Davis. This 20-point score sheet evaluates different aspects of mead. It is used to identify production defects, and it is advised to be applied to samples of varying quality (Jackson 2009).

2.4 Experiment design and statistical analysis

The employed design was completely random, due to the levels in every factor (three for the yeasts and four for the nitrogen sources). Data is presented as mean values with their standard deviation. One-way analysis of variance and means comparison for the physicochemical parameters were executed using MATLAB and EXCEL. Twelve treatments with the following variations were elaborated for the study: **E1** (Lalvin QA23 – Mix Pollen-ADP), **E2** (Lalvin QA3 – Yeast Extract), **E3** (Lalvin QA23 – Pollen), **E4** (Fermiblanc – Mix Pollen-ADP), **E5** (Fermiblanc – Yeast Extract), **E6** (Fermiblanc – Pollen), **E7** (Uvaferm – Mix Pollen-ADP), **E8** (Uvaferm – Yeast Extract), **E9** (Uvaferm – Pollen), **E10** (Lalvin QA23 – Pretreated Pollen), **E11** (Uvaferm – Pretreated Pollen), **E12** (Fermiblanc – Pretreated Pollen).

3. Results and Discussion

3.1 Sugar consumption, glycerol and ethanol production during the fermentation process

During the fermentation of mead with Lalvin QA23, there are discrepancies among the profiles of sugar consumption (Glucose + Fructose), for the different nitrogen sources (Figure 1). Lalvin QA23 shows a faster intake (sugars) if yeast extract (E2) is present in the medium, finishing the fermentation earlier (16 days), compared to the other treatments (20 days). At the beginning E1 (mix) exhibited the same trajectory as E2 but that changed on the second day. At the end, E1 had more sugar compared to E3. E10 showed the slowest consumption and the highest sugar content when finished. Šturd et al. (2001) reported differences in sugar intake profiles when using three commercial nitrogen sources and the same yeast, as those found here. They explain that these differences are due to the level of other elements contained in the nitrogen source.

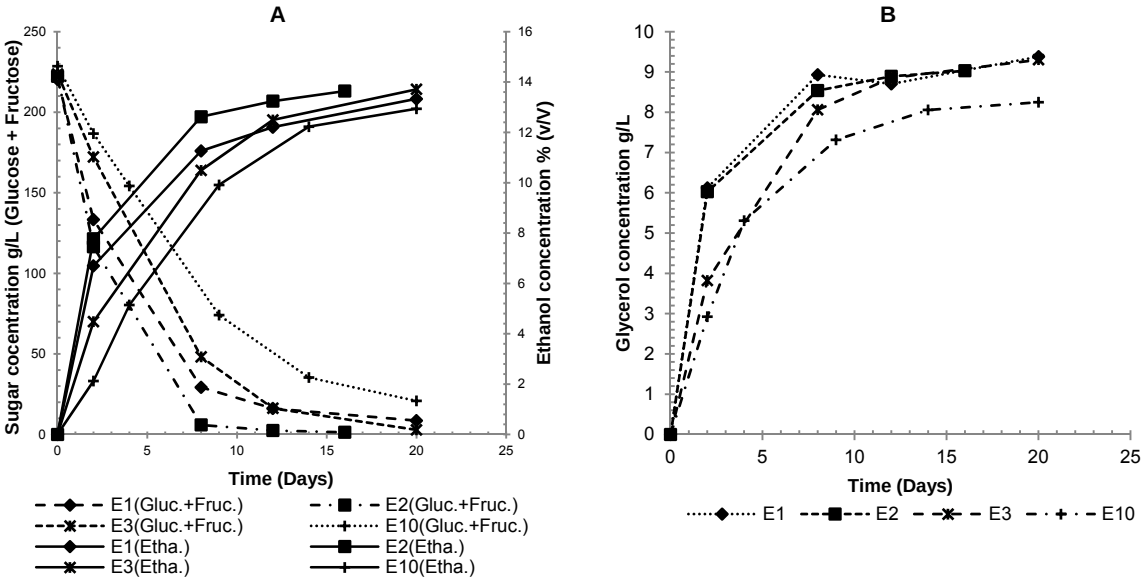

Figure 1: Fermentation profiles of Lalvin QA23 using different nitrogen sources. A) Consumption of reducing sugar and ethanol production. B) Production of glycerol.

The production of Ethanol and Glycerol show the same pattern as sugar intake (Figure 1). E1, E2 and E3 are statically higher than E10 at the end of the fermentation, for both Glycerol and Ethanol concentration. Due to the high initial concentration of reducing sugars, production of those compounds were not affected. Gomes et al. (2013) reported in their study that low nutrient levels can lead to final glucose and fructose concentration higher than 3.5 and 10 g/L, respectively. In regard to sugar profiles of Fermiblanc (Figure 2), the same is seen when identical nitrogen sources were used compared to Lalvin QA23, the only difference is that, the final concentration of sugars is higher for Fermiblanc Arom (E4 and E6). E5 shows a lower final concentration of sugars. E5 exhibited the higher concentration of ethanol at the end of the fermentation, next to it are E4 and E6; E12 shows the lowest level. The ethanol production (Figure 2) tells that there is a close relationship between the amount of sugar the yeast takes into and the amount or volume of produced ethanol. Likewise, the rate of sugar consumption depends on the type of added nitrogen sources; making the ethanol dependent

on the nutritional supplement as well. Regarding glycerol production, yeast extract (E5) stimulates a fast production compared to the others, but E6 (pollen) showed the higher concentration at the end.

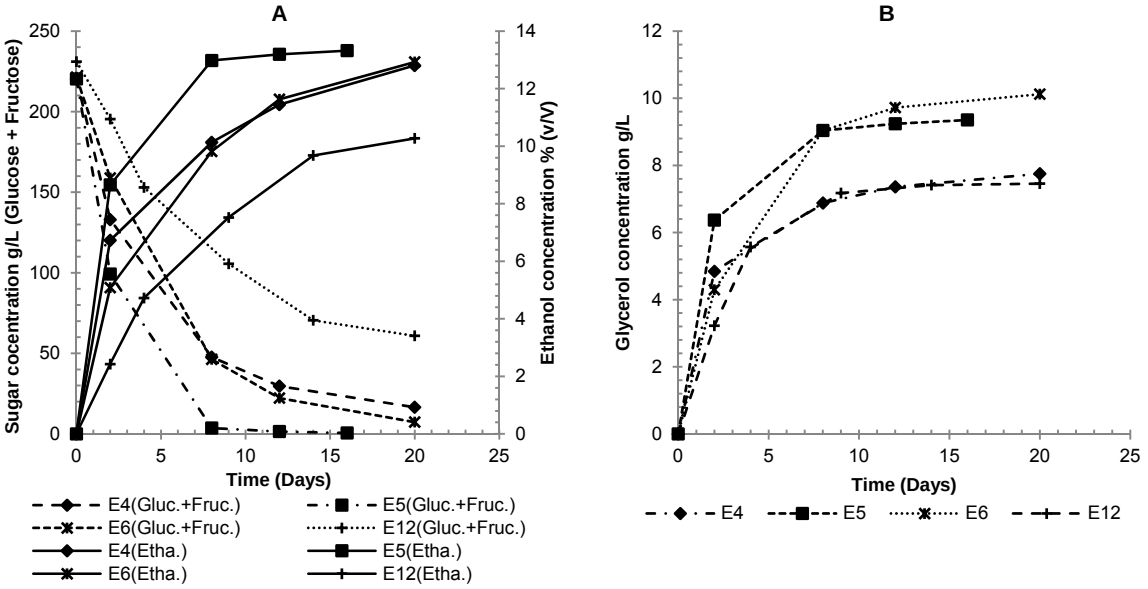

Figure 2: Fermentation profiles of Fermiblanc Arom using different nitrogen sources. A) Consumption of reducing sugar and ethanol production. B) Production of glycerol.

Uvaferm shows the same dynamic of consumption and production as the other yeasts (Figure 3). But it is clear that, this responds much better to the mix of pollen-ADP and pollen (E7 and E9) since the treatments with that supplementation finished in 16 days. The sample with yeast extract (E8) still presents the fastest rates (Figure 3); however, the trajectories exhibited by E7 are closed (E8), specially the sugar consumption. This means that the production of glycerol, ethanol and sugar consumption are influenced by the type of yeast and the type of nitrogen source (as well as the concentration) used during the elaboration of mead.

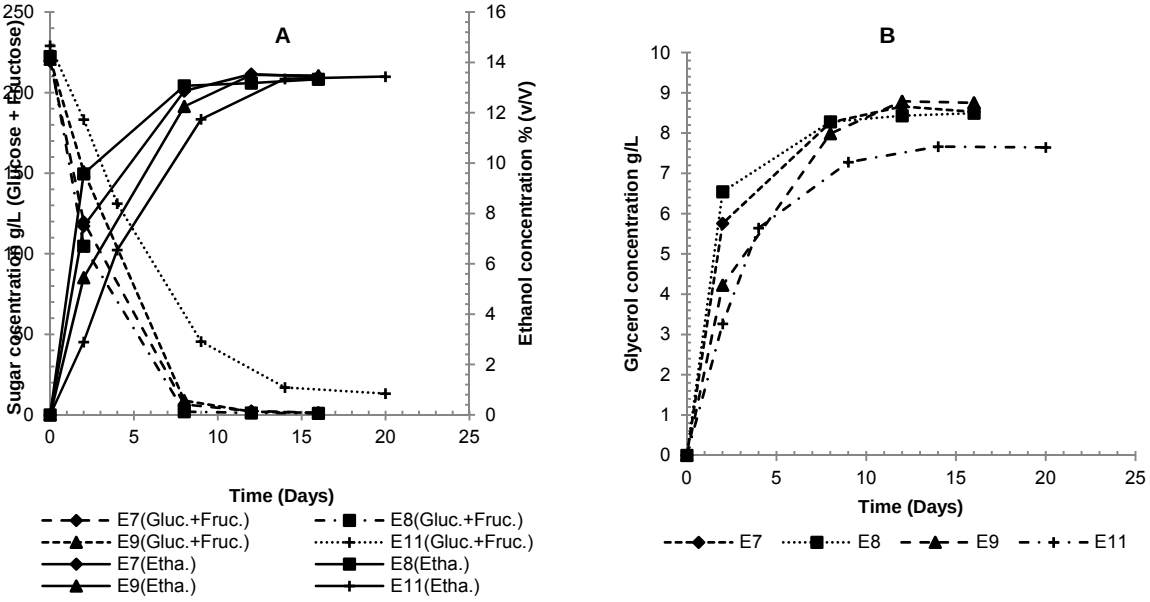

Figure 3: Fermentation profiles of Uvaferm using different nitrogen sources. A) Consumption of reducing sugar and ethanol production. B) Production of glycerol.

3.2 Ethanol yield and organic acids production

Table 1, shows the ethanol yield of all evaluated treatments. Although, there were not significant differences, it can be seen that exists a link between the nitrogen source and the value of the ethanol yield. The samples with yeast extract displayed the lowest level in almost all the yeasts tested. Conversely, the experiments with the mix of Pollen-ADP exhibited the highest value for every one of the employed yeasts. The reported organic acids were those produced by yeast during the fermentation time. Mendes-Ferreira et al. (2010) showed that, the dominant acids, responsible for the increase of the titrable acidity are: succinic and acetic acid. In this research the two major acids obtained were those mentioned above. This was confirmed by the results of Table 1. Table 2 shows that there are significant differences among experiments in terms of the succinic acid production. E6 presents the highest level of succinic acid, compared to the others treatments. It can also be seen that the concentration of this acid (at the end of fermentation) is influenced by the nitrogen source present in the honey-must. All the experiments with pollen, exhibited the major production for all the commercial yeast. Lactic acid is only produced in small quantities by yeast. Any difference (> 300 mg/L) clearly indicates the presence of lactic acid bacteria. Experiment E12 presents the highest concentration of lactic acid. From Table 1 it can be inferred that, the production of lactic acid depends on the type of yeast employed and to a lesser degree, the type of nitrogen source added. Fermiblanc produces more lactic acid compared to Uvaferm and Lalvin QA23, moreover pretreated pollen enhances even more its production. Volatile acidity (acetic acid) increases during fermentation as a result of yeast metabolism. The acetic acid production depends mainly on the concentration of carbohydrates, on the nitrogen source, as well as on pH (Sroka & Tuszyński 2007). From the results of Table 1 we conclude that that the addition of pretreated pollen stimulates the production of acetic acid. Treatments E10 and E11 shows the highest levels of acetic acid, at the end of the fermentation process.

Table 1: Ethanol Yield and organic acids produced at the end of the fermentation process, (Mean ± SD. n=3). Values in the same column shown with different letters differ significantly from one another (p-value <0.05).

Treatments	Commercial Yeast	Nitrogen Source	Ethanol Yield (%)	Succinic Acid (g/L)	Lactic Acid (g/L)	Acetic Acid (g/L)
E1	Lalvin QA23	Pollen-ADP	49.69 ± 0.25^{a}	0.48 ± 0.08^{b}	0.05 ± 0^{b}	0.66 ± 0.2^{a}
E2	Lalvin QA23	Yeast Extract	48.72 ± 0.12^{a}	0.82 ± 0.29^{b}	0.05 ± 0^{b}	0.47 ± 0.22^{b}
E3	Lalvin QA23	Pollen	48.99 ± 1.03^{a}	0.95 ± 0.18^{b}	0.1 ± 0^{b}	0.41 ± 0.03^{b}
E4	Fermiblanc	Pollen-ADP	49.61 ± 2.22^{a}	0.55 ± 0.07^{b}	0.15 ± 0.03^{b}	0.45 ± 0.01^{b}
E5	Fermiblanc	Yeast Extract	47.83 ± 0.19^{a}	1.14 ± 0.01^{a}	0.21 ± 0.01^{b}	0.42 ± 0.05^{b}
E6	Fermiblanc	Pollen	47.63 ± 0.49^{a}	1.4 ± 0.12^{a}	0.19 ± 0.02^{b}	0.54 ± 0.02^{b}
E7	Uvaferm	Pollen-ADP	48.56 ± 0.18^{a}	0.58 ± 0.05^{b}	0.05 ± 0^{b}	0.31 ± 0.04^{b}
E8	Uvaferm	Yeast Extract	47.53 ± 0.48^{a}	0.8 ± 0.03^{b}	0.03 ± 0.01^{b}	0.36 ± 0.13^{b}
E9	Uvaferm	Yeast Extract	48.44 ± 0.41^{a}	1.03 ± 0.01^{a}	0.05 ± 0.01^{b}	0.22 ± 0.01^{b}
E10	Lalvin QA23	Pretre. Pollen	49.16 ± 1.01^{a}	1.04 ± 0.04^{a}	0.16 ± 0.01^{b}	0.97 ± 0.01^{a}
E11	Uvaferm	Pretre. Pollen	49.13 ± 1.04^{a}	0.94 ± 0.01^{b}	0.09 ± 0.01^{b}	0.54 ± 0^{b}
E12	Fermiblanc	Pretre. Pollen	47.63 ± 0.35^{a}	1.23 ± 0.03^{a}	0.33 ± 0.06^{a}	0.98 ± 0.07^{a}

3.3 Sensory evaluation of meads produced by different nitrogen sources and commercial yeasts

The sensory evaluation methodology developed by the University of California, Davis was useful to assess eight important organoleptic aspects of mead. This approach gives two points to the visual performance of a sample (appearance), six points to the olfactory (Aroma), and twelve points to the gustatory (Acidity, Balance, Body, Flavor, Finish and Overall quality), resulting in a 20-point evaluation scale. Besides, it gives a framework of reference to place the quality of meads according to the final score obtained (Table 2). In this manner a 20 – 17 score defines the mead as superior, a 16 – 13 score as standard, a 12 – 9 score as under the standard, and an 8 – 1 score as no acceptable.

Table 2 shows that E4 scored the highest value in all the organoleptic aspects, but one (finish). The resulting mead (E4) elaborated under these conditions can be qualified as standard (Total = 14). Due to the fulfillment of the physicochemical requirements (Gomes et al. 2013), and the attainment of the highest score made on the sensory evaluation, the conditions in E4 seem to be the best among the investigated ones.

Table 2: Results of the sensory evaluation applied to mead samples by eight trained panellists (Median. n=8)

Treatments	Appearance	Aroma	Acidity	Balance	Body	Flavor	Finish	Overall quality	Total
E1	1	3.5	0.5	0.5	1	2	2	1	11.5
E2	2	3.5	1	1	1	2	1	2	13.5
E3	1	3.5	1	1	1	2	1	1.5	12
E4	2	4.5	1	1	1	2	1	1.5	14
E5	2	3	1	0.5	0	1.5	1	1	10
E6	1	3.5	0.5	1	1	2	1.5	1	11.5
E7	1	3	1	0.5	1	1.5	1	1	10
E8	2	3.5	1	1	1	1	1	1	11.5
E9	1	3.5	1	1	1	2	1	1	11.5
E10	1.5	2	0	1	0	1	1	1	7.5
E11	1.5	3.5	1	1	1	2	1	1	12
E12	1	1.5	1	0.5	1	1	0.5	1	7.5

4. Conclusions

According to the results, the rate of sugar intake is affected by the type (and concentration) of nitrogen source applied to the fermentation medium. The commercial yeast employed can affect the kinetics of sugar fermentation. The nitrogen source has an effect on the sugar consumption as well as the rate of production for both glycerol and ethanol. The yeast extract and the mix of pollen-ADP have a positive impact on such rates. Uvaferm seems to have the less nitrogen requirements since exhibit higher rates of sugar intake compared to the others commercial yeasts. With regard to organic acids production, succinic acid is affected positively by pollen addition. Besides, Fermiblanc produces more lactic acids than the other two yeasts. Finally, pretreated pollen stimulates the acetic acid production. Due to the score obtained by E4 on the sensory analysis, and the compliance of the physicochemical requirements, it was selected as the treatment giving the best conditions for mead production.

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