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Study of the Possibility of Honey Wine Production Using New Active Dry Yeasts and Yeasts Derivatives

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ABSTRACT

The current article is dedicated to the study of the possibility of obtaining alcoholic beverages made by fermenting natural honey (honey wine) using different types of dry active yeast and yeast autolysis derivatives. The data obtained from the results of the research will be interesting both from the scientific and production point of view and allow us to conclude that the selected yeasts can be used for the production of such alcoholic beverages.

Introduction

These research activities were carried out with the support of the Innovative Agriculture Training and Learning Camp (AGRI CAMP) Program which is financed by The United States Agency for International Development (USAID) and implemented by International Center for Agribusiness Research and Education Foundation (ICARE).

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Honey wine is probably considered to be the first alcoholic beverage in human history, the first mentions of which date back to 7000 years BC. As a result of excavations in China, Egypt, India, and other countries, many clay containers (karas) and jars were found, the remains of which were found to contain honey (Bayon, 1997, Jorczyk, et al., 1977).

Honey is a natural product rich in biologically active substances, the composition of which depends on the floral origin – climate, environmental and seasonal conditions – terroir (Al-Mamary, et al., 2002). The aroma and taste of honey are due to the nectar of plants. Honey contains useful trace elements: iodine, iron, potassium, phosphorus, magnesium, folic acid, and vitamins B_2 and B_6 . The viscosity of honey depends on the content of sugars; honey with high fructose content is thicker than honey with glucose and other sugars. The amount of fructose is predominant over other sugars in honey, resulting in the fermentation process of honey wine taking longer (Carol, et al., 2006).

Honey wine is made from the alcoholic fermentation of diluted honey. As a result, the beverage is containing 8 to 18 % by volume of ethanol (Antonio Iglesias, et al., 2014). Honey fermentation is a time-consuming process that lasts from weeks to months, and the quality of the final

product is highly variable and depends on several factors: the type and composition of honey, the yeast strain, and fermentation conditions, the fermentation improvers used, etc. (Navrátil, et al., 2001).

Materials and methods

To carry out research, the company “Greenwoods” LLC supported us by providing natural Armenian mountain honey (AST 228-2003), which was selected for its unique relatively weakly expressed delicate and neutral taste and smell, to better identify the characteristics of the yeasts.

Active dry yeast and fermentation improvers used for this research were provided by LabCare&Consulting company, which is the official distributor of Fermentis in Armenia. Samples were made according to yeast strains: 1. SafCeno™ VR-44 (control) 2. SafCider™ AB-1, 3. SafCider™ AC-4, 4. SafCider™ AS-2, 5. SafCider™ TF-6 (www.fermentis.com).

During the fermentation, we also added yeast derivatives: SpringFerm™ and SpringCell™. SpringFerm™ is a fermentation activator based on partially autolyzed yeasts, around 3 times richer in available nitrogen than basic inactivated yeast. Directly issued from yeast, it brings amino acids, sterols, minerals & vitamins. The absence of these compounds can be harmful to complete fermentation (www.fermentis.com).

SpringCell™ is the original pure yeast cell hull. Yeast cell hulls are performing fermentation aids that allow acting efficiently against stuck & sluggish fermentation. SpringCell™ yeast hulls are the original cell hulls patented by the university of Bordeaux (Lafon-Lafourcade, et al., 1984, www.fermentis.com). Prepared honey was dissolved in pure drinkable water at 20-25 °C. According to the refractometer, the total sugar content of the obtained honey juice (must) was 21 %. Then it must be sulfited by potassium metabisulfite in a dosage of 60 mg/l.

The active acidity pH of the honey was 3.32. Although the pH indicator is considered acidic and it is the same as in grape juice, we added 50 g/hl of DL-malic, and citric acids to the honey must, bringing the pH level of the juice to 3.00, as well as Spring Ferm™ at the rate of 4 g/hl for supplying nutrients for yeast growth and fermentation.

The honey was evenly filled in glass jars, to each of which 30 g/hl of dry active yeast strains were added and were closed with fermentation airlock valves. The fermentation process was carried out at a temperature of 16-18 °C. After one week, we added the calculated amount of Spring Ferm

which is necessary to provide the necessary amount of fermentable nitrogen (YAN) for each yeast in the jars.

During the fermentation process when 2/3 of the sugar was fermented we also added SpringFerm™ and Spring Fell™ concentration of 20 g/hl as suggested on Fermentis protocols.

OIV and EAEU GOSTs methods were used to evaluate the physicochemical indicators of wines. Sugar content was determined by refractometry and Bertrand methods (GOST 13192-73). Alcohol content: OIV-MA-AS312-01A, Total acidity: OIV-MA-AS313-01, Volatile acidity: OIV-MA-AS13-02, Free and total sulfur dioxide: OIV-MA-F1-07, Chromatic characteristics: OIV-MA-AS2-07B, Folin Ciocalteu index with OIV-MA-AS2-10 methods (International Organization of Vine and Wine, 2022). Organic acids were determined by liquid chromatography with an Agilent 1100 Series instrument equipped with an Agilent 1260 Infinity DAD detector (Schneider, et al., 1987). Color characteristics were determined using a UNICO 2802 UV/VIS photo spectrometer at 420, 520, and 620 nm in a 1-cm-thick cuvette.

Results and discussions

The content of reducing sugars (fructose and glucose) in the honey sample was 801.8 g/l, and the number of total sugars was 827.3 g/l. The difference in the obtained results, 25.48 g/l can be considered the amount of sucrose, because sucrose is reducing sugar.

Once or twice a week, the containers were mixed and the sugar content of the samples was measured (Figure 1).

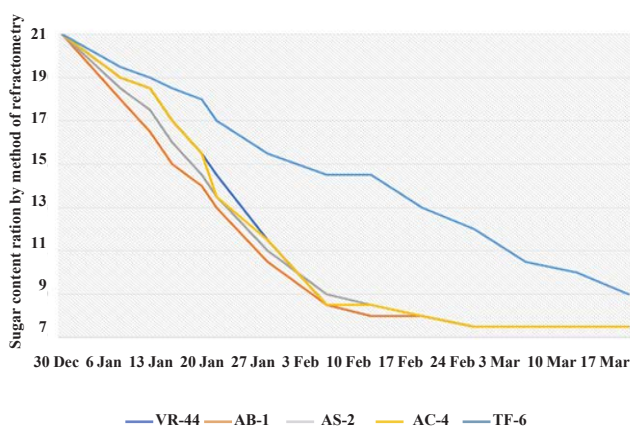


Figure 1. Fermentation graph of honey must (30.03.2022) (composed by the authors).

As it has been shown in the graph, the four types of yeast strains we selected for the research: VR-44, AB-1, AS-2, and AC-4 completed fermentation in 45 days, and only TF-6 strain completed fermentation in 75 days (Navrátil, M., et al, 2001).

After the end of alcoholic fermentation, we did sampling for physicochemical analysis. For the rest of the wines, samples for further preservation and maturation potassium metabisulphite at 20 mg/l was added.

The results of laboratory analyses were studied and presented in Table 1. The amount of titratable acidity in the honey was 0.32 g/l. The titratable acidity of the samples used VR-44, AB-1, and AS-2 yeast is 3.97 g/l, and AC-4 – 3.3 g/l and TF-6 – 3.75 g/l. The increase in titratable acidity is explained by the formation of new acids as a result of fermentation, as well as by the amount of malic acid and citric acid added before the fermentation.

Since the fermentation process was quite prolonged during our research, the high content of volatile acidity obtained as a result of the research in all samples is also legitimate, which is possibly the result of undesirable microbial processes, since the medium contained sugars and exhausted yeast (Navrátil, et al., 2001). The maximum amount of volatile acidity in the samples was recorded at

TF-6: 0.94 g/l, the minimum at VR-44: 0.56 g/l, and at AB-1, AC-4, and AS-2, respectively: 0.72 g/l, 0.64 g/l, and 0.86 g/l.

The active acidity pH of honey was 3.44, then after adjusting the acidity and carrying out fermentation, the pH of the wines decreased to 3.0. After fermentation, the active acidity in the samples was 3.0-3.04. The amount of alcohol produced by the tested yeasts was interesting, because the tested yeast, according to the manufacturer's Fermentis brand, is intended for the production of apple cider.

According to the results of the research, the alcohol content was the highest at 12.3 vol. % (AB-1, AS-2, AC-4) and the lowest indicated: 11.9 % in TF-6, and for the control – VR-44 – 12.2 vol. % (Antonio Iglesias, et al., 2014). Residual sugar content significantly affects the taste and quality of the drink. The sugar content of honey was 227.5 g/l. As a result of yeast activity, most of the sugar was fermented, as a result of which the most fermented sample was VR-44, the residual sugar was 11.58 g/l, and the highest content of residual sugar was observed in sample TF-6 – 19.55 g/l. In other samples AB-1, AS-2, AC-4, respectively: 13.0 g/l, 12.45 g/l, 12.1 g/l. The sugar content in those types of alcoholic beverages is usually classified as semi-dry.

Table 1. The results of the physicochemical analysis *

Parameters	unit	Honey must	SafOENO VR-44	SafCider AB-1	SafCider AS-2	SafCider AC-4	SafCider TF-6
Free sulfur dioxide	mg/l	11.17	4.07	4.7	5.02	2.8	4.07
Total sulfur dioxide	mg/l	51.53	35.4	36.37	41.39	37.6	41.39
Reductions SO ₂	mg/l	2.48	3.13	3.13	3.76	3.13	4.07
Titratable acidity	g/l	0.32	3.97	3.97	3.97	3.3	3.75
Volatile acidity	g/l	-	0.56	0.72	0.86	0.64	0.94
Active acidity pH	-	3.44	3.00	3.02	3.04	3.02	3.03
Alcohol, by vol.	%	-	12.2	12.3	12.3	12.3	11.9
Sugars	g/l	227.5	11.58	13	12.45	12.1	19.55
Yeast assimilable nitrogen (YAN)	mg/l	53.2	42,0	44,8	42,0	42	36.4
Total phenols	mg/l	61.66	112.61	123.36	124.28	114.76	113.8
Folin Ciocalteu index	-	1.40	2.56	2.8	2.82	2.61	2.59
Aldehydes	mg/l	3.96	30.8	36.96	89.76	34.76	47.08
Acetals	mg/l	42.48	38.9	21.24	49.56	29.5	50.74

*Composed by the authors.

Yeast assimilable nitrogen (YAN) or fermentable nitrogen is the combination of free amino nitrogen (FAN), ammonia (NH_3), and ammonium (NH_4^+) that is available for yeast. Outside the fermentable sugars glucose and fructose, nitrogen is the most important nutrient needed to carry out a successful fermentation that doesn't end prior to the intended point of dryness or sees the development of off-odors and related wine faults. As a result of nitrogen metabolism during fermentation, the wine's bouquet and style are also formed. Depending on the characteristics of the yeast, the ratio of fermentable nitrogen (mg/l) to sugar (g/l) according to the selected yeast is 0.7 for VR-44, AB-1, AS-2, AC-4, and 0.9 for TF-6. In the honey must concentration of YAN was 53.2 mg/l, 120 mg/l of SpringFerm was added before starting the fermentation, which helped to increase the amount of organic nitrogen by 120 mg/l.

The results of the analysis showed that the amount in the samples after fermentation was: VR-44 – 42.0 mg/l, AB-1 – 44.8 mg/l, AS-2 – 42.0 mg/l, AC-4 – 42.0 mg/l, TF-6 – 36.4 mg/l, which is sufficient for the stability of the obtained wine (Ribereau-Gayon, et al., 2006).

There is a lack of data on phenolic content in honey (Al-Mamary, et al., 2002). We studied the content of total phenols concentration in honey and recalculated it according to gallic acid, which was 61.66 mg/l. Taking into account that the honey was diluted 4 times, the content of total phenols in honey would be 246.64 mg/l.

After fermentation, the concentration of total phenols increased twice, even though no additional tannins were added to the must. The number of total phenols in fermented samples was: VR-44 – 112.61 mg/l, AB-1 – 123.36 mg/l, AS-2 – 124.28 mg/l, AC-4 – 114.76 mg/l, TF-6 – 13.8 mg/l. The increased amount of total phenols could have occurred either as a result of the biological activity of the yeast or from the addition of yeast autolysates, in which there may also be gallic acid derivatives.

The Folin Ciocalteu index is an international method for determining the total content of phenolic compounds, which is used in winemaking (International Organization of Vine and Wine, 2022). Folin Ciocalteu index in the honey must was: 1.4, in wine samples: VR-44 – 2.56, AB-1 – 2.8, AS-2 – 2.82, AC-4 – 2.61, TF-6 – 2.59, respectively.

The concentration of aldehydes in honey beverages usually ranges from 18.2 to 125.5 mg/l (Antonio Iglesias, et al., 2014, Victoria Moreno-Arribas, et al., 2009). According to the results of our research, the number of aldehydes in the honey was 3.96 mg/l. The minimum amount of aldehydes after fermentation recorded in the wine

samples was 30.8 mg/l for VR-44, which corresponds to the technical sheet data of this strain. The VR-44 strain is used in the production of slightly oxidized champagne wines. The number of aldehydes among the investigated strains according to the strain by growth order was: AC-4 – 34.76 mg/l, AB-1 – 36.96 mg/l, TF-6 – 47.08 mg/l, and the maximum – AS-2 – 89.76 mg/l.

The amount of acetals in the must was 42.48 mg/l, and the amount of acetals in wines decreased by: AB-1 – 21.24 mg/l, AC-4 – 29.5 mg/l, VR-44 – 38.9 mg/l, and increased: AS-2 – 49.56 mg/l and TF-6 – 50.74 mg/l.

Organic acids are also important components of wine, which are important for the evaluation of wine's microbiological stability, aroma, and quality (Table 2). As a result of yeast activity, new acids were found in honey wine, which were not found in honey must, for example, malic acid, ascorbic acid, lactic acid, citric acid, and fumaric acid (Ribereau-Gayon, et al., 2006).

DL-malic acid, and citric acid were added at the amount of 0.5 g/l to increase the acidity of the honey must. After fermentation, malic acid concentrations were changed under the influence of yeast strains VR-44, AB-1, AS-2, and AC-4, respectively: 0.63 g/l, 0.66 g/l, 0.55 g/l, 0.59 g/l, and decreased respectively up to 0.47 g/l in TF-6 yeast sample. The amount of formic acid in must was 0.1255 g/l, but the amount in wines decreased up to VR-44 – 0.055 g/l, AB-1 – 0.0509 g/l, AS-2 – 0.066 g/l, AC-4 – 0.0454 g/l, TF-6 – 0.0739 g/l (Kazumyan, et al., 2022).

In our experiments, even though the fermentation process took quite a long time, a decrease in the amount of malic acid was observed only in the sample TF-6 up to 0.470 g/l, the decrease in the amount of malic acid is only 0.03 g/l. In all other samples, no decrease in the amount of malic acid was observed, although the manufacturer describes some yeasts as consuming malic acid. The amount of malic acid in the samples increased from 0.5 g/l up to VR-44 – 0.6347 g/l, AB-1 – 0.6674 g/l, AS-2 – 0.5558 g/l, and AC-4 – 0.59 g/l.

Although no ascorbic acid was added to the honey must, fermentation revealed trace amounts of ascorbic acid in both wine samples. It can be assumed that it is related to the characteristics of the yeast AS-2 – 11.0 mg/l, TF-6 – 13.8 mg/l.

Lactic acid is produced during alcoholic fermentation in the amount of up to 1.0 g/l. In young, unaged wines, lactic acid can also be produced from malic acid under the influence of certain types of lactic acid bacteria. Taking into account the fact that lactic acid was not found in the honey must, after fermentation it was newly formed in all samples in the following amounts: VR-44 – 0.8419 g/l, AB-1 – 0.4365 g/l, AS-2 – 0.7405 g/l, AC-4 – 1.062 g/l, TF-6 – 0.4946 g/l.

The content of acetic acid in wines is limited: no more than 1.2 g/l. Depending on the yeast strain, some amount of acetic acid is produced during fermentation by the oxidation of acetaldehyde, as a result of the activity of lactic acid and acetic acid bacteria under aerobic conditions (Victoria Moreno-Arribas, et al., 2009). Trace amounts of acetic acid were found in the honey must 0.0173 g/l, after fermentation its amount increased up to VR-44 – 0.4527 g/l, AB-1 – 0.6608 g/l, AS-2 – 1.0212 g/l, AC-4 – 0.672 g/l, TF-6 – 1.0434 g/l.

Citric acid is produced as a by-product during alcoholic fermentation. It is also added to adjust the acidity and pH of the must. Since we added 0.50 g/l of citric acid to honey must, we have interesting results after fermentation: in the case of VR-44, the amount of citric acid decreased up to 0.4573 g/l, in samples AB-1, AS-2, AC-4, TF-6 citric acid content increased respectively: 0.5683 g/l, 0.7157 g/l, 0.6133 g/l, 0.5422 g/l.

The amount of succinic acid prevails over the amount of all acids. Formed during alcoholic fermentation from glutamic acid by deamination and decarboxylation 0.4232 g/l of succinic acid was detected in the must, after fermentation

its amount significantly increased. The minimum quantity was recorded in TF-6 sample: 0.7756 g/l, maximum: AC-4 – 1.2406 g/l, VR-44 – 1.2334 g/l, AB-1 – 1.0757 g/l, AS-2 – 0.9871 g/l.

Fumaric acid ensures the microbial stability and freshness of the wine, which also allows to reduce the amount of SO₂ used. Fumaric acid is effective in reducing or preventing the growth of lactic acid bacteria in wine. It is present in small amounts in a wide range of biological systems because it is an intermediate metabolite of the citric acid cycle. Fumaric acid is produced by the dehydration of malic acid and succinic acid. As a result of our research, it became clear that there is no fumaric acid in honey must, but as a result of yeast activity, VR-44 – 1.1 mg/l, AB-1 – 0.7 mg/l, AS-2 – 0.6 mg/l, AC-4 – 1.0 mg/l, TF-6 – 1.1 mg/l.

To determine the color characteristics of the samples, photoelectric colorimetry was carried out. Color characteristics in wine samples were determined according to the three main colors, yellow, red, and blue absorption coefficients (International Organization of Vine and Wine, 2022). The data calculated as a result of the processing of the results obtained by the device are presented in Table 3 and Figure 2.

Table 2. Organic acids*

Organic acids	unit	Honey must	SafCEnoTM VR-44	SafCider AB-1	SafCider AS-2	SafCider AC-4	SafCider TF-6
Formic acid	g/l	0.1255	0.0551	0.0509	0.0662	0.0454	0.0739
Malic acid	g/l	-	0.6347	0.6674	0.5558	0.59	0.4707
Ascorbic acid	mg/l	-	-	-	11.0	-	13.8
Lactic acid	g/l	-	0.8419	0.4365	0.7405	1.062	0.4946
Acetic acid	g/l	0.0173	0.4527	0.6608	1.0212	0.672	1.0434
Citric acid	g/l	-	0.4573	0.5683	0.7157	0.6133	0.5422
Succinic acid	g/l	0.4232	1.2334	1.0757	0.9871	1.2406	0.7756
Fumaric acid	mg/l	-	1.1	0.7	0.6	1.0	1.1

Table 3. Color characteristics of honey wine *

Parameter	Honey must	SafCEnoTM VR-44	SafCider AB-1	SafCider AS-2	SafCider AC-4	SafCider TF-6
Absorption coefficient						
A420 Yellow	0.33	0.4027	0.218	0.2145	0.2201	0.2408
A520 Red	0.18	0.2444	0.1069	0.1054	0.1081	0.1258
A620 Blue	0.12	0.1793	0.0747	0.0723	0.0714	0.0714
Color intensity	0.63	0.83	0.4	0.39	0.4	0.44
Color shade	1.91	1.65	2.04	2.04	2.04	1.91
Color composition %						
A420 Yellow	53.09	48.73	54.56	54.7	55.07	54.98
A520 Red	27.81	29.57	26.74	26.87	27.06	28.71
A620 Blue	19.09	21.69	18.69	18.43	17.87	16.3

*Composed by the authors.

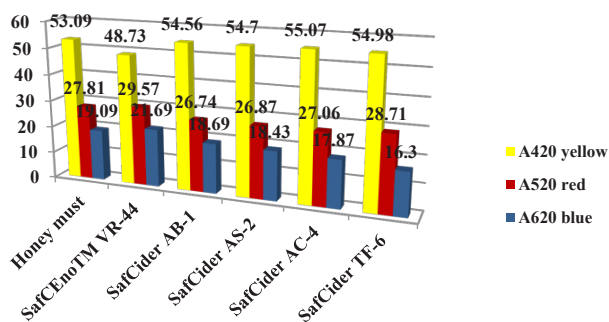


Figure 2. Color composition (%) (composed by the authors).

According to the received data, the color intensity coefficient compared to the hunger indicator decreased in all samples except for VR-44. The color intensity decreased due to exposure to other yeasts. In the case of Color Hue, the opposite picture is observed: it decreased in the case of VR-44, remained the same in the case of TF-6, and increased in the case of other yeasts.

There was also a change in color composition, the yellow shade (53.09 %) increased in AB-1, AS-2, AC-4, and TF-6 yeasts. In the case of red color, the change is small: minimum 26.87 %, maximum 29.57 %. The blue color was 19.09 % in the sample, but increased to 21.69 % in VR-44 and decreased to 6.3 in other samples.

Conclusion

Analyzing the results of the research, especially physicochemical tests it became clear that the use of the mentioned yeasts is appropriate in the production of honey wine. The main issue in our opinion is sluggish and slow fermentation, as mentioned in the literature data (Carol, et al 2006). For this reason, we made new trials to speed up the fermentation process, which will be presented in the following articles.

It is well known that for the evaluation of wine or other alcoholic beverage quality the most important thing is organoleptic tasting. Physicochemical tests are very important for controlling fermentation, aging, and another process in wine and beverage production, but till now there is not any analytical equipment that can measure human feeling when they drink good wine.

The results of the tasting of the samples will be presented in the following articles to study the organoleptic properties of the samples and we are planning to make aroma wheels for each sample which will show the specific properties of the yeasts.

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