



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

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ABSTRACT

Honey wine, also known as a fermented beverage made from natural honey, is believed to be one of the earliest alcoholic drinks created by ancient humans thousands of years ago, long before the discovery of grape wine. Our research aims to produce wine from honey collected in the Armenian mountains. A series of articles 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 research results will be interesting from both the scientific and production points of view. This will allow us to conclude that the selected yeasts can be used for producing such alcoholic beverages.

Introduction

This is the second part of our research on honey wine samples produced using different yeasts and yeast autolysates from the alcoholic fermentation of diluted mountain Armenian honey. The first part of the Study describes the entire technological process of making honey wine, as well as the results and comments on the physicochemical investigations of the honey wine samples after fermentation. This research will compare the physicochemical results of the honey wine samples obtained after six months of storage and aging (Bakhshetsyan, et al., 2022). The second part of the article describes not only the physicochemical analysis of the samples but also includes the most important aspect of our research: tasting, to understand the flavors and profiles of our honey wine samples.

Materials and methods

This study aims to compare the physicochemical parameters of honey wine samples after fermentation and storage, as well as assess their tasting results. For an accurate representation of the tasting experience, we've crafted a specialized tasting form tailored for honey wine (mead). This process proved intricate due to the diverse ingredients often used in mead production, such as fresh fruit juice, berries, or other aromatic substances. In our research, the above supplements were not used, which is why the process is more complicated. These graphs visually represent the diverse aromatic profiles, allowing for a more nuanced understanding of the tasting results. The inclusion of such graphs enhances the clarity and precision of our evaluation process.

OIV and EAEU GOSTs methods were used to evaluate honey wine samples' physicochemical indicators. 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 Chekoltau index with OIV-MA-AS2-10 methods (International Organization of Vine and Wine, 2022). 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

As a result of laboratory research, the following physicochemical indicators of honey wine were studied: free sulfur dioxide, total sulfur dioxide, reductants, titratable, volatile and active acidity, amount of ethyl alcohol and sugars, total and dry extract, specific gravity, the total content of phenolic compounds, aldehydes, acetals, and Folin Chekoltau index (Table 1).

At low pH, the predominant species is molecular sulfur dioxide (SO_2), which exhibits germicidal properties. However, at the wine pH, the major species is the bisulfite anion, which acts as an antioxidant (Stockley, 2005). Sugar-sulfur dioxide complexes break down as sugar is metabolized during fermentation. All binding reactions can significantly reduce the free sulfur dioxide concentration in wine (Ronald and Jackson, 2008). The amount of free sulfur dioxide in the samples ranges from 23.65 to 34.41 mg/dm^3 .

During storage and maturation, we added potassium metabisulphite to all samples to preserve honey wine from oxidation. This was to prevent changes in organoleptic and quality characteristics. As a result, sulfur dioxide-free and total amounts increased in five samples after six months of storage. As a result, in the VR-44 sample total concentration of sulfur dioxide became 129.94 mg/dm^3 from 35.4 mg/dm^3 , AB-1 from 36.37 to 131.17 mg/dm^3 , in the AS-2 sample from 41.39-126.87 mg/dm^3 , TF-6 from 41.39 to 115.2 mg/dm^3 . The highest sulfur dioxide total concentration was recorded for AC-4: from 37.6 to 132.65 mg/dm^3 .

It is common to consider only the molecular sulfur dioxide content when assessing antimicrobial action.

Table 1. The results of the physicochemical analysis*

Parameters	Unit	SafOENO VR-44	SafCider AB-1	SafCider AS-2	SafCider AC-4	SafCider TF-6
Free sulfur dioxide	mg/dm^3	23.96	23.96	34.41	25.49	23.65
Total sulfur dioxide	mg/dm^3	129.94	131.17	126.87	132.65	115.2
Reductons SO_2	mg/dm^3	2.15	2.45	3.07	2.15	3.38
Molecular SO_2	mg/dm^3	1.58	1.62	2.27	1.61	1.34
Titratable acidity	g/dm^3	4.05	3.9	3.97	3.97	3.82
Volatile acidity	g/dm^3	0.64	0.67	0.95	0.75	1.07
Active acidity, pH	-	2.96	2.95	2.96	2.98	3.03
Alcohol, by vol.	%	12.4	12.4	12.5	12.5	12.5
Sugars	g/dm^3	12.45	13.0	13.02	12.72	13.1
Total Extract	g/dm^3	44.70	43.60	44.90	39.60	41.8
Dry Extract	g/dm^3	32.25	30.60	31.88	26.88	28.7
Density	g/dm^3	1.0173	1.0169	1.0174	1.0153	1.0162
Total phenols	mg/dm^3	143.16	142.21	136.85	136.47	137.95
Folin Chekoltau Index	-	3.25	3.23	3.11	3.10	3.13
Aldehydes	mg/dm^3	43.12	49.28	36.96	39.16	35.64
Acetals	mg/dm^3	29.5	25.96	29.5	63.72	9.44

*Composed by the authors.

Of the free states of sulfur dioxide, molecular SO_2 is the most readily absorbed by microbes. However, as the cytoplasm typically has a pH of around 6.5, the molecular form quickly changes into its ionic states, bisulfite and sulfite. The binding of these ions with various cellular constituents enhances the continued uptake of sulfur dioxide. The level of free sulfur dioxide required to obtain a desired amount of molecular SO_2 can be partially estimated by dividing the desired value by the percentage of molecular SO_2 at the wine's pH. As sulfur dioxide binds with carbonyls and phenolics, the percentage of free and total SO_2 declines, until it reaches a new equilibrium between its bound and free forms (Ronald, 2008). The amount of molecular SO_2 in our samples was as follows: VR-44: 1.58 mg/dm^3 , AB-1: 1.62 mg/dm^3 , AS-2: 2.27 mg/dm^3 , AC-4: 1.61 mg/dm^3 , TF-6: 1.34 mg/dm^3 . Based on the obtained data, we can say that the wines were stable. The presence of ascorbic acid can affect reductones. Reductones decreased by almost one mg/dm^3 . The minimum amount was 2.15 mg/dm^3 in the VR-44 and AC-4 samples. Samples AS-2 and TF-6 had the maximum amounts, 3.07 mg/dm^3 , and 3.38 mg/dm^3 respectively.

There was no significant change in titratable acidity results in the studied samples. The increase in titratable acidity in samples AS-2 and TF-6 can be attributed to residual sugar. An increase in titratable acidity and residual sugar occurred in sample VR-44. The titratable acidity results were as follows: minimum amount observed in sample TF-6, 3.82 g/dm^3 , maximum amount observed in VR-44, 4.05 g/dm^3 .

Volatile acids can increase due to oxidation or lactic acid fermentation. Because there was residual sugar in the samples, the decrease in volatile acidity may be due to the formation of new acetic acid esters. The amount of volatile acids changed irregularly: AB-1 sample decreased from 0.72 g/dm^3 to 0.67 g/dm^3 , VR-44 increased from 0.54 to 0.64 g/dm^3 , AS-2: 0.86-0.95 g/dm^3 , AC-4 increased from 0.64 g/dm^3 to 0.75, TF-6 increased from 0.94 g/dm^3 to 1.07 g/dm^3 . The change was 0.05-0.13 g/dm^3 .

During honey wine maturation and storage, the pH decreases. The amount of mineral matter in the samples was low, salts of weak acids were also low and the slight change in titratable acidity during aging affected the pH. Active acidity in the samples before storage was 3.00-3.04. The acidity in the samples after storage was 2.95-3.03.

Interestingly, the amount of ethyl alcohol increased by 0.2 % in the four investigated samples and by 0.6 % in the TF-6 sample. This means that the amount of residual

sugar during storage was converted into alcohol by slowly ongoing fermentation.

There was also a change in residual sugar amounts. Sample AB-1 did not change. In samples VR-44, AC-4, and AS-2 there was an increase in residual sugar by 0.87 g/dm^3 , 0.57 g/dm^3 , and 0.62 g/dm^3 , respectively, which can be explained by the breakdown of polysaccharides contained in honey, which was realized as a result of the autolysis of the yeast, as a result of the breakdown of the enzymes that pass into the wine. A decrease in residual sugar occurred only in sample TF-6, from 19.55 g/dm^3 to 13.2 g/dm^3 , which is explained by alcoholic fermentation.

The total and dry extracts of honey wine samples have also been determined. All substances that dissolve in wine and remain after distillation (evaporation) of the water-alcohol part are called total extracts, which is the total amount of all non-volatile substances dissolved in the wine, because we used different yeasts, which were originally intended for the production of fruit wine, and cider (Bakhshetsyan, et al., 2022), including carbohydrates, glycerin, non-volatile acids, nitrogen compounds, tannins and dyes, higher alcohols, and minerals. Since we used different yeast and the raw materials were the same, the difference depends on the difference in the amount of glycerol and succinic acid produced by the yeast.

Glycerol ranks as the most important fermentation product after ethanol and carbon dioxide. It is involved in osmotic cell regulation (Blomberg and Adler, 1992). Many growth and environmental factors influence the amount of glycerol produced by yeast in wine, e.g., sulfite concentration, pH, and nitrogen composition (Ough, et al., 1972). The amount of dry extract in the investigated samples was as follows: VR-44: 44.70 g/dm^3 , AB-1: 43.60 g/dm^3 , AS-2: 44.9 g/dm^3 , AC-4: 39.60 g/dm^3 , TF-6: 41.8 g/dm^3 .

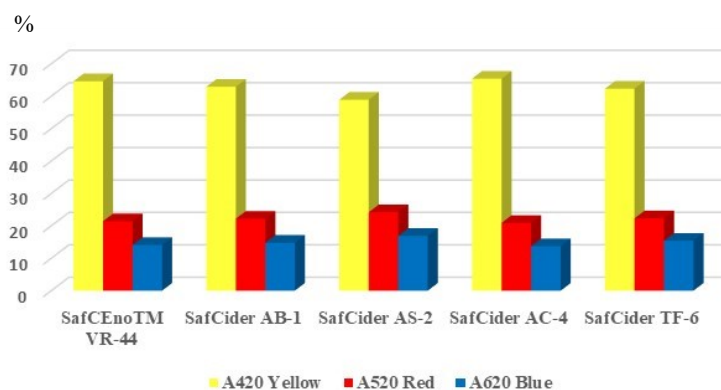
The content of phenolic substances in honey wine samples varied from 136.47 mg/dm^3 to 143.16 mg/dm^3 . Because no additional tannins were added, phenolics after storage increased by 30 mg/dm^3 to 12.57-30.55 mg/dm^3 . We assume that the increase in the total concentration of phenolic compounds could have occurred due to yeast cell autolysis (Bakhshetsyan, et al., 2022).

The Folin-Chekoltau index is an international method for determining the total content of phenolic compounds (International Organisation of Vine and Wine). In mature samples, this indicator increased. The results were as follows: VR-44 – 3.25, AB-1 – 3.23, AS-2 – 3.11, AC-4 – 3.10, TF-6 – 3.13 (Table 2).

Table 2. Color characteristics of honey wine*

Parameter	SafCeno TM VR-44	SafCider AB-1	SafCider AS-2	SafCider AC-4	SafCider TF-6
Absorption coefficient					
A420 Yellow	0.1194	0.1311	0.1494	0.1019	0.1106
A520 Red	0.0395	0.0463	0.0615	0.0325	0.0395
A620 Blue	0.0261	0.0309	0.0429	0.0214	0.0272
Color intensity	0.18	0.21	0.25	0.16	0.18
Color shade	3.02	2.83	2.43	3.13	2.8
Color composition (%)					
A420 Yellow	64.6	62.9	58.9	65.4	62.3
A520 Red	21.4	22.2	24.2	20.9	22.3
A620 Blue	14.1	14.8	16.9	13.7	15.4

*Composed by the authors.

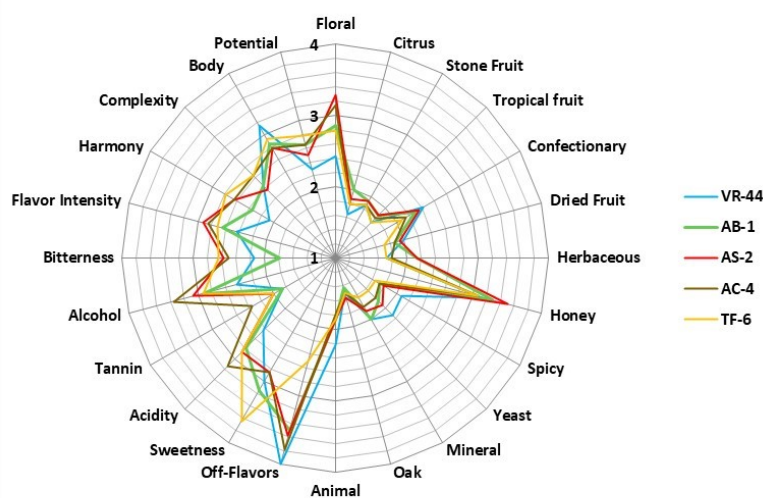
**Figure.** Color composition (composed by the authors).

Chemical processes of the natural storage mechanism can lead to the oxidation of phenolic compounds and other reducing agents, the formation of aldehydes, acetals, and esters, and the hydrolysis of polysaccharides and glucosides (Ribereau-Gayon, et al., 2006). After storage, the total amount of aldehydes in the VR-44 sample increased from 30.8 to 43.12 mg/dm³, AB-1 from 36.96 to 49.28 mg/dm³, AS-2 from 89.76 mg/dm³ to 36.96 mg/dm³, AC-4 from 34.76 -39.16 mg/dm³, TF-6: from 47.08 mg/dm³ to 35.64 mg/dm³.

The total amount of acetals in the samples changed significantly. In samples VR-44, AS-2, TF-6 it decreased, respectively 29.5 mg/dm³, 29.5 mg/dm³, 9.44 mg/dm³. The

quantity of AB-1 and AC-4 increased by 25.96 mg/dm³ and 63.72 mg/dm³, respectively. There are many shades of honey wine, from transparent to dark amber.

The chemical changes that occurred during storage also became the basis for the change in the wine color. In all five samples, the percentage of yellow color in the color composition increased, while the percentage ratio of red and blue colors decreased. The color intensity changed significantly during storage. Intensity decreased in all samples. It decreased from 0.83 to 0.18 in the VR-44 sample, from 0.4 to 0.21 in the AB-1 sample, AS-2 from 0.39 to 0.25, AC-4 from 0.4 to 0.16, and TF-6 from 0.44 to 0.18 (Table 2, Figure).



Wheel graph. Honey wine aroma wheel (composed by the authors).

The tasting form is thoughtfully designed to capture the nuanced flavors, and aromas inherent in each honey wine sample. To accommodate this complexity, our tasting form provides a comprehensive assessment framework. One distinctive feature of our tasting form is the inclusion of aroma wheel graphs for each honey wine sample (Wheel graph).

During the tasting, the participants appreciated the aroma and taste of the wines and left a general impression of the samples. For the tasting, a special evaluation sheet was compiled according to a five-point system, in which the following indicators were evaluated: from aromas: floral, stone, citrus, tropical, dried fruit, honey, confectionery, herbal, yeast, spicy, oak, animal, off-flavors, mineral, to taste: sweetness, acidity, tannins, alcohol, bitterness, intensity of taste. The indicators of the overall rotation are harmony, diversity, physicality, and potential.

Based on the opinions of the tasting participants, we have compiled a wheel of flavors from five samples. The wheels of aromas provide an opportunity to better describe wine's peculiarities the olfactory characteristics. VR-44, being a test version of the study samples, showed the following positive results: honey shades are well expressed, as well as a floral bouquet, yeasty aromas are also noticeable, the most unpleasant aromas were found in this sample, it is fleshier compared to other samples. The VR-44 sample has

the highest body and spicy notes. Alcohol is not strongly noticeable during tasting, due to glycerin.

The AB-1 yeast sample used is distinguished by its high floral and herbaceous aromas, with well-defined honey notes. pastry aromas are also noticeable, and mineral-origin aromas are faintly noticeable. This sample stands out for its citrus tones and interesting sweetness, with no bitterness. As a negative aroma, tasters noted the presence of off-flavors, which is acceptable in honey wine's aromatic bouquet. International experience shows that any wine made from honey has characteristic side aromas (Kay Senn, et al., 2020).

The AS-2 sample did not change significantly during the study, but after storage, it acquired different properties. The Honey, flowers, and stone fruit aromas were well expressed. The intensity of taste and bitterness complements each other. Among the used yeasts, AS-2 showed the best results.

AC-4 has pleasant acidity and spirit, and a fresh floral aroma is noticeable. Among the samples, the quantity and variety of tannins are mentioned only here. The highest level of acidity is expressed in this sample.

According to the participants of the tasting, the best option is TF-6, because the unpleasant aromas are not clearly expressed, and the aromas of stone fruits are pleasantly emphasized, although the honey or floral aromas are not

significantly expressed here. Let's take into account the fact that this sample was fermented for the longest without losing its quality characteristics. The data show that this sample is the wine with the greatest potential.

Taking into account the fact that honey wines are not produced in Armenia, the participants of the tasting, not having experience of evaluating honey wine, gave their opinions on the studied samples according to the same pattern as wines made from grapes.

Conclusion

According to studies, Ferment is dry yeast and derivatives can be used to produce honey wine. The laboratory tests confirmed that the wines have high-quality characteristics, and the tasting results indicated that they had rich olfactory characteristics. A wide range of quality and taste characteristics can be achieved with the yeast used. Based on the research results, the AS-2 sample showed the best taste indicators. The AS-2 sample stands out for its maximum floral, herbaceous, and honey tones, strongly expressed aromas of tropical fruits and stone fruit, as well as dry aromas. The unique style of the wines has aroused great interest. As a result, in the future, we will clarify the possible options for reducing the fermentation period and improving the wine quality. We will also carry out new studies on the drinks obtained from the alcoholic fermentation of honey.

In conclusion, our specialized tasting form, complete with aroma wheel graphs, serves as a sophisticated tool to capture honey wine flavors. This innovative approach ensures that the tasting results are not only comprehensive but also visually accessible, making it an invaluable resource for enthusiasts and connoisseurs alike.

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Declarations of interest

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