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Investigating the Effect of Yeasts and their Derivatives on the Qualitative Indices of Red Wine

K.N. Kazumyan, M.N. Mikaelyan, E.R. Gevorgyan, A.A. Jraghatspanyan
Armenian National Agrarian University
kkazumyan@yahoo.com, mikayelyan.m@mail.ru, elyanora.gevorgyan@mail.ru, artem.jraghatspanyan@mail.ru

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ABSTRACT

The study on the influence of yeast and yeast derivative autolyzes on the wine qualitative and quantitative characteristics has been conducted upon the first article of the series. Red grapes contain more than 1500 components, which are forming the taste and aroma of red wines, as well as the biological value of the product. One of the main technological operations in the production of red wines, which is related to the creation of favorable conditions for extracting phenolic and coloring compounds from the solid parts of the grape berry that are responsible for the color, taste, and aroma preserving them at the different stages of the wine formation and maturation, has been researched and analyzed.

Introduction

Red wines are macerated wines (Ribereau-Gayon, et al., 2006). Crushed grape is forming pomace (the mixture of skin, seeds, and grape juice and pulp) from which the coloring and aromatic phenolic compounds are released as a result of extraction (Busse-Valverde, et al., 2011, Kazumov, et al., 2013). For this purpose, red grapes are crushed in crusher comb separators, and the pomace is sent to the fermentation tanks. Extraction processes in the pomace depend on fermentation temperature, while maceration of the skin cells and seeds occurs with the extraction of color and phenolic substances. Many phenolic compounds in grape berries have antimicrobial and antiviral properties (Edi Maleti, et al., 2009). Even in ancient times, it was practiced to mix water with wine to disinfect water contaminated with bacteria. The beneficial effects of red wines on human health has recently raised significant interest in oenotherapy.

Materials and methods

Grape variety is the base of the future wine (Cabezas, et al., 2003). A large supply of technologically important components and hygienic indicators of grapes are the main factors that determine the appropriateness of choosing a variety. Actual research in the field of development and production of additional materials for winemaking has made it possible to create effective preparations for fermentation of pomace and must. Among those materials active dry yeasts (Tikhonova, et al., 2011), natural fermentation activators, color stabilizing agents for increasing the color intensity and stability of the polyphenolic complex of the wine are involved (Ageeva, 2007).

Our studies in this field aim to investigate the characteristics of new yeast strains and yeast derivatives used in winemaking to significantly improve the wine's quality. For the first time in the field of winemaking in Armenia, an investigation of the impact of Fermentis products (by Lesaffre Group) provided by "LabCare&Consulting" LLC – the official distributor of Fermentis in Armenia, on the quality of the red wines has been carried out. The following yeast strains have been applied:

1. SafOeno™ NDA 21: a new active dry yeast strain. Alcohol resistance – up to 16 % by vol., sugar to alcohol ratio: 17.6 g/1% vol.

2. SpringFerm™ Xtrem: activator of alcoholic fermentation, consisting of 100 % fully autolyzed yeast, containing 9 times more soluble nitrogen than basic inactivated yeast.

3. SpringGel l™ Color G2: color stabilizer. Based on pure inactivated yeast from Saccharomyces cerevisiae particularly rich in polysaccharides, it improves the action on the intensity and the stability of the polyphenolic profile of red wines.

For the study the following Armenian grape varieties have been used: Tigrani, Karmrahyut and Charentsi (red-colored pulp), which are widespread in the Armavir region of the Republic of Armenia. The selection of these grape varieties is not occasional because wines made therefrom sometimes have problems, such as unstable color and sediment of coloring matter (Jacobson Jean, 2006).

The process of wine samples preparation has been done in the Jraghatspanyan Winery during the 2020 grape harvest season. Jraghatspanyan Winery is located in the Bambakashat village, Armavir region, Armenia.

The OIV and EAEU GOST methods were used to assess the physicochemical indicators of wines. The sugar content in grapes was determined by refractometry and densitometry methods. Alcohol content was measured by the OIV-MA-AS312-01A method, total acidity – through OIV-MA-AS313-01, volatile acidity – through OIV-MA-AS13-02, free and total sulfur dioxide – by OIV-MA- F1-07, chromatic characteristics – by means of OIV-MA-AS2-07B, Folin-Ciocalteu Index – via OIV-MA-AS2-10 (Hrazdina, et al., 1970, International Organization of Vine and Wine, 2021). Organic acids were determined by HPLC (system: Agilent 1100 Series, detector: Agilent 1260 Infinity) (Ribereau-Gayon, et al., 2006).

Grapes were harvested at the end of September and beginning of October 2020 at the stage of full technical maturity, i.e., Tigrani sugar content making 260 g/l, Karmrahyut sugar content – 215 g/l, Charentsi – 220 g/l. The grapes were crushed in a crusher comb separator and the pomace was transferred to the tanks for fermentation and maceration for extracting coloring and phenolic substances from skin cells and seeds. Maceration of pomace is the main way of making high-quality red wines. The temperature of fermentation was + 28 ^{0}C which is the optimal one for extraction of phenolic components from the solid parts of the fermenting grape must (Ribereau-Gayon, et al., 2006).

It is well known that during the maceration of the pomace, one of the most freely isolated compounds is the seeds oenotannin, which takes part in the formation of the color and taste of the future wine (Bagaturia, et al., 2011). The floating cap of the pomace formed during alcoholic fermentation was well mixed four times per day. This process also supplies oxygen from the air in the intervals between the immersions of the cap, which ensures the occurrence of oxidative processes with the formation and accumulation of esters (substances that are responsible for the basis of the wine bouquet).

The study seeks to investigate the effect of using active dry yeast – NDA-21 on the wine quality (Tikhonova, et al., 2011) with the combination of fermentation activator: SpringFerm™ Extrem and color stabilizing agent: G-2 during alcoholic fermentation. Yeast and yeast derivatives were introduced to the pomace according to the producer requirements: active dry yeast in the amount of 20 g/hl, SpringFerm™ Extrem at the rate of 20 g/hl, G-2 color stabilizer – 20 g/hl.

Maceration with the pomace cap lasted 4 days, after that the pomace was pressed (separated from solid parts) and young wine was sent for further fermentation. To ensure the natural occurrence of malolactic fermentation, no sulfur dioxide was added. SpringFerm™ Extrem has a good influence on malolactic fermentation and it can be used as an activator for malolactic fermentation too.

After the complete alcoholic fermentation, the young wines were stored on the yeast lees and periodically stirred for the enrichment of young wine with the yeast autolysis in order to enrich the taste of young wine. As the wine was clarified, it was removed from the yeast lees in December of 2020. Sulfur dioxide was added in the form of potassium metabisulfite at the rate of 60 mg/l for each sample of the wines. The wine samples were bottled in February 2021. The mentioned samples have been tested in the wine laboratory and via tasting evaluation.

Results and discussions

The results of the laboratory physicochemical tests are shown in the presented table. Investigation of the data shows that the residual sugar content in all three samples is less than 1 g/l, the lowest amount of residual sugar is in the Karmrahyut sample – 0.16 g/l, the highest amount of sugar is in the Tigrani sample – 1 g/l. It should be noted that the concentration of sugar in the grape juices Karmrahyut was 215 g/l, Charentsi – 220 g/l, and the greatest amount of sugar was in Tigrani – 260g/l. This circumstance points out the good fermentable activity of the introduced strain of yeast SafOeno NDA 21, and SpringFerm™ Xtrem – a fermentation activator for promoting alcoholic fermentation (Kazumov, et al., 2013, Tikhonova, et al., 2011).

One of the main indicators of the wine quality is the alcohol content (Yanniotis, et al., 2007). High alcohol content gives the wine microbiological stability and improves its organoleptic characteristics. The alcohol content in the wine samples was, respectively: in Tigrani – 14.5 % vol. (17.93 g sugar consumption for 1% vol. alcohol production), Karmrahyut – 11.4 % vol. (18.86 g sugar for 1% vol. alcohol production), Charentsi – 12.1 % vol. (18.18 g sugar per 1 % vol. alcohol production). The fermentation process was most efficient in the sample of Tigrani, where the alcohol yield was closest to the indicator of 17.6 g of sugar for 1 % vol.; alcohol and the yield was over 98.6 %.

The amount of titratable or total acidity in the tested samples was at the level of 4.04 g/l for Charentsi, 3.6 g /l for Karmrahyut and Tigrani. Low total acidity and, as a result, higher level of pH in all the samples are respectively typical for wines produced in geographically southern wine regions (pH: 4.08 – Charentsi, 4.20 – Karmrahyut, and 4.33 – Tigrani). All enzymatic processes occurring at the different stages of wine production depend on the pH level of the must and wine. The level of pH of wine is responsible for its antibacterial effect and its ability to neutralize the course of undesirable microbiological processes (Ribereau-Gayon, et al., 2006), as evidenced by the low level of the obtained volatile acids: 0.46 g/l for Charentsi, 0.66 g/l for Karmrahyut, and 0.59 g/l for Tigrani.

The total concentration of sulfur dioxide in the wine samples is from 47.04 mg/l to 64.28 mg/l, which protects and provides the microbiological stability of the wine. The synergistic action of phenolic compounds with a high content of sulfur dioxide is also evidence of their joint presence in red wines which inhibits the development of cells of harmful microorganisms and their vital activity.

Organic acids play an important role in the development of wine quality. Total acidity content determines the grape's suitability for the production of the appropriate wine type. The main acids of the grape are tartaric and malic; their content is responsible for the pH value of wine (Ribereau-Gayon, et al., 2006). Tartaric acid salts affect the organoleptic characteristics and are responsible for the tartaric stability (crystal precipitation) of wine. Potassium and calcium salts of tartaric acid are less soluble in the presence of alcohol; therefore, they can precipitate forming small crystals causing the cloudiness of wine (Ribereau-Gayon, et al, 2006). Malic acid is the most labile acid in grape berries. It can participate in the respiratory processes, in the metabolic processes and can take part as an intermediate product for the synthesis of many other compounds. The high concentration of malic acid in wine can cause the appearance of a sharp taste of wine, which is also called "green acidity" (Kazumov, et al., 2013). Spontaneous malolactic fermentation can start during or after the main alcoholic fermentation of the grape pomace or must, under favorable conditions (temperature of wine could be + 20 ^{0}C or above it). Malolactic fermentation reduces the content of malic acid increasing the level of pH, which gives the wine a soft and creamy taste and a specific aroma of the young wine (Ribereau-Gayon, et al., 2006, Schneider, et al., 1987).

Organic acid concentrations were tested in all wine samples. The result shows the presence of tartaric acid in all samples in the range of 1.46 g/l – 1.74 g/l. Malolactic fermentation was efficient. In the tested samples, malic acid presence was detected only in the Tigrani variety with a small amount (0.29 g/l). Acetic acid concentration is fixed within the range of volatile acid values. The citric acid content is in the range of 0.13-0.11 g/l. The low citric acid content can be associated with an indicator of the biological stability of the wine. In the Krebs Cycle after the addition of acetyl-coenzyme A to oxalic-acetic acid, citric acid is formed through several derivatives, which are converted into succinic acid. Succinic acid was identified in all tested samples. The highest concentration of succinic acid was identified in the Charentsi sample – 0.88 g /l, the lowest was in the Karmrahyut – 0.38 g/l and in Tigrani sample – 0.70 g/l. The Krebs Cycle ends with dehydrogenation to fumaric acid. Fumaric acid was identified only in the Tigrani sample in a very little amount – 7.77 mg/l.

Shikimic acid was detected in all the samples with fairly great amounts. So, in the sample of Charentsi the lowest amount of shikimic acid is recorded – 0.021 mg/l and the highest amount in Karmrahyut – 79.70 mg/l, while in Tigrani it was 16.31 mg/l. Grape berry contains mainly tartaric, malic and citric acids, the rest of the acids are formed as a result of alcoholic fermentation through a series of chemical transformations. Their role is important for the formation of wine taste and bouquets. Thus, the biosynthesis of aromatic amino acids is based on an important discovery that shikimic acid is an indispensable intermediate product in the biosynthesis of aromatic compounds.

Studies on carbon 14 (C^{14}) have shown that in plants shikimic acid can be converted to phenylalanine and tyrosine. According to the existing data, the average content of aldehydes in wines is 12-220 mg/l (Gerzhikova, 2009). Aldehydes are formed as a result of a non-enzymatic process of oxidative-deamination of amino acids (alanine) and in the Cycle of Krebs. In small concentrations, they have a pleasant smell and are involved in creating a specific aroma of aged wine. Aldehydes can positively be associated not only with aromatic alcohols but also be involved in the significant reaction for red wine: polymerization of the anthocyanin-tannin complex is as a bridge between anthocyanins and tannins (Ribereau-Gayon, et al., 2006, Yakimenko, et al., 2012). The total amount of aldehydes in wine samples is low: Tigrani – 9.24 mg/l, Karmrahyut – 8.8 mg/l, and Charentsi – 6.2 mg/l, which corresponds to the data mentioned in the literature and indicates the quality of wine, and quality of ADY (NAD-21) which is used for alcoholic fermentation.

Acetals have a direct, positively significant correlation with methanol, ethers, and aromatic acids. The increase of acetals during fermentation increases their presence in the wine. The total amount of acetals in wine samples is respectively: in Tigrani – 15.34 mg/l, Karmrahyut –16.52 mg/l, and Charentsi – 12.98 mg/l.

Dry extract represents the concentration of the total extract (non-volatile content) by subtracting the concentration of sugars. It includes all non-volatile acids (tartaric, lactic, malic, citric, and succinic), some of them in a certain amount are allowed for addition to wine. In this case, it becomes necessary to use an additional indicator: residual extract (reduced extract-titratable acids) (Bagaturia, 2011, Yanniotis, 2007).

Table. Physicochemical analysis of red wines*

№	Parameters	Unit	Charentsi	Karmrahyut	Tigrani
	Standard chemical parameters				
1.1.1	Sulfur dioxide-free	mg/l	10.98	15.68	9.40
1.1.2	Sulfur dioxide total	mg/l	61.15	64.28	47.04
1.1.2	Sulfur dioxide reductions	mg/l	4.70	4.70	4.70
1.2.1	Volatile acidity	g/l	0.46	0.66	0.59
1.2.2	Total acidity	g/l	4.04	3.60	3.60
1.2.3	pH		4.08	4.20	4.33
1.3.	Residual sugar	g/l	0.39	0.16	1.00
1.4.	Alcohol	Vol.%	12.10	11.40	14.50
1.5.1	Total aldehydes	mg/l	6.20	8.80	9.24
1.5.2	Total acetals	mg/l	12.98	16.52	15.34
1.6.1	Total extract	g/l	31.30	38.00	34.10
1.6.2	Dry extract	g/l	30.91	37.84	33.10
1.7	Density	g/ml	0.994252	0.997493	0.993292
	Organic acids				
2.1.	Tartaric acid	g/l	1.46	1.50	1.74
2.2.	Malic acid	g/l	-	-	0.29
2.3.	Shikimic acid	mg/l	0.02	79.70	16.31
2.4.	Lactic acid	g/l	2.51	2.74	1.43
2.5.	Acetic acid	g/l	0.46	0.79	0.52
2.6.	Citric acid	g/l	0.13	0.11	0.12
2.7.	Succinic acid	g/l	0.88	0.38	0.70
2.8.	Fumaric acid	mg/l	-	-	7.77

*Composed by the authors.

It is known that the concentration of the dry extract is an important indicator of the wine quality. In dry white wines, it contains tartaric and malic acids, nitrogen compounds, and other substances of the grape must and non-volatile compounds formed during alcoholic fermentation. The extract of red wine compared to white additionally contains non-volatile substances extracted from the solid parts of the grape pomace (skin, seeds) (Gerzhikova, 2009). The speed of extraction of extractive substances from the skin and seeds of grapes depends on their diffusion coefficient. For example, this value for anthocyanins equals to 0.03-0.331 X 10^{-7} m^2/s, for leuco-anthocyanins – 0.024-0.310 X 10^{-7} m^2/s, for tannins – 0.018-0.310 X 10^{-7} m^2/s. The extractive substances accumulation dynamics in red wine is maximum within 7-10 days of the pomace maceration (Ribereau-Gayon, et al., 2006). Enrichment of must with the extractive compounds of skin and seeds has a positive effect on the future wine quality up to a certain limit, and later the wine acquires rough tones. Maceration of the cap for more than 10 days (Yakimenko, et al., 2012) as a result of changing dissolving ability of the must, is caused by an increased alcohol content, as well as due to the polymerization and condensation reaction of phenolic compounds; some of them are insoluble and can precipitate, thereby reducing the extract of the wine.

Stirring the pomace cap during maceration and fermentation has a significant effect on the extract and quality of the wine. Aeration during the stirring process intensifies the multiplication and yeast metabolism, which is a positive effect on the wine quality. The aging of wine can decrease the amount of wine extract. The terroir has a significant impact on the quality of the wine, as evidenced by the amount of accumulated extract. Concentration of dry extract in wine samples ranges from 30.91 g/l to 37.84 g/l. Increased content of extracts favorably contributes to quality indicators of wine, also increasing its hygienic properties.

Conclusion

The tasting evaluation of the investigated wines showed that at this stage of production, all three samples of red wines are corresponding to the quality requirements, however, the potential for the development of quality indicators, depending on numerous factors, can reveal the character of the investigated wines in a new way, after a period of aging and the formation of wine. Therefore, repeated physicochemical analysis and wine tasting assessment will be provided in 6 months.

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