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The study of the biochemical composition of grape and wine from the Armenian selection variety Nrneni

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ABSTRACT

Background: In Armenia, autochthonous, modern cultivars, and wild grape populations are highly diverse. Different types of wine can be produced due to the climatic zones. Grapes are a popular fruit all over the world, consisting of bioactive compounds and antioxidants, such as proanthocyanidins, anthocyanins, flavonols, organic and phenolic acids, and stilbenes. It is well known that grape varieties have different yields as well as quantitative and qualitative characteristics of the berry, which impact the quality of wine.

Objective: Conduct an in-depth study of Indigenous grape varieties and clones is essential in the development of the best wine. Our focus is to specifically study Nrneni, an artificially selected Armenian grape variety. P.K. Ayvazyan, G.P.Ayvazyan created this variety by crossing a combination of Alikante Bouchet and Cabernet Sauvignion with Saperavi. The resulting product has pulp and juice that are an intense red color.

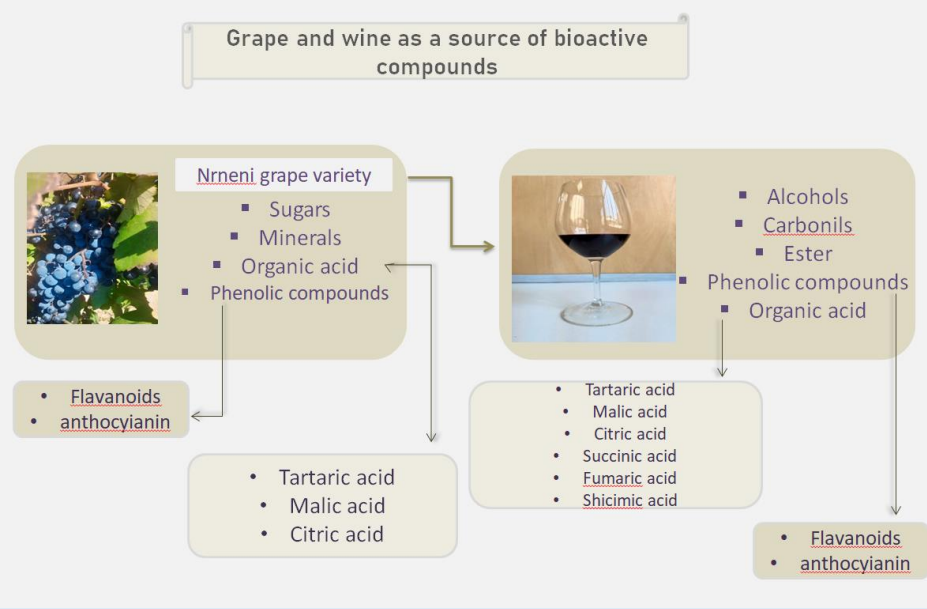
Methods: In the Ararat Valley region, the grapes were hand-harvested at the end of September once they reached full technical maturity. The wine was made by the classic red winemaking method. Physicochemical analyses of grape and wine were carried out by OIV methods. Organic acids were separated and identified using liquid chromatography. A photocalorimetric method was used to determine the total phenolic compounds.

Results: The chemical composition of red wine made from Nrneni grapes was assessed. In the process of wine production, storage, and maturation, grapes produce a variety of other substances, including organic acids,

polysaccharides, and polyphenols, some of which contribute to the wine's sensory properties (such as flavor, color, and taste) and health benefits. Grapes are excellent sources of bioactive compounds, especially polyphenols. The total and extractable anthocyanin and flavonoid content of the Nrneni grape variety was determined. The conducted studies showed that Anthocyanins were present in high amounts of 2366.4 mg/kg. Flavonoids were present in 10183.0 mg/kg in the 2022 vintage, and 3159.9 mg/kg and 12563.7 mg/kg in the 2023 vintage. Extractable anthocyanin and flavonoid amounts were 1135.87 and 4684.18 mg/kg in 2022, 1540.32 and 5836.67 mg/kg in 2023 respectively. These indicators are also high in the wine: 502.18mg/l and 2971.15mg/l in 2022 vintage, while 975.7and 4208 mg/l in 2023 vintage. Theoretical varieties are present in 8.9 g/l and 2.02 g/l in 2022, and 6.5 g/l and 1.6 g/l in 2023. Licosaccharides, acetic acids, citric acids, and succinic acids are produced by alcoholic and malolactic fermentation.

Conclusion: There is a wide range of bioactive compounds in red grapes and red wine that have been shown to have potential health benefits. According to studies, the Nrneni grape variety and the wine produced with these grapes have functional and oenological potential due to their high levels of biologically active substances, such as phenolic compounds and organic acids.

Keywords: bioactive compounds, grape, organic acids, phenolic compounds, red wine.



Graphical Abstract: Grape and wine as a source of a bioactive compounds.

INTRODUCTION

In Western Asia and the Caucasus, grapevines were domesticated about 11,000 years ago [1]. In comparison, the Republic of Armenia has a wide variety of autochthonous and modern grape cultivars, in addition to wild grape populations. this is due to its relatively small and mountainous terrain with a total area of 29,740 km^2. Located at the junction of the biogeographic zones of the Lesser Caucasus and the Iranian and Mediterranean zones, Armenia also exhibits great altitudinal and climate variation that is conducive for growing different grape varieties for wine production [2].

Among Armenian agriculture's basic sectors, viticulture is one of its main exports, along with brandy and wine production. During the wine industry renaissance in recent years, a limited number of traditional varieties were used for wine production, which resulted in a shift to single-variety vineyards. Since only 30–35 of 400 native grapevine varieties is used in wine and brandy production, intensive cultivation has resulted in an alarming loss of genetic diversity.

Functional food decreases the risk of cardiovascular diseases, cancer, and other diseases, and has health benefits [3].

Grape contains various bioactive compounds, especially phenolic compounds and organic acids. Phenolic compounds and organic acids have antioxidant, antiinflammanory, antimicrobial properties.

As the “French paradox” reveals, people regularly using moderate quantities of wine can prevent coronary, heart disease, despite the daily intake of high-fat diets. The wine also can prevent other diseases, such as obesity, neurodegenerative diseases, and cancers.[4]

After cultivation, the grapes undergo production, storage, and maturation to be made into wine. Organic acids, and polyphenols that are produced or transported from grapes during this process contribute to sensory properties, like flavor, color, and taste, and health benefits of wine [5]. Red wine's health benefits are mostly attributed to its resveratrol content and phenolic compounds, which are mostly found in red grape skins that enter the into wine through the maceration process [6].

Since polyphenols are naturally present in fruits and vegetables, there has been a great deal of interest in plant polyphenols in recent years. These free radical scavengers can interact with biological systems and prevent neurodegenerative diseases, cardiovascular disorders, and cancer [7-8]. Phenolic compounds come in a wide range of structures, from simple hydroxylated aromatic rings to highly complex polymers. There are many flavonoids and non-flavonoids in grape skins and seeds [9-10]. Viniculture and grapevine variety influence the concentration of phenolic compounds in grapes [11]. These compounds are considered to have antioxidant, anti-cancer and anti-inflammatory properties [12-13] and they are also responsible for some sensory properties like color, aroma, flavor, bitterness and astringency in grapes and wine [14]. These characteristics contribute to the grapes’ potential as a functional food.

We studied Armenian grape varieties' genetic potential, specifically their bioactive compounds and quantitative indicators. Through the use of this information, new wine varieties can be discovered, selected, and distributed, resulting in high-quality wines that are rich in biologically active compounds. In our research, we found that Nrnani grapes and wines made from them are particularly rich in these compounds, which makes them excellent choices for wine and beverage production. The importance of studying indigenous and selection grape varieties and clones in wine production cannot be overstated. The research aims to determine the biochemical composition, especially phenolic compounds and organic acids of Nrneni grape variety and wine. The first-time oenological properties of this selection variety have been also observed.

METHODS

The grapes were collected from the Ptxunk village, in the Ararat Valley region. The vineyards (0.5 ha) are located at an altitude of 870m. Vines were planted at 2.5 mx1.5 m spacing (between row and within row distance), respectively. Vines were trained to the fun system without trank. In winter, the vineyard was buried. They were hand-harvested in the end of September, at full technical maturity. Grape sampling and harvest were conducted in 2022 and 2023. Nrneni is Armenian grape variety created by artificial crossing in 1979, and the authors were P.K. Ayvazyan, G.P. Ayvazyan. It was the result of crossing (Alikante Bouchet+Cabernet Sauvignion) and Saperavi.Nrneni is a red grape variety that ripens late and yields 25-45 tons per hectare. The leaves are medium in size and round in shape. Bunches are medium in size, cylindrical, and medium in density.

The pulp and juice of Nrneni have an intense red color. It is relatively resistant to fungi. The sugar content is 22-26% and the titratable acidity is 7.5-9.5 grams per liter [15].

Picture 1. Picture Shoot tip, Grape bunch of Nrneni, photo by author.

Winemaking: The classic red winemaking method using fermentation on pulp, the wine was made on a mini scale. All wine samples were made at the 2022-2023 winemaking season. SafOeno NDA 21 ADY was used for fermentation. SpringGel l™ Color G2 color stabilizer also was added before adding yeast. In order to improve color and phenol extraction, manual punch downs were performed twice a day. As soon as the alcoholic fermentation was finished, the wines were racked off and the pomace gently pressed using a hydraulic press. After 30 days of malolactic fermentation, the wines were racked and potassium metabisulphite at 50 mg/L was added. For analysis, the wine was bottled in 750 mL glass bottles with cork caps and stored at 15°C. SafŒno™ NDA 21 is recommended for harmonious, strong & fruity red wines with short vatting times. Indeed, with its high glycerol production, SafŒno™ NDA 21 brings good roundness and general balance to the finished product. SafŒno™ NDA 21 has a strong fermenter and quick fermentation kinetic, very tolerant to alcohol: up to 16% vol./vol m and low to medium nitrogen requirements [16].

SpringCell™ Color G2: color stabilizer. Pure inactivated yeast from Saccharomyces cerevisiae rich in polysaccharides improves the intensity and stability of the polyphenolic profile of red wines. [17]

Chemical analyses: Grapes and wines were analyzed using OIV methods [18]. Grape sugar content was determined using a refractometer. OIV-MA-AS312-01A method was used to measure alcohol content, OIV-MA-AS313-01 method for total acidity; OIV-MA-AS13-02 method for volatile acidity; OIV-MA-F1-07 method for measuring free and total sulfur dioxide, and liquid chromatography method for determining organic acids [19]. HPLC system configuration and method conditions were as follows: Mobile phase/eluent, H2O with 0.5 % ethanol/0.0139 % conc. Thermostatic column compartment (460C); Variable wavelength detector (210 nm); and Refractive index detector. H2SO4; vacuum degasser; Binary pump, Flow rate: 0.6 ml/min; Auto sampler, injection volume: 0.01 mL.

Total phenolic compounds determination: Total phenolic compounds were determined by photo-colorimetry [20]. Following homogenization of 100 grape berries by a high-speed Ultra-Turrax T25 for 3 minutes, 10 grams of the homogenate were suspended in 10 mL of hydrochloric ethanol solution for 30 minutes at 20°C.

For 10 minutes, the supernatant was centrifuged at 5000 x g in a volumetric flask of 10 mL.To make the volume 50 ml, a hydrochloric ethanol solution was used. For wine all samples diluted with hydrochloricethanol solution. By recording UV-visible spectra from 230 to 700nm, anthocyanin and flavonoids were quantified spectrophotometrically.

Color measurement in wine: A color assessment of the wine was conducted to determine its intensity and hue. Cuvettes with a 1mm optical path were used to measure the spectrophotometric absorption values at 420nm and 520nm of the undiluted wine.

The climate data were provided by the official meteomonitoring website [21].

Statistical analysis: The data were analyzed by method of data statistical processing for quantitive variables.

RESULTS

Weather conditions and phonological stages: Vintage 2022, and 2023 marked the main rain period from April to June, with a maximum temperature of 26.7°C, and 27.6°C in August respectively. The low rainfall during the ripening period allowed the Nrneni grapes to ripen regularly

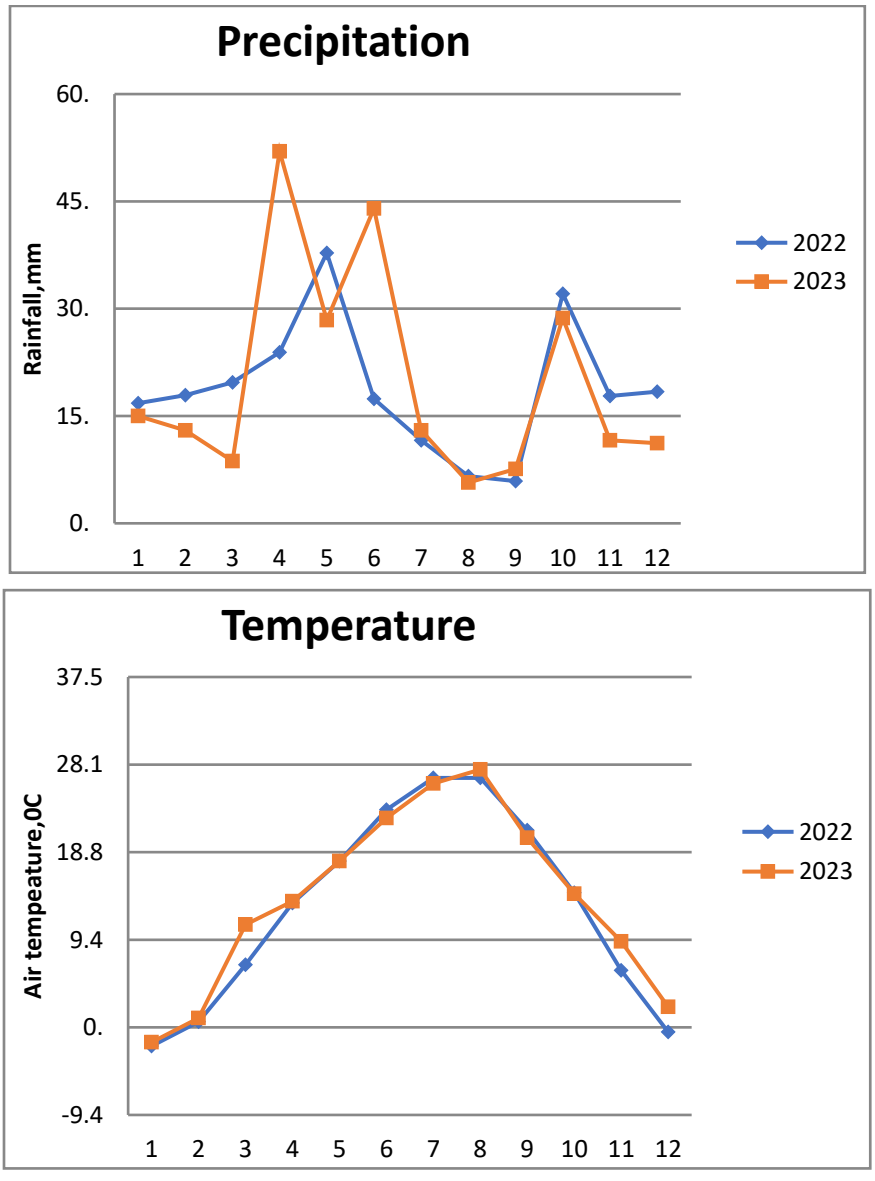


Figure 1. Weather conditions

Table 1. Phenological stages of the grape variety of Nrneni.

Year	Budburst	Time of Flowering		Ripening		Vegetation period, days	The sum of active temperature, ^{0}C
		beginning	ending	Time of beginning of berry ripening	Time of full physiological maturity of the berry		
2022	24.04	30.05	09.06	04.08	24.09	153	3938.2
2023	20.04	26.05	15.06	26.07	30.09	163	4209.4

In both seasons, none of the experimental vines showed symptoms of Downy mildew and Powdery Mildew at harvest.

The results showed that phenological stages are dependent on climate conditions flowering time in 2022 was from the end of April to the first decade of June, and in 2023 the flowering time was almost the same, despite hotter climates and earlier bud burst spring of 2023. Full ripening was finished in the third decade of September. Therefore, these results demonstrate the potential for using these grapes as a source of raw materials for producing high-quality wines.

Table 2. Chemical composition of Nrneni grapes harvested in 2022, and 2023 in the Ararat Valley region.

Parameters	unit of measurement	Grape	
		2022	2023
Sugar	Brix, %	24±0.35	25.2±0.28
Total acidity	g/l	6.74±0.02	4.67±0.02
(pH)	-	3.49±0.01	3.65±0.01

Values are expressed as average ± standard deviation (n = 2)

Standard physicochemical compounds of grape and wine: Sugar content in 2022 vintage was 24 and acidity was 6.74 g/L. In 2023 vintage, the sugar content increased, and total acidity decreased from 2022 vintage with 25.20 g/L of sugars and 4.67 g/L of total acidity. In 2023 vintage, the pH of the grape juice ranged from 3.49 to 3.65. (Table 2).

Table 3 data demonstrates that all the wine samples were dry with < 4 grams of residual sugar per liter. The 2022 vintage had an alcohol level: of 14.2%, and 2023: of 15.6%. The pH of the wine's samples was slightly higher, ranging from 3.9-4.05. The total acidity of the wine samples were between 4.94-4.37 g/l; volatile acidity was 0.62-0.9 g/l. Volatile acidity in wine is limited to 1.2 grams per liter by the OIV. The amounts of aldehydes and acetals in the 2022 vintage were 36.08-38.94, and in 2023, they were 40.27-55.46, respectively, higher than in the 2022 vintage.

Armenia has some regulations regarding the dry extract content of wines. For red wines, a minimum actionable level of 17 g/l is required. The total and dry extract amounts exhibited in our wine samples are quite high, which is appropriate for the total phenolic content. Other indicators were within normal ranges.

Table 3. Physicochemical parameters of the wines

Parameters	unit of measurement	Wine	
		2022	2023
Alcoholic strength	%	14.2±0.14	15.6±0.28
Total acidity	g/l	4.94±0.03	4.37±0.01
(pH)	-	3.91±0.01	4.05±0.02
Reducing sugar	g/l/	0.54±0.02	1.16±0.02
Volatile acidity	g/l	0.62±0.01	0.9±0.04
Aldehydes	mg/l	36.08±0.01	40.27±0.01
Acetals	mg/l	38.94±0	55.46±0.03
Total Extract	g/l	33.9±0.14	45.4±0.07
Dry extract	g/l	33.4±0.21	44.2±0.35
Free SO2	mg/l	23.83±3.25	19.6±0.21
Total SO2	mg/l	96.9±0.4	101.5±0.5
Reductions SO2	mg/l	6.27±0.21	6.3±0.21

Values are expressed as average ± standard deviation (n = 2)

Anthocyanins, Flavonoids, and Polyphenols: Anthocyanins was found in high concentrations (2366.4 mg/kg) in the 2022 vintage, and flavonoids were present (10183mg/kg) in the Nrneni variety at higher concentrations than in the previously observed variety (Charentsi grape variety [11]). In 2023 vintage, anthocyanins were present at even higher concentrations of 3159.9 mg/kg and flavonoids were present 12563.7 mg/kg. Approximately 48% of the grape content was extracted and transferred from grape to wine. The total anthocyanins, flavonoids, and phenolic content in the 2022 vintage wine were 502.18 mg/l and 2971.15 mg/l , 3824.47mg/l, respectively. Comparatively, the total anthocyanins, flavonoids, and phenolic content in the 2023 vintage wine were975.7 mg/l, 4208 mg/l, and5813 mg/l, respectively. The results showed that 60% of anthocyanin and flavonoids from the extractable content of grapes was transferred into the wine. Folin-Ciocalteu's, an international measure of the amount of phenols in wine, was used [18]. The index is for 2022 – 91.4, for 2023 138.92 (Table 4).

Anthocyanin concentrations depend on cultivar, season, area, and viticulture practices [26-27]. Samples of grapes and wine are intensely red because they contain high concentrations of red pigments, such as anthocyanin. Additionally, they are found in the thick epidermal layer of the skin. Their colors range from red to blue and they absorb light [28-29]. Wine quality estimation and wine characterization are both influenced by the color of the wine. In addition to providing information about wine quality and type, it can also provide insight into its chemical composition. According

to the color results: the largest in 2023 was 36, and the smallest in 2022 was 16.9. In the 2022 vintage, the highest color shade indicator was 0.8, and in the 2023 vintage, it was 0.6. Colors are defined by wavelengths: 420 nm for yellow, 520 nm for red, and 620 nm for blue. The 2023 vintage has the highest combination of red (51.5%) and yellow (33.4%) colors.

Polyphenols are abundant in red grapes and red wine. In plants, phenolic compounds play a crucial role in growth, reproduction, and protection against pathogens and radiation [23].

Moreover, they provide cardiovascular protection, anti-inflammatory properties, and anti-cancer effects. [24]. It has been shown that phenolic compounds have health benefits because of their antioxidant activity [25]. Due to these compounds, grape and red wine can be used as functional food.

Table 4. The total anthocyanin, flavonoids, phenolic compounds, and color composition of the Nrneni grapes and wines

Compounds	**unit of measurement**	**Grape**				**Wine**	
						2022	**2023**
		2022	**extractable**	**2023**	**extractable**		
Total anthocyanins	mg/l	2366.4±2.7	1135.87±4.2	3159.9±1.9	1540.3±3.2	502.18±1.6	975.7±6.2
Total flavonoids	mg/l	10183±3.5	4684.18±3.1	12563.7±9.1	5836.67±5.2	2971.15±11.4	4208±7.7
The total content of phenolic compounds	mg/l	-	-	-	-	3824.47±11.6	5813±3.5
Folin checaltau index	-	-	-	-	-	91.4±2.3	138.92±2.5
Chromatic characteristics							
Color intensity	-	-	-	-	-	16.9±0.4	36±2.8
Color shade	-	-	-	-	-	0.8±0.2	0.6±0.28
Color composition, %							
Yellow	%	-	-	-	-	38±3.5	33.4±0.07
Red	%	-	-	-	-	47.7±2.1	51.5±1.3
Blue	%	-	-	-	-	14.3±0.35	15.2±1.2

Values are expressed as average ± standard deviation (n = 2)

Organic acids: Among the various compounds in grape juices and wines, organic acids are one of the most important. Wine contains three main organic acids: tartaric acid, citric acid, and malic acid. Other fermentation products (e.g., lactic acid, succinic acid, or acetic acid) affect wine sensory characteristics, such as color, and microbiological stability [30]. In addition, they contribute directly or indirectly to wine color and stability [31]. As well as promoting moderate wine consumption as part of a balanced diet, determining the organic acid content of wine is important because of its impact on consumers' health. The compounds found in the human diet bind free radicals [32]. Table 5 shows the concentrations of organic acids in the wine and grape juice samples.

Tartaric acid: The chemical stability of wines is maintained by tartaric acid, including their color and taste [33]. Tartaric acid is formed during berry cell division as berries mature [34-35]. Tartaric acid levels in the grape variety Nrneni were 8.9g/l in 2022 vintage, and 6.5g/l in 2023 vintage. Samples of wine presented at different years ranged from 2.07-1.98g/l. Various oscillations are seen in the tartaric acid concentrations during the alcoholic phase, depending on the moment of sampling. Tartaric acid concentrations increased significantly (p 0.05) in all variants during the primary fermentation phase but decreased continuously after that.[36]

Table 5. An analysis of the organic acids in the Nrneni grape and wine.

Organic acids	unit of measurement	Grape		Wine	
				2022	2023
		2022	2023		
Tartaric acid	g/l	8.9 ±0.09	6.5±0.03	2.07±0.10	1.98±0.02
Formic acid	g/l	0.26±0.11	0.1±0.01	0.29±0	2.26±2.62
Malic acid	g/l	2.02±0.03	1.55±0.01	-	-
Shicimic acid	mg/l	25.5±0	11.0±0	69.4±0	34.8±0
Lactic acid	g/l	-	-	2.79±0.02	2.18±0.07
Acetic acid	g/l	-	-	0.85±0.01	0.99±0
Citric acid	g/l	0.67±0.06	0.53±0.02	0.6±0.02	0.4±0.02
Succinic acid	g/l	0.22±0.05	0.11±0	0.82±0.04	0.9±0.01
Fumaric acid	mg/l	3.0±0	2.8±0	5.5±0	4.8±0

Values are expressed as average ± standard deviation (n = 2)

Malic acid: The level of malic acid in grape berries is influenced by grape maturation and microclimate conditions. As a result of malolactic fermentation, malic acid concentrations in wines are affected by its conversion to lactic acid [33,35,37,41]. In different years, malic acid concentrations vary from 2.02 to 1.55 g in these grape juices. As a result of fermentation, malic acid content in Nrneni changed from 2.02g/l in the 2022 vintage to 1.55g/l in the 2023 vintage, and in the wine, it was not detected. In wines, malic acid is important because of its high antibacterial activity due to synergistic effects between organic acids (especially malic and

tartaric acids), alcohol, and acidic pH [38].

Citric acid: Citric acid concentrations in wines can range from 0.10 to 1.08 grams per liter, and in grape juices, from 0.13 to 0.41 grams per liter. Citric acid is converted to malic acid during berry maturation, resulting in low citric acid concentrations [36,38-39]. The amount of citric acid in Nrneni was 0.67 g/l in 2022, and 0.53 g/l in 2023. Citric acid in wine varies between 0.6 and 0.4 grams per liter. During alcoholic and malolactic fermentations, lactic acid, acetic acid, citric acid, and succinic acid are produced [35]. It was found that lactic acid concentrations in these wines ranged from 2.79 to 2.18 g/L. Lactic acid contributes to the smooth, creamy taste of some wines and may promote the growth of beneficial bacteria in the gut. In addition to its essential functions in wine quality, its determination has health benefits as well. The benefits include improved lactose digestion, the prevention of cancer, and the maintenance of cholesterol levels [42].

The antioxidant and antimicrobial properties of citric acid make it important to monitor its level. As an integral mediator in the tricarboxylic acid (TCA) cycle, citric acid is also involved in the metabolism of most microorganisms [43].

Succinic acid: In grapes and wine, succinic acid is a natural component. Wine's flavor and aroma are influenced by this dicarboxylic acid. Studies suggest that succinic acid may be able to protect cells from oxidative stress and reduce inflammation. As part of yeast metabolism, this acid is one of the most important acids during fermentation [44-45]. Usually, white cultivars have a low concentration of succinic acid, while red cultivars have a higher concentration. Results showed that 0.22-0.11g/l were formed in the grape, 0.82g/l in the wine of the 2022 vintage, and 0.9g/l in the wine of the 2023 vintage. During the TCA cycle, succinic acid can be formed because of oxidative decarboxylation [46].

Shikimic acid (SHA): The Shikimic acid levels in all color wines, produced on a semi-industrial scale with 12 strains of Romanian grapes ranged from 3 to 36 mg per liter-1 and 4 to 34 mg per liter-1, respectively [47]. According to our study, the amount of shikimic acid in Nrneni was 25.5 mg/l in 2022, 11 mg/l in 2023, and 69.4-34.8 mg/l in wine.

Fumaric acid: The human body uses this organic acid in a variety of metabolic processes. As a result of malolactic fermentation, fumaric acid is mainly produced by lactic acid bacteria in wine [48-49]. Fumaric acid levels range from 3-2.8mg/l in grape variety Nrneni, and 5.5-4.8mg/l in wine samples.

CONCLUSION

Bioactive compounds found in red grapes and red wine have been shown to have potential health benefits. Using Armenian cultivars, we can produce good-quality wine and enhance the grape characteristics. The Nrneni grape variety and wine also contain biologically active substances, including phenolic compounds and organic acids, which have functional and oenological properties. Nrneni grapes have high levels of anthocyanins, which can produce deeply colored red wines.

Abbreviations: International Organization of Vine and Wine: OIV, shikimic acid: SHA, active dry yeast: ADY

Competing interests: Authors declare no conflict of interest.

Author Contributions: BG and MM designed this study, and other authors also carried out the experimental part. All participants read and agreed with the final version of the manuscript.

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