

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 hydrochloric ethanol 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 meteorological website [21].

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

RESULTS

Weather conditions and phenological 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 Nreni grapes to ripen regularly

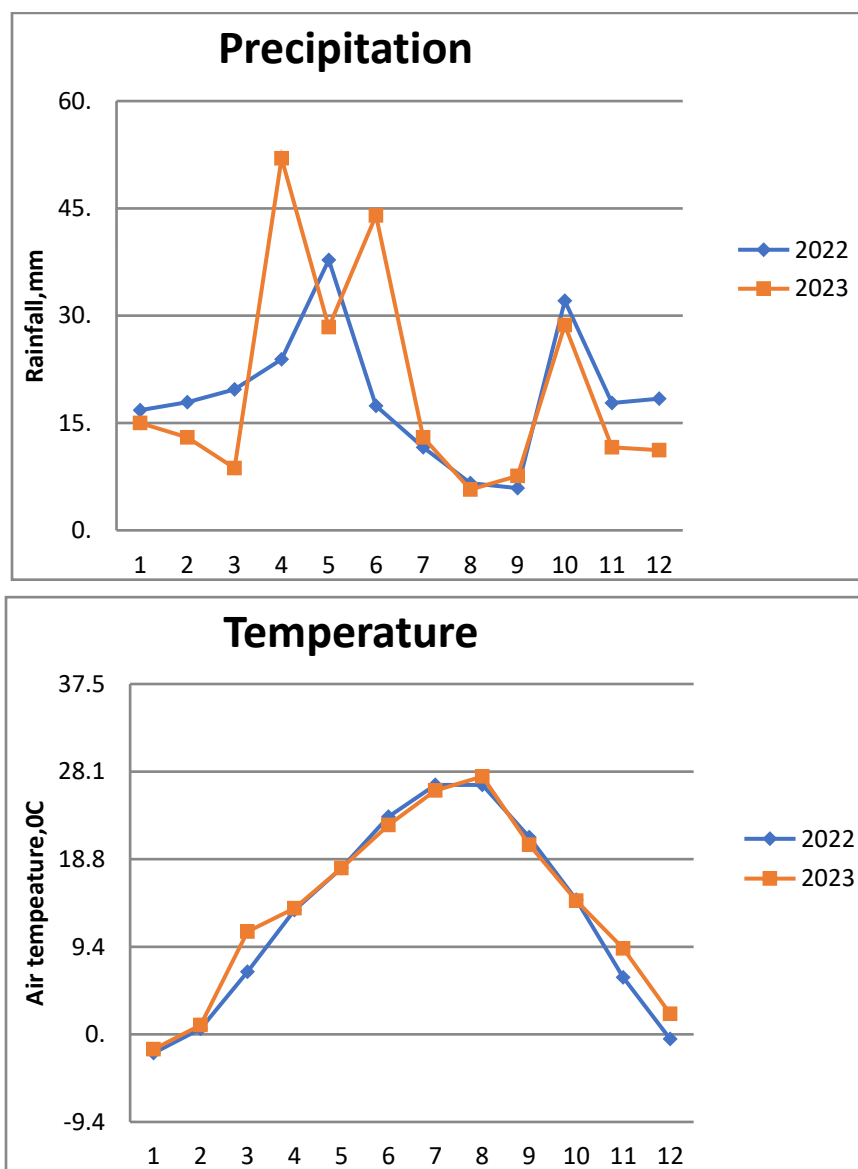


Figure 1. Weather conditions

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

Year	Budburst	Time of Flowering		Ripening		Vegetation period, days	The sum of active temperature, °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 SO ₂	mg/l	23.83±3.25	19.6±0.21
Total SO ₂	mg/l	96.9±0.4	101.5±0.5
Reductions SO ₂	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 were 975.7 mg/l, 4208 mg/l, and 5813 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	extractable	2023	extractable	2022	2023
		Total anthocyanins	mg/l	2366.4±2.7	1135.87±4.2	3159.9±1.9	1540.3±3.2
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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