



The investigation of bioactive compounds in the Charentsi grape variety and its derived wines

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ABSTRACT

Background: Grapes contain thousands of chemical compounds, including sugars, organic acids, phenolic compounds, minerals, organic nitrogen, amino acids, and aromatic compounds.

Objectives: The study investigates the possibility of producing high-quality red wines from the Charentsi grape variety and observes the extraction of bioactive compounds formed during fermentation.

Results: The organic acids in grapes and wine were also studied. There were significant differences in the compounds mentioned above between wine samples based on the yeasts used.

Our observations showed that the amount of tartaric acid in the Charentsi grape variety was 7.26 g/l, and after fermentation by using different yeasts in the wine samples, the content of tartaric acid was: BCS:103 2.06 g/l, AC-4: 1.79 g/l. The grape sample contained 3.01g/l malic acid, while the selections of wine BCS103 and AC-4 contained 2.01g/l and 3.00g/l, respectively. In the case of citric acid, the grape contained 0.5 g/l a, while the wine samples with BCS103 and AC-4 yeasts contained 0.52 g/l and 0.57 g/l respectively.

The grape sample did not contain lactic acid or succinic acid. Wine samples were fermented by a variety of newly formed yeasts. In BCS103 yeast, these values were 1.89 g/l and 1.63 g/l, and 1.3 g/l and 1.61 g/l in AC-4.

The values of anthocyanin and flavonoids in Charentsi grape variety are 2084.5 mg/kg and 8828.6 mg/kg, respectively. Wine samples contained high quantities of these compounds as well. The anthocyanin and flavonoids concentrations in BCS103, were 842.7 mg/l and 4898.22 mg/l, while they were 783.3 mg/l and 5025.5 mg/l in AC-4. The

total phenolic compounds detected in the wine samples were 4957.9 mg/l and 5102.6 mg/l, in BCS103 and AC-4 respectively. According to the analysis, 37%-40% of anthocyanin and 55-56% of flavonoids were transferred to the wine.

Conclusion: Charentsi grapes and wine are highly functional due to their high phenolic content and organic acids. These compounds are biologically active and influenced by the yeast strain used for wine fermentation.

Keywords: grapes, red wine, phenolic compounds, organic acids, bioactive compounds



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INTRODUCTION

Armenia is the cradle of winemaking. Archaeological excavations revealed evidence of grape cultivation and winemaking in ancient Armenia going back thousands of years. The latest scientific research suggests that grapevine domestication was done more than 11,000 years ago, and archaeological evidence shows that industrial winemaking in Armenia dates back to at least 6,000 years ago [1-2]. The Areni-1 cave complex in the Vayots Dzor region of Armenia is believed to be one of

the oldest winemaking sites in the world. Excavations at this site have uncovered grape seeds, fermentation vessels, and other artifacts associated with winemaking.

Throughout history, Armenia has been ruled by various civilizations and empires that have influenced the region's winemaking tradition. These civilizations include the Urartians, Persians, Greeks, Romans, and Ottomans. Each of these influences has contributed to the development of unique winemaking techniques and grape varieties in Armenia. The country's unique

geographical location, diverse climate, and fertile soil contribute to high-quality grapes and wines. Armenian wineries are adopting modern winemaking techniques while also preserving traditional methods such as aging wines in clay jars called "karas".

Armenian wines have gained recognition and awards in international competitions, raising their global profile. The country's wine tourism industry is also growing, with visitors from around the world exploring Armenia's vineyards, wineries, and winemaking culture. There has traditionally been a great degree of interest in red wine in Armenia, and therefore red wine production has increased. Viticulture and winemaking requirements have also been changed. Rather than quantitative approaches, qualitative ones are promoted today, along with an importance placed on wine and grape quality. [1].

Since sweet and fortified wines along with brandy were the main wine-making products before One unique region is Vayots Dzor, where Sev Areni grapes were traditionally grown for thousands of years. Due to this objective, many black-colored and frost-resistant grape varieties were selected by great Armenian scientists. A limited number of studies are available that concern the bioactive compounds present in grapes and wine.

The Charentsi grape variety is a late-ripening wine grape variety. It is a European-Amurian hybrid, obtained from the "Amurakan x Zhemchug Saba" elite seed plant and a cross of the Karmrahyut variety. It is widely spread in the Ararat and Armavir regions of Armenia and is characterized by strong growth and high frost resistance, up to -28°C . The berries are medium-sized, round or oval, and black. The fruit skin is thick, and the juice is intensely red-colored [3].

The organic acids found in grapes and wines can have important human health benefits. A wine's flavor, aroma, and acidity are derived from a complex mixture of organic acids.

In metabolic pathways, organic acids are intermediates produced from the catabolism of amino

acids. Several types of food and beverages contain organic acids (juice, coffee, tea, and wine) [4-5], which support antioxidants' ability to manage free radicals. Aside from having antibiotic and antimicrobial properties, these acids improve the stability of wine [6,7,8]. Several organic acids are found in wine, including tartaric acid, oxalic acid, succinic acid, malic acid, acetic acid, and lactic acid. They can be derived from raw materials (over 90% of the total concentration of organic acids in grapes is derived from malic and tartaric acids), or they can be derived from yeast metabolism during winemaking [9-11].

The quantitative and qualitative composition of organic acids and the total amount of phenolic compounds were first investigated in the grape and wine from the Charentsi variety in Armenia. This study aims to investigate the production of high-quality red wines from the Charentsi grape variety and analyze its organic acid and phenol compound compositions.

METHODS

The grapes were collected from the Nalbandyan Research Collection Vineyard of the Voskehat Educational and Scientific Center of ANAU. They were hand-harvested in October, at the stage of full technical maturity. The wine was made on a mini-production scale using the classic red winemaking method: fermentation on pulp. The fermentation was done at $25-28^{\circ}\text{C}$. All wine samples were made in the EVN wine lab during the 2022-2023 winemaking season. Two types of fermented yeasts were used for fermentation: SafOeno BCS103 and SafCider AC-4.

BC S103 has excellent fermentation characteristics and has very good resistance to extreme wine making conditions. Its metabolic characteristics are killer factor, sensitive but excellent settlement strength, high consumption of malic acid, low volatile acidity production (generally below $0.2\text{g/L H}_2\text{SO}_4$), medium-low SO_2 production / medium SO_2 combination, high production of acetate esters (amylic notes) at low

temperature with a good nutrition, and promotion of terpenes and thiols [12].

AC-4 is used for sweet and dry ciders from fresh or concentrated apple juices. It has an intensely fresh aromatic profile, with a crisp mouthfeel enhancing cider structure, it mixes with sugar syrups, and its suitable for extreme fermentation conditions [13].

OIV methods were used to analyze grapes and wines [4]. A refractometer determined the sugar content of grapes. The alcohol content was measured by the OIV-MA-AS312-01A method, total acidity was measured by OIV-MA-AS313-01; volatile acidity – through OIV-MA-AS13-02; free and total sulfur dioxide were measured by OIV-MA- F1- 07; and organic acid determination was done with the liquid chromatography method [14]. An analysis of grape and wine organic acids (fumaric acid, succinic acid, citric acid, acetic acid, lactic acid, shikimic acid, ascorbic acid, malic acid, formic acid, and tartaric acid) was conducted using high-performance liquid chromatography. Configuration of the HPLC system and condition of the method were as follows: Mobile phase/ eluent, H₂O with 0.5 % ethanol/ 0.0139 % conc. H₂SO₄; vacuum degasser; Binary pump, Flow rate: 0.6 ml/min; Autosampler, injection volume: 0.5 mL; Therostated column compartment (46 °C); Variable wavelength detector (210 nm); and Refractive index detector. Total phenolic compounds were determined by the photometric method [15]. Total anthocyanin and flavonoids were extracted as follows: One hundred grape berries were homogenized by a high-speed Ultra-Turrax T25 for 3± 0.05 min, then 10g of homogenate was suspended in 10 mL of hydrochloric ethanol solution and kept for 30 min at 20 ±1°C. The sample was centrifuged at 5000 * g for 10 min and the supernatant and suspended in a 10 mL volumetric flask. The hydrochloric ethanol solution was used to bring the volume to 50 ml. The quantification of anthocyanin and flavonoids was carried out spectrophotometrically by recording UV-visible spectra in the range of 230 to

700nm. The wine color was assessed for intensity and hue, and the spectrophotometric absorption values at 420nm and 520nm of the undiluted wine were recorded using cuvettes with a 1mm optical path.

RESULTS AND DISCUSSION

Standard physicochemical compounds of grape and

wine: The results of the standard physicochemical analysis of both grapes and wines are reported in Table 1. According to Brix, the grapes contained 25% sugar. All wine samples were dry with less than 4 grams of residual sugar per liter. We found that the wines had alcohol levels between 15.8% and 16%. The pH level in the grape was 3.5, and in the wines, it was slightly higher with values between 3.73 and 3.78. The grape acidity by tartaric acid was 7.85 g/l. Acidity values in the wine samples ranged from 6.51 to 7.27 g/l. For BCS103 and AC-4 yeasts, wine volatile acidity levels are between 0.36 and 0.37 g/l, which is very low. By OIV standards, the maximum acceptable level of volatile acidity in wine is 1.2 g/l. In BCS103 and AC-4, the amounts of aldehydes and acetals were 21.12 and 33.04, respectively. While both yeast strains produce low levels of aldehydes, AC-4 produces 59.0 mg/l.

The SO₂ was added after sampling wine samples and before bottling, which means that the fermentation and maturation of wines were not affected by SO₂. Wine samples can be kept in bottles for long periods of time without losing quality because of the amount of free and total SO₂ in the wine. We plan to store and taste the wine samples to determine the aroma wheels of the wine's simplest dry and total extracts to be successful. The Armenian Government has some regulations regarding wine dry extract minimum quantities. For red wines, the minimum actable level should be not less than 17 g/l. compared with our wine samples, the total and dry extract amounts are quite high, which is appropriate for the total phenolic content of the samples.

Table1. Grape and wine concentrations of standard physicochemical analysis parameters

Parameters	unit of measurement	Grape	Wine	
			BCS103 (yeast)	AC-4 (yeast)
Sugar	Brix, %	25.0	-	-
Total acidity	g/l	7.85*	6.51	7.27
(pH)	-	3.5	3.73	3.78
Alcoholic strength	%	-	15.8	16.1
Reducing sugar	g/l/	-	2.50	3.25
Volatile acidity	g/l	-	0.36	0.37
Aldehydes	mg/l	-	21.12	12.76
Acetals	mg/l	-	33.04	59.0
Total Extract	g/l	-	36.5	40.4
Dry extract	g/l	-	34.0	37.15
Free SO ₂	mg/l	-	15.20	11.8
Total SO ₂	mg/l	-	67.80	54.32
Reductions SO ₂	mg/l	-	3.10	3.72

*g/kg for grape

Organic acids: In wine, tartaric, lactic, and acetic acid concentrations varied at about 2-fold, malic and citric acid concentrations about 3-fold, and succinic acid concentrations about 4-fold. The concentrations of tartaric and citric acids in grape juices varied about threefold, and malic acid concentrations about eightfold [5]. The results of the organic acids analysis of grape and both wines are reported in (Table 2).

Tartaric acid: Observation showed that the amount of tartaric acid in the Charentsi grape variety is 7.26 g/l. The samples presented different concentrations of tartaric acid, according to the inoculated yeast, the highest value being registered in the sample of yeasts of BCS103 2.06 g/l, in the sample of the yeast of AC-4: 1.79 g/l. In wines and grape juices, tartaric acid concentrations ranged from 0.63 to 9.94 grams per liter of wine, and from 0.79 to 15 grams per liter of grape juice [16].

Tartaric acid is the most abundant organic acid in wine, and it gives the wine its characteristic tart taste. It has been shown to have antioxidant properties and may help protect against oxidative stress and inflammation. Tartaric acid degradation is also related to yeast species [17] and fungi [18-19].

Malic acid: Malic acid is another important organic acid in wine. It contributes to the sour taste of grapes and wine and can help regulate pH levels in the body. Malic acid has also been shown to have antimicrobial properties and may help improve digestion [20]. The amount of malic acid in grape variety Charentsi is 3.01 g/l, but during the fermentation, the content of malic acids almost doesn't change in the wine of AC-4 yeast strains trial. This is confirmed by literature that reported concentrations ranged from 0.22 to 0.68 g L⁻¹ for Malic acid in wine [16].

Citric acid: The fresh, citrusy taste of some wines, can be attributed to citric acid, which is found in small amounts in wine. Before malolactic fermentation, citric acid concentrations in must and wine are usually between 0.5 g/l and 1.0 g/L [21,22]. The amount of citric acid in the Charentsi grape variety is 0.5 g/l, in the wine the level of this molecule ranges from 0.52 g/l

(yeast`BCS103) to 0.57 g/l (yeast`AC-4). According to the literature, citric acid concentrations vary from 0.10 up to 1.08 g L⁻¹ for wines and from 0.13 up to 0.41 g L⁻¹ for grape juices. It has been suggested that the low citric acid concentrations are due to the conversion of citric acid to malic acid during berry maturation [5,16,23]

Table 2. Organic acids in the Charentsi grape and wine

Organic acids	Unit of measurement	Grape	Wine	
			BCS103 (yeast)	AC-4 (yeast)
Tartaric acid	g/l	7.26	2.06	1.79
Formic acid	g/l	0.51	0.57	0.78
Malic acid	g/l	3.01	2.01	3.00
Shicimic acid	mg/l	5.3	22.6	14.59
Lactic acid	g/l	-	1.89	1.30
Acetic acid	g/l	-	0.69	0.63
Citric acid	g/l	0.50	0.52	0.57
Succinic acid	g/l	-	1.63	1.61
Fumaric acid	mg/l	2.48	7.36	9.59
Total		11.19	9.40	9.70

RSD%= max.2.0%

Acetic acid: Acetic acid is the primary acid in vinegar and is also found in small amounts in wine. It contributes to the sharp, pungent taste of some wines and may have antibacterial properties. The content of acetic acid in the wine made from Charentsi grape variety in the case of yeasts of BSC-103 fermentation it was 0.69, and in the case of yeast AC-4, it was 0.63 g/l. Acetic acid concentrations are capped at 1.4 g L⁻¹ for red wine and 1.20 g L⁻¹ for white wine in the USA [6,24,25].

Lactic acid: The production of lactic acid in some wines, particularly red wines, occurs during alcoholic fermentation and mainly during malolactic

fermentation. Its concentration significantly increases according to inoculated yeasts and how malolactic fermentation was carried out primarily in red wines. Charentsi grape wine made with BCS103 yeast contained 1.89 g/l of lactic acid, while AC-4 yeast contained 1.3 g/l. Some authors reported lactic acid concentrations from 0.13 up to 5.71 for wines [24, 26]

Lactic Acid contributes to some wines' smooth, creamy taste and may have probiotic properties, promoting the growth of beneficial bacteria in the gut. Its determination is important not only for essential functions in wine quality but also due to its health benefits. These benefits include better lactose digestion, favorable effects against cancer, and maintaining cholesterol levels [29].

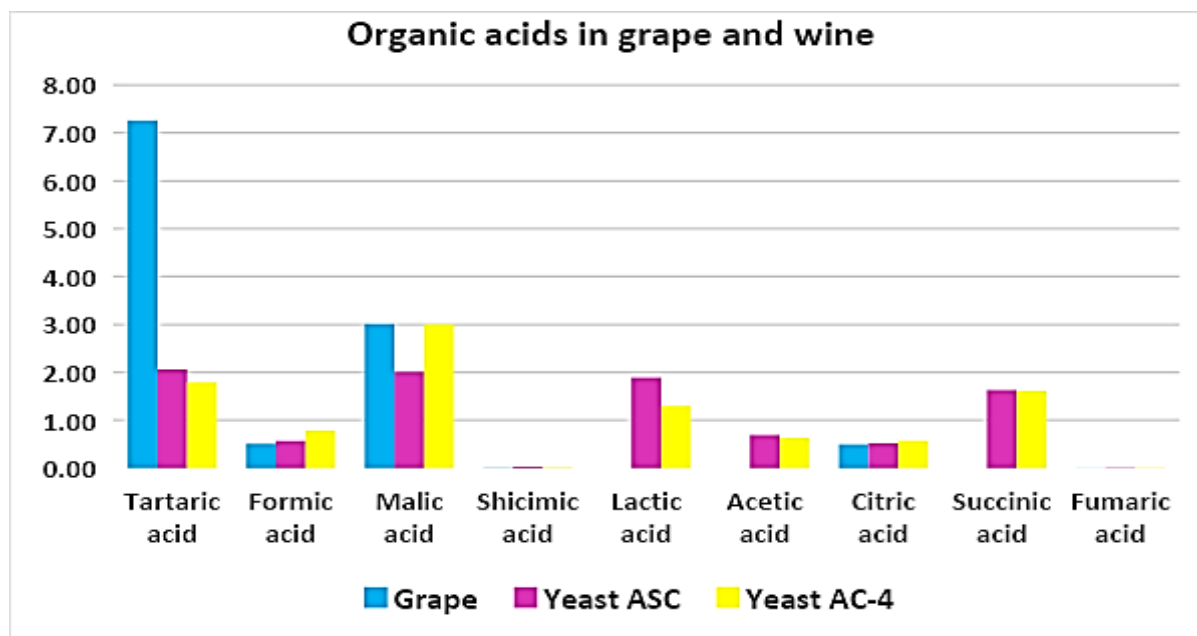


Figure1. Organic acids of grape and wine

Shikimic acid (SHA): Our study showed that the amount of shikimic acid in grape variety Charentsi is 5.3mg/l, in the wine which used BCS103 yeast fermentation it was 22.6mg/l. When the AC-4 yeast was used the concentration was 14.59 mg/l. SHA level is quite high in our trials and corresponds to the data in the literature.

A survey on mono-variety white wines from Abruzzo (Italy) reported values ranging from 8 to 60 mg·L⁻¹, confirming 'Char Donnay' among the SHA richest cultivars, irrespective of harvest year [27], However more research is needed to fully understand the health benefits of shikimic acid and its role in wine. SHA levels from 3 to 36 mg·L⁻¹ and from 4 to 34 mg·L⁻¹ have been respectively found in red and white wines produced on a semi-industrial scale with 12 grape varieties native to Romania [28]. Shikimic acid is an organic acid that is found in a variety of plants, including grapes. It is an intermediate in the biosynthesis of many aromatic compounds, including the anti-influenza drug oseltamivir (Tamiflu).

Fumaric acid: Fumaric acid is a natural organic acid that is involved in several metabolic processes in the human body. In wine, fumaric acid is mainly produced by lactic acid bacteria during malolactic fermentation [30,31].

The amount of fumaric acid in grape variety Charentsi is 2.5mg/l, in the wine samples: BCS103 was 7.36 mg/l, and AC-4: 9.59 mg/l. Fumaric acid is a dicarboxylic acid that is found in small amounts in wine. Fumaric acid has also been shown to have antioxidant properties and may help protect against oxidative stress, which is a major contributor to aging and the development of chronic diseases [19,32].

Formic acid: In wine, formic acid contributes to the overall acidity of the wine and can affect its taste and aroma. While formic acid is generally considered safe for human consumption at low levels, it can be toxic at high levels and may cause skin irritation or respiratory issues. Formic acid is a colorless, pungent-smelling organic acid that is found in very small amounts in wine. It is typically produced by yeast during fermentation and can also be found in some fortified wines. Formic acid is also used as a food preservative and is found in some condiments and dressings [33].

The content of formic acid in grape was 0.51g/l, in the wine samples: BCS103 was 0.57g/l, and in AC-4: 0.78 g/l.

Succinic acid: Succinic acid is one of the most important acids that develop during fermentation due to yeast metabolism, with concentrations averaging approximately 0.5 to 1.5 g/L in wine [6, 34]. Succinic acid levels vary between grape varieties, as concentrations are usually very low in white cultivars but slightly higher in red grapes.

The obtained results show that 1.63g/l was formed in the case of fermentation with BCS103 yeast, and 1.61g/l in the case of AC-4. Due to the significant content of organic acids, the grape variety of Charentsi and the wine produced from it can be used as an important component in a balanced diet. In the literature it ranged from 0.20 to 1.70 g/l [18,20-21,26].

Succinic acid is a natural component found in grapes and can therefore be present in wine. It is a dicarboxylic acid that is formed during the fermentation process and contributes to the flavor and aroma of the wine. Succinic acid can provide a fruity, slightly sweet taste to wine.

Some studies have suggested that succinic acid may have antioxidant properties, which could potentially help to protect cells from oxidative stress and reduce inflammation [35].

Polyphenols: Phenolic compounds have many positive contributions to health and have been investigated in many studies for this purpose [36-37]. Interest in phenolic compounds has increased due to their antioxidant properties. Their free radical scavenging properties help to prevent chronic diseases associated with oxidative stress, such as cardiovascular, cancer, and neurodegenerative diseases [38-41].

The result of the total phenolic content and color composition of wines are presented in (Table 3).The results indicated that there is a high amount of total anthocyanin (2084.5 mg/kg) and flavonoids (8828.6

mg/kg) in the Charentsi grape variety, and the content of these compounds are also high in the wine: in the case of yeasts of BCS103 it was 842.7 mg/l, 4898.22 mg/l, with 4957.9 mg/l total content of phenolic compounds, and in the case of the yeast of AC-4 the concentrations were 783.3 mg/l, 5025.5 mg/l, 5102.6 mg/l respectively. The results show that 37-40% of anthocyanin and almost 55-56% of flavonoids from grapes were transferred into the wine.

In yeast samples BCS103 and AC-4, the Folin-Ciocalteu Index was 118.48 and 121.93, respectively. Red grapes and red wine are rich in polyphenols. Polyphenols are phenolic compounds that are ubiquitous secondary metabolites in plants essential for growth, reproduction, and protection against pathogens and radiation [18]. The health effects of phenolic compounds are often attributed to antioxidant activity, mediated by a variety of mechanisms, including the reduction, or scavenging of reactive oxygen species, chelation of transition metal ions, and inhibition of enzymes involved in oxidative stress [42].

Red wine polyphenols include anthocyanin, flavanols, and flavan-3-ols. These compounds have anti-inflammatory, anti-cancer, and cardiovascular protective properties [37]. Anthocyanins are the pigments of red grapes; with few exceptions, they are in the skin of the berries, within the vacuoles [43]. Anthocyanins accumulate in grape berries at veraison. Their concentration increases up to a maximum value as a function of some factors such as the cultivar, seasonal conditions, production area, and viticulture practices [44-46]. Due to the high concentrations of red pigments such as anthocyanin in grape and wine samples, both wine samples are intensely red-colored. As they age, wine samples change in color because free anthocyanins are significantly active and can polymerize alongside tannins using aldehydes as a bridge [47].

Table 3. The total anthocyanin, flavonoids, phenolic compounds, and color composition of the Charenci grapes and wines

Compounds	Unit of measurement	Grape	Wine	
			BCS103 (yeast)	AC-4 (yeast)
Total anthocyanins,	mg/l	2084.5	842.7	783.3
Total flavonoids,	mg/l	8828.6	4898.22	5025.5
The total content of phenolic compounds,	mg/l	-	4957.9	5102.06
Folin checaltau index	-	-	118.48	121.93
Chromatic characteristics				
Color intensity	-	-	30.0	29.0
Color shade	-	-	0.59	0.63
Color composition, %				
Yellow	%	-	32.1	33.2
Red	%	-	54.8	53.6
Blue	%	-	13.1	13.2

RSD%= max.2.0%

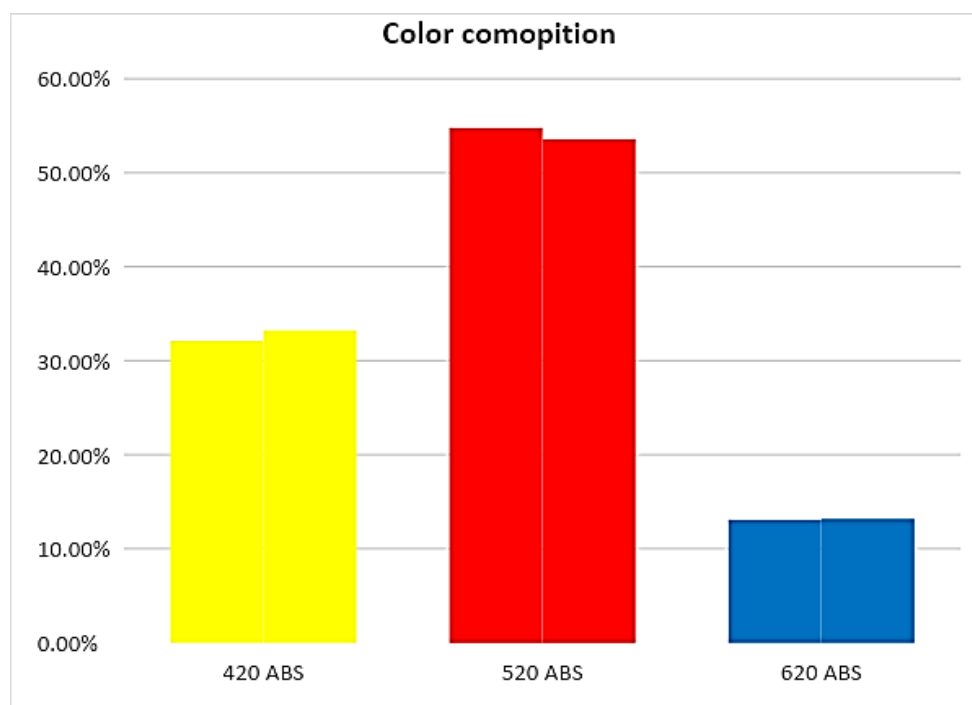


Figure 2. Color compositions of wine samples

CONCLUSION

According to our research, the Charentsi grape variety and the wine produced from it have a high functional potential, because of high quantities of bioactive substances such as organic acids and phenolic

compounds. The yeast strain used for grape pomace fermentation also affects the extraction of phenolic compounds and organic acids from the wine. After the alcoholic fermentation and maceration, the pressed

grape pomace can be used for the extraction of the rest of the bioactive compounds such as anthocyanin and phenolic compounds. This is possible because, as shown in our study, these wines contain 45-50% of the total phenolic compounds of the unprocessed grape.

Abbreviations: International Organization of Vine and Wine: OIV, shikimic acid: SHA

Competing interests: Authors declare no conflict of interest.

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

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