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Phenolic Compounds of Autochthonous Grape Varieties of Western Georgia

(Submitted for the academic degree of Doctor of Chemical Sciences

Specialty: Bioorganic Chemistry)

A N N O T A T I O N

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The defense of the thesis will be held on ..... …….. 2019 at the meeting of the dissertation council of the Faculty of Natural Sciences and Public Health of Batumi Shota Rustaveli State University.

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The dissertation work can be found in the library of Batumi Shota Rustaveli State University, as well as on the university website - www.bsu.edu.ge.

Introduction

The relevance of the thesis. Such wine-producing regions of Western Georgia, as Imereti, Adjara, Samegrelo and Guria have shaped and created Georgian wine culture over the centuries. The quality indicators of grapes and wines in different zones of Georgia's viticulture are affected by various factors. Among them, a special importance is given to varieties, but no less significant is the soil-climatic factor, rainfall and air temperature. The soil-climatic factor is manifested in the chemical composition of grapes, as well as in the wine obtained from it.

Phenolic compounds of grapes and their transformation products are actively involved in the formation of wine. They make a significant contribution to the formation of organoleptic characteristics of wine, its color, taste, aroma and antimicrobial activity. Phenolic compounds have a multilateral biological activity, including cardioprotective, anti-inflammatory and anti-cancer effect, due to their antioxidant properties (Ketskhoveli N. ....., Ramishvili M. .... Nutsubidze M. ....)

The aim of the research is to study the chemical composition of autochthonous grape varieties of Western Georgia and wine, obtained from them by various technologies, as well as to allocate and identify individual compounds by high pressure liquid chromatography method (mass spectral detection); to establish a correlation between the quantitative content of phenolic compounds and antioxidant activity; to determine the influence of the vine location on the chemical composition of grapes and wine; to identify the influence of winemaking technology on its chemical composition.

Scientific novelty. Using the HPLC and UPLC-MS methods, there have been analyzed 16 autochthonous grape varieties common in Western Georgia, and identified 9 anthocyanins, 5 aglycones, 3 flavonol glycosides, 1 catechin and 1 proanthocyanidin in the obtained wine. The qualitative and quantitative composition of common phenols, flavonoids, catechins and anthocyanins was determined and compared; the antioxidant activity of grapes and wine has also been determined.

The practical significance of the work. On the basis of the data obtained, it is possible to determine the origin of the wine variety, as well as to establish its falsification.

Research object, materials and methods of study: The object of study is pink, red and white grapes of cultivated vine (Vitis vinifera L.) spread in the four regions of western Georgia (Adjara, Imereti, Samegrelo, Guria), as well as wines prepared by local and European technology.

The samples of *Chkhaveri* - pink grape variety, have been gathered in Adjara region, Western Georgia (Vaio, Ortsva, Koromkheti, Jalabashvilebi, Gvara – the Agroservice Center of Vine and Fruit Tree Nursery) and in the village of Erketi in Guria.

10 grape varieties have been selected from the red grapes in different regions of western Georgia: *Aleksandrouli* and *Mujuretuli* – the Ambrolauri district (vil. Khvanchkara), *Usakhelauri* – the Agroservice Center of Vine and Fruit Tree Nursery (Adjara), *Dzvelshavi* – the Baghdati district (vil. Phersati), *Otskhanuri Sapere* and *Tolis Sapere* – the Zestafoni district (vil. Zeda Sakara), *Ojaleshi* – the Tsageri district (vil. Tvishi), *Kachichi* – the Keda district (vil. Kharaula), *Satsuri* – the Keda district (vil. Kokotauri).

The samples of white grape varieties have been obtained from *Tsolikouri, Tsitska, Krakhuna, Klarjuri* and *Kutaturi* grapes, growing at the territory of Ajara, Samegrelo and Imereti: in Adjarian districts of Keda (vil. Kokotauri) and Kobuleti (Gvara - the Agroservice Center of Vine and Fruit Tree Nursery), in Imereti in the Baghdati district (vil. Opcha), in Samegrelo in some villages of the Martvili district (Bandza, Najakhao, Mukhurcha, Lekhaindravo, Nagvazao, Vedidkari, Salkhino). The wine was prepared according to European technology.

The juice obtained from 5 - 10 kg of grapes, taken together with the peel, was placed in a glass dish; the enzyme yeast (Saccaromyces cerevisiae) was used for fermentation. The fermentation process with systematic stirring lasted 5-10 days; a valve was used to protect the fermenting mass from the air entering. Then the wine was cleaned of pulp, filtered, fermented again, and then placed in a refrigerator at the temperature of 8 degrees.

The following physical and chemical methods have been used for the research:

1. The phenolic compounds were allocated and identified by high-pressure liquid mass spectromatic chromatography (UPLC) method.
2. Qualitative and quantitative analysis of flavonols, anthocyanins and catechins was made by high-pressure liquid chromatography method.
3. The antioxidant activity (using stable radicals of 2.2-diphenyl-1-picryl hydrazyl was determined by the DPPH method.
4. The quantity of catechins was identified through vanilla reagent, by the spectral method.

5. The quantity of flavonols was determined by the spectral method ($AlCl_3$ reagent, with the calculation of routine).
6. The total of monomeric anthocyanins was identified by the pH differential method (AOAC Official Method 2005).
7. The number of common phenols was determined by the Folin-Ciocalteu method (Folin-Ciocalteu) OIV-MA-AS2-10 (with the calculation of gallic acid);
8. The sugar content was determined by the refractometer method (OIV-MA-AC2-02);
9. pH was determined by the OIV-MA-AS313-15 method;
10. The titrated acids were identified by calculating the wine acidity, while the sugar content and the acidity of wine were identified by the acidometer method (OIV-MA-AS313-01).

The approbation of the thesis. The results of the research are submitted in 3 scientific articles and 5 international scientific conferences.

The volume and structure of the thesis.

The dissertation paper consists of 112 printed pages, based on the instructions for the dissertation, submitted for the academic Doctoral degree, and includes a title page and signed pages, the summary in Georgian and English languages, the content, the list of tables - 15, the diagrams - 16, the list of literature - 85 units. The main text includes: Introduction, Literature Review, Analysis of Results, Experimental Part, Conclusions, List of Used Literature and Appendix.

Literature Review - The first chapters of the paper discuss the distribution of phenolic compounds in plants, their physiological activity and biological characteristics of the autochthonous grape varieties spread in Western Georgia. The list of used literature is attached to the dissertation paper.

Chapter1. Physical and chemical features of autochthonous grape varieties spread in Western Georgia.

1.1. ***Chkhaveri*** is a promising and popular autochthonous **pink grape variety** of the Black Sea basin. It is cultivated at different heights from the sea level. The samples were taken in Adjara region (during the technical maturity – in November, since *Chkhaveri* ripens in late period) at an altitude of 5 m above the sea level (Kobuleti), 300 m (Koromkheti), 360 m (Erketi), 380 m (Vaio) 400 m (Ortsva), 780 m (Jalabashvilebi) within 2014, 2015 and 2016 years.

Chkhaveri is characterized by an average and at the same time unstable yield (5.5-8 t / ha), especially in the high-mountainous regions of Adjara. The bunches of this variety are of medium or less than the medium size. Their length is 13.0-15.8 cm, and the width is 8.0-16.0 cm. The number of berries in bunches reaches 90-100. The mass of bunches varies from 126.0 to 383.6 g (Table 1).

Technical indicators of Chkhveri grape variety

Table 1

The name of a sample Chkhaveri	technical indicators of grapes						
	Colour of berries	Shape of berries	Taste	Mass of bunch, g	Length of bunch, sm	Width of bunch, sm	Mass of berries, g
Vaio	Dark red	Round	sweetish	150,8	14,8	16, 0	1,43
Ortsva	Dark red	round	sweetish	192,5	14,0	8,0	1,63
Koromkheti	Dark red	round	sweetish	178,5	13,0	8,75	1,6
Jalabashvilebi	Dark red	round	sweet-sour	126,0	15,8	8,66	1,31
Kobuleti	Dark red	round	sweetish	129,4	13,25	10,25	1,28
Guria	Dark red	round	sweetish	383,6	13,6	11,0	1,41

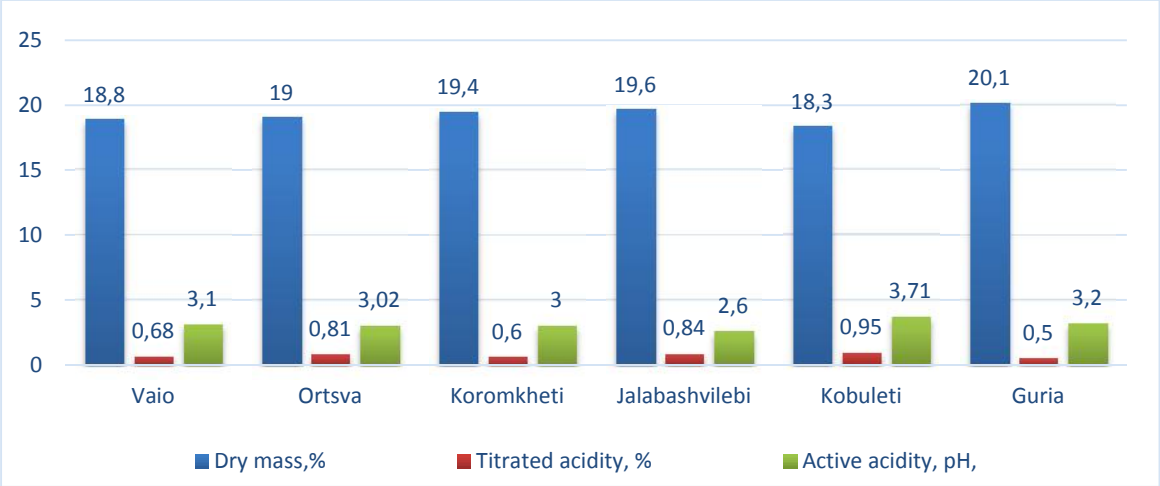
All *Chkhaveri* samples, taken for analysis, were characterized by a dark red color, round berries and sweetish taste, but the variety of *Chkhaveri*, collected in Jalabashvilebi, was distinguished by a sweet-sour taste. To begin the grape harvest and processing or storage, it is not enough to determine only the presence of sweetness in the grapes. It is also very important to

identify the titral acidity (total acidity) and pH of active acidity (hydrophobic concentration). The ratio of sweetness and acidity is one of the most important criteria for assessing the quality of grapes, so that the wine, obtained from it, has a unique aroma and taste.

In order to determine these components, the samples of grapes (each 1 kg) were firstly separated from grape peels, then, crushed and placed in a homogenizer. Next they were diluted with ethyl alcohol and placed in a refrigerator. The grapes harvested in the Erketi region (360 m above the sea level), were distinguished by a large mass of grapes (383.6 g) and a high sugar content (20.1%), while *Chkhaveri* grapes, harvested in 0.95% of the territory of Kobuleti (5 m above the sea level), were distinguished by a high acidity. There have also been determined the concentration of sugar, titral acidity and active acidity were also determined. Unlike other grape varieties, a relatively low concentration of sugar and a relatively high concentration of titral acid ultimately give *Chahaveri* its unique individuality.

Physical and chemical indicators of *Chkhveri* grape juice.

Diagram1



1.2. White grapes are: *Tsolikouri, Tsitska, Krakhuna, Klardzhuri and Kutaturi*. According to the cultivation area of *Tsolikuri* in Georgia, it ranks second after *Rkatsiteli*. It has high agricultural and technological qualities. The high-quality natural semi-sweet wines, made according to the European and Imeretian traditional methods, are characterized by moderate alcohol content and acidity, excellent taste qualities. *Tsitska* variety is distributed mainly in western Georgia (the upper and middle Imereti), where it represents the main production grape variety. The most important varieties of grape for viticulture are the ancient ones –*Klardjuli* and

Krakhuna; they are preserved only in small quantities in the villages of the mountainous regions of Guria-Adjara due to the vine phylloxera and other fungal diseases prevalent there (Beridze K., A. A. Dmitrieva ...)

The five types of grape samples were taken in October-November in 2016-2017, including *Tsolikouri* gathered in Adjara, Imereti and Samegrelo, *Tsitska* - in Adjara and Imereti and *Klarjuri* and *Kutatura* – in the Agroservice Center of Vine and Fruit Tree Nursery (Adjara). All the five varieties are represented in Table 1.

Among the samples of *Tsolikouri* varieties, the largest berries were peculiar to the grape fruits gathered in the Kobuleti Agroservice Center; the berries collected in Keda, were characterized by a smaller size of berries, while the relatively small bunches of samples were collected in Samegrelo (Lekhaindrao).

The fruits of *Tsitska*, collected in Imereti and Kobuleti, differ by technical features from the samples collected in other regions. The fruits of *Klardjuli* and *Krahuna* varieties, grown in Kobuleti, are significantly different and are almost one and a half times more than the samples of industrial grape varieties of *Tsitska* and *Tsolikouri*, collected in Imereti and Samegrelo (Table 3.4).

Samples of grapes taken for analysis

Table 3

Variety	region	district	Village	Name of sample
Tsolikuri	Adjara	Kobuleti	Gvara	G.1
Tsolikouri	Adjara	Keda	Kokotauri	G.2
Tsolikouri	Imereti	Baghdadi	Opcha	G.3
Tsolikouri	Samegrelo	Martvili	Bandza	G.4
Tsolikouri	Samegrelo	Martvili	Najakhao	G.5
Tsolikouri	Samegrelo	Martvili	Mukhurcha	G.6
Tsolikouri	Samegrelo	Martvili	Lekhaindravo	G.7
Tsolikaouri	Samegrelo	Martvili	Nagvazao	G.8
Tsolikouri	Samegrelo	Martvili	Vedidkari	G.9
Tsitska	Imereti	Baghdadi	Opcha	G.10
Tsitska	Adjara	Kobuleti	Gvara	G.11

Klarjula	Adjara	Kobuleti	Gvara	G.12
Krakhuna	Adjara	Kobuleti	Gvara	G.13
Kutatura	Adjara	Kobuleti	Gvara	G.14

Technical indicators of white grape varieties

Table 4

Sample name	White Grape Varieties						
	Grain colour	Grain shape	Taste	Bunch mass, g	Bunch length, sm	Bunch width, sm	Grain mass, g
G.1	green	round	sweetish	552,03	22	18	3,07
G.2	Greenish-amber	Round	Sour	199,92	17	12	2,0
G.3	Greenish-amber	Round	Sweet	188,65	13,83	9,16	2,0
G.4	Green	Round	Sweetish	139,7	12,75	9,5	2,4
G.5	Amber	Round	Sweet	185,0	17,5	11	2,6
G.6	green	Round	Sweetish	130,66	14,5	10,16	2,52
G.7	Greenish-amber	Round	Sweet	104,75	14,75	9,87	2,5
G.8	Yellowish-green	round	Sourish	151,66	22,7	12,16	2,4
G.9	green	Round	Sweetish	169,83	24,16	10,5	2,46
G.10	Amber	Round	Sweet	225,25	16,66	12,33	2,3
G.11	Green	Round	Sweetish-sourish	258,83	16,8	10	2,94
G.12	Green	Round	Sweet	364,95	17	12	3,31
G.13	Green	Round	sour-sweetish	345,94	15,5	12	3,08
G.14	Greenish-amber	Round	Sourish	144,85	9,25	7	1,97

The high content of sugar and low acidity in the ripe fruit are the important components for wine production. Almost all the samples collected in Samegrelo, are high in dry substance (21,0 to 23.8%); however, this indicator is relatively low in the grapes, grown on Kobuleti territory, -

19.0%, while in the regions of Keda and Imereti the average indicator is 20.0 - 21.3%. Among the technological characteristics of the wine grapes, the most important ones are titrated acidity and dry substance. These indicators differ in the samples of *Tsolikouri* variety, grown in different climatic conditions. Titrated acidity varies between 0,23 - 0,76%. The grapes harvested in Imereti, are high in dry substance and low in acidity (acidity - 0.23%, dry matter - 21.3%). The Kobuletian *Tsolikouri* (pH 3.15) is characterized by high active acidity, while the Imeretian *Tsolikouri* (pH 4.2) has relatively low active acidity (Table 5).

Physical and chemical indicators of white grape juice

Table 5

Sample	Physical and Chemical indicators of grape juice		
	Dry substance according to %	Titrated acidity, %	Active acidity, pH
G.1	19	0,62	3,15
G.2	20,0	0,74	3,72
G.3	21,3	0,23	4,20
G.4	23,6	0,45	3,76
G.5	23,2	0,43	3,95
G.6	21,2	0,61	3,63
G.7	23,8	0,51	3,65
G.8	21,9	0,76	3,46
G.9	21,0	0,62	3,66
G.10	21,2	0,34	3,86
G.11	20,3	0,85	3,22
G.12	19,6	0,99	2,98
G.13	19,8	0,90	3,35
G.14	19,4	0,73	3,09

As for *Tsitska, Klarjuri, Krakhuna* and *Kutatura* grape varieties, the indicators of dry substances are relatively high in *Tsitska* variety collected in Imereti and Adjara regions - 20,3 to 21,2%, while their content in *Klarjuri, Krakhuna* and *Kutatura* varieties is almost identical - 19,4

- 19,8%. Titrated acidity varies between 0,34 - 0,99%, while active acidity rates from pH 2,98 to 3,86 (Table 3,5).

1.3. ***Aleksandrouli, Usakhelauri, Dzvelshavi, Mujuretuli, Ojaleshi, Kabistoni, Kachichi, Toluri Sapere, Otskhanuri Sapere and Satsuravi*** are the red grape varieties, spread in different regions of western Georgia (Adjara, Guria, Imereti). These red grape varieties have a late period of maturity. They are promising wine grape varieties, and the wine, made from these varieties, is characterized by a beautiful and rich colour, harmony and a normal alcohol content and acidity, as well as a pronounced varietal aroma and high taste properties.

The grape samples were taken in different districts of Western Georgia: *Aleksandrouli* and *Mujuretuli* - in the Ambrolauri district (vil. Khvanchkara), *Usakhelauri* – the Agroservice Center of vineyards and fruit trees nursery (Adjara), *Dzvelshavi* – in the Baghdati district (vil. Persati), *Otskhanuri Sapere* and *Tolis Sapere* - in Zestaponi district (vil. Zeda Sakara), *Ojaleshi* – in the Tsageri district (vil. Tvishi), *Kachichi* – the Keda district (vil. Kharaula), *Satsuri* – in the Keda district (vil. Kokotauri).

The brunches of grapes under study have an average size of 86-156 g, black and round grains (0.9-2 g). The length of the grapes is 11–16 cm, and the width is 5-10 cm. In order to start harvesting, it is necessary to determine the ripening period of the grapes, which must meet the technical requirements of the product, made from it (dry wine, natural semi-sweet wine, sparkling wine, etc. (Table 6).

Technical indicators of red grape varieties

Table 6

Sample name	Technical indicators of grapes						
	Grain colour	Grain shape	taste	Branch mass, g	Branch length, sm	Branch width, sm	Grain mass, g
Aleksandrouli	black	Round	sweet	144,52	15,7	9,1	2,0
Usakhelauri	black	Conical	sweet	87,72	12,16	9,6	1,8
Dzvelshavi	black	Round	sweet	137,522	11,6	7,2	1,89
Mujuretuli	black	Round	sweet	89,908	14,8	8,9	1,58
Ojaleshi	black	Round	sweetish	103,12	11,75	8,37	1,99
Kabistoni	Dark purple	Round	sweet	86,03	15,5	9,5	1,46

Kachichi	black	Round	Sweetish -sourish	156,5	11,1	6,4	1,22
Tolis Saperavi	black	Round	Sweetish -sourish	87,32	13,89	5,16	1,93
Otskhanuri Sapere	black	Round	Sweet	84,01	11	7,75	0,9
Satsuravi	black	Conical	Sweet	14,12	6,87	6,02	2,0

Samples of grapes for analysis (*Usakhelauri, Dzvelshavi, Kabistoni*) were taken during the period of technical maturity, at the end of September, when the amount of sugar was 23.8, 25.7 and 22.0%, respectively; in the middle of October – *Otskhanuri Sapere* (23.0%), *Tolis Sapere* (22.5%), *Aleksandrouli* (24.7%), *Mujuretuli* (26.0%) and *Kachichi* (24.0%); in the second half of November - *Ojaleshi* (23.5%) and *Satsuravi* (19%) (Table 7).

Physical and chemical indicators of red grape varieties

Table 7

Sample	Physical and chemical indicators of grape juice		
	Dry mass, %	Titrated acidity, %	Active acidity, pH
Asleksandrouli	24,7	0,49	4,08
Usakhelouri	23,8	0,72	3.87
Dzvelshavi	25,7	0,70	3,99
Mujuretuli	26	0,54	4,24
Ojaleshi	23,5	0,68	3,85
Kabistoni	22.0	0,76	3,88
Kachichi	24,0	0,71	3,92
Tolis Saperavi	22,5	0,75	3,91
Otskhanuri Sapere	23,0	0,76	3,65
Satsuravi	19,0	0,74	3,64

There have been studied **10 different types of red grape varieties**, grown in different parts of western Georgia: *Aleksandrouli, Usakhelauri, Dzvelshavi, Mujuretuli, Ojaleshi, Kabistoni, Kachichi, Tolis Sapere, Otskhanuri Sapere* and *Satsuravi*; **5 types of white grape varieties:** *Tsolikouri, Tsitska, Klarjuli, Krakhuna and Kutaturi*; technical characteristics (dry substance, titrated acidity, active acidity) of 6 samples of pink grape variety - *Chkhaveri*, grown in different places.

Chapter 2. Allocation and identification of phenolic compounds of autochthonous grape varieties of Western Georgia using HPLC and UPLC-MS method.

2.1. The allocation and identification of white grape phenolic compounds.

Phenolic compounds and the products, derived from them, are actively involved in the formation of wine type at all stages of its preparation and storage, and have an uneven effect on taste, color and transparency of wine.

The aim of the work is:

- to allocate and identify the phenolic compounds of the wine, made from the white grape varieties of vines (Vitis vinifera L.) cultivated in three regions (Adjara, Imereti, Samegrelo) - *Tsolikouri, Tsitska, Klarjuli, Krakhuna* and *Kutatura*, using the European technology and high-pressure liquid mass spectromatic chromatography;
- to study the total number of phenolic compounds, catechins, flavonols and to identify their antioxidant activity.

Tsolikouri is a local Imeretian variety, which is widely spread in almost all districts of western Georgia. Different types of wine are produced from *Tsolikouri* variety. They are characterized by excellent taste and rich chemical composition. *Tsolikouri* wine is rich in alcohol and is characterized by a sufficient amount of acids, which ultimately improve the aging process of wine and its shelf life.

Tsitska is a high quality grape variety widely spread in Imereti. A high-quality material for sparkling wine is obtained from this grape variety. The wine, made from *Tsitska* variety, has a light beige-greenish colour, and is characterized by a rich composition, delicate and harmonious taste. After the aging, it acquires a softer and more pleasant taste.

Krakhuna variety gives high quality wine, prepared by the European technology, which has a yellowish-beige colour and is characterized by a delicate and pleasant taste. The wine, prepared by the Imeretian technology, is darker and is characterized by saturation and a peculiar to this variety aroma.

Klarjula belongs to a group of white grape varieties. Due to excellent grape taste, transparency, excellent ability to store, external beauty of bunches and berries, as well as rich

yield, *Klarjula* is considered to be one of the best grape varieties spread in Georgia (M. Ramishvili ..., A. Shalashvili ...).

In October-November 2016-2017, the following five types of grape samples were taken for analysis: *Tsolikouri* - in Adjara, Imereti and Samegrelo, *Tsitska* - in Adjara and Imereti, and *Krakhuna, Klarjuli* and *Kutatura* - in the Agroservice Center of Vine and Fruit Tree Nursery (Adjara). The wine was made by the European technology. The samples of all five types of grapes (per 5 kg) were cleared of stalks and the juice, squeezed out of them, was filtered into a glass dish. The yeast was added to the juice (with a calculation of 10 CB 2000/25 g / hL (10 CB 2000/25 g / hl.) After the end of alcoholic fermentation, the wine was placed in a refrigerator. The analysis was carried out 5 months after the preparation of the wine (Table 8).

Samples of wine taken for analysis

Table 8

№	variety	Region	district	village	wine
1	Tsolikouri	Adjara	Keda	Kokotauri	w. 1
2	Tsolikouri	Adjara	Kobuleti	Gvara	w. 2
3	Tsitska	Adjara	Kobuleti	Gvara	w. 3
4	Klarjula	Adjara	Kobuleti	Gvara	w. 4
5	Krakhuna	Adjara	Kobuleti	Gvara	w.5
6	Kutatura	Adjara	Kobuleti	Gvara	w. 6
7	Tsolikouri	Samegrelo	Martvili	Bandza	w. 7
8	Tsolikouri	Samegrelo	Martvili	Najakhao	w. 8
9	Tsolikouri	Samegrelo	Martvili	Mukhurcha	w. 9
10	Tsolikouri	Samegrelo	Martvili	Lekhaindravo	w. 10
11	Tsolikouri	Samegrelo	Martvili	Nagvazao	w. 11
12	Tsolikouri	Samegrelo	Martvili	Vedidkari	w. 12
13	Tsolikouri	Imereti	Baghdadi	Opcha	w. 13
14	Tsitska	Imereti	Baghdadi	Opcha	w. 14

The isolation and identification of phenolic compounds were carried out using High Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography

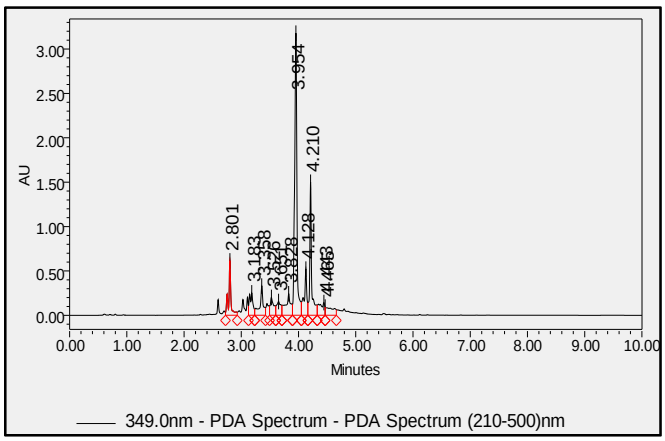
with Mass Spectrometry Coupled to Photodiode Array Detection (UPLC-MS-PDA). Thus, several compounds have been identified. Before chromatographic separation, a sample was subjected to solid phase extraction, which involves activating the column with methanol. Then the activated sorbent was equilibrated with distilled water. After that the sample was transferred to the cartridge, using vacuum. At the next stage, the unwanted components, remaining on the sorbent, were washed off with water. Concentrated substances were eluted with methanol.

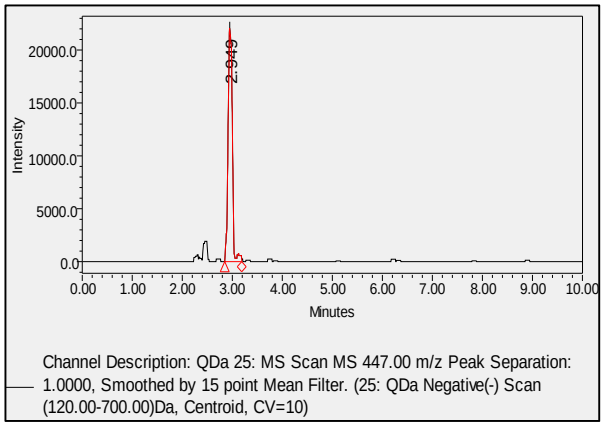
The following compounds have been identified in the wine by the UPLC-MS method: Procyanidin B_2 – (release time - 2,315 min; MW-578, m / z-577, fragment 289, λmax 80 nm); (-) - Epicatechin (release time - 2.426 min, MW -290, m / z-289, (fragment 245) λmax 280 nm); Flavonols: Quercetin-3-Glucuronide (release time - 2.828 min, MW-478, m / z-477, fragment 301, λmax 256, 354 nm); Quercetin-3-Glucoside (release time 2.833 min, MW-464, m / z-463, fragment 301, λmax 256, 356 nm); Quercetin-3-Rhamnoside (release time 2949 min, MW-448, m / z-447, fragment 301, λmax 256, 354 nm); (Table 9).

UPLC-PDA-MS spectrum of the white wine

Table 9

Substance name	RT (min)	MW	[M-H]- (fragment m/z)	UV maximum (nm)
(-)-Epicatechin	2.426	290	289 (245)	280
Quercetin-3-Rhamnoside	2.949	448	447 (301)	256 (max), 352
Quercetin-3-Glucoside	2.833	464	463 (301)	256 (max), 356
Quercetin-3-Glucuronide	2.828	478	477 (301)	256 (max), 354
Procyanidin B_2	2.315	578	577 (289)	280





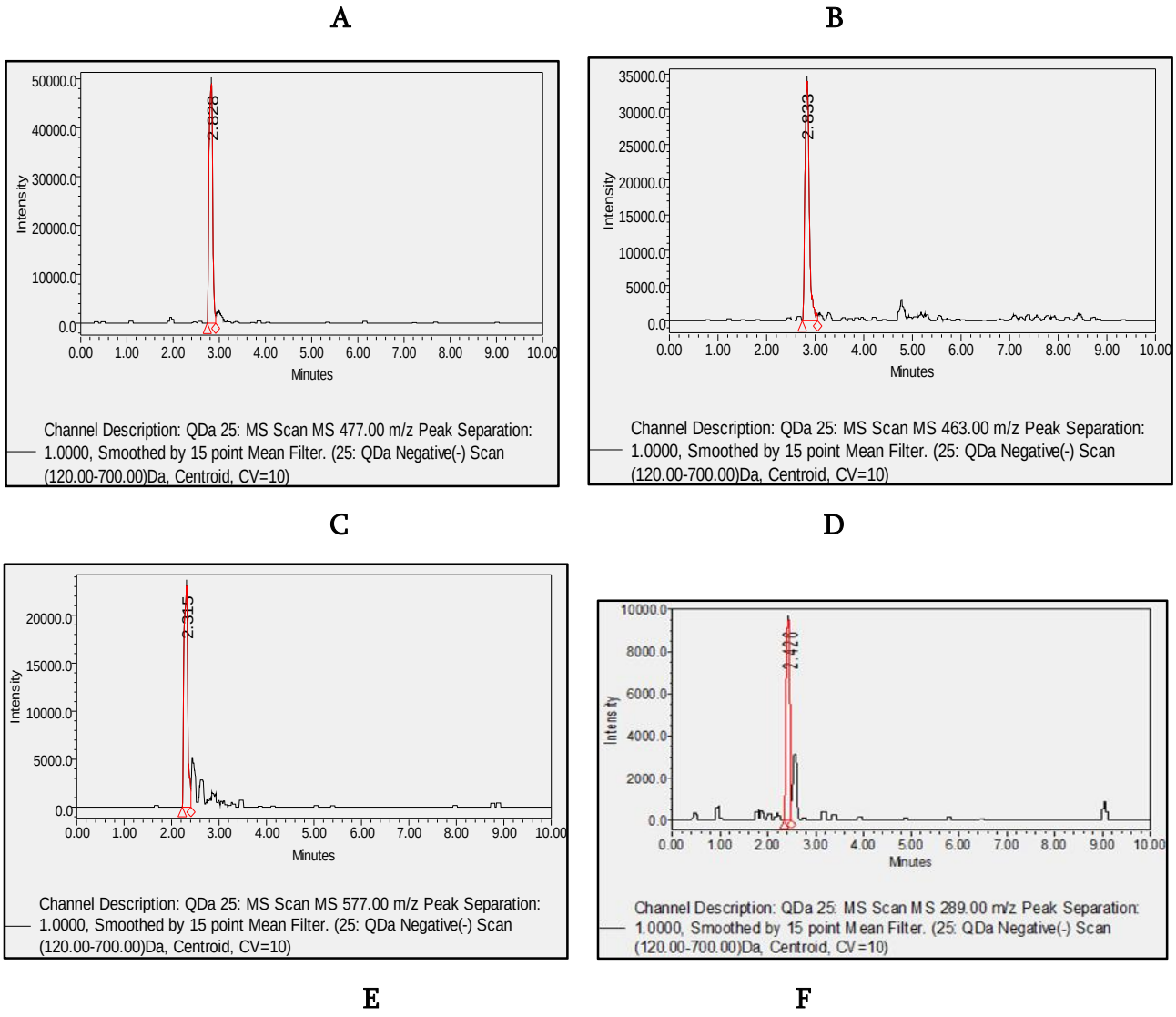
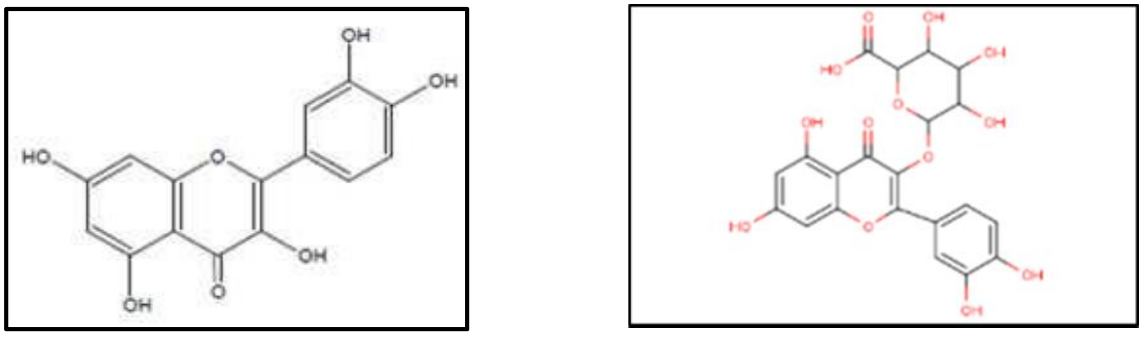


Fig. 9. Wine UPLC-PDA-MS Chromatogram; A - Common Chromatogram: B- Quercetin-3-Rhamnoside; C - Quercetin-3-Glucoside; D - Quercetin-3-Glucuronide; E - Procyanidin B_2; F - (-) – Epicatechin



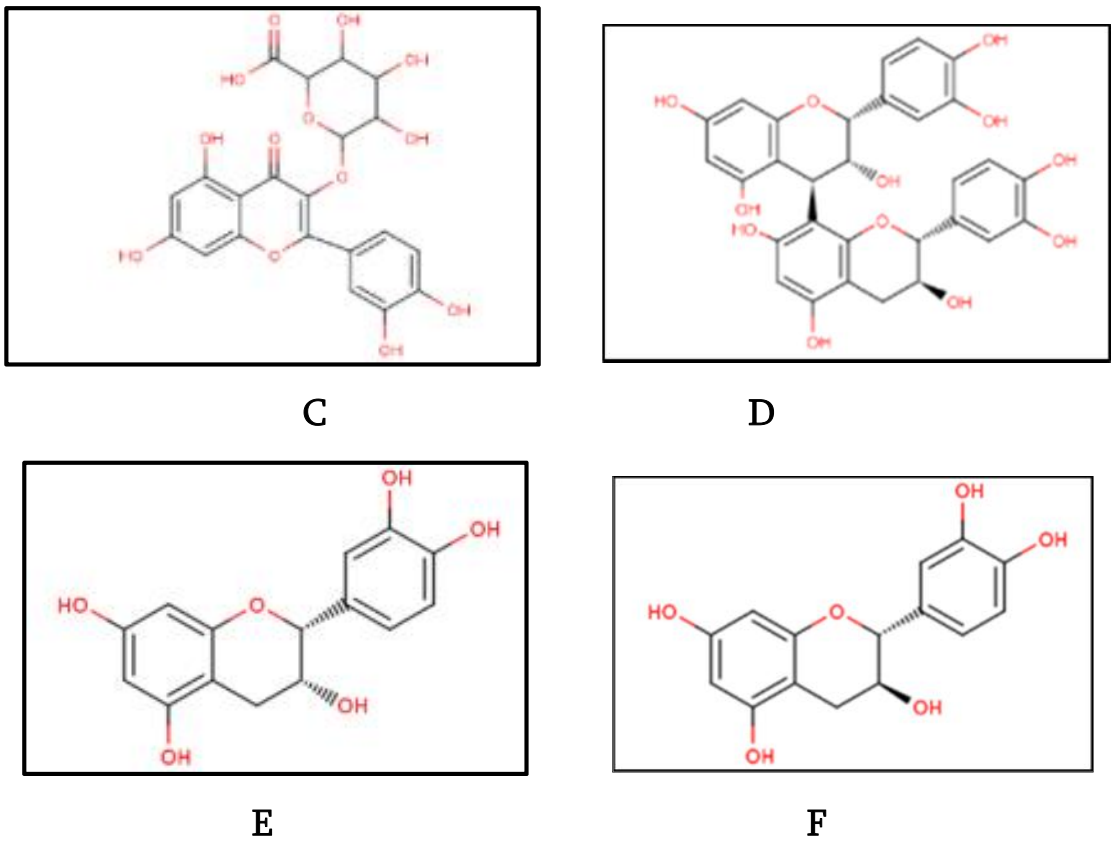


Fig. 10. **A** - Quercetin-3-Rhamnoside; **B** - Quercetin-3-Glucoside; **C** - Quercetin-3-Glucuronide; **D** - Procyanidin B_2; **E** - (-) - Epicatechin; **F** - Catechin- (); (

2.2. Allocation and identification of red wine anthocyanins and their aglycons

Red wine production is a priority in many countries around the world, and the demand for them is growing every day. Red wines, in addition to good organoleptic characteristics, are characterized by significant and diverse biological activity.

Organic compounds with a variety of antioxidant properties have been found in red wine, made from various grape varieties. They are mainly found in the skin, seeds and grains of grapes. These include: stilbene, flavonols, anthocyanins, catechins, polymeric proanthocyanidins, phenolic acids, etc. According to recent studies, the composition of polyphenols, the content of the phenol complex, their quantity, antioxidant and anti-radical properties of wine depend on many factors: grape variety, vineyard location, climatic conditions, soil type and winemaking technology. The red grape pigments are anthocyanins, which are mostly monoglycosides *(Burin V...., Danila Di Majo...).*

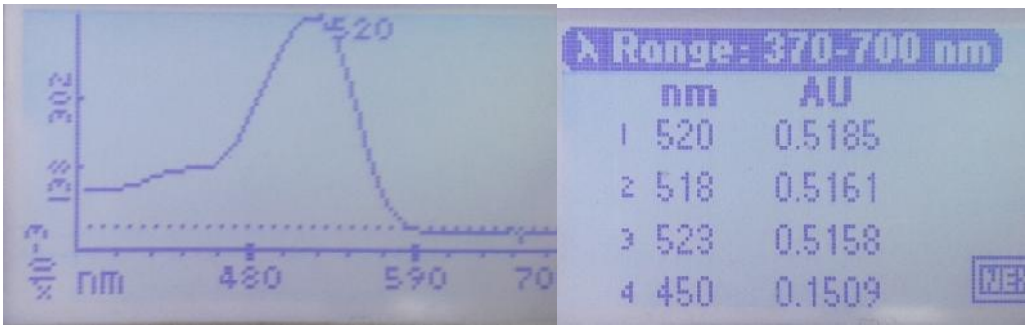
The aim of the work is the qualitative study of monomeric anthocyanins of red wines, made from grape varieties (*Aleksandrouli, Mujuretuli, Saperavi, Otskhanuri Sapere, Ojaleshi*) spread in different regions of Georgia.

The wine samples were prepared from 10 kg of each grape variety according to local technology (grape stalks participate in the process of alcoholic fermentation) within 10 days in 2015. The analysis of the quantitative content of anthocyanins and the antioxidant activity was done after a year of aging of the wine samples of *Saperavi, Mujuretuli, Ojaleshi* and *Otskhanuri Sapere*; while the wine sample, obtained from the Alexandrouli grape variety, was analyzed before aging (after the completion of alcoholic fermentation, the name of the sample is Aleksandrouli 1) and after a year of aging (sample name is Alexandrouli 2).

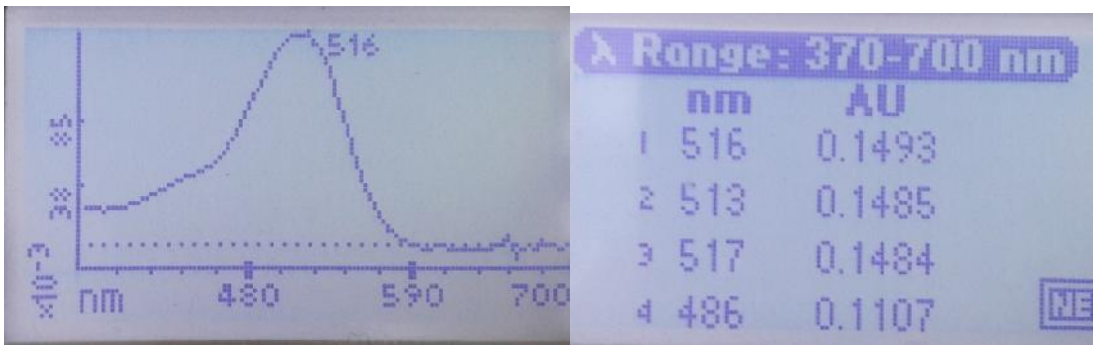
The wine samples were placed in 3-5 ml of the Waters Sep-Pak C18 (500 mg) column. The remaining pigments were eluted by acetonitrile. All samples have been filtered before analysis. Filter Waters Acrodisc LC PVDF Filter 13 mm 0,45μm was used for filtering.

Anthocyanin analysis was conducted with HPLC, on the C18 analytical and preparatory column. The Eluent A: water / formic acid / acetonitrile (87: 10: 3); Eluent B: water / formic acid / acetonitrile (40:10:50); gradient (0-15 min from 6% to 30% B, 30 min 50% B, 35 min 60% B, 41-45 min 6% B). Detecting 518 nm. UPLC-MS analysis BEN C18, 1.7μm, BENAmide1.7μm, column. Eluent acetonitrile, formic acid, (gradient), flow 0,4 ml / min, column temperature 50 ^{0}C, MS- scan 200-1200 da, Probe 500 ^{0}C, Positive 0,8 kV, capillary 1,5 kV,CV -15.

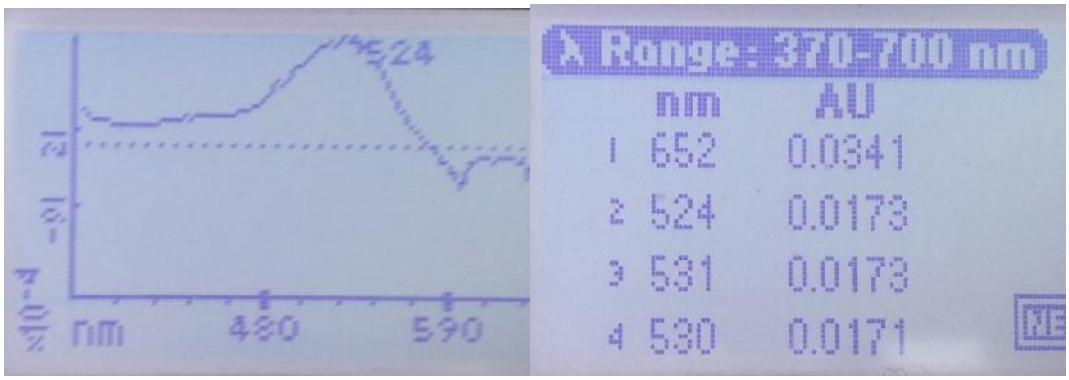
Detection 518 nm. UPLC-MS analysis BEN C18, 1.7μm, BENAmide1.7μm, column, eluent acetonitrile, formic acid, (gradient), flow 0,4 ml / min, column temperature are identified as aglycones and glycosides. Aglycones identification was performed by hydrolysis 6M HCl with separate individual compounds of acids.



A


516
85
38
x10-3
nm
480
590
700
λ Range: 370-700 nm
nm AU
1 516 0.1493
2 513 0.1485
3 517 0.1484
4 486 0.1107

B


524
21
-01
x10-4
nm
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nm AU
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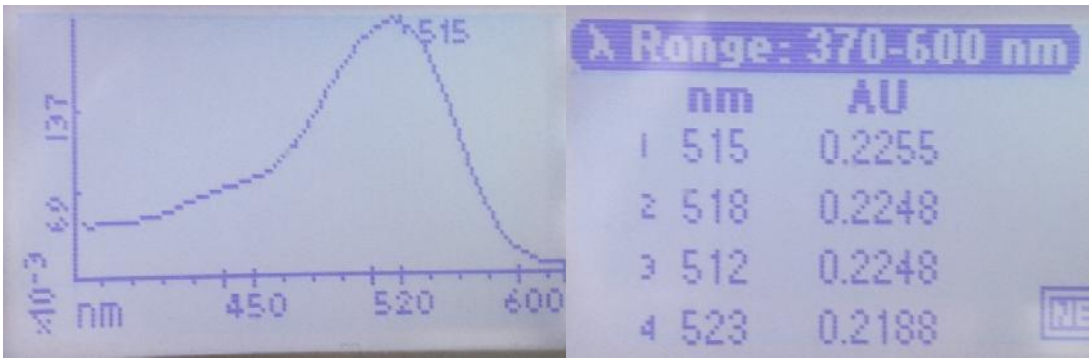
2 524 0.0173
3 531 0.0173
4 530 0.0171

C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nm
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600
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nm AU
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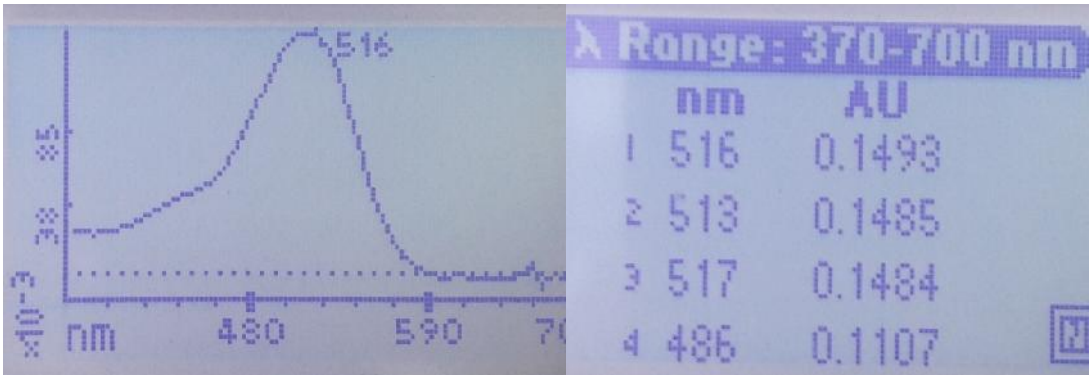
2 518 0.2248
3 512 0.2248
4 523 0.2188

D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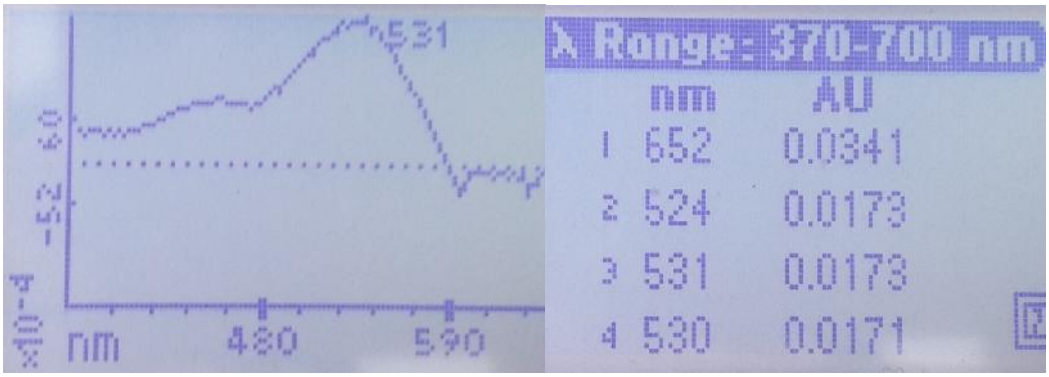
2 513 0.1485
3 517 0.1484
4 486 0.1107

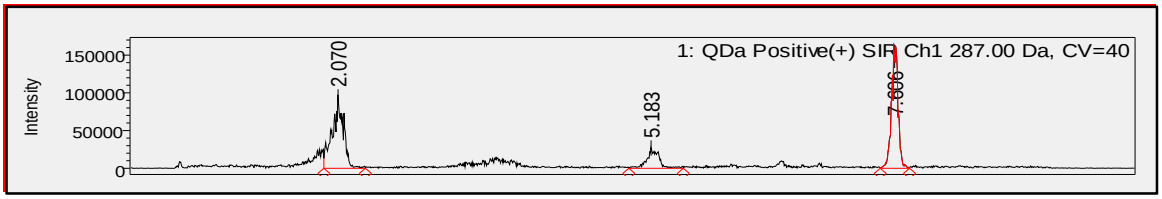
E



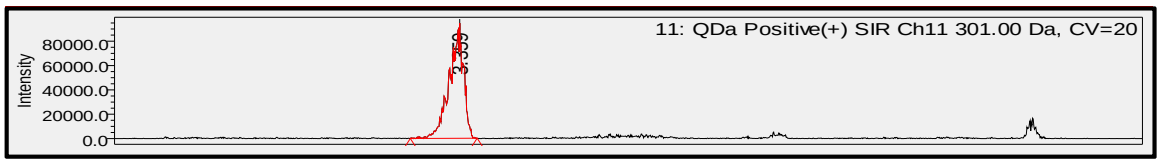
F

Fig. 11: The maxima of anthocyanins and the absorption of their glycones were detected using high performance liquid chromatography: - A-malvidin-3-0-glucoside; B is cyanidin-3,5-0-diglucoside; C-Malvidin-3,5-0-diglucoside; D-peonidin-3,5-0-diglucoside; E- cyanidin; F-peonidin.

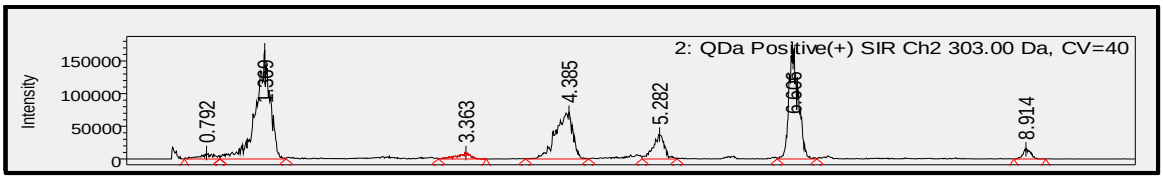
For greater accuracy of the results, the anthocyanins and aglycones have also been subjected to mass spectrometry analysis. The presence of 5 aglycones was established: cyanidin (m / z287), peonidine (m / z301), delphinidin (m / z303), petunidine (m / z317) and malvidin (Fig. 12).



A



B



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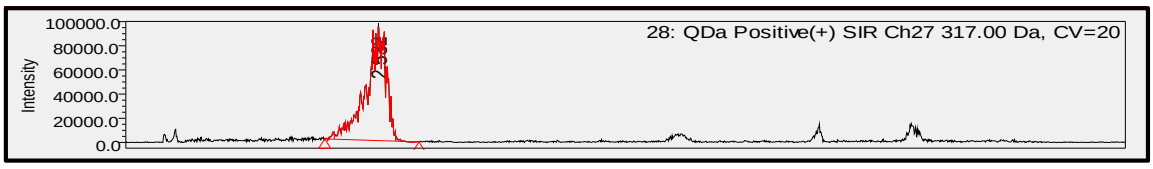

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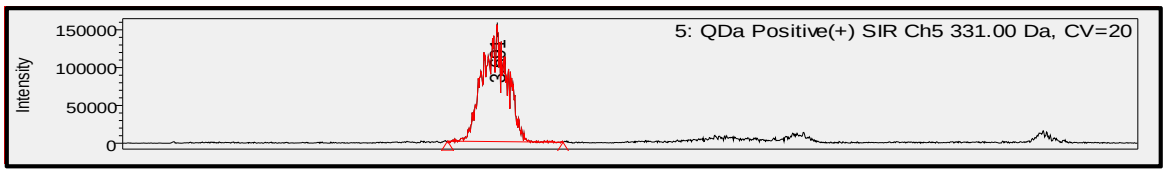
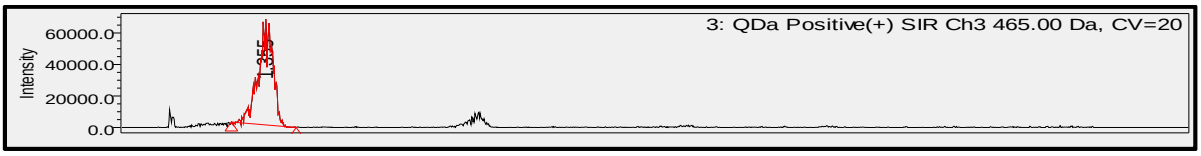


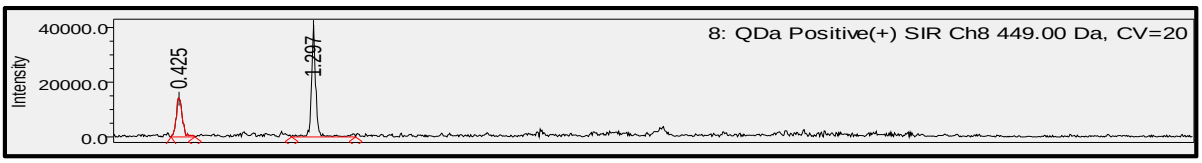
E

Fig. 12. UPLC-MS spectrum of aglycones of anthocyanins: **A**- cyanidin (m /z 28), **B**- peonidine (m/z301), **C**- delphinidin (m/z303), **D**- petunidine (m/z317) **E**- malvidin (m/z331).

Using the UPLC-MSB method under the same conditions, 9 anthocyanins were identified in the wine samples (Fig. 13): delphinidin-3-O-glucoside (m / z465 / 303), cyanide-3-O-glucoside (m / z449 / 287); petunidin-3-O-glucoside (m / z 479/317); peonidin-3-O-glucoside (m / z463 / 301); Malvidin-3-O-Glucoside (m / z493 / 331); peonidin-3-O-acetylglucoside (m / z505 / 301); Malvidin-3-O-acetylglucoside (m / z 595/331); Peonidin-3-O-coumarilglucoside (m / z 609/301); Malvide 3-O-coumaryl glucoside (m / z 611/331).



A



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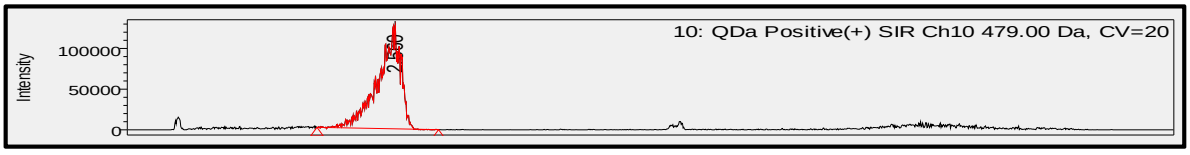

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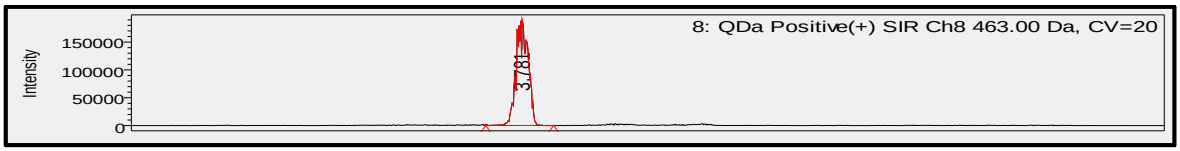

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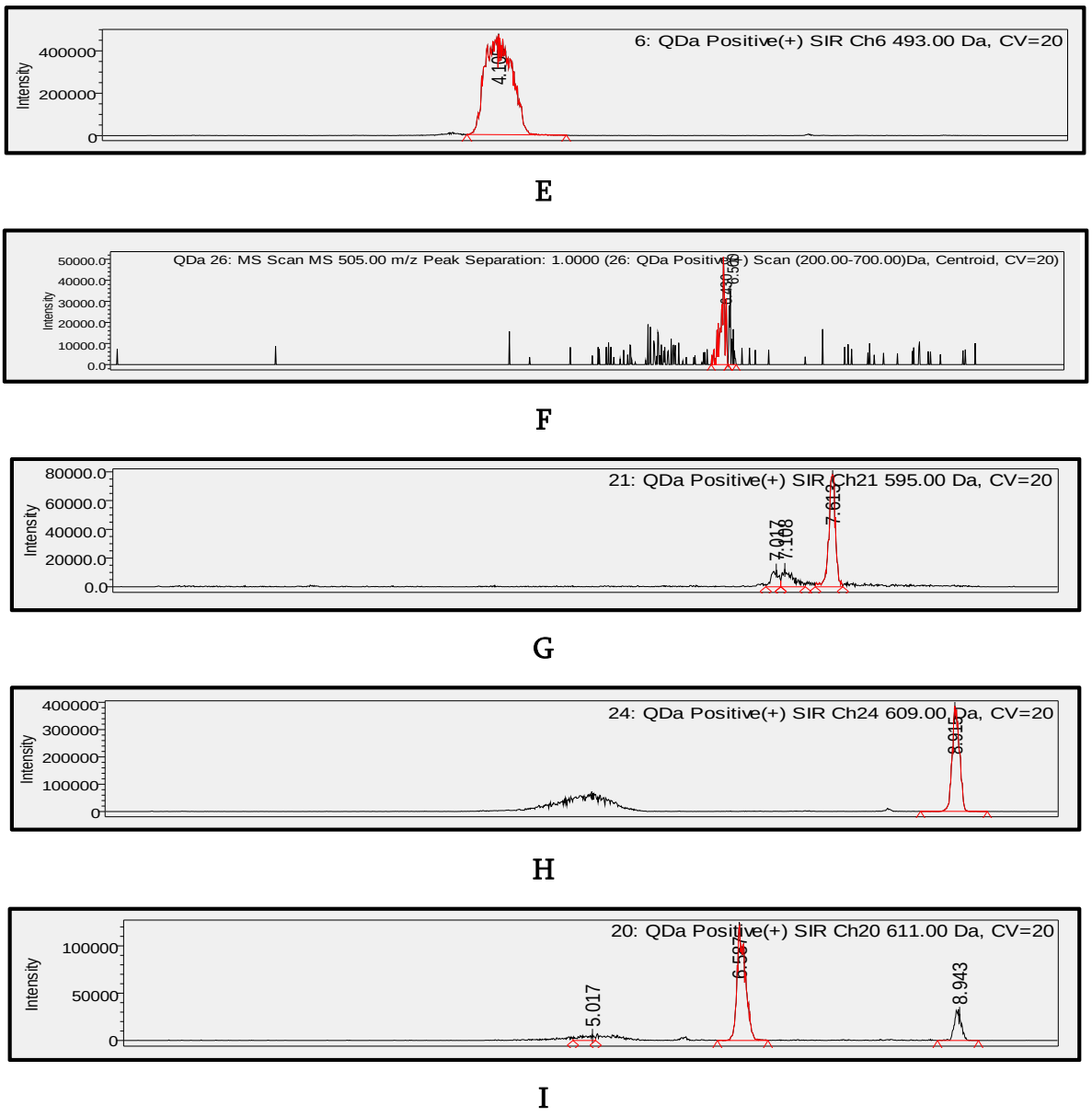
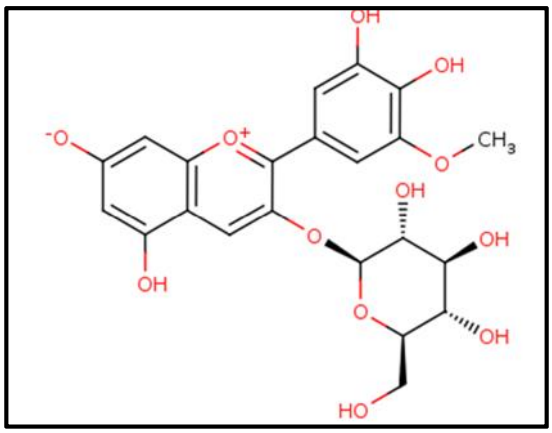
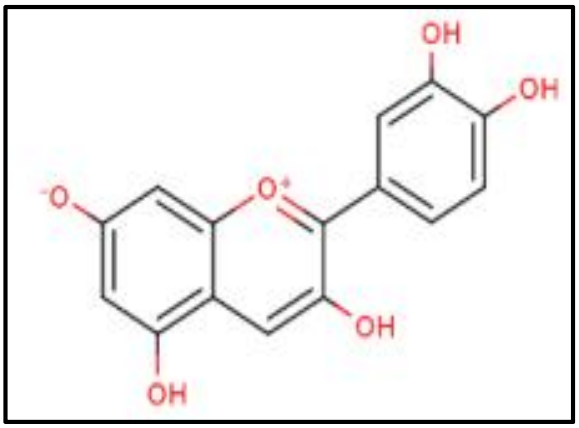


Fig. 13. UPLC-MS spectrum of aglycones of anthocyanins: **A –** delphinidin 3-O-glucoside; **B –** cyanidin -3-O- glucoside; **C -** petunidine 6-3-O- glucoside; **D** - peonidine -3-O- glucoside; **E -** malvidin -3-O- glucoside; **F –** peonidine -3-O- acetylglucoside; **G –** malvidin -3-O- acetylglucoside; **H –** peonidine -3-O- coumarilglucoside; **I -** malvidin - 3-O- coumarilglucoside.

The content of malvidin-glucoside quantitatively predominates in all samples. The samples of wine differ in the number of monomeric anthocyanins, depending on grape variety.



A



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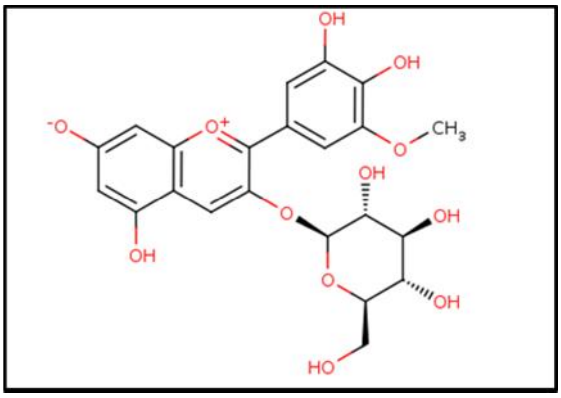

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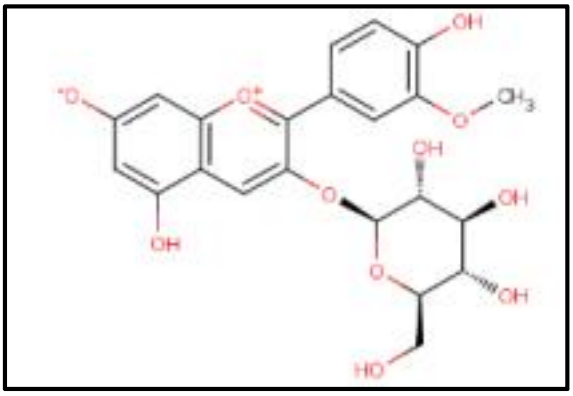

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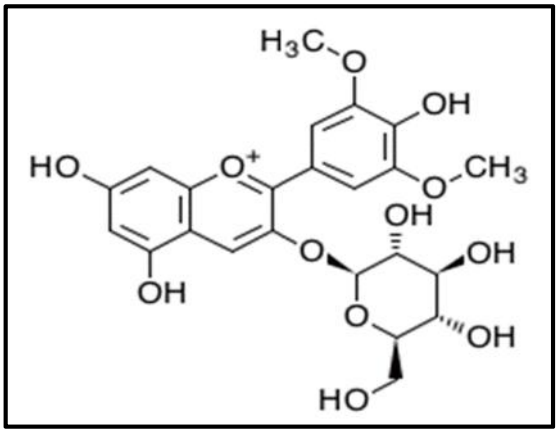

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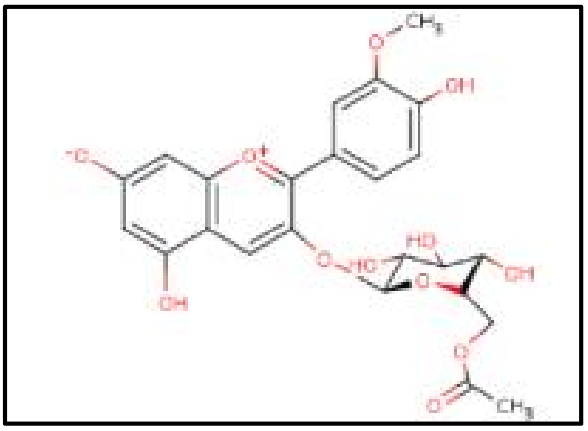

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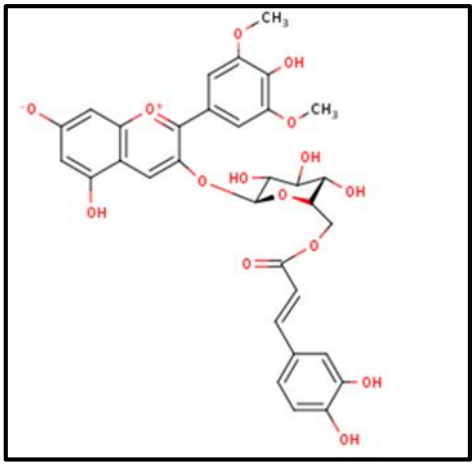

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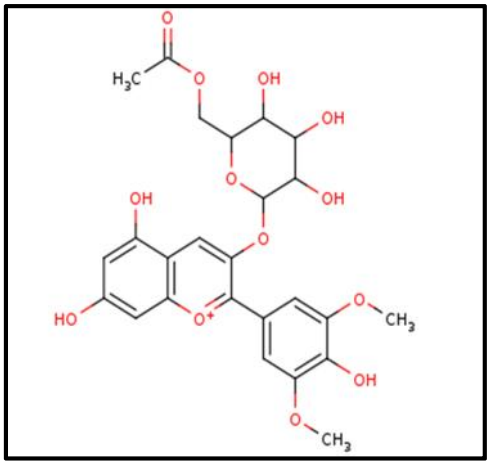

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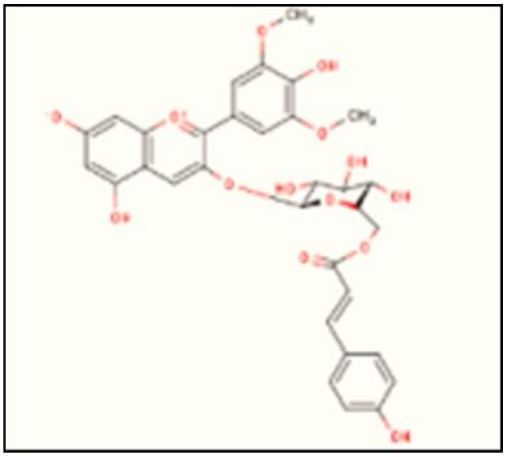


I

Fig. 14. Formulas of anthocyanin glucosides: A – delphinidin 3-O- glucoside; **B –** cyanidin -3-O- glucoside; **C -** petunidine 6-3-O- glucoside; **D -** peonidine -3-O- glucoside; **E -** malvidin -3-O- glucoside; **F –** peonidine -3-O- acetylglucoside; **G –** malvidin -3-O- acetylglucoside; **H –** peonidine -3-O- coumarilglucoside; **I -** malvidin - 3-O- coumarilglucoside.

Chapter 3. Quantitative analysis of phenolic compounds of grapes and wine of autochthonous varieties of Western Georgia and determination of their antioxidant activity using the DPPH method.

3.1. Quantitative analysis and antioxidant activity of common phenols, flavonols, anthocyanins and catechins of grapes and pink wine of Chkhaveri variety.

The content of common phenols, flavonols and anthocyanins was identified in *Chkhaveri* grape variety and their number was compared according to location. For the quantitative analysis, 5 g of grapes without seeds have been extracted in 90% alcohol (200 ml) at the temperature of -

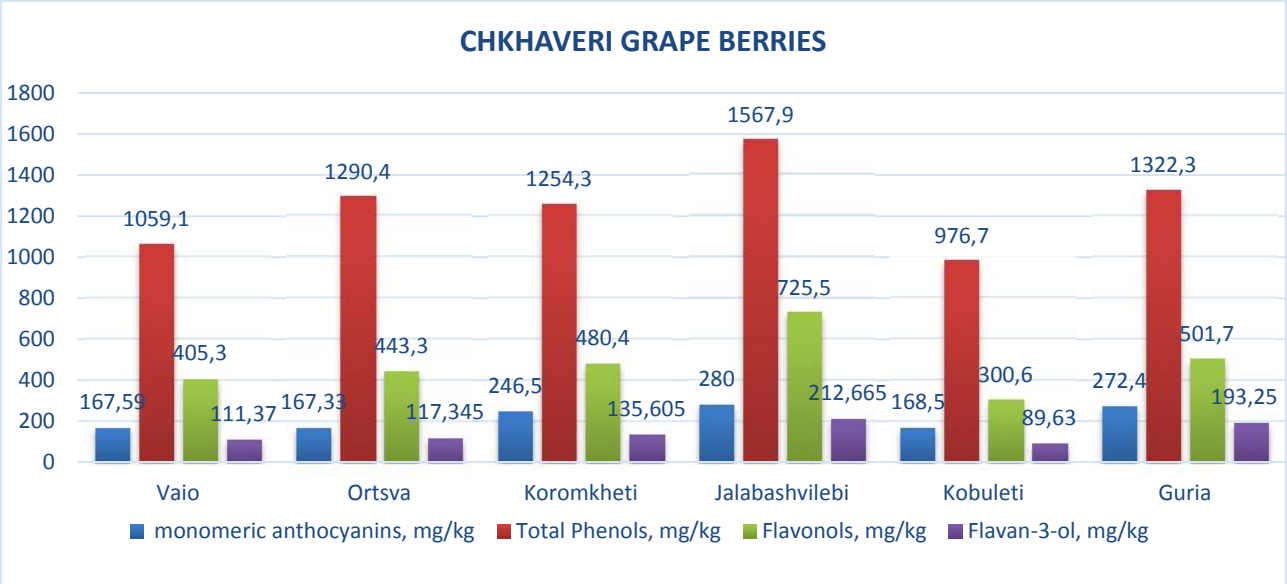
20^{0}C until a complete bleaching of the extract; thereafter, the presence of the substances in the obtained extract was determined by appropriate analysis methods. The research lasted for three years (2014, 2015, 2016), at different heights from the sea level and in different climatic conditions.

The number of common phenols in *Chakveri* grapes in 2016 varies between 976.7-1567.9 mg / kg per raw weight, monomeric anthocyanins - 168.5-280.0 mg / kg per raw weight, flavonols - 300,6-725,5 mg / kg and catechins - 89.63-212.665 mg / kg. The relatively high content of monomeric anthocyanins - 280.0 mg / kg was observed in *Charkveri* grapes, collected at 780 m above the sea level (vil. Jalabashvilebi).

The taken samples are rich in common phenols (1567.9 mg / kg), flavonols (725.5 mg / kg) and catechins (212.665 mg / kg). *Chakveri* grape variety, grown on the test area territory of Kobuleti (5 m above the sea level), is characterized by their relatively low content (monomeric anthocyanins - 168.5 mg / kg, common phenols - 976.7 mg / kg, flavonols - 300.6 mg / kg and catechins - 89.63 mg / kg per raw weight), while the biologically active compounds of *Chkhaveri* collected in Guria, namely village Erketi (360 m from the sea level), are distinguished by an average indicators (Diagram 2).

Quantitative content of common phenols, flavonols, anthocyanins and catechins in Chkhaveri grapes

Diagram 2

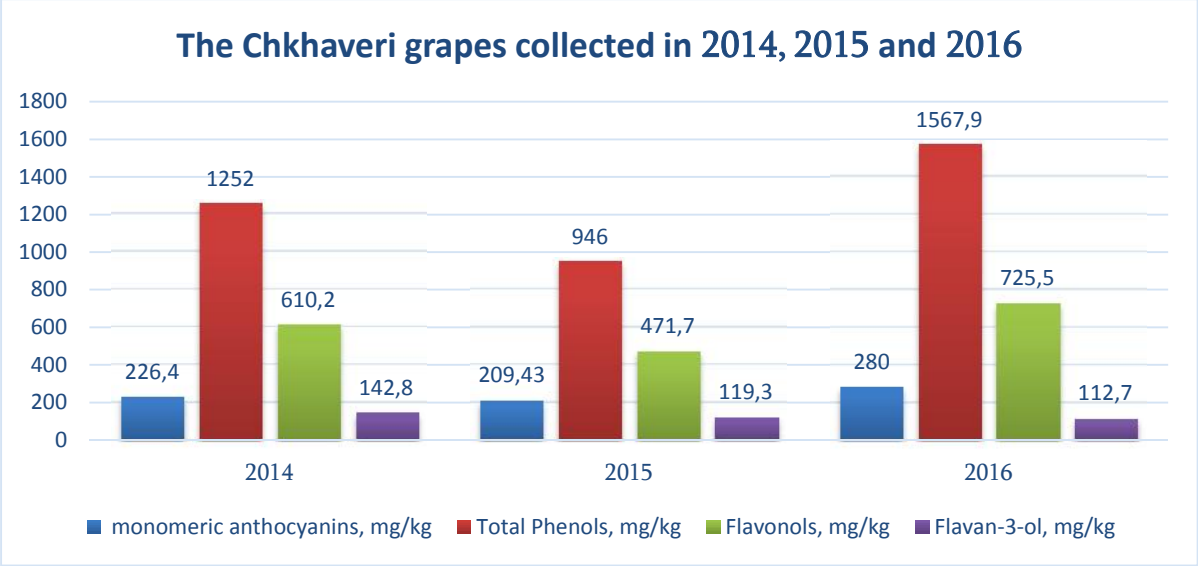


Such a content of the compounds in samples is apparently caused by a location of vine. Namely, the villages - Vaio, Ortsva, Koromkheti and Jalabashvilebi belong to the Keda municipality and

are situated on the left side of the Adjaristskali River, but at different altitudes (300-780 m) above the sea level. Thus, the content of the biologically active compounds of *Chkhaveri* from Jalabashvilebi, is also high, as with increasing height, environmental conditions become more stringent, and the plant strengthens its immunity due to the accumulation of phenolic compounds (Diagram 3).

Quantity content of common phenols, flavonols, anthocyanins and catechins in *Chkhaveri* grapes, collected in 2014, 2015, 2015

Diagram 3



Comparing the obtained results, it has been found that the maximum number of common phenols was recorded in the yield of 2016: common phenols - 1567,9 mg / kg, flavonols 725.5 mg / kg, catechnis 212,665 and monomeric anthocyanins - 280,0 mg / kg per wet weight. This can be explained by the fact that 2016 was distinguished by a long period of vegetation.

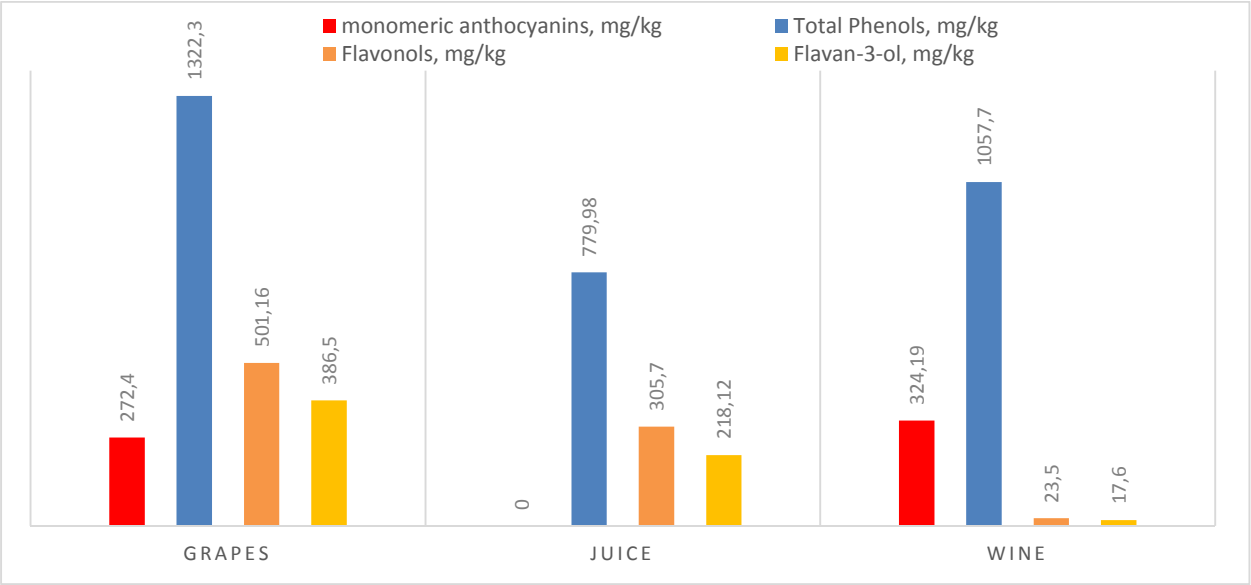
Grain phenolic compounds and their transformation products are actively involved in the formation and preparation of wine type - complex biochemical processes at all stages of storage. They directly affect the taste, colour, transparency and stability of wine.

The maceration is a technological process of wine making, which considers interacting of the solid and liquid phases of grape for some time, in order to get a drink with more extract, saturation and color. One of the main tasks in developing pink wine making technology from *Chkhaveri* grapes is to maintain a pleasant, typical pink color in the process of maceration and infusion. It should also be taken into consideration that the abundance of extractive components

or their insufficient amount may deteriorate the colour and the taste of the obtained wine, as well as the other indicators of its quality. Therefore, the establishment of the procedure for the implementation of this process should be determined individually for each specific case, taking into account the parameters of the final product, which should be prepared from a particular grape variety and macerated wine material.

Phenolic compounds of Chkhaveri grapes, juice and wine.

Diagram 4

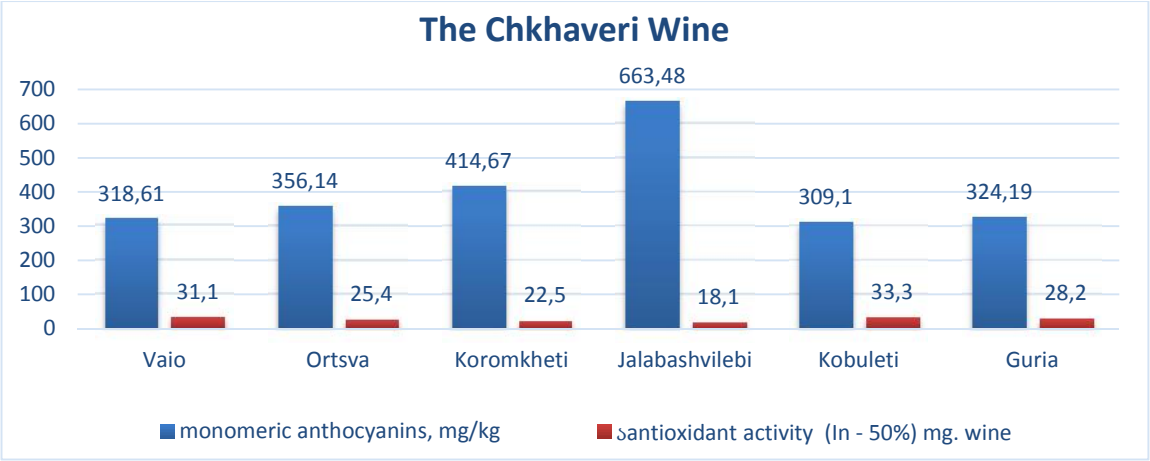


Anthocyanins are not found while straining the juice of the Chkhaveri grape. Their number increases in the process of maceration. (Diagram 4).

During the maceration process, the concentration of anthocyanins was identified every day in order to determine the optimal period. At the same time, a grape pulp was treated with enzyme preparations to ensure equal fermentation. The 5th day appeared to be the optimal one for obtaining the pink wine. After this time the color intensity increases, the pulp acquires darker tone, but the number of monomeric anthocyanins decreases and, therefore, the pigmentation (the polymerization) of anthocyanins occurs. After the pulp fermentation, 55% of the extracted anthocyanins are observed in wine, what represents 324.19 mg / kg; 80% or 1057.7 mg / kg of the common phenols; 4% or 23.5 mg / kg of flavonols; 3% or 17.6 mg / kg of catechins.

The quantitative content of anthocyanins and antioxidant activity in Chkhaveri wine

Diagram 5



The antioxidant activity (the sample amount in milligrams, which inhibits the 50% DPPH) has also been identified in the *Chkhaveri* wine samples. The results are presented in the diagram and point to the fact that all the six samples of wine are characterized by high antioxidant activity, in particular, the *Chkhaveri* wine is distinguished by a relatively high antioxidant activity - 18,1 mg in Jalabashvili (780 m above the sea level), while in Kobuleti territory (5 m above the sea level) it is relatively low - 33.3 mg (Diagram 5).

There is a correlation between the number of monomeric anthocyanins and the antioxidant activity. The antioxidant activity increases with an increase of anthocyanins.

Depending on the altitudes, the presence of common acidity, sugar, common phenols, monomeric anthocyanins and flavonols varies in *Chkhaveri* grapes. It is caused by the climatic conditions. Among the 6 analyzed samples, the grapes, collected in the high area above the sea level (780 m), are distinguished by the highest content of compounds.

The juice of *Chakveri* grape variety, unlike the grape skin, does not contain anthocyanins. Their number increases in the process of maceration in wine produced by the Imeretian technology. 5 days are the most optimal time of maceration. There has been determined a directly proportional correlation between antioxidant activity and monomeric anthocyanins.

3.2 Quantitative analysis of common phenols, catechins, flavonols and antioxidant activity of Tsolikouri, Tsitska, Klarjuli, Krakhuna and Kutatura grape varieties and wine.

The samples of *Tsitska, Klarjuri, Krakhuna* and *Kutatura* grapes, collected in Imereti (vil. Opcha) are high in common phenols (1748.98 mg / kg), catechins (1147.73 mg / kg) and flavonols (453.92 mg / kg). The samples, collected in Keda, are close to them in quantity: common phenols (1578.0 mg / kg), catechins (1006.0 mg / kg) and flavonols (420.8 mg / kg). Although these two areas belong to different regions, they are similar in climatic conditions and the both are located

at the same altitudes above the sea leve. The samples, taken in Kobuleti, are distinguished by a relatively high content of flavonols, what can be explained by the concentration of flavonols in grapes, which increases according to how much these compounds are exposed to sunlight. Despite the fact that according to technical and biochemical data, the samples of grapes from Samegrelo (Vedidkari) are not inferior to other samples, they are distinguished by a relatively low level of all types of phenolic compounds. It is interesting to note that the content of biologically active compounds in the samples of *Tsitska*, collected in Imereti (Opcha), is higher than in the samples of grapes, collected in Adjara. The ratio 3: 2: 1 between the quantitative content of common phenols, catechins and flavonols, respectively, is maintained in all samples. The concentration of catechins in grapes grown in wetter conditions is higher than in dry and sunny areas. It is observed in the *Tsitska* samples taken in Adjara. This is observed in the Tsitska samples collected in Adjara.

The samples of Imereti region are distinguished with high antioxidant activity, determined by the DPPH method. There has been established a correlation between the content of phenolic compounds and antioxidant activity (Table 10).

Common phenols, catechins, flavonols and antioxidant activity of Tsolikouri, Tsitska, Klarjuli, Krakhuna and Kutatura grape varieties

Table 10

Grape name	Common phenolic compounds based on gallic acid calculation mg / kg	Flavonoids based on Rutin calculation, mg / kg	Catechins based on (+)-Catechin calculation, mg / kg	Antioxidant activity mg 50% of the sample inhibition
G.1	1347,58	449,50	964,67	35
G.2	1578, 00	420,80	1006,7	33.3
G.3	1748.98	453.92	1147.73	30.1
G.4	1135,55	339,70	828,00	34.0
G.5	988,70	317,90	778,5	38.4
G.6	1137,0	340	827,7	34.2
G.7	1098,3	337,2	799,8	35.1

G.8	998,9	325,89	779,8	37.3
G.9	976,56	315,7	750,8	44.5
G.10	1582,68	540,0	1052,82	32.6
G.11	1410,0	481,5	1001,5	36.1
G.12	1280,56	420,0	918,43	39.1
G.13	1265,92	346,9	954,97	40.2
G.14	902,91	196,5	772.0	37.4

The *Tsitska* grape samples, collected in the regions of Adjara and Imereti, are distinguished by a high content of common phenols 1410.0 - 1582.0 mg / kg, therefore, their antioxidant potential is high as well - 30.1-44.5 mg.

The quantitative content of phenolic compounds and antioxidant activity have been determined in 14 samples of wine made from five white varieties of grapes (*Tsolikouri, Tsitskha, Klarjula, Krakhuna* and *Kutaturi*), grown in three regions of western Georgia (Adjara, Samegrelo, Imereti). In Adjara, the largest total amount of phenolic compounds is determined in two wines - W_1 (686.0 mg / kg) and W_2 (633.4 mg / kg), prepared from *Tsolikouri* grape variety. They are followed by *Tsitska* (W_3), *Klarjula* (W_4) and *Krakhuna* (W_5), respectively, 611.0, 488.88, 405.8 and 386.68 mg / kg. The number of catechins in all five varieties vary from 32.5 to 42.53 mg / l and flavonols - from 105 mg to 272 mg per liter.

It is noteworthy that the wine (W_1), made from *Tsolikouri* grapes from mountainous Adjara (the Keda municipality), contains more quantities of phenols (682 mg / l) and catechins (42.35 mg / l) than the wine (W_2), prepared from the same grape variety, collected in Kobuleti, - 633.4 and 37.96 mg / l, respectively, while the amount of flavonols in both wines is almost equal (220.2 and 220.8 mg / l, respectively). The sum of wine phenols (653.22 and 845.0 mg / l), catechins (44.85 and 45.25 mg / l) and flavonols (392 and 380 mg / l) in *Tsitska* wine (W_{14}) and *Tsolikouri* wine (W_{13}), made from grapes collected in Imereti (the Baghdadi municipality), exceeds the amount of phenolic compounds (633.4 and 611.0 mg / l, respectively), catechins (37.96 and 40.0 mg / l) and flavonols (220.8 and 272 mg / l) in the wines (W_2, W_3,), prepared from the same varieties of grapes, collected in Adjara (the Kobuleti municipality).

The total number of phenols in wines, prepared from *Tsolikouri* grapes, collected in six villages of the Martvili municipality of Samegrelo region (Table 3), varies by 476.6 mg / l (W_{12})

504.2 mg / l (W_9), catechins 45.25 mg / l (W_{12}) 38 l 96 mg / l (W_9), while flavonols are 145.9 mg / l (W_{12}) 162. 5 mg / l (W_9). In the samples taken from all three regions, the wines W_1, W_2, W_{13}, W_{14}, prepared from the Adjarian, Imeretian Tsolikouri and the Imeretian Tsitska, are distinguished by high antioxidant activity; the wine samples of 24.3; 25.2; 22.4; 22.3 mg of wine samples, respectively, are enough for 50% inhibition of DPPH radicals.

Phenolic compounds and antioxidant activity of Tsolikouri, Tsitska, Klarjula, Krakhuna and Kutatura wine

Table 11

№ wine	Common phenolic compounds based on gallic acid calculation, mg/m	Catechins (+)- based on catechin calculation, mg/l	Flavonoids based on Rutin calculation, mg/l	Antioxidant activity (In - 50%) mg of a sample
W.1	686.0	42.35	220.2	24,3
W.2	633.4	37.96	220.8	25,2
W.3	611.0	40.0	272	27,6
W.4	488.88	33.81	170	29,1
W.5	405.8	32.5	109	30,1
W.6	386.68	35.78	105	28,05
W.7	499.7	37.8	161.3	26,5
W.8	488.7	35.5	151.9	30,2
W.9	504.2	38.96	162.5	26,3
W.10	497.8	36.9	159.7	27,2
W.11	490.5	36.4	155.6	28,1
W.12	476.6	33.0	145.9	37,1
W.13	845.0	45.25	380	22,4
W.14	653.22	44.85	392	22,3

The quantitative content of phenolic compounds in wines, prepared from white grape varieties common in other countries, is of particular interest. For example, the sum of phenols in 24 wines, prepared from different grape varieties, spread in the Czech Republic, varies from 292 mg to 858 mg per liter. Moreover, the total number of phenols in 8 white wines, produced in the Czech Republic, ranges from 90 mg to 166 mg (Stratil P., Kuban V ...), while their number in two white wines, produced in Greece, is 450 mg / l and 267 mg / l (Roussis G.I., ambropoulos I.L....).

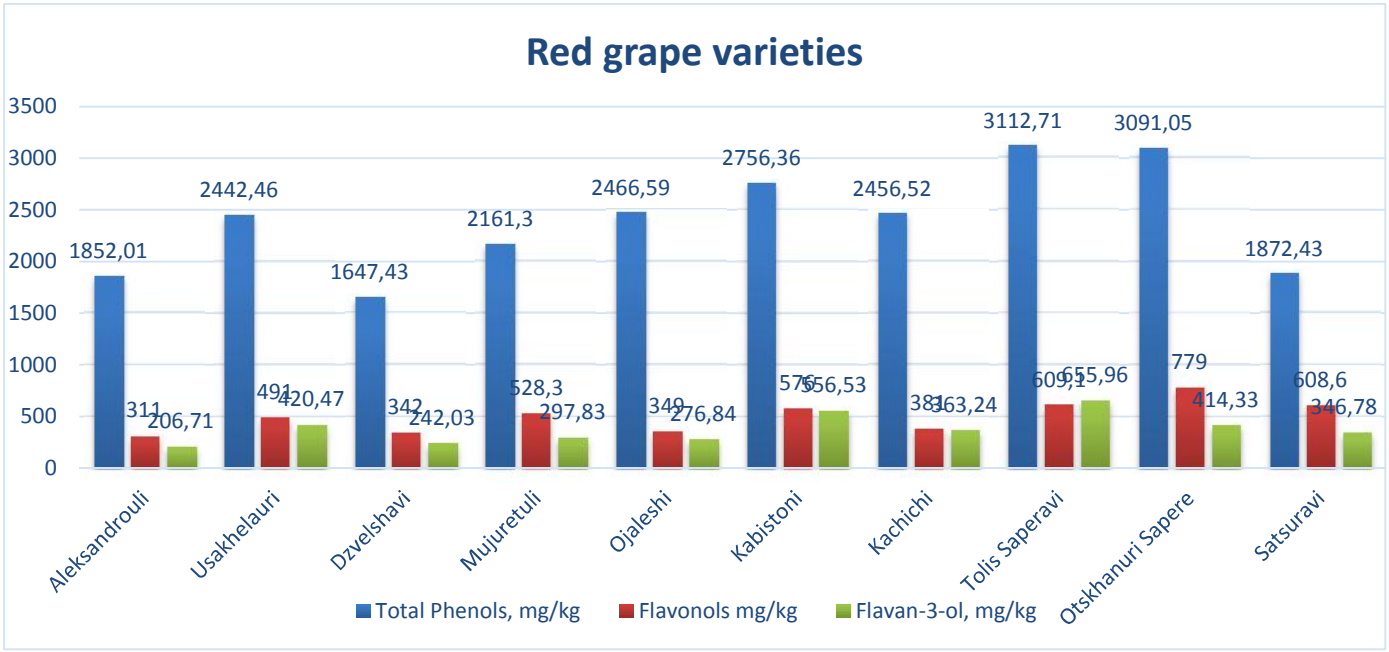
3.3. Quantitative content of common phenols, monomeric anthocyanins, catechins, flavonols and antioxidant activity in red grape variety and wine.

The phenolic compounds of five red grape varieties cultivated in different regions of Georgia, have been studied: *Aleksandrouli* (Racha), *Mujuretuli* (Racha), *Saperavi* (Kakheti), *Otskhanuri Sapere* (Imereti), *Ojaleshi* (Samegrelo). The quantitative content of common phenols, catechins and flavonols and antioxidant activity were determined in grapes.

The grapes taken for analysis (the harvest of 2015-2016), are characterized by high content of biologically active compounds, the presented samples differ in the content of phenolic compounds. In particular, the total phenol content is 1647,43 - 3112.71 mg / kg. The grape samples of *Tolis Sapere* (3112.71 mg / kg) and *Otskhanuri Sapere* (3091,05 mg / kg) are comparatively high in phenolic compounds. In the presented samples, the concentration of phenolic compounds in *Dzvelshavi* and *Satsuravi* varieties is relatively low - 1647,43-1872,43 mg / kg. Similarly to the phenolic compounds, flavonols are also represented in a similar ratio. Their content is 311,0 - 609,1 mg / kg, and the flavon-3s are within 206,71-655,96 mg / kg. The correlation between phenolic compounds and flavonols is expressed in 1: 5- 1: 6 ratio.

The Content of Common Phenols, Flavonols and Catechins in Red Grape Varieties

Diagram 6

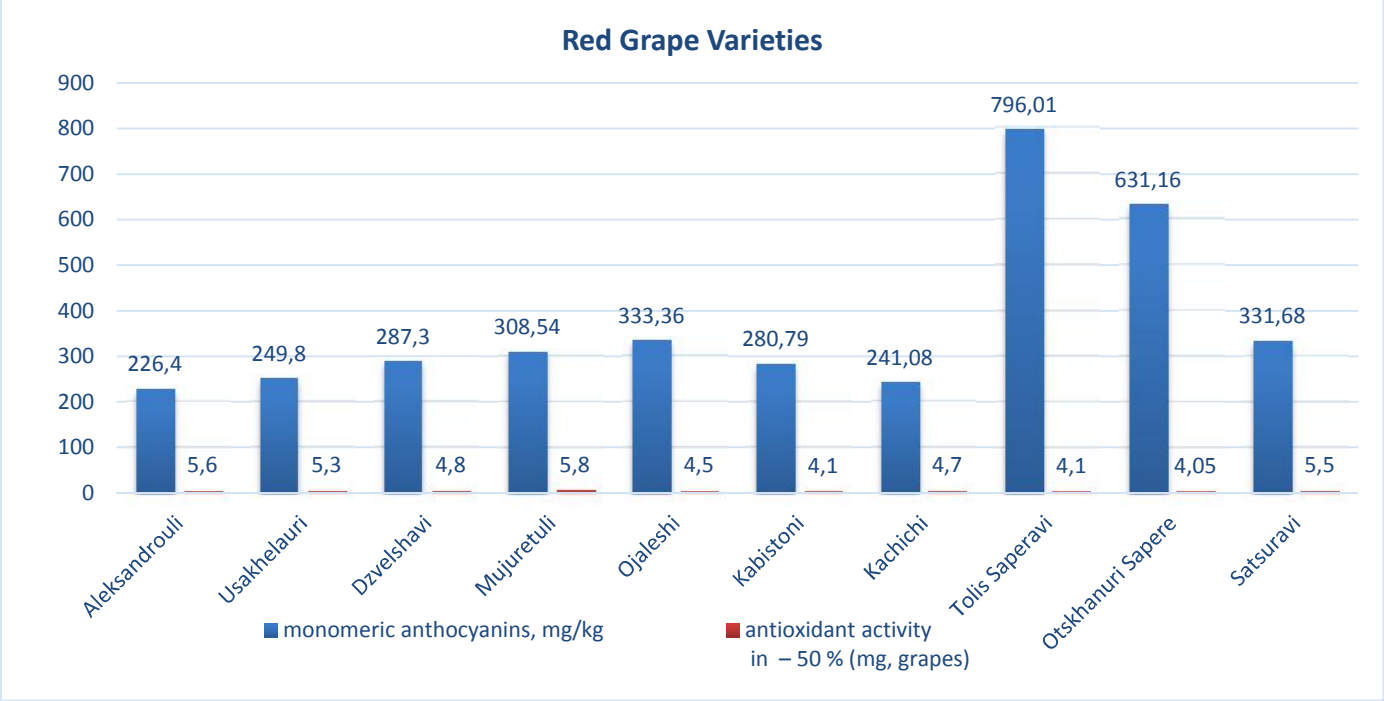


The anthocyanins are localized in grape grains in almost all red grape varieties – *Usakhelauri, Dzvelshavi, Kabistoni, Otskhanuri Sapre, Tolis Sapere, Aleksandrouli, Mujuretuli, Kachichi, Ojaleshi* and *Satsuravi*. In particular, most of them are in the area adjacent to the softness. Anthocyanins determine the colour of berries during the ripening period and the colour of wine after fermentation.

Different varieties of grapes have individual peculiarities of the formation and accumulation of anthocyanins. The content of monomeric anthocyanins is 796,01 mg / kg in *Tolis Sapere* grapes; it is followed by O*tskhanuri Sapere* - 631,16 mg / kg. The number of anthocyanins in *Ojaleshi* and *Satsuravi* is almost equal - 333,36 and 331,68 mg / kg (Diagram 7). Among the analyzed samples, a relatively low content of anthocyanins is in *Aleksandrouli* - 226.4 mg / kg, *Usakhelauri* - 249.8 mg / kg, *Dzvelshavi* - 287.3 mg / kg, *Mujuretuli* - 308.54 mg / kg and *Kachichi* - 241,08 mg / kg of per raw weight.

The content of monomeric anthocyanins and antioxidant activity
in grape varieties of red vine

Diagram 7



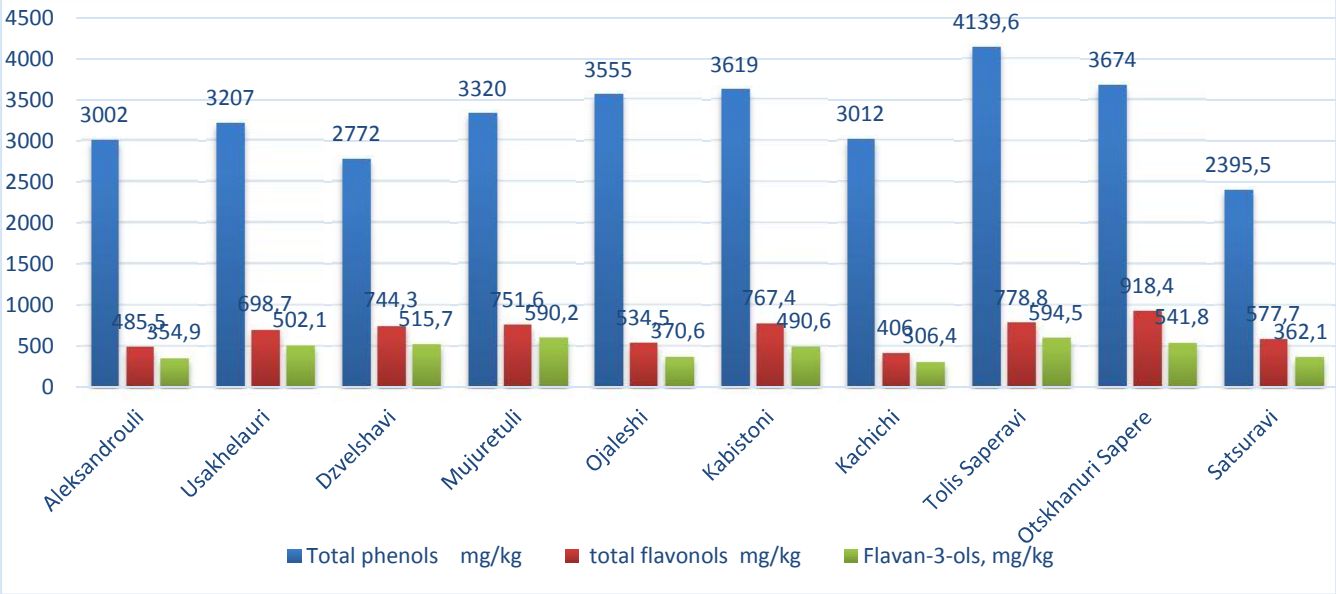
The extracts of analyzed grapes are characterized by high antioxidant activity. In order to inhibit 50% of the biologically active ingredients by a radical, it is necessary to dilute the extract 1g / 100ml in a ratio of 1: 2. According to the obtained results, *Tolis Sapere, Otskhanuri Sapere* and *Kabistoni* are distinguished by a high antioxidant activity – 4; 4; 4.1 mg, respectively. The antioxidant activity in the extracts of *Dzvelshavi, Ojaleshi* and *Kachichi* grapes is 4.8, 4.5 and 4.7 mg. The samples of *Aleksandrouli, Usakhelauri, Mujuretuli* and *Satsuravi* are characterized by a similar activity - 5.6, 5.3, 5.8 and 5.5 mg (Diagram 7).

There have been studied the wines, made from red grape varieties (*Aleksandrouli, Usakhelauri, Dzvelshavi, Mujuretuli, Ojaleshi, Kabistoni, Kachichi, Tolis Sapere, Otskhanuri Sapere* and *Satsuravi*), grown in different places of the three regions of western Georgia - Adjara, Imereti and Samegrelo.

The content of the common phenolic compounds in the presented wine samples varies between 2395.5-4139.6 mg / l. The wine samples of *Tolis Sapere, Kabistoni* and *Otskhanuri Sapere* are characterized by a relatively high content - 4139.6 mg / l, 3619.0 mg / l and 3674.0 mg / l, respectively. Diagram 8).

The content of common phenols, catechins and flavonols in the wine of red grape varities

Diagram 8



The concentration of phenolic compounds in *Dzvelshavi* and *Satsuravi* is relatively low - 2772.0-2395.5 mg / l, respectively. Similarly to the phenolic compounds, the content of flavonols also varies between 406.0 - 918.4 mg / l, and the number of catechins is between 354.9- 594.5 mg / l (Diagram 8).

There is a directly proportional correlation between the quantitative content of antioxidant activity and monomeric anthocyanins (Table 20). The samples of unaged wine of the variety *Aleksandrouli* are characterized by high content of monomeric anthocyanins - *Aleksandrouli 1* (871.7 mg / l); they contain high antioxidant activity - 59.6%. In the samples of *Mujuretuli* wine, the total amount of monomeric anthocyanins is lower than in the other samples (327.1 mg / l) and, therefore, the antioxidant activity is lower as well - 36.4%.

The quantitative content of identified anthocyanins in wine samples is different and varies according to the variety and the wine age (Table 12). In the studied samples mavidine-3-glucoside is a dominant. At the same time, the samples of *Saperavi* wine are characterized by its highest number (264.05 mg / l), while the samples of *Mujureteli* and *Ojalesheli* grape varieties have its lowest content - 125,44 and 126,99 mg / l, respectively. After malvidine-3-glucoside, cyanidine-3-glucoside dominates in wine samples (89.36 mg / l in *Ojaleshi* wine), then comes petunidine-3-glucoside, which prevails in the wines made from the remaining varieties. A low content of peonidin-3-glucoside (12.95 mg / l) was recorded in samples of *Otskhanuri Sapere* wine; the lowest content of malvidine-3-O-acetylglucoside (2.48 mg / l) was observed in samples of *Mujuretuli.*

The results of the study have shown that the number of anthocyanins in wine, prepared from *Alexandrouli* grape variety, decreased after a year of aging: in 7.8 times - malvidine-3-O-acetylglucoside (from 144.27 mg / l to 18.40 mg / l), in 4.6 times - dolphinidine -3-glucoside (from 52.13 mg/l to 11.22 mg/l), in 2.7 times - malvidin-3-cumaryglucoside and peonidin-3-cumaryglucoside (from 19.96 mg / l to 7.44 mg / l and from 94.58 mg / l to 34.36 mg / l, respectively), in 2 times - petunidine-3-glucoside and peonidine-3-acetylglucoside (from 101.55 mg / l to 43.14 mg / l and from 15.78 mg / l to 7.03 mg / l, respectively), 51.4% - malvidine-3-glucoside (from 353.13 mg / l to 171.49 mg / l), 44% - cyanidine-3-glucoside (from 11.72 mg / l to 6.59 mg / l), 42% of peonidine-3-glucoside (from 47.68 mg / l to 27.48 mg / l), while the total number of anthocyanins decreased in 2.4 times (from 871.7 mg / L to 370 mg / l).

Content of anthocyanins in wine samples

Table 12

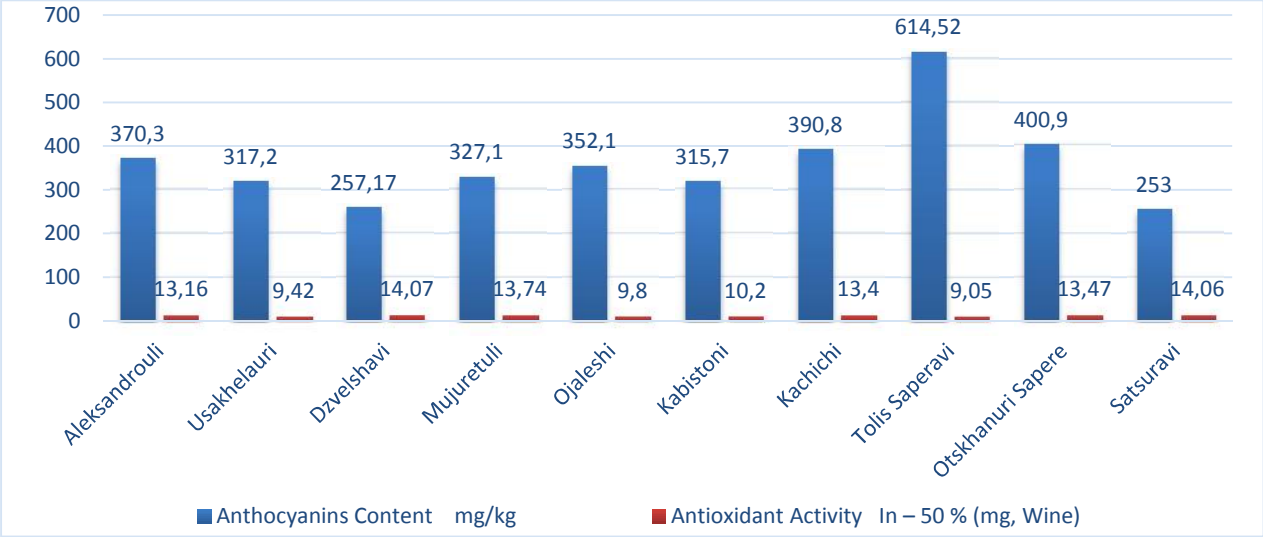
#	Anthocyanins, mg/l	Alexandrouli–1	Alexandrouli –2	Saperavi	Mujuretuli	Ojaleshi	Oskhanuri Sapere
1	dolphinidine -3-glucoside	52.13	11.22	47.75	13.57	41.30	34.60
2	cyanidine-3-glucoside	11.72	6.59	6.88	5.40	89.36	3.89
3	petunidine-3-glucoside	101,55	43.14	92.42	39,28	77.25	53.24
4	peonidine-3-glucoside	47.68	27.48	27.65	21.92	46.26	12.95
5	malvidine-3-glucoside	353.13	171.49	264.05	125.44	126.99	136.83
6	peonidin-3-acetylglucoside	15.78	7,03	33.06	13.74	19.69	4.69
7	malvidin-3-acetylglucoside	144.27	18.40	13.76	2.42	23.16	34.60
8	malvidin-3-cumaryglucoside	19.96	7.44	9.40	4.97	2.48	3.25
9	peonidin-3-cumaryglucoside	94.58	34.36	34.10	25.02	9.44	14.55
	Total	871.7	370.3	614.5	327.1	621	400.9

The total amount of the identified anthocyanins in the samples of *Saperavi* and *Ojaleshi* is almost two times higher than that of *Alesandrouli* and *Mujuretuli* samples.

The aging of *Aleksandrouli* wine throughout a year, along with a decrease of anthocyanins in the sample of Aleksandrouli 2, causes a decrease of antioxidant activity (from 59.6 to 38.0%).

Anthocyanins Content and Antioxidant Activity of Wine Samples

Diagram 9



The direct proportional correlation between quantitative content of anthocyanins and antioxidant activity has been revealed. The amount of monomeric anthocyanins and the antioxidant activity decreases during a year of wine aging.

CONCLUSIONS

1. The phenolic compounds of wine and 16 autochthonous grape varieties of the western Georgia have been studied.
2. There have been identified 9 anthocyanins in the wine, produced from the grape varieties (*Aleksandrouli, Mujuretuli, Saperavi, Otskhanuri Sapere* and *Ojaleshi*) spread in the winemaking regions of Georgia: Dolphinidine-3- O-Glucoside, Cyanidin-3- O-Glucoside, Petunidine-3- O-Glucoside, Peonidine-3 O-glucoside, Malvidine-3-O-glucoside, Peonidine-3-O-Acetylglucoside, Malvidine-3-O-Acetylglucoside, Peonidin-3-O-Coumaryglucoside and Malvidine-3-O-Coumaryglucoside. Malvidine-3-O-glucoside quantitatively dominates in all samples.
3. 5 flavonols have been identified in wine of white grape varieties of *Tsolikouri, Tsitska, Klarjula, Krakhuna* and *Kutatura* spread in Adjara, Imereti and Samegrelo: (-) - epicatechin, procyanidin B2, quercetin-3-0-glucoside, quercetin-3-0-rhamnoside and quercetin-3-0-glucuronide.
4. Among the grapes grown at different altitudes from the sea level, the maximum amount of common phenols, catechins, flavonols and monomeric anthocyanins has been fixed in *Chakveri* grape variety, grown at the altitude of 780 m above the sea level (the Jalabashvilebi village), while their minimum amount is fixed at the altitude of 5 meters above the sea level (Kobuleti). The wine, prepared from *Chkhaveri* grape variety, cultivated at the altitude of 780 m above the sea level, is distinguished by a high antioxidant activity; the maximum accumulation of common phenols, catechins, flavonols and monomeric anthocyanins in *Chakveri* grape samples, harvested in 2014-2016, was in 2016, which was distinguished for a long period of vegetation; comparing the monomeric anthocyanins of *Chkhaveri* grape berries, juice and wine, there has been found that they are much more in wine than in grape berries; the juice does not contain them at all, while common phenols, catechins and flavonols prevail in berries.
5. Among the white grape varieties (*Tsolikouri, Tsitska, Klarjula, Krakhuna and Kutatura),* spread in Adjara, Imereti and Samegrelo, *Tsolikouri* grapes, grown in Imereti (Opcha), are distinguished in phenolic compounds, where the total number of phenols, catechins and flavonols is 1748.98, 1147.73 and 453.92 mg / kg, respectively, while the antioxidant activity

is 30.1 units. The wines, prepared from *Tsolikouri* and *Tsitska* grape varieties, are distinguished by the composition of phenolic compounds and the antioxidant activity.

6. Among the red vine grape varieties (*Aleksandrouli, Usakhelauri, Dzvelshavi, Mujuretuli, Ojaleshi, Kabistoni, Kachichi, Tolis Sapere, Otskhanuri Sapere* and *Satsuravi*), spread in Adjara, Samegrelo and Imereti, the maximum number of common phenols, catechins and flavonols is collected in *Tolis Sapere* - 3112.71, 655.96 and 609.1 mg / kg, respectively, while the minimum number is in *Dzvelshavi* - 1647.43, 420.47 and 491.0 mg / kg. *Tolis Sapere* is also distinguished by the content of common phenols, catechins and flavonols - 4139.6, 594.5 and 778.8 mg / l, respectively.
7. The amount of anthocyanins in red wine samples is different and varies according to the grape variety.
8. A correlational change in phenolic compounds between grape seed, grape juice and wine has been established.

The works published on the basis of Dissertation:

M. Kharadze, I. Japaridze, A.Kalandia, M. Vanidze. Anthocyanins and antioxidant activity of red wines made from endemic grape varieties. Annals of Agrarian Science, volume 16, Issue 2, Agricultural Universityof Georgia. Publishedby Elsevier B.V. Pp. 181-184 Elsevier B.V. June 2018;

M. Kharadze, I. Djaparidze, M. Vanidze, A. Kalandia, Chemical Composition and Antioxidants of 14 Varieties of White Grape spreadin Western Georgia. Global Journal of Current Research Vol. 6 No. 1, ISSN: 2320-2920, Online version available at: Pp. 31-35, 2018;

M. Kharadze; I. Djaparidze; A. Shalashvili, M.Vanidze, A. Kalandia, Phenolic Compounds and Antioxidant Properties of Some White Varieties of Grape Wines Spreadin Western Georgia, Bulletin of the Georgian National Academy of Sciences. Tbilisi 2018.

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M. Kharadze, I. Djaparidze, M. Vanidze, A. Kalandia. Antioxidant Activity of Grape Chkhaveri and Its Wine Cultivated in West Georgia (Adjaria). 2017.19th International Conference on Chemistry ICC: Dec 25-26, 2017 in Dubai, UAE.

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