საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 14, №4, 2020
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 14, no. 4, 2020

Biotechnology

Characterization of Phenolic Acids in Several Autochthonic Wines

Maia Kharadze*, Maia Vanidze*, Indira Djaparidze*, Givi Kalandia*, Merab Ardzenadze*, Aleko Kalandia*

*Batumi Shota Rustaveli State University, Batumi, Georgia

(Presented by Academy Member Giorgi Kvesitadze)

Phenolic acid content of some autochthonous grape varieties common in Western Georgia has been studied. Samples were collected from different grapes such as Ojaleshi, Aladasturi, Aleqsandrouli, Mujuretuli, Chkhaveri, Tsolikouri, Tsitska, and wines produced from them. Phenolic acids were analyzed using HPLC-UV (column C18, solvent 0.1% Formic acid , 0.1% Formic acid in Acetonitrile, gradient), UPLC-MS methods (Column BEN C18, 1.7 μm), C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg).); 12 phenolic acids were identified. The amount of phenolic acids varies in different grape varieties. Ojaleshi has the highest indicator of phenolic acids – 1.13 g kg^{-1}, while the lowest is in Tsolikouri–0.09 g kg^{-1}. It has also been determined that only 10% of the initial phenolic acids content is passed into the grape juice. Besides this, during the alcohol fermentation the amount of phenolic acids almost doubles. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Autochthonic, wine, phenolic acid, identification

Wine is a complex substance, obtained from grapes, which contains ethanol, organic acids, sugar, aromatic, polyphenolic compounds and others. Phenolic compounds are found in large quantities both in grapes and in wine. Considerable attention has recently been paid to the study of phenolic compounds, including phenolarboxylic acids, which determine the quality of wine (color, taste, etc.) and its therapeutic and prophylactic value (antioxidant, antitumor effect, etc.) [1-3]. Phenolic compounds control the oxidation-reducing processes in the human body [4-6]. They protect the body from the effects of free radicals of oxygen, enhance the protection of biological systems and protect against macromolecules such as carbohydrates, proteins, lipids, as well as the harmful effects of oxidative DNA processes [4].

The products and raw materials, containing flavonoids and phenolic acids, are widely used to prevent atherosclerosis and ischemic diseases [7-9]. Phenolic acids are medium-level metabolites of aromatic polyphenol compounds, widely spread in the kingdom of plants [10].

The content of phenolarboxylic acids changes during the process of fruit ripening and fermentation of grape juice [7,10]. Phenolcarbonic acid content also depends on such factors as grape varieties, maturity, climatic and geographical

conditions [8,9,11]. The accumulation of phenolic compounds during maturation, introduced as "phenolic maturity", is of a particular interest [9,12,13]. There are several analytical methods but we have focused on using Amberlite XAD-16 resin column chromatography and mass spectrometry analysis due to their reliability and cost effectiveness.

Georgian grapes and, respectively, wine, especially the one prepared according to the Kakheti method, are rich in phenolic acids, including phenol carbonic acids. The phenolic acids were identified in the presented varieties of grapes and their wines. Unfortunately, the information about these compounds in Georgian wines is very small, one exception is Kekelidze N., et al. [14] that discusses the wines produced in Eastern Georgia that differ greatly from the wines produced in Western Georgia due to the differences in temperature, and soil.

Some phenolic compounds of Georgian wine (Tsolikuri and Tsitska) [15] and their antioxidants (Alexandrouli, Mujuretuli, Saperavi, Otskhanuri Sapere, Ojaleshi) [16] have been studied, although there is still a lot to be studied.

The purpose of the article was to identify and quantify phenolic acids, contained in popular autochthonous grape varieties common in Western Georgia, such as Ojaleshi, Aladasturi, Aleksandrouli, Mujuretuli, Chkhaveri, Tsolikuri and Tsitska..

Materials and Methods

HPLC-UV Waters (Breeze, USA) HPLC system equipped with a model 1525 pump and a UV detectors (2489), preparation collector. The UPLC analyses were carried out using an UPLC system model Waters Acquity H MS-QDa and PDA detectors with electrospray ionization and ion trap analyzer. C_{18} reversed-phase column was used (100×2.1 mm, ACQUITE BEH C18, 1.7 μm). Methanol and ethyl acetate were used as a solvent and during extraction (Merc, Germany HPLC grade). For the preparation of mobile phases for chromatography water with 0.1% formic acid as **solvent A** and acetonitrile with 0.1% formic acid as **solvent B** were used (Merc, Germany HPLC grade). In order to filter the samples before chromatography C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg) was used. Amberlite XAD-16 resin (Sigma-Aldrich) was used for separating phenolic acids from other compounds in wine and grape juice.

At the first stage of wine sample preparation, the wine was concentrated from 100 to 90 mL in vacuum at a temperature of not more than 40^0C (before alcohol removal). The obtained wine concentrate was subjected to Sep-pak C18 filter. The Amberlite XAD-16 column was equilibrated with the mixtures of 4 mL methanol and 0.1% formic acid and 4 mL water and 0.1% formic acid, before adding 5 mL sample. Elution was performed with the mixture of water and 0.1% formic acid. Elution of phenolic acid was performed with 4 mL of ethyl acetate. The process allowed us to greatly reduce the presence of non-target compounds. Final fraction was concentrated and dissolved in 90-10 ratio A-B solvent mixture mentioned above.

The grape sample was extracted with ethanol. 10 grams of grape was placed in 90 mL of ethanol 30 min. The sample was subjected to ultrasonic mixing and filtration. The process was repeated once again and final filtrate was concentrated using vacuum evaporator. In order to obtain the phenolic compounds column chromatography with Amberlite XAD-16 was repeated.

Grape juice was obtained by pressing and centrifuging (subjected Sep-pak C18 preparation). Juice was filtered and as in previous two cases Amberlite XAD-16 Column was used for acquiring final samples.

2.0 μL of each sample was injected and analyzed at 30°C. The elution program at 0.20 mL/min was 10% B (0-2 min), 10-60% B (2-14 min), 60% B (14-16 min) followed by a 2 min wash with 100% B and a 5 min reequilibration step.

Table 1. Phenolic acid MS, PDA characterization

Compounds	MW	m/z	m/z	ʎ max
		-	+	nm
Caffeic acid	180	179	181	220,325
Caftaric acid	312	311	313	328
p-Coumaric acid	164	163	165	226,310
p-Coutaric acid	296	295	297	310
Ferulic acid	194	193	195	218,325
Fertaric acid	326	325	327	322
Gallic acid	170	169	171	217,272
Protocatechuic acid	154	153	155	218,260
Gentisic acid	154	153	155	213,332
Vanillic acid	168	167	169	219,320
Syringic acid	198	197	199	218,328
Chlorogenic acid	354	353	355	325

The detection wavelengths were set at 290 and 306nm. Electrospray ionization in positive and negative mode was used. Samplers temperature 10^0C; MS- scan 100- 1100 da; probe 600^0C; negative 0.8kV, Capilarity 1.5kV, C -20, 40V)

Results and Discussion

Using HPLC preparation column, making PDA chromatography of the obtained fractions UPLC-MS and comparing the results of the https://metlin.scripps.edu database as well as using literary data, it became possible to identify the following compounds:

Compound 1-m/z 179, [M-H]-, a fragment m/z 135 [M-H]- ʎ max 220,325; which correlates to Caffeic Acid MW 180 (Table 1, Fig. 1);

Compound 2-m/z 311, [M-H]-, fragment m/z 179 [M-H]- ʎ max 328; which correlates to Caftaric Acid MW 312 (Table 1, Fig. 2);

Compound 3-m/z 163, [M-H]-, fragment m/z 119 [M-H]- ʎ max 226, 310; which correlates to p-Coumaric acid MW 164 (Table 1, Fig. 3);

Compound 4 m/z 295, [M-H]-, fragment m/z 163 [M-H]- ʎ max 310, which correlates to is p-Coutaric acid, MW 296 (Table 1, Fig. 4);

Compound 5 m/z 193, [M-H]-, fragment m/z 179 [M-H]- ʎ max 218,325; which correlates to is Ferulic acid, MW 194 (Table 1, Fig. 5);

Compound 6 m/z 325, [M-H]-, fragment m/z 193 [M-H]- ʎ max 322; according to the obtained results the compound is Fertaric acid, MW 326 (Table 1, Fig. 6);

Compound 7 m/z 169, [M-H]-, fragment m/z 125 [M-H]- ʎ max 217,272; which correlates to Gallic acid, MW 170 (Table 1, Fig. 7);

Compound 8 m/z 153, [M-H]-, fragment m/z 109 [M-H]- ʎ max 218,260; which correlates to Protocatechuic acid MW 154 (Table 1, Fig. 8);

Compound 9 m/z 153, [M-H, ʎ max 213,332; which correlates to Gentistic acid MW 154 (Table 1, Fig. 8);

Compound 10 m/z 167, [M-H]-, fragment m/z 123 [M-H]- ʎ max 219,320; which correlates to Vanillic acid MW 168 (Table 1, Fig. 9);

Compound 11 m/z 197, [M-H]-, fragment m/z 153 [M-H]- ʎ max 218,328; which correlates to Syringic acid MW 198 (Table 1, Fig. 10);

Compound 12 m/z 353, [M-H], fragment m/z 191 [M-H]- ʎ max 325, which correlates to Chlorogenic acid MW 354 (Table 1, Fig. 11). Phenolic acids are unevenly distributed in different varieties, as well as in different parts of the grape

(juice, peel) (Table 2). Their content in the grapes of the Ojaleshi variety is higher (1.13 mg kg^{-1}), than in other varieties. Only a small part of them gets into the juice (up to about 10%), and most of them remain in the skin, pulp, and seeds, where during fermentation they enter the wine (up to 50%) and change the properties of the wine. Mujuretuli (0.75 mg kg^{-1}) is also distinguished by the high content of phenolic acid, while other varieties, such as Aleqsandrouli (0.51 mg kg^{-1}), Tsolikouri (0.49 mg kg^{-1}), Tsitska (0.47 mg kg^{-1}), Aladasturi (0.47 mg kg^{-1}) contain it in almost equal amounts; the content of these compounds in Chkhaveri is relatively low (0.37 mg kg^{-1}). As for Ojaleshi, the regularity of the transition of phenolic acids into grape juice remains.

Table 2. Phenolic acid content in grape berry, juice and wine

Samplers name	Berry	Juice	Wine
	mg kg^{-1}	mg L^{-1}	mg L^{-1}
Ojaleshi	1.13	0.13	0.45
Aleqsandrouli	0.51	0.08	0.16
Aladasturi	0.47	0.09	0.30
Mujuretuli	0.75	0.10	0.24
Chkhaveri	0.37	0.06	0.18
Tsolikouri	0.49	0.15	0.09
Tsitska	0.47	0.10	0.08

In all cases, about 10% of phenolic acids pass into juice, and in wine, their transition depends on the fermentation time, when phenolic acids pass from the grape skin to wine, due to an increase in the alcohol content in this area. The same reason causes the increase of phenolic acids' content in wine, that varies in different grape varieties: Aladasturi (0.30 mg kg^{-1}), Mujuretuli (0.24 mg kg^{-1}), Chkhaveri (0.18 mg kg^{-1}), Aleqsandrouli (0.16 mg kg^{-1}), some of the phenolic acids undergo oxidation, as a result of which in Tsolikouri and Tsitska, prepared using European technologies, in which the fermentation of wine proceeds without the addition of skin, pulp, and seeds, there is observed a decrease in the amount of phenolic acids: almost twice in Tsolikouri (0.15; 0.09 mg kg^{-1} in juice and in wine, respectively), and in Tsitska it decreases slightly (0.10; 0.08 mg kg^{-1} in juice and in wine, respectively).

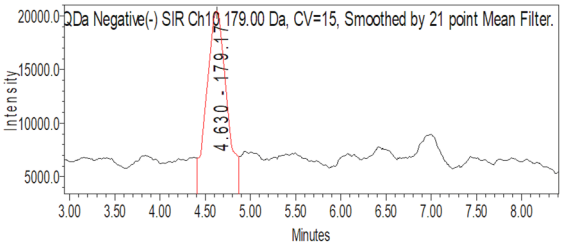

Fig. 1. Caffeic acid MW 180, m/z 179, [M-H]-

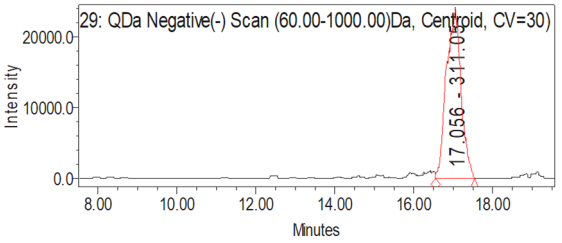

Fig. 2. Caftaric acid MW 312, m/z 311, [M-H]-

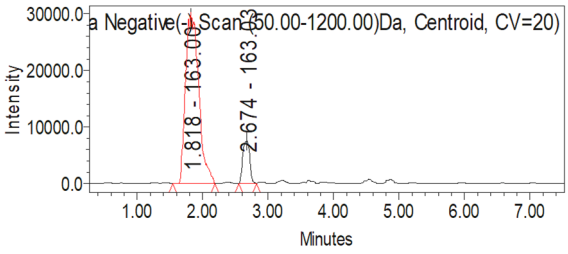

Fig. 3. p-Coumaric acid MW 164, m/z 163, [M-H]-

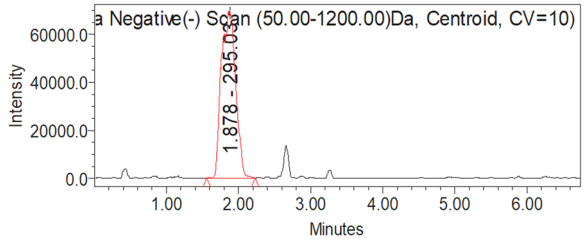

Fig. 4. p-Coutaric acid, MW 296, m/z 295

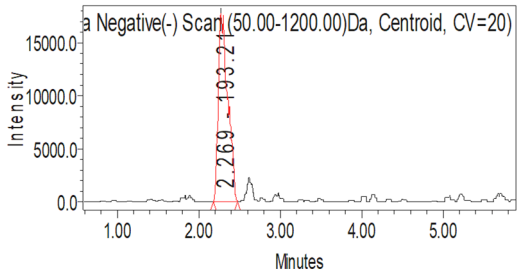

Fig. 5. Ferulic acid, MW 194, m/z 193, [M-H]-

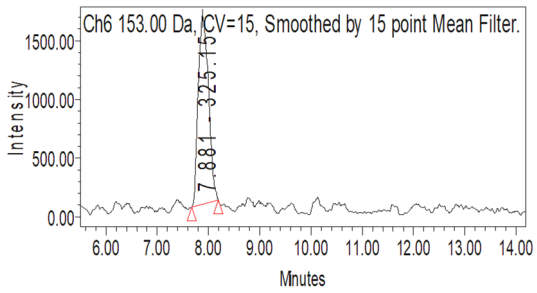

Fig. 6. Fertaric acid, MW 326, m/z 325, [M-H]-

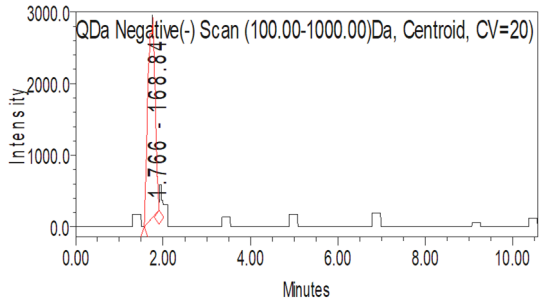

Fig. 7. Gallic acid, MW 170, m/z 169, [M-H]− m/z

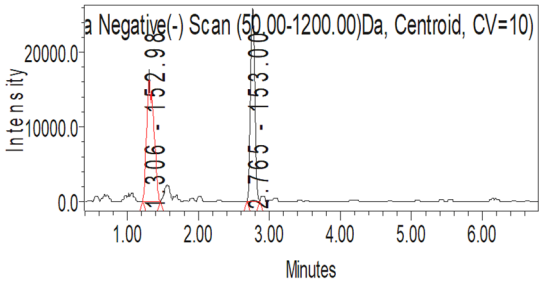

Fig. 8. Protocatechuic acid MW 154, m/z 153, [M-H]- Gentisic acid MW 154, m/z 153, [M-H]-

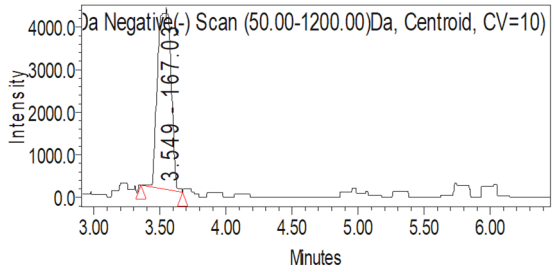

Fig. 9. Vanillic acid MW 168, m/z 167, [M-H]-

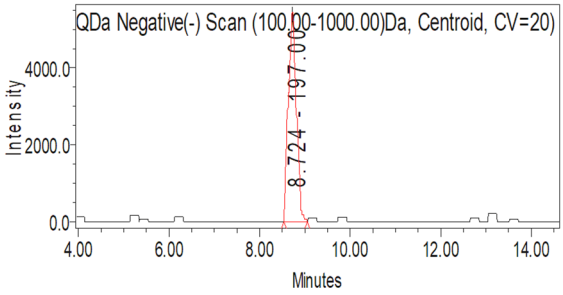

Fig. 10. Syringic acid MW 198, m/z 197, [M-H]-

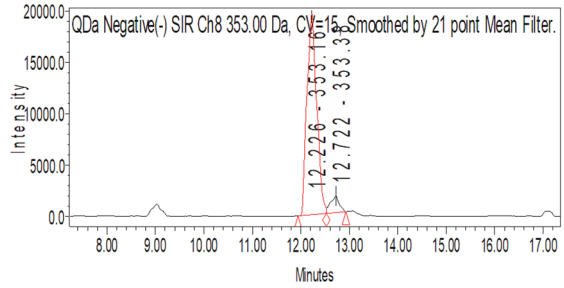

Fig. 11. Chlorogenic acid MW 192, UPLC/ESI-MS (−) m/z 191, [M-H]-

Conclusions

Using the UPLC –PDA, MS methods, 12 phenol acids (Caffeic acid, Caftaric acid, p-Coumaric acid, p-Coutaric acid, Ferulic acid, Fertaric acid, Gallic acid, Protocatechuic acid, Gentisic acid, Vanillic acid, Syringic acid and Chlorogenic acid) have been identified in 7 autochtonic wine samples of Ojaleshi, Aladasturi, Aleqsandrouli, Mujuretuli, Chkhaveri, Tsolikouri and Tsitska grape varieties. The phenol acids content was between 0.37-1.13mg kg^{-1} in grape, 0.08-0.15mg L^{-1} in juice and 0.1-

0.45mg L^{-1} in wine samples. Their content in the grapes of the Ojaleshi variety is higher (1.13mg kg^{-1}), than in other varieties. Only a small part of them gets into the juice (up to about 10%), and most of them remain in the chacha, where during fermentation they enter the wine (up to 50%) and change the properties of the wine. The lowest phenol acids content was found in Chkhaveri samples (0.37mg kg^{-1} in grape, 0.06mg L^{-1} in juice and 0.18mg L^{-1} in wine). The content of the other samples was almost equal.

The above project was fulfilled by financial support of the Georgian National Science Foundation (Grant 216816, Grant AP/96/13). Any idea in this publication is possessed by the authors and may not represent the opinion of the Georgian National Science Foundation.

ბიოტექნოლოგია

ზოგიერთი ქართული ავტოქტონური ღვინის ფენოლური მჟავების დახასიათება

მ. ხარაძე*, მ. ვანიძე*, ი. ჯაფარიძე*, გ. კალანდია*, მ. არძენაძე*, ა. კალანდია*

*ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტი, ბათუმი, საქართველო

(წარმოდგენილია აკადემიის წევრის გ. კვესიტაძის მიერ)

შესწავლილია დასავლეთ საქართველოში გავრცელებული ზოგიერთი ავტოქტონური ყურძნის ჯიში ოჯალეში, ალექსანდროული, მუჯურეთული, ალადასტური, ჩხავერი, ციცქა, ცოლიკოური და მისგან წარმოებული ღვინის ფენოლური მჟავები HPLC-UV (სვეტი C18, გამხსნელი 0,1% ჭიანჭველმჟავა (A), 0,1% ჭიანჭველმჟავა აცეტონიტრილში, გრადიენტი), UPLC-PDA, MS მეთოდების გამოყენებით (სვეტი BEN C18, 1.7μm), ნიმუშის კონცენტრირება C18 მშრალი ფაზით ექსტრაქციის კარტრიჯით (SPE) Waters Sep-Pak C18 (500mg), ელუირება აცეტონიტრილი 0,1% ჭიანჭველმჟავა, 0,1% ჭიანჭველმჟავა (გრადიენტი); ღვინოებში იდენტიფიცირებული იქნა 12 ფენოლური მჟავა, სხვადასხვა ჯიშის ყურძენში ფენოლურ მჟავათა რაოდენობა განსხვავებულია, ყველაზე მაღალი ოჯალეშია 1,13გ $კგ^{-1}$, ხოლო დაბალი ცოლიკოურში 0,09გ $კგ^{-1}$. ყურძნის წვენში გადადის ფენოლური მჟავების მხოლოდ 10%-მდე. სპირტული დუღილის დროს მათი რაოდენობა თითქმის ორმაგდება.

REFERENCES

1. Abu-Amsha, R., Croft, K.D., Puddey, B., Proudfoot, J.M. and BeilinN, J. (1996) Phenolic content of various beverages determines the extent of inhibiton of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine, *Clinical Science*, 91: 449–458.
2. Frankel, E.N.,Waterhouse, A.L. and Teissedre, P.L. (1995) Principal phenolic phytochemicals in selected California wines and their antioxidant activity inhibiting oxidation of human low-density lipoproteins, *Journal of Agricultural and Food Chemistry*, 43: 890–894.
3. Jayaprakasha, G.H., Singh, R.P. and Sakariah, K.K. (2001) Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro, Food Chemistry, Vol. 73, p.285–290.
4. Gonzalez-Paramas, A.M.; Esteban-Ruano, S.; Santos-Buelaga, C.; De Pascual-Teresa, S. Rivas-Gonzalo, J.C. (2004) Flavanol content and antioxidant activity in winery byproducts. *J. Agric. Food Chem*, Vol.52, p. 234-238.
5. Guendez, R.; Kallithraka, S.; Makris, D.P.; Kefalas, P.(2005) Determination of low molecular weight polyphenolic constituents in grape (vitis vinifera sp.) seed extracts: correlation with antiradical activity. *Food Chem.* Vol. 89, p.1-9.
6. Monagas, M.; Bartolome, B.; Gomez-Cordoves, C(2005) Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci. Nutr.* Vol, 45, p. 85-118.
7. Ramos, R., Andrade, P. B., Seabra, R. M., Pereira, C., Ferreira, M. A., & Faia, M. A. (1999). A preliminary study of non-coloured phenolics in wines of varietal white grapes (codega, gouveio and malvasia fina); effects of grape variety, grape maturation and technology of winemaking. *Food Chemistry*, Vol 67,p. 39–44
8. Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L(2000) Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* , Vol.*55*, p.77-85.
9. Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L(2002) Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Viticult.* Vol. 53,p. 268-278.
10. Rong-Rong Tian, Qiu-Hong Pan, Ji-Cheng Zhan, Jing-Ming Li, Si-Bao Wan, Qing-Hua Zhang and Wei-Dong Huang(2009) Comparison of Phenolic Acids and Flavan-3-ols During Wine Fermentation of Grapes with Different Harvest Times *Molecules* Vol. 14, p.827-838; doi:10.3390/molecules14020827.
11. Dokoozlian, N.K.; Kliewer, W.M.(1996) Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hortic. Sci.* Vol. 121, p. 869-874.
12. Bocco, A., Cuvelier, M.E., Richard, H. and Berset, C. (1998) Antioxidant activity and polyphenolic composition of citrus peel and seed extracts, Joural of Agricultural and Food Chemistry, Vol.46,p. 2123–2129.
13. Pérez-Magariño, S.; González-San José, M. L.(2006) Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chem.*,Vol. 96,p. 197-208
14. Kekelidze N, Kekelidze T, Akhalbedashvili L, et al.(2018) The content of antioxidants - Phenolic compounds, in red wines of Georgia “Kindzmarauli” and “Saperavi”. Appl Food Sci J. Vol.2(2):p.18-22.
15. Kharadze Maia, Indira Djaparidze, Armaz Shalashvili, Maia Vanidze, Aleko Kalandia (2018). Phenolic Compounds and Antioxidant Properties of some White Varieties of Grape Wines Spread in Western Georgia. *Bull. of Georg. Natl. Acad. Scie.,* vol. 12, no.3.
16. Kharadze M., I. Japaridze, A. Kalandia, M. Vanidze (2018). Anthocyanins and antioxidant activity of red wines made from endemic grape varieties. Annals of Agrarian Science 16 181–184.

Received July, 2019