Химия растительного сырья. 2024. №3. С. 138–143.
Khimiya rastitel'nogo syr'ya, 2024, no. 3, pp. 138–143.
DOI: 10.14258/jcprm.20240314482

UDC 615.322: 547.972+543.544

THE *VITIS LABRUSCA* (FOX GRAPE) FAMILY'S REDGRAPE SEED EXTRACTS AND LIQUID CONCENTRATES

© ***T.N. Gvinianidze***

Akaki Tsereteli State University, Tamar Mepe Street, 59, Kutaisi, 4600, Georgia, Temur.ghvinianidze@atsu.edu.ge

The skin and seeds of red grapes varieties of the Vitis labrusca (Fox Grape) family are environmentally friendly raw materials for the production of strong antioxidant, polyphenolic concentrates, because no pesticides and various chemical agents are used in their cultivation.

The aim and objectives of our scientific studies were to determine the uvological characteristics of red grapes raw materials of the Vitis labrusca (Fox Grape) family's vine varieties "Zeibel 5455", "Ojaleshi" and "Jvarisula" growing in the viticulture-winemaking zone of Imereti (Georgia) and study of phenolic compounds, flavonoids, catechins, leucoanthocyanins, cations of mineral substances and antioxidant activity of liquid hydrophilic concentrates containing 65–67% dry matter.

We used modern methods of gravimetric, extraction, spectral and chromatographic research.

Scientific studies have shown that all three grape-seed concentrates contain large amounts of phenolic compounds, potassium, calcium and magnesium cations, and are characterized by high antioxidant activity (49.8% – Zeibel 5455 seed concentrate; 51.5% – Ojaleshi seed concentrate; and 50.2% – Jvarisula seed concentrate).

The research is of practical importance, because the developed strong antioxidant, polyphenolic grape seed concentrates are the best raw materials for the production of functional food supplements.

Keywords: antioxidant activity, DPPH, polyphenols, Vitis labrusca (Fox Grape).

For citing: Gvinianidze T.N. *Khimiya Rastitel'nogo Syr'ya*, 2024, no. 3, pp. 138–143. (in Russ.). DOI: 10.14258/jcprm.20240314482.

Introduction

Due to the high content of phenolic compounds and strong antioxidant activity, red grape seed extracts and concentrates have long been the field of intensive research of scientists from many countries around the world [1–5].

According to their chemical structure, phenolic compounds can be divided into flavonoids and non-flavonoids. 60–70% of phenolic compounds in grape seeds are in the seed, 30–35% in the skin, and 7–9% in the juice and pulp [2, 6, 7].

Grape seeds are rich in fatty acids, tocopherols, flavonoids, especially proanthocyanidin oligomeric complex [8, 9]. It is therefore necessary to create such technologies of grape seed extracts and concentrates, in which biologically active compounds of seeds are represented in the maximum amount [10].

M. Hertog and his colleagues studied 12000 people in 7 countries over a 25-year period and found that people regularly taking bioflavonoid seed extracts had 25% less cardiovascular and oncological diseases [11].

Our area of interest was the study of uvological characteristics of red grape clones and hybrids of the *Vitis labrusca* (Fox Grape) family growing in private vineyards in the Baghdati (Georgia) viticulture microzone, phenolic compounds and antioxidant activity of grape-seed extracts and liquid concentrates.

The raw materials of red grapes of the *Vitis labrusca* (Fox Grape) family is rarely used for the production of natural and special wines. Moreover, in the process of their cultivation, different types of pesticides and chemical plant protection products are not used, and therefore they are the best raw material for the production of environmentally friendly juices and strong antioxidant, polyphenolic extracts and concentrates [12].

Studies of Georgian and foreign scientists have found that the raw materials of red grapes of clones and hybrids contain relatively higher amounts of both mono and diglycoside forms of anthocyanins than the raw materials of industrial varieties of redgrapes [13–15]. The high content of phenolic compounds increases immunity of clones and hybrids vines, and therefore these varieties do not actually get diseases that are typical for industrial grape varieties.

Experimental part

The study tested the raw materials of red grapes of the *Vitis labrusca* (Fox Grape) vine varieties "Zeibel 5455", "Ojalesh" and "Jvarisula" growing in private vineyards in the Baghdati (Georgia) viticulture microzone in Imereti region (Georgia).

Samples of the raw materials of "Zeibel 5455" grape variety selected for the experiment were taken on September 17, 2022, when sugar content was 22.6% and titratable acidity was 6.85 g/l. Samples of the raw materials of "Ojaleshi" grape variety were taken on September 22, 2022, when the sugar content was 21.5% and the titratable acidity was 6.57 g/l, and samples of the raw materials of "Jvarisula" grape variety were also taken on September 22, 2022, when sugar content was 21.2% and titratable acidity was 6.62 g/l.

The raw materials of red grapes were passed through the destemmer-crusher of Baby INOX company (manufacturer – Fratelli Marchisio, Italy), while the crushed and de-stemmed pomace was passed through the Atollo company's hydraulic press (manufacturer – Fratelli Marchisio, Italy). The newly pressed or the so-called sweet crush containing 36–43% dry matter is supplied to a Quincy Lab 20GC-type convection oven at no more than 36–43 °C for drying to reach 7–10% moisture content. We separated the skin and seed of the dried crush in the laboratory using the special-purpose (N4, N3 and N2) sieves. The average yield of the dried seed is 16–21% of the amount of dried crush.

The seeds, dried to 7–10% moisture content, are delivered for crushing in the MM-10 laboratory micro-mill to an average of 0.1–0.2 mm fraction.

Determination of water and dry substances in the samples was carried out by standard thermogravimetric method (GOST 28561-90). The method is based on the principle that the moisture-containing material loses moisture under certain pressure and temperature conditions. We place 3–5 grams of the analysis sample in a weighing bottle previously brought to constant weight and dry it at a temperature of 100–105 °C in a cupboard drier until constant weight. To calculate moisture content and dry substances, we used the following formula:

$$X= [(m – m_1)/m]100\%,$$

where X – the percentage of water in the sample; m – initial mass of the sample; m_1 – the dried sample mass.

Determination of the total amount of phenolic compounds was carried out with Folin-Ciocalteu reagent by spectrophotometric method. 0.5 or 1 ml taken from the total volume of the analyzed filtered extract was placed in a 25 ml volumetric flask, added 5 ml of H_2O, 1 ml of Folin-Ciocalteu reagent, settled for 8 min at room temperature, then 10 ml of 7% Na_2CO_3 is added, the flask filled with H_2O, and settled for 2 h in the dark at room temperature. We determined the optical density at λ=750 nm. with a cuvette thickness of 1 cm. As a control, we take 1 ml of the appropriate extracting agent and go through the same process. Conversion of the data obtained from the determination was carried out on the calibration curve of gallic acid.

The total phenol content shall be calculated in accordance with the formula:

$$X = (D\cdot K\cdot V\cdot F)\cdot 1000/m,$$

where X – the total phenol content, mg/kg; D – optical density; K – gallic acid conversion factor; F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Quantification of total flavonoids was carried out with $AlCl_3$ reagent by spectral method – 1 ml of sample taken from the total volume of extract obtained from was placed into a 10 ml flask, then we added 5 ml of H_2O, 0.3 ml of 5% $NaNO_2$ was settled for 5 minutes, and then we added 0.3 ml of 10% $AlCl_3$ and settled for 6 minutes, then we added 2 ml of 1N NaOH- R and the determination was carried out at 510 nm. As a control, we took 1 ml of the appropriate extracting agent and then went through the same process.

Conversion of the data obtained from the determination was carried out on the rutin calibration curve. The total flavonoid content shall be calculated in accordance with the formula:

$$X = (D\cdot K\cdot V\cdot F)\cdot 1000/m,$$

where X – the total flavonoid content, mg/kg; D – optical density; K – rutin conversion factor; F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Quantification of catechins was carried out by spectral method. 1 ml taken from the total volume of extract is added with 3 ml of vanillin reagent and, 3 minutes later, we determine the optical density of red test sample at 500 nm [13]. As a control, we take 1 ml or 3 ml of vanillin reagent. Conversion of the data obtained from the determination was carried out on the (+)catechin calibration curve. The catechin content shall be calculated in accordance with the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000/m,$$

where X – the catechin content, mg/kg; D – optical density; K – 35.0 ((+) catechin conversion factor); F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Quantification of leucoanthocyanins was carried out by spectral method. 1 ml of alcohol solution taken from the total volume of extract is added with 1 ml of water so that the concentration of alcohol in the test sample does not exceed 50%. Then, 8 ml of leucoanthocyanidin reagent (25 ml of concentrated hydrochloric acid and 475 ml of butanol) are added. The mixture is mixed well and heated in a boiling water bath for 3 minutes, then the vessel is tightly closed and heated again for 40 minutes. After that, the solution is cooled under a water jet. The obtained dark pink solution is filled up to 10 ml with the leucoanthocyanidin reagent and the optical density of the sample is measured at 550 nm [13]. The control is the extract of the test sample and the leucoanthocyanidin reagent without heating.

Conversion of the data obtained from the determination shall be carried out on the cyanidin calibration curve. The catechin content shall be calculated in accordance with the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000/m,$$

where X – the leucoanthocyanin content, mg/kg; D – optical density; K – 85.0 (cyanidin conversion factor); F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Antioxidant activity was determined by DPPH method, which is a DPPH free radical colorimetry with 50%-radical inhibition. DPPH is a rapid, simple and accurate test method for determining antioxidant activity.

DPPH – ($C_{18}H_{12}N_5O_6$ M=394.33) is a stable free radical with maximum absorption at 515–517 nm, and purple-violet coloration of its methanol extracts changes to bright yellow as a result of the recovery [16–18].

To determine the antioxidant activity or radical binding activity of the sample, we added 3 ml of DPPH alcohol solution to 1 ml of the test extract (0.1 mM DPPH – 0.004 g/100 ml of ethyl alcohol) and after 30 minutes, we carried out spectrophotometric determination of the optical density of the test sample at 515 nm. DPPH solution is a control solution, while the 96%-ethyl alcohol is a background.

Inhibition of free radical (DPPH) activity shall be calculated by the following formula:

$$\text{In } \% = AC\text{-}AS/AC100,$$

where AC is the absorbance of the alcohol solution of DPPH, and AS is the absorbance of the test extract.

Determination of cations of mineral substances was carried out by high-performance liquid chromatography. Detector Waters 432 (Conductivity) Column IC-Pak Cation MD, Eluent 3 mM HNO_3/0.1 mM EDTA, Back conductivity 1250±50 μS, Base Sensitivity 2000 μS, Integrator Sensitivity μS, column temperature 35 °C, Polarity-negative.

Results and Discussion

We studied the uvological characteristics of the raw materials of red grapes of the *Vitis labrusca* (Fox Grape) family vine varieties "Zebel 5455", "Ojaleshi" and "Jvarisula" by the method of Professor Prostoserdov [19].

The results of the study are presented in Table 1.

The structural characteristic of a bunch of grapes is equal to the ratio of the mass of juice and flesh to the mass of the skeleton (the total mass of the stalk and the skin).

Studies have confirmed that the redgrape varieties with less structural index and more solid parts are distinguished by the content of phenolic compounds [20].

Table 1. Uvological characteristics of the selected grape varieties

Grape bunch components	Grape bunch mechanical composition, %		
	Zeibel 5455	Ojaleshi	Jvarisula
Juice and flesh	79.85	79.28	80.45
Grape stalk	4.45	4.36	4.62
Grape skin	11.38	11.9	10.75
Grape seed	4.32	4.46	4.18
Skeleton (stalk and skin together)	15.83	16.26	15.37
The sum of solid components	20.15	20.72	19.55
Structural characteristic	5.04	4.47	5.23
Date of taking samples	17.09.2022	22.09.2022	22.09.2022

A study of the uvological characteristics of grape bunches of *Vitis labrusca* (Fox Grape) family vines showed that "Ojaleshi" has relatively lower structural characteristic, and the sum of solid components (stalk, skin and seed) of a bunch of grapes is higher in "Ojaleshi" compared to other varieties.

Studies have confirmed that the solid parts of grapes contain water-soluble and alcohol-soluble phenolic compounds [21–24]. It is not coincidental therefore that the phenolic complex extraction from the grape seed crushed to 0.1–0.2 mm fraction was carried out with the water-alcohol extracting agents of different concentrations in two stages, at the initial (first) stage – using an extracting agent with a higher-concentration, and at the next (second) stage – using an extracting agent with a lower-concentration.

At the first stage (*seed I – extract*): we extracted seed powders of *Vitis labrusca* (Fox Grape) family vines crushed to 0.1–0.2 mm fraction with a 40–43% ethyl alcohol hydrophilic extracting agent. We diluted ethyl alcohol to 40–43% with "Borjomi" acidic-mineral drinking water, the pH of which is 3.6–6.3, and the mineralization varies within 7–14 g/dm^3.

This mineral water contains sodium (as well as potassium) bicarbonate and boric acid. Peliminary experiments suggest that ethyl alcohol as an extracting agent mixed with mineral water can successfully replace the extracting agent diluted with water containing the 40–43% alcohol, which is acidified with hydrochloric acid.

At the second stage (*seed II – extract*): we extracted the remaining cake again with drinking water acidified with 2% citric acid.

We have experimentally determined that the extraction of polyphenols from seed powders is relatively effective a water-alcohol extracting agent with a low-concentration (Fig.).

We determined the optimal extraction parameters experimentally (Table 2).

We collected the I and II stage extracts according to the vine varieties of the *Vitis labrusca* (Fox Grape) family, cooled to 7–10 °C, settled at a specified temperature for 7–9 hours, removed from the sediment and filtered using a MINI 6–20x20 wine filter (manufacturer: Fratelli Marchisio – Italy).

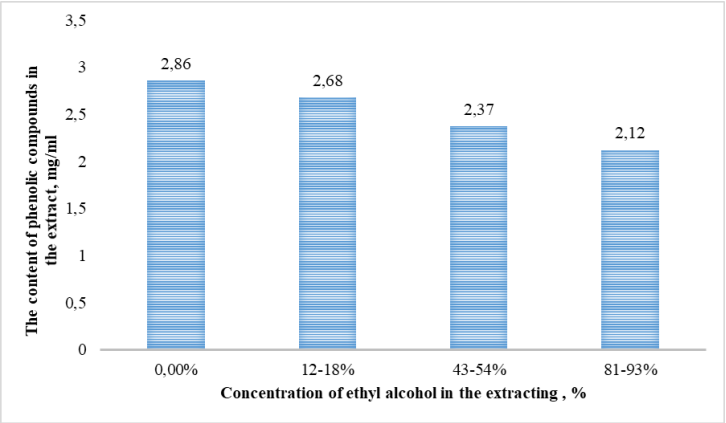
An overview of processes for concentrating the composition of extracts. The filtered skin and seed extracts contained up to 3.4–3.6% of dry substances on average, so we carried out their concentration in two stages: - at the initial stage, on a vacuum-rotary thickener of Chinese production (R-1010 10L vacuum rotary evaporator concentrator with additional chiller and vacuum pump) up to 27–30% dry matter content, and at the second stage, we concentrated the extract thickened on a rotary thickener to 65–67% dry matter content on a Chinese-made TOPT-10A-type lyophilic dryer intended for laboratory research.

We pumped the compositions of concentrated liquid hydrophilic extracts of seeds into enameled collectors from which we took the test samples for the content of biologically active compounds and antioxidant activity (Table 3).

There are many methods available for evaluating the antioxidant activity of plant extracts: free radical binding (DPPH), ability to absorb oxygen radicals (ORAC), antioxidant ability to restore iron (FRAP), etc. [16–18, 25, 26].

One of the widespread methods is the free radical colorimetry with 50% inhibition of the radical or DPPH method. It is a relatively simple, fast and accurate test method.

The amount of bioflavonoids in the extracts and concentrates of seeds and skins in some cases does not determine the amount of antioxidant activity of the extracts and concentrates and depends on the qualitative composition of flavonoids.



The influence of the concentration of ethyl alcohol in the extracting agent on the content of phenolic compounds in the grape seed extracts

Table 2. Optimum parameters of the grape seed extraction process

Characteristics	Grape seed	
Crushing quality, mm	0.1–0.2	0.1–0.2
Extraction stages	I	II
pH	3.8–5.6	4.1–6.2
Optimal parameters of the extraction process		
Concentration of ethyl alcohol in an extracting agent, vol.%	40–43	0.00
The hydro-module (sample : extracting agent), kg/l	1 : 5	1 : 3
Extraction temperature, °C	54–57	54–57
Extraction time, hr.	4.5	4.0
Pulsation frequency	4 sec^{-1}	4 sec^{-1}
Pulsation amplitude, mm	2–3	2–3
Processing of extracts	Collecting the I and II extracts, cooling up to 7–10 °C, settling for 7–9 hours, removing from the sediment and filtration	

Table 3. Biologically active compounds and antioxidant activity of grape seed composition

Composition of hydrophilic extracts	Biologically active compounds, mg/100 g on dry weight basis				AOA, % (F=100), In, %
	Phenolic compounds	Flavonoids	Flavan-3-ols	Leukoanthocyanins	
Zeibel 5455	2628.75	833.44	1243.9	378.5	49.8
Ojaleshi	2845.16	955.30	1579.4	397.4	51.5
Jvarisula	2523.28	687.65	1193.6	394.2	50.2

Conclusion

The studied environmentally clean varieties of red grapes are the best raw materials for the production of strong antioxidant, polyphenolic concentrates, because all three studied hydrophilic liquid grape seed concentrates contain a large amount of phenolic compounds, potassium, calcium and magnesium cations, and all three concentrates are characterized by high antioxidant activity ("Zeibel 5455" seed concentrate – 49.8%; "Ojaleshi" seed concentrate – 51.5%; "Jvarisula" seed concentrate – 50.2%).

Funding
This work was supported funding Akaki Tsereteli State University. No additional grants to carry out or direct this particular research were obtained.

Conflict of Interest
The author of this work declares that he has no conflicts of interest.

References

1. Denev P., Lojek A., Ciz M., Kratchanova M. *Bulg. J. Agric. Sci.*, 2013, vol. 19, pp. 22–27.
2. Jayaprakasha G.K., Selvi T., Sakariah K.K. *Food Research International*, 2003, vol. 36(2), pp. 117–122.
3. Fraga C.G., Galleano M., Verstraeten S.V., Oteiza P.I. *Mol. asp. Med*., 2010, vol. 31, pp. 435–445.
4. Rajha H.N., Darra N., Vorobiev E., Louka N., Maroun R. *Food and Nutrition Sciences*, 2013, vol. 4(6), pp. 650–659.
5. Yamagata K., Tagami M., Yamori Y. *Nutrition*, 2015, vol. 31, pp. 28–37.
6. Gvinianidze T.N., Karchava M.S., Jabnidze R.H. *Open Access J. ARTOAJ*, 2018, vol. 16(2), pp. 56–63.
7. Romer J., Lund L.R., Dano K. *Nature Medicine*, 1997, vol. 3, pp. 1195–1196.
8. Kammerer D., Claus A., Carle R., Schieber A. *J. Agric. Food Chem*., 2004, vol. 52, no. 14, pp. 4360–4367.
9. Zharskaya O.M., Gorgun Yu.V., Karaseva G.A., Ulasevich D.N., Usov G.M. *Meditsinskie novosti*, 2014, vol. 4, pp. 16–20. (in Russ.).
10. Vuolo M.M., Lima V.S., Maróstica M.R. Jr. *J. Bioactive Compounds*, 2019, pp. 33–50.
11. Hertog M.G., Kromhout D., Aravanis C. et al. *Arch. Inter. Med*., 1995, vol. 155(4), pp. 381–386.
12. Gvinianidze T.N., Chikovani P.M., Gvinianidze T.T., Jabnidze R.H., Mindeli V.A. *Scientific Journal "Annals of Agrarian Science"*, 2017, vol. 15, pp. 472–475.
13. Durmishidze S., Khachidze O. *Chemical composition of grapes*. Publishing House "Science," Tbilisi, 1981, 189 p.
14. Nilov V.I., Skurikhin I.M. *Khimiya vinodeliya*. [Chemistry of winemaking]. Moscow, 1967, 450 p. (in Russ.).
15. Rubilar M., Pinelo M., Shene C., Sineiro J., Nunez M.J. *J. Agric. Food Chem*., 2007, vol. 55, pp. 10101–10109.
16. Assraoui K., Rochd T. *Journal of Biosciences and Medicines*, 2023, vol. 11, pp. 79–95.
17. Okawa M., Kinjo J., for Nohara T., Ono M. Biological and Pharmaceutical Bulletin, 2001, vol. 24, pp. 202–211.
18. Oszmiański J., Wojdyło A. *European Food Research and Technology*, 2005, vol. 221(6), pp. 809–813.
19. Prostoserdov N.N. *Izucheniye vinograda dlya opredeleniya yego ispol'zovaniya (Uvologiya)*. [The study of grapes to determine its use (Uvology)]. Moscow, 1963, 79 p. (in Russ.).
20. Gvinianidze T.N. *Wine-technology and technochemical control. Monograph*. Kutaisi, 2023, 450 p.
21. Pütz H. *Winemaking*. Tbilisi, 2020, 400 p.
22. Kopaliani R., Gvinianidze T., Jabnidze R. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*, 2019, vol. 4, pp. 93–104.
23. Rosch D., Bergman M., Knorr D., Kroh L. *J. Agric. Food Chem*., 2003, vol. 51, pp. 4233–4239.
24. Temur G., Teona G. *Open Acc. J. Envi. Soi. Sci*., 2018, vol. 1(4), pp. 83–87.
25. Huang D., Ou B., Prior R.L. *J. Agric. Food Chem*., 2005, vol. 23, no. 53(6), pp. 1841–1856.
26. Pellegrini N., Serafini M., Colombi B., Del Rio D., Salvatore S., Bianchi M., Brighenti F. *J. Nutr*., 2003, vol. 133(9), pp. 2812–2819.

Received December 20, 2023

Revised January 14, 2024

Accepted January 14, 2024

Information about author
Gvinianidze Temur Nikolaevich – Doctor of Technical Sciences, Professor, Department of Food Technology, Beverage Coordinator, Temur.ghvinianidze@atsu.edu.ge; temur1951@mail.ru