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Biochemistry

Study of the Phenolic Complex of Secondary Resources of Colored Grapes Grown in Georgia ("Zeibel 5455" and "Otskhanuri Sapere")

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(Presented by Academy Member Gia Khatisashvili)

Abstract. The study is devoted to the analysis of the phenolic composition and antioxidant activity of secondary raw materials (skins and seeds) of colored grapes ("Zeibel 5455" and "Otskhanuri Sapere" varieties) widely planted in Western Georgia. A high content of phenolic compounds was determined in the extracts obtained from the skins and the seeds of the "Otskhanuri Sapere" and "Zeibel 5455" varieties. The extract obtained from the skins of "Zeibel 5455" contains phenolic compounds – 4212.5 mg/100 g; the extract obtained from the seeds – 4577.5 mg/100 g; in the case of extracts obtained from "Otskhanuri Sapere" – 4156.9 mg/100 g and 4111.0 mg/100 g; in the anthocyanins of "Zeibel 5455", the diglucoside forms of phenolic compounds prevail, while in "Otskhanuri Sapere", the monoglucoside forms prevail. The study of the antioxidant activity of phenolic extracts revealed that the antioxidant activity of the skin extract of "Zeibel 5455" is 68%; and in the seed extract – 47%; the antioxidant activity of the skin extract of "Otskhanuri Sapere" is 42 in % (Inhibit 42% of the free DPPH radical); and antioxidant activity of the seed extract of „Otskhanuri sapere" is 41.5 in %; the exceptionally high antioxidant activity of the skins of "Zeibel 5455" is due to the abundance of diglucoside forms of phenolic compounds (malvidin 3-5-0 diglucoside, peonidin 3-5-0 diglucoside, etc.), which is why these extracts can be used as biologically active ingredients with antioxidant activity in food technology. © *2025 Bull. Georg. Natl. Acad. Sci.*

Keywords: colored grapes, phenolic compounds, diglucosides, anthocyanins, antioxidant activity

Introduction

The history of vine growing and winemaking spans 8 thousand years. The use of wine for medicinal and preventive purposes also has ancient history, due to the high content of biologically active substances in it, especially phenolic compounds, which have a unique ability to inhibit oxidative reactions (Artem et al., 2014; Ronkainen, 2016; Nel, 2018; Kumar & Pandey, 2013).

Environmental degradation, elevated radionuclide background, critically high levels of chemicalization of agriculture and the food industry, and many other constantly acting negative factors lead to the accumulation of free radicals and the

initiation of chain oxidative reactions in the body, and oxidative stress develops, and a whole cascade of pathologies begins, up to the formation of tumor cells, and early aging occurs.

It is known that the ability to prevent the above processes is possessed by substances that inhibit oxidative reactions – antioxidants. The advantage of natural, plant antioxidants over chemical preparations of similar action is also known, especially with their long-term use. In modern conditions, when enhanced oxidation is an almost constant accompanying process of life, the use of natural antioxidants is of particular importance due to their "mild" effect on the body without side effects.

Today, the ability of plant phenolic compounds (phenolic acids, flavonoids) to inhibit oxidative reactions is widely recognized, neutralize free radicals, chelate metals that catalyze oxidative processes, activate endogenous antioxidant enzymes, and inhibit peroxidative enzymes; they have anti-inflammatory, cardioprotective, and neuroprotective properties. In this regard, the raw material of colored grape varieties and wines derived from them is distinguished, which is a natural source of natural antioxidants for the body (Georgiev et al., 2014; Tyagi, 2013) .

The study is devoted to the analysis of the phenolic composition and antioxidant activity of secondary raw materials of colored grapes widely planted in Western Georgia in order to obtain a preventive agent for the development of oxidative stress, which can be used as a natural ingredient with antioxidant activity in food technology.

To this end, the study and use of the phenolic complex of secondary resources (skins and seeds) of colored grapes cultivated in Georgia is particularly relevant.

When processing the grape raw materials, approximately 20% remains unused as secondary resources. This "waste", especially the "waste" of colored grapes, is no less valuable in its composition than the grape raw materials due to the high content of phenolic substances known for their strong antioxidant activity (almost 80-90% of the phenolic compounds in grape raw materials are localized in the skin, seeds, and stem).

The research aims to investigate the phenolic complex of secondary resources (skins and seeds) of colored grapes cultivated in Georgia ("Zeibel 5455" and "Otskhanuri Sapere" varieties) with a view to creating a natural food ingredient with antioxidant activity.

Materials and Methods

The non-industrial variety of colored grapes cultivated in the viticulture and winemaking zones of Imereti ("Zeibel 5455") and the industrial variety ("Otskhanuri Sapere") were selected for the research. The grapes were harvested in the technical ripeness phase, when a high glucoacidometric (sugar-acid) index and a high phenolic ripeness index were recorded in the grape raw material, which was a guarantee of a high content of phenolic compounds in studied material ("Zeibel 5455" from September 26 to September 29, and "Otskhanuri Sapere" – from October 11 to October 16 in 2020-2022).

To obtain extracts containing phenolic compounds from selected colored grapes (skins and seeds), the raw material was processed, the juice and the must were separated, the freshly squeezed, destemmed must was dried, the dried must was separated and sieved, the skins and seeds were separated, and they were ground separately. We extracted phenolic compounds from the obtained micropowders under the following conditions: type of extractant – for the first extraction – 54% ethanol, – for the second extraction – 18% ethanol. Extraction temperature – for both the skins and the seeds – 54-57°C; hydromodulus – for the first extraction – 1:3; for the second extraction – 1:2; extraction duration – for both the skins and the seeds – 180 minutes (Lamparadze et al., 2018; Gvinianidze, 2018; Gvinianidze & Chikovani, 2017, 2018; Gvinianidze et al., 2017; Kharadze et al., 2018).

We performed identification and quantitative analysis of phenolic compounds in colored grape

skin and seed extracts by ultra-high performance liquid chromatography (UPLC) using photodiode array (PDA) and mass (MS) detectors (Surmanidze et al., 2024; Danak et al., 2022) (UPLC PDA MS method Waters, UPLC Acquity, QDa Detectore). Total phenols were determined spectrophotometrically using Folin-Ciocalteu reagent. Extraction of phenolic compounds from the raw material taken for analysis was carried out with 80% ethyl alcohol at a temperature of 70-75°C. 0.5 or 1 ml of the total extract volume was placed in a 25 ml volumetric flask, 5 ml of H_2O was added, 1 ml of Folin-Ciocalteu was left for 8 minutes at room temperature, then 10 ml of 7% Na_2CO_3 was added, the flask was filled with H_2O and left for 2 hours in the dark at room temperature. These parameters were determined on a spectrometer (Mettler Toledo uv 5 a) – at 750 nm.

Total flavonoids were quantified by the spectral method: the sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75°C. 1 ml of the total extract was placed in a 10 ml test tube, 5 ml of H_2O and 0.3 ml of 5% $NaNO_2$ solution were added, the mixture was left for 5 minutes, then 0.3 ml of 10% $AlCl_3$ solution was added, the mixture was left for 6 minutes, then 2 ml of 1N NaOH solution was added and the measurement was performed on a spectrometer at 510 nm.

For the quantitative determination of leucoan-| thocyanins, the sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75°C. 8 ml of leucoanthocyanidin reagent was added to 1 ml of the total extract. A parallel sample was prepared for each sample, which was not heated. The second sample was subjected to heating for 40 min. After this time, the samples were determined at 550 nm.

To determine the antioxidant activity, the radical bonding ability was determined using the stable radical 2,2-diphenyl-1-picrylhydrazil DPPH. To 1 ml of the analysis extract, we added 3 ml of an alcoholic solution of DPPH (0.1 mM DPPH – 0.004 g/100 ml of ethyl alcohol) and 30 minutes later, we determined the absorption of the test sample at 515 nm. The reference solution was a DPPH solution, and the background was 96%-ethyl alcohol. The antioxidant activity of stable free radical (DPPH) is calculated by the following formula: in % = (AC – AS)/AC*100 (1), where in % is the degree of inhibition of the free DPPH radical by the antioxidant in percent; AC – absorption of 0.1 mM DPPH in alcohol, and AS – absorption of the test extract and 0.1 mM DPPH in alcohol. To determine the antioxidant activity of the product directly, we used the following formula: 2) C = m/V*V in %, where C is the mg of sample that inhibits 0.1 mM DPPH by 50%; m – mass of the sample taken in milligrams; V – volume of the test extract (ml); F – dilution factor; in %-the sample amount for 50% inhibition of 0.1 mM DPPH.

The statistical analysis was conducted by calculating the standard error for each set of data utilizing the Microsoft Excel software. A confidence level was established, with a significance threshold set at $p \leq 0.05$.

Results and Discussion

The analysis of phenolic compounds in the extracts obtained from the skins and seeds of the ”Otskhanuri Sapere” variety and “Zeibel 5455” revealed that the extract obtained from the skins of the “Zeibel 5455” variety contains phenolic compounds – 4212.5 mg/100 g; the extract obtained from the seeds – 4577.5 mg/100 g; in the case of the extracts obtained from the skins of the “Otskhanuri Sapere” variety contains 4156.9 mg/100 g phenolic compounds and the extract obtained from the seeds 4111.0 mg/100 g.

Both the seeds and the skins of “Zeibel 5455” and “Otskhanuri Sapere” are characterized by a high content of phenolic compounds. Flavonoids account for 86% and 73% of the total phenols in both grape varieties, flavon-3-ols – for 22% and 29%, and leucoanthocyanins – for 7.5% and 7.3% of the total phenols for “Zeibel 5455” and “Otskhanuri Sapere”, respectively.

The skins of both grape varieties are characterized by a higher content of anthocyanins than the seeds – 5.5 times more in the case of "Zeibel 5455" and 3.3 times more in the case of "Otskhanuri Sapere". Flavonoids account for 91% and 64% of the total phenols in the skins of both grapes, respectively.

Table 1. Phenolic compounds of grape seeds of the varieties of "Zeibel 5455" and "Otskhanuri Sapere"

Compounds	"Zeibel 5455"	"Otskhanuri Sapere"
Total amount of phenolic compounds mg/100 g on dry matter basis	4577.5	4111
Total amount of flavonoids mg/100 g on dry matter basis	3944.6	3042.5
Flavan-3-ols mg/100 g on dry matter basis	1048.5	1195.7
Leucoanthocyanins mg/100 g on dry matter basis	338.89	304.06

As a result of the identification and quantitative analysis of individual forms of leucoanthocyanins in the phenolic compound concentrates of the seeds and skins of the varieties of "Zeibel 5455" and "Otskhanuri Sapere", it was determined that the skins of both test samples are rich in mono- and diglucoside forms of anthocyanins. Both monoglucoside forms of leucoanthocyanins (peonidin 3-0-monoglucoside, malvidin 3-0-monoglucoside, petunidin 3-0 acetylmonoglucoside, peonidin 3-0 acetylmonoglucoside, malvidin 3-0-acetylmonoglucoside, peonidin 3- (6-0-P conmatroyl) 5-0 monoglucoside,) and diglucoside forms (delphidin -3-5-0 diglucoside, cyanidin 3-5-0 diglucoside, petunidin 3-5-0 diglucoside, peonidin 3-5-0 diglucoside, malvidin 3-5-0 diglucoside, delphidin 3 (6-0-P conmatroyl)5-0 diglucoside, petunidin 3 (6-0-P conmatroyl)5-0 diglucoside, peonidin 3-0 (6-0-P conmatroyl)5-0 diglucoside, malvidin3-0 (6-0-P conmatroyl) 5-0 diglucoside). It is noteworthy that the anthocyanins of "Zeibel 5455" are prevailed by diglucoside forms, while those of "Otskhanuri Sapere" are dominated by monoglucoside forms.

Table 2. Phenolic compounds of grape seeds of the varieties of "Zeibel 5455" and "Otskhanuri Sapere"

Biologically active compounds	"Zeibel 5455"	"Otskhanuri Sapere"
Total amount of phenolic compounds mg/100 g on dry matter basis	4577.5	4111
Total amount of flavonoids mg/100 g on dry matter basis	3944.6	3042.5
Anthocyanins mg/100 g on dry matter basis	1887.5	1019

From literary sources, it is known that diglucoside forms of phenolic compounds are characterized by particularly high antioxidant activity (Danak et al., 2023; Manohar et al., 2017).

Diglucoside forms of phenolic compounds predominate in the variety of "Zeibel 5455", which may be the basis for its high antioxidant activity, which was confirmed by the study of the antioxidant activity of the studied extracts

The analysis of the antioxidant activity of the extracts obtained from the skins and seeds of "Otskhanuri Sapere" and "Zeibel 5455" revealed

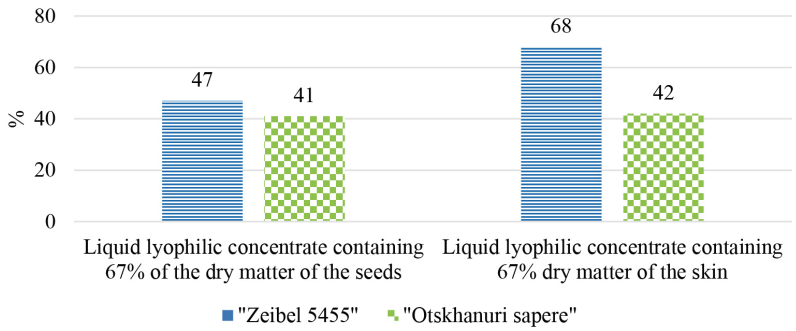

Figure. Antioxidant activity of the phenolic concentrates of "Zeibel 5455" and "Otskhanuri Sapere".

that the antioxidant activity of the phenolic extract of the skins of the variety of "Zeibel 5455" is 68 in %; the phenolic extract of the seeds of "Zeibel 5455" is 47 in %; the phenolic extract of the skins of the variety of "Otskhanuri Sapere" is 42 in %; and the phenolic extract of the seeds of "Otskhanuri Sapere" is 41.5 in % (Figure).

As can be seen from the data presented in figure 2, the skin extracts of the "Zeibel 5455" variety are characterized by exceptionally high antioxidant activity, which is due to the abundance of diglucoside forms of phenolic compounds in them.

Conclusions

Analysis of phenolic compounds in the extracts obtained from the skins and seeds of the varieties of "Otskhanuri Sapere" and "Zeibel 5455" revealed that the obtained extracts are characterized by a high content of phenolic compounds. In the phenolic extracts of the skins of "Zeibel 5455", diglucoside forms of phenolic compounds prevail, while in the skins of "Otskhanuri Sapere" monoglucoside forms are dominant. "Zeibel 5455" skin extracts are characterized by exceptionally high antioxidant activity, which is due to the abundance of diglucoside forms of phenolic compounds in them. The concentrates of phenolic compounds obtained from secondary raw materials of colored-skinned grape varieties of "Zeibel 5455" and "Otskhanuri Sapere" can be used as a biologically active ingredient with antioxidant activity in food technology.

ბიოქიმია

საქართველოში კულტივირებული ფერადი ყურძნის („ზეიბელ 5455“-ისა და „ოცხანური საფერე“-ს) მეორეული რესურსების ფენოლური კომპლექსის კვლევა

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(წარმოდგენილია აკადემიის წევრის გ. ხატისაშვილის მიერ)

კვლევა ეხება დასავლეთ საქართველოში გავრცელებული ფერადი ყურძნის – „ოცხანური საფერე“ და „ზეიბელ 5455“-ის მეორეული ნედლეულის – კანისა და წიპწის – ფენოლური შემადგენლობისა და ანტიოქსიდანტური აქტივობის ანალიზს. კვლევისათვის შერჩეულია იმერეთის მევენახეობა – მეღვინეობის ზონებში კულტივირებული ფერადი ყურძნის არასამრეწველო ჯიში – „ზეიბელ 5455“ და სამრეწველო ჯიში – „ოცხანური საფერე“. საკვლევი

ნედლეულის კანისა და წიპწისაგან მიღებული ექსტრაქტების ფენოლურ ნაერთთა ანალიზმა აჩვენა, რომ „ზეიბელ 5455“-ის კანისაგან მიღებული ექსტრაქტი შეიცავს ფენოლურ ნაერთებს – 4212,5 მგ 100 გ-ზე; წიპწისაგან მიღებული ექსტრაქტი – 4577,5 მგ 100 გ-ზე; „ოცხანური საფერეს“ ექსტრაქტების შემთხვევაში კი 4156,9 მგ 100 გ-ზე და 4111,0 მგ 100 გ-ზე; ორივე საკვლევი ნიმუშის კანი მდიდარია ანტოციანების მონო- და დიგლუკოზიდური ფორმებით. ამასთან, „ზეიბელ 5455“-ში ჭარბობს ფენოლურ ნაერთთა დიგლუკოზიდური ფორმები, რაც მისი მაღალი ანტიოქსიდანტური აქტივობის საფუძველი უნდა იყოს, ეს დაადასტურა კიდეც საკვლევი ექსტრაქტების ანტიოქსიდანტური აქტივობის კვლევამ. „ოცხანური საფერე“-ს და „ზეიბელ 5455“-ის კანისა და წიპწისაგან მიღებული ექსტრაქტების ანტიოქსიდანტური აქტივობის ანალიზმა აჩვენა, რომ „ზეიბელ 5455“-ის კანის ფენოლური ექსტრაქტის ანტიოქსიდანტური აქტივობა შეადგენს 68 in %-ს; „ზეიბელ 5455“-ის წიპწის ფენოლური ექსტრაქტისა კი 47 in %-ს; „ოცხანური საფერე“-ს კანის ფენოლური ექსტრაქტის ანტიოქსიდანტური აქტივობა 42 in %-ია; ხოლო „ოცხანური საფერე“-ს წიპწისა კი – 41,5 in %. კვლევამ დაადასტურა, რომ „ზეიბელ 5455“-ის კანის ექსტრაქტები ხასიათდება გამორჩეულად მაღალი ანტიოქსიდანტური აქტივობით, რაც მათში ფენოლურ ნაერთთა დიგლუკოზიდური ფორმების სიჭარბით აიხსნება.

REFERENCES

Artem, V., Geana E., & Antoce, A. (2014). Study of phenolic compounds in red grapes and wines from Murfatlar wine center, *25*(1), 47-52.

Craft, B., Kerrihard, A., Amarowicz, R., & Pegg, R. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety, 11*(2), 148-173.

Danak, P., Spiegel, M., & Gabrielska, G. (2022). Interference of malvidin and its mono- and di-glucosides on the membrane – Combined in vitro and computational chemistry study. *Journal of Functional Foods, 99*, 22-25.

Danak, P., Spiegel, M., & Gabrielska, G. (2023). Malvidin and its mono- and di-glucosides forms: A study of combining both in vitro and molecular docking studies focused on cholinesterase, butyrylcholinesterase, *COX-1 and COX-2 activities. Molecules, 28*(23).

Georgiev, V., Ananga, A., & Tsolova, V. (2014). Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients, 6*(1), 391-415.

Gvinianidze, T. (2018). Characteristic of the bone and skin of colored eco-grape varieties. *Bulletin of Science and Practice, 4*(11).

Gvinianidze, T., Chikovani, P. (2017). Colored grape polyphenol concentrate. *Annals of Agrarian Science*, *15*(4).

Gvinianidze, T., Chikovani, P. (2018). Polyphenolic extracts of red grapes. *Agricultural Research & Technology: Open Access Journal, 16*(2).

Jiang, B., Sun, Z. (2019). Phenolic compounds, total antioxidant capacity and volatile components of Cabernet Sauvignon red wines from five different wine-producing regions in China. *Food Science and Technology, 39*(3), 735-746.

Kharadze, M., Abashidze, N., Djaparidze, I., Vanidze, M., Kalandia, A. (2018). Antioxidant activity of chestnut honey produced in Western Georgia. *Bull. Georg. Natl. Acad. Sci., 12*(2), 145-151.

Kumar, S., Pandey, A. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal,* Article ID 162750.

Lamparadze, Sh., Gvinianidze, T., & Gamkrelidze, E. (2018). Polyphenolic extracts of red grapes. *Bulletin of Akaki Tsereteli State University,* 1. Kutaisi, Georgia.

Manohar, C., Xue, J., Murayyan, A., Neethirajan, S., Shi, J. (2017). Antioxidant activity of polyphenols from Ontario grown onion varieties using pressurized low polarity water technology. *Journal of Functional Foods, 31*, 52-62.

Nel, A. P. (2018). Tanins and anthocyanins: From their origin to wine analysis. *South African Journal of Enology and Viticulture, 39*(1), 1503-1523.

Niina, J. R. (2016). Determination of trace elements in wine by atomic spectroscopy and electroanalytical methods. *In Grape and wine biotechnology* (pp. 417-439).

Shakulashvili, N., & Chikvaidze, E. (2018). Free radicals, antioxidants, resveratrol, vine and Georgian wine. *Food Science Journal, 2*(2), 1-3.

Surmanidze, N., Vanidze, M., Djafaridze, I., Davitadze, R., Qarcivadze, I., Khakhutaishvili, M., Kalandia, A. (2024). Optimization of the method of ultrasonic extraction of lycopene with a green extract from the fruit of Elaeagnus umbellata, common in Western Georgia. *Food Science and Nutrition, 12*(5), 35-36.

Tyagi, A. (2013). Differential effect of grape seed extract against human non-small-cell lung cancer cells: The role of reactive oxygen species and apoptosis induction. *Nutrition and Cancer, 65*(1) 44-53.

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