Biologically valuable components of grape stems

Yuriy Grishin[1], *Nikolay* Oleinikov[2,1], *Marina* Ryndach[3], *Anatoliy* Kazak[2], *Alexey* Musakin[4], *Irina* Churilina[5], *Mariya* Chernenok[5] and *Anastasia* Kryukova[2]

[1]All-Russian National Research Institute of Viticulture and Winemaking "Magarach" RAS, Kirova street 31, Yalta, 298600, Russian Federation
[2] V.I. Vernadsky Crimean Federal University, Prospekt Vernadskogo 4, Simferopol, 295007, Russian Federation
[3]Kherson State Pedagogical University, st. Universitetskaya, 27, Kherson, 273003, Russian Federation
[4]Saint Petersburg State University of Economics; St. Petersburg, nab. Griboyedov Canal, 30-32 liter A, 191023,Russian Federation
[5]Herzen University, St. Petersburg, nab. Moika River, 48, 191186, Russian Federation

Abstract. The current problem of recycling winemaking waste is largely determined by the lack of technological cycles and waste-free technology schemes that can ensure the rational use of natural resources, including grape stems, when creating functionally new composite compositions designed to support human immunity and health. The most effective way to recover raw materials from grape stems is to produce extracts from them. As part of the study, the qualitative and quantitative phenolic composition of grape stem extracts was established. The methodology and technological regimes for the preparation of grape stems, as well as the ranges for varying the content of biologically valuable components in extracts from the stems, have been established. The use of the proposed scheme for obtaining extracts will allow us to separate the process of extracting the components of grape stems from infusion and will allow us to develop a number of technological advantages: targeted regulation of the quality of grape processing products, more complete use of raw materials, and reduction of losses of basic and auxiliary materials.

1 Introduction

One of the most common types of waste when processing grapes into white, rose and red wines is grape stems. Thus, in the process of processing 100 kg of grapes into grape must, up to 6.3 kg of ridges are formed as waste. According to a number of studies, it has been established that grape stems are a valuable source of trans-resveratrol, which plays an important role in the prevention of cardiovascular diseases, preventing the blockage of blood vessels, cholesterol oxidation and the formation of malignant tumors [1-9]. A high content of these representatives of phenolic substances characterizes one of the most widespread types of waste in the viticulture and wine industry - grape stems, which can act

[1] Corresponding author: oleinikov1@mail.ru

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

as a valuable source of biologically active substances and antioxidants with a phenolic structure [10-12].

Conducted in vivo studies on the antioxidant activity and biological value of phenolic substances have established that representatives of phenolic substances such as (+)-D-catechin, gallic acid and *trans*-resveratrol are characterized by high antioxidant and biological activities [13]. As a result of numerous demographic studies, significant numbers of test populations have been analyzed and the most applicable norms for daily consumption of phenolic substances with high antioxidant activity have been established. The average daily intake of catechins varies widely, from 77 to 182 mg/day and varies depending on the geographical features and botanical diversity that determine the composition and structure of the respondent's diet [14]. According to Dutch researchers, the main bioavailable representative of catechins is (+)-D-catechin, the average consumption of which was about 50 mg/day [15]. The detoxifying properties of catechins are most pronounced in (+)-D-catechin, which inhibits DNA damage caused by potential food carcinogens such as heterocyclic amines [16]. It has been established that the average daily intake of phenolic acids in the human diet is about 200 mg/day, while this value is largely averaged and depends on the individual characteristics of the diet [17, 18]. In particular, the daily intake of hydroxybenzoic acids. coming from food is estimated at 25-100 mg/day [19, 20].

According to D. Harman, the founder of the free radical theory of aging, according to which premature aging of the human body occurs as a result of an increase in the amount of damage caused by free radicals, the use of supplements in the daily diet containing substances that exhibit antioxidant properties will not only increase the quality of human life - reduce the rate of accumulation of age-related changes, chronic disability in older people, but also reduce mortality from cancer and cardiovascular diseases [21, 22].

An analysis of the literature data on the total content of phenolic compounds in the solid components of a bunch of grapes showed that the highest values of this indicator were noted in samples of grape stems, which indicates that they represent the most valuable source of bioactive phenolic substances [23, 24].

2 Materials and Methods

2.1. Reagents

2.1.1. Spectrophotometric method

Spectrophotometric analysis to determine the mass concentration of phenolic sub-stances was carried out using the Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent was prepared as follows: 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) were dissolved in 700 cm^3 of distilled water. Added 50 cm^3 of concentrated orthophosphoric acid (H_3PO_4) 85% ($\rho 20$ = 1.71 g/cm^3) and 100 cm^3 of con-centrated hydrochloric acid (HCl) ($\rho 20$ = 1.19 g/cm^3). Bring to a boil and reflux for 10 hours. Then add 150 g of lithium sulfate ($Li_2SO_4 \cdot 2H_2O$) and a few drops of bromine and boil for 15 minutes. After cooling to a temperature of (20±0.5) °C, the volume of the solution was brought to 1 dm^3 with distilled water.

Aqueous-alcohol solution of tartaric acid: measured 8.0 g of tartaric acid and placed it in a volumetric flask with a volume of 2.0 dm^3, dissolved in a small amount of distilled water, added 200 cm^3 of ethyl alcohol, brought the volume of the solution to 2.0 dm^3.

Standard solution of gallic acid: 1.2 g of gallic acid were measured and placed in a flask with a volume of 0.5 dm^3, dissolved in a small amount of an aqueous-alcohol solution of tartaric acid, and the volume of the solution was brought to 0.5 dm^3.

2.1.2. Chromatographic method

To carry out the chromatographic analysis of phenolic substances, an eluent was used with the following composition: solution A - methyl alcohol, solution B - an aqueous solution of trifluoroacetic acid ($C_2HF_3O_2$) with a mass concentration of 0.6 g/100 cm^3.

The *trans*-resveratrol, (-)-epicatechin, syringic acid (Sigma-Aldrich) were used as standards when determining the individual phenolic composition by HPLC.

2.2. Samples

The samples for the research were alcoholic extracts of grape stems of 11 white grape stems of the Vitis vinifera species (Aligote, Rkatsiteli, Colombard, Shabash, Tashly, Soldaiya, Abla, Aurora, Pervenetz Magaracha, Podarok Magaracha, Kok Pandas) from the ampelographic collection of grapes of the FSBSI Institute Magarach of the RAS. To prepare experimental extracts, grape stems isolated from the technological process of preparing white wines during the 2012-2019 winemaking seasons were used.

Experimental samples of extracts were prepared according to the scheme presented in Figure 1.

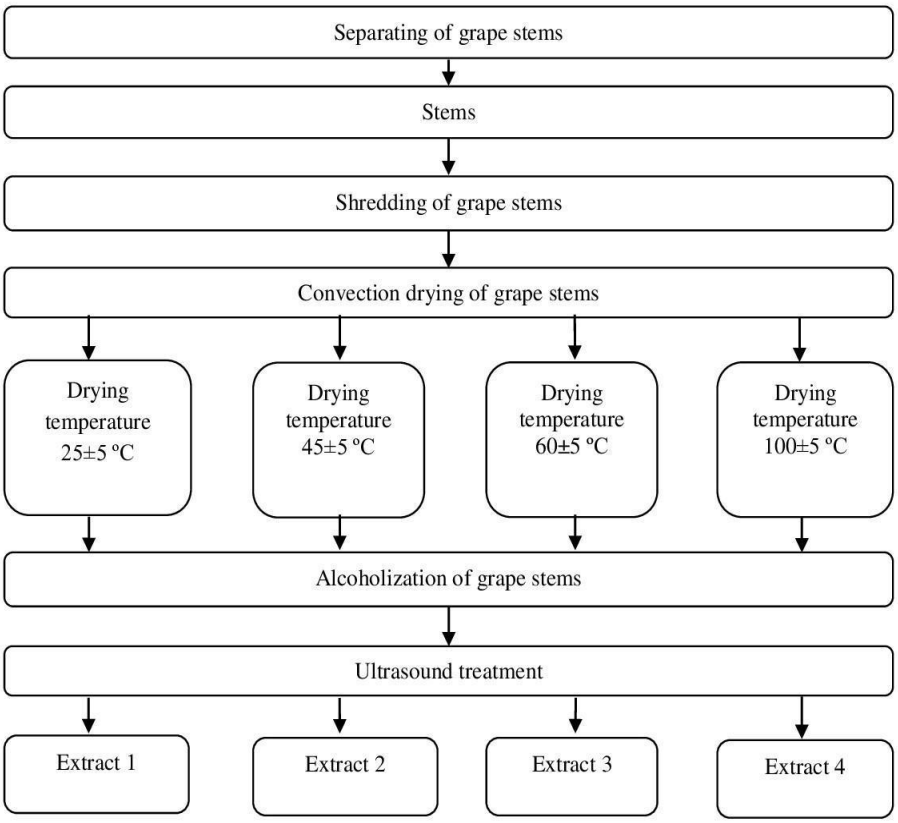

Fig.1 Scheme for the preparation of experimental samples of grape stems extracts

2.3. Instrumentation

The mass concentration of phenolic substances was identified using a Specord 40 Analytik single-beam scanning spectrophotometer Jena with a working wavelength range of 190-1100 nm.

The composition of phenolic substances was analyzed by high-performance liquid chromatography (HPLC) using an Agilent Technologies chromatography system (model 1100) with a diode array detector. For separation, we used a Zorbax SB-C18 chromatographic column with a size of 2.1×150 mm, filled with silica gel grafted with an octadecylsilyl phase with a sorbent particle size of 3.5 μm.

2.4. Measurements

2.4.1. Determination of the mass concentration of phenolic substances by the colorimetric method with the Folin-Ciocalteu reagent

The method is based on the ability of phenolic substances, when oxidized, to reduce phosphotungstic and phosphomolybdic acids, which are part of the Folin-Ciocalteu reagent, to their reduced forms. The resulting tungsten oxide (W_8O_{23}) and molybdenum oxide (Mo_8O_{23}) are colored blue, the intensity of which is measured colorimetrically, and the resulting values are proportional to the mass concentration of phenolic substances. Measurements were made in quartz cells with an optical path length of 1 cm, in automatic mode. The calibration graph was based on calibration solutions prepared by adding to 9 volumetric flasks with a volume of 100 cm^3 – 2.5; 5.0; 7.5; 10.0; 12.5; 15.0; 17.5; 20.0; 25.0 cm^3 of a standard solution of gallic acid and bringing them to a volume of 100 cm^3 with an aqueous alcoholic solution of tartaric acid. A calibration graph was constructed based on the obtained absorption values. Based on the obtained absorption values, a calibration graph was constructed. The arithmetic mean of the results of two parallel measurements was taken as the final result.

2.4.2. Identification of mass concentration of phenolic substances using high-performance liquid chromatography

The qualitative and quantitative composition of phenolic substances in the test samples was determined by HPLC using an Agilent Technologies chromatographic system (model 1100) with a diode array detector [25,26]; chromatography was carried out in gradient mode. During the chromatography process, the composition of the eluent underwent changes in the content of component B, according to the following scheme: 0 minutes 8 %; 0-8 minutes 8-38 %; 8-24 minutes 38-100 %; 24-30 minutes 100 %. Eluent flow rate 0.25 cm^3/min.

Chromatograms were recorded at the following wavelengths:

280 nm - gallic acid, (+)-D-catechin, (-)-epicatechin and procyanidins;

313 nm - derivatives of hydroxycinnamic acids;

371 nm - quercetin.

Individual compounds were identified by comparing their spectral characteristics with the spectra described in the literature and by matching the retention time of the detected peak and the peak of the standard sample. The spectral characteristics of individual substances were confirmed using literature data [27]. Calculation of the quantitative content of individual components was carried out using calibration graphs of the dependence of the peak area on the concentration of the substance, constructed using solutions of standard substances. All determinations were carried out in triplicate.

3 Results and Discussion

During the experiment, after separation from the pulp, the combs were crushed using an AXT Rapid 2000 unit and dried in a stationary oven programmed for drying at 45, 60 and

100 ºC and under normal conditions at a temperature of 25±5 ºC (control). The process of drying the stems was stopped at a relative humidity value of no more than 15%, in accordance with the data of C. Leal [28]. The duration of drying the stems at a temperature of 25±5 ºC was ~8 hours. Increasing the drying temperature to 45, 60 and 100 ºC contributed to a reduction in the duration of stem processing from 6 hours to 20 minutes. Crushed and dried grape stems were preserved in alcohol with a water-ethanol extractant with a volume fraction of ethanol of 70%, the ratio of solid and liquid phase was 1:3. Samples of stems preserved in alcohol were placed in a Bandelin Sonorex RK 225 H unit, in which they were treated with ultrasound at an oscillation frequency of 35 kHz for 10-20 minutes.

Studies of the influence of temperature regimes for convection drying of crushed grape combs have shown that increasing the temperature of convection drying of ridges to 60 ºC promotes the accumulation of all forms of phenolic substances, and the content of monomeric forms of phenolic substances increases by 4.3 times. There is an increase in the content of hydroxybenzoic acids by 35 %, flavanols by 43 %, hydroxycinnamic acids by 47.5 %, flavonols by 38 %, stilbenes by 16 %, compared to the control. A further increase in temperature leads to a decrease in the concentration of phenolic compounds, which is apparently associated with the activation of oxidative processes.

The content of biologically active substances with a phenolic structure in experimental grape stem extracts is presented in Table 1.

Table 1 The content of biologically active substances with a phenolic structure in experimental grape stem extracts

Name	**Indicator value,** $\frac{min-max}{avg}$ **, mg/dm³**
Mass concentration (MC)	Extract of grape stems
MC of hydroxycinnamic acids	58.49-270.78 163.22
MC of hydroxybenzoic acids, including:	65.75-186.29 93.63
- gallic acid	60.70-171.10 95.70
MC of flavanols, including.	209.40-350.55 303.63
- (+)-D-catechin	192.10-309.70 275.88
MC of flavonols	50.44-217.10 97.63
MC of stilbenes, including:	25.80-31.24 28.71
- *trans*-resveratrol	2.60-9.30 7.16

Analysis of the qualitative and quantitative phenolic composition of water-ethanol extracts of the stems of white technical grape varieties indicates that the prepared water-ethanol extract of the stems of the Rkatsiteli grape variety was superior to samples of extracts from the stems of other varieties in terms of the content of such biologically active compounds as: flavanols (27.1 %), hydroxybenzoic acids (by 75.7 %), stilbenes (by 16.1 %), oligomeric (by 34.1 %) and polymeric procyanidins (by 13.5 %).

Using the method of regression analysis, a regression equation was obtained expressing the relationship between the technological reserve of phenolic substances in the stems of white technical grape varieties and the mass concentration of sugars in grape berries:

$$y = 0.4209x - 50.465, r = 0.8908,$$

where *y* is the value of the technological reserve of phenolic substances, g/kg dry weight, *x* is the value of the mass concentration of sugars in grapes, g/dm^3, *r* is the value of the approximation reliability.

According to the data obtained, the approximation reliability value for our sample was 0.8908, which indicates a high degree of relationship between the technological reserve of phenolic substances and the mass concentration of sugars. It has been established that in grapes with a sugar content of 160 to 180 g/dm^3, the technological reserve of phenolic substances in the stems varies in the range from 15-30 g/kg dry weight.

4 Conclusions

It has been established that for the production of stem extracts of white technical grape varieties, characterized by the highest content of biologically active substances, in particular (+)-D-catechin, gallic acid and *trans*-resveratrol, it is advisable to use grape stems containing at least 160 g/dm^3 of sugars and characterized by a technological reserve of phenolic substances at the level of 15-30 g/kg dry weight. Preliminary preparation of stems is carried out by grinding the stems to sizes (20±10) mm on an AXT 2000 Rapid chopper and subsequent convection drying of the stems at a temperature not exceeding 60±5 ºC using drying equipment to a relative humidity level not exceeding 15 %.

Acknowledgements
The study supported by the Russian Science Foundation grant No. 24-25-20001.

References

1. M. Anastasiadi, H. Pratsinis, D. Kletsas, A.L. Skaltsounis, S.A. Haroutounian, LWT Food Sci. Technol. **48** (2012). DOI: 10.1016/j.lwt.2012.04.006.
2. M.R. Gonzalez-Centeno, M. Jourdes, A. Femenia, S. Simal, C. Rossello, P.L. Teissedre, J. Agric. Food Chem. **60**, 48 (2012). DOI: 10.1021/jf303047k.
3. T. Pussa, J. Floren, P. Kuldkepp, A. Raal, J. Agric. Food Chem. **54**, 20 (2006). DOI: 10.1021/jf061155e.
4. M.J. Ruiz-Moreno, R. Raposo, J.M. Cayuela, P. Zafrilla, Z. Pineiro, Ind. Crop. Prod. **63** (2015). DOI: 10.1016/j.indcrop.2014.10.016.
5. J.M. Souquet, B. Labarbe, C. Guerneve, V. Cheynier, M. Moutounet, J. Agric. Food Chem. **48**, 4 (2000). DOI: 10.1021/jf991171u.
6. I. Esparza, J.A. Moler, M. Arteta, N. Jimenez-Moreno, C. Ancin-Azpilicueta, Biomolecules **11**, 8 (2021). DOI: 10.3390/biom11081221.
7. J.A. Nieto, S. Santoyo, M. Prodanov, G. Reglero, L. Jaime, Foods. **9**, 5 (2020). DOI: 10.3390/foods9050604.
8. M. Blackford, M. Comby, L. Zeng, A. Dienes-Nagy, G. Bourdin, F. Lorenzini, B. Bach, Molecules. **26**, 5 (2021). DOI: 10.3390/molecules26051240.
9. Y. Zeng, W. Zhou, J. Yu, L. Zhao, K. Wang, Z. Hu, X. Liu, Antioxidants (Basel) **12**, 2 (2023). DOI: 10.3390/antiox12020418.

10. D.P. Markis, G. Boscou, N.K. Andrikopoulos, J. Food Comp. Anal. **20** (2007). DOI: 10.1016/J.JFCA.2006.04.010.
11. D.P. Markis, G. Boscou, N.K. Andrikopoulos, Bioresour. Technol. **98**, 15 (2007). DOI: 10.1016/J.BIORTECH.2006.10.003.
12. G. Spingo, D.M. Faveri, J. Food Eng. **78**, 3 (2007). DOI: 10.1016/j.jfoodeng.2005.11.020.
13. K.I. Priyadarsini, S.M. Khopde, S.S. Kumar, H. Mohan, J. Agric. Food Chem. **50**, 7 (2002). DOI: 10.1021/jf011275g.
14. A. Vogiatzoglou, A.A. Mulligan, M.H. Lentjes, R.N. Luben, J.P.E. Spencer, H. Schroeter, PLoS One. **10**, 5 (2015). DOI: 10.1371/journal.pone.0128132.
15. I.C. Arts, B. van de Putte, P.C. Hollman, J. Agric. Food Chem. **48**, 5 (2000). DOI: 10.1021/jf000025h/
16. A.I. Haza, P. Morales, J. Appl. Toxicol. **31** (2011). DOI: 10.1002/jat.1559.
17. K. Herrmann, C.W. Nagel, Crit. Rev. Food Sci. Nutr. **28**, 4 (1989). DOI: 10.1080/10408398909527504.
18. M.N. Clifford, J. Sci. Food Agric. **80**, 7 (2000). DOI: 10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T.
19. R. Zamora-Ros, J.A. Rothwell, A. Scalbert, V. Knaze, I. Romieu, N. Slimani, Br. J. Nutr. **110**, 8 (2013). DOI: 10.1007/s00394-015-0950-x.
20. X. Han, M. Li, L. Sun, X. Liu, Y. Yin, J. Hao, Nutrients. **14**, 24 (2022). DOI: 10.3390/nu14245383.
21. D. Harman, Mutat. Res. **275**, 3-6 (1992). DOI: 10.1016/0921-8734(92)90030-s.
22. D. Harman, AGE. **23** (2000). DOI: 10.1007/s11357-000-0017-6.
23. F. Bonilla, M. Mayen, J. Merida, M. Medina, Food Chem. **66** (1999). DOI: 10.1021/jf0006269.
24. G.K. Jayaprakaska, T. Selvi, K.K. Sakariah, Food Res. Int. **36** (2003). DOI: 10.1016/S0963-9969(02)00116-3.
25. B. Yuan, D.R. Byrnes, F.F. Dinssa, J.E. Simon, Q. Wu, J. Food Sci. **84**, 2 (2019). DOI: 10.1016/j.foodchem.2019.126030 .
26. A. Kazak, Y. Plugatar, J. Johnson, Y. Grishin, P. Chetyrbok, V. Korzin, P. Kaur, T. Kokodey, Appl. Syst. Innov. **5** (2022). DOI: 10.3390/asi5050104.
27. J.B. Johnson, R. Timofeev, A. Kazak, Y. Grishin, L. Solovyova, M. Rudenko, Front. Biosci. (Elite Ed) **16**, 2 (2024). DOI: 10.31083/j.fbe1602016.
28. C. Leal, I. Gouvinhas, R.A. Santos, E. Rosa, A.M. Silva, M.J. Saavedra, A.I.R.N.A. Barros, Ind. Crop. Pod. **154** (2020). DOI: 10.1016/j.indcrop.2020.112675.