Bulgarian Journal of Agricultural Science, 32 (No 2) 2026, 401–410

Study of the content of total phenols and antioxidants in Bulgarian grape varieties after freeze-drying

Georgi Angelov

Agricultural Academy, Institute of Cryobiology and Food Technologies, Cheni Vrah 53 blvd., Sofia, Bulgaria
Corresponding author: eng.angeloff@gmail.com

Abstract

Angelov, G. (2026). Study of the content of total phenols and antioxidants in Bulgarian grape varieties after freeze-drying. *Bulg. J. Agric. Sci., 32*(2), 401–410

The study aims to investigate the physicochemical parameters as well as the antioxidant activity and the total amount of phenols of different grape varieties, selected on the territory of the Institute of Viticulture and Enology – Pleven at the Agricultural Academy, located in the Central Danube Plain, Bulgaria. Three different grape varieties were presented: a local variety Gamza, an introduced – Cabernet Sauvignon, and a hybrid – Kailashki Rubin (Pamid x Hybrid IV-2-15 x Gamay Noir x Vitis amurensis). The study investigated parameters such as pH, moisture content, color characteristics, antioxidant activity and total phenolic number of fresh and freeze-dried grapes of the three varieties, and a comparative characteristic between the two types of processing was made. After the conducted studies, it was found that fresh and freeze-dried grapes retain approximately the same results of the physicochemical parameters.

Keywords: grapes; freeze-drying; physicochemical parameters; Gamza; Kailashki rubin; Cabernet Sauvignon

Introduction

Research in the field of food technology on a global scale places emphasis on the demand and supply of foods with intensified and increased health capacity. Starting from the point of view of maintaining a stable health status of the human individual, foods with the presence of components with a high health effect and activities exerted on the metabolism and systems of the human body, provide prevention against a number of diseases. Research in the field of food technology on a global scale places emphasis on the demand and supply of foods with intensified and increased health capacity. Starting from the point of view of maintaining a stable health status of the human individual, foods with the presence of components with a high health effect and activities exerted on the metabolism and systems of the human body, provide prevention against a number of diseases.

Phenols are chemical compounds whose hydroxyl group is directly bonded to the benzene ring. In the phenol molecule, the oxygen atom located in the hydroxyl group is in the sp2– hybrid state. One of the p-AO remains in an unhybridized state and spatially occupies a perpendicular position with respect to the plane of the benzene ring. The unhybridized p-AO conjugates with the p-AO of the carbon atom of the benzene ring. This process leads to the presence of a positive mesomeric effect (+M/ conjugation effect), from which the significant differences between the properties of alcohols and phenols could be defined.

Scientists in the food industry, cosmetics industry and pharmacy have conducted scientific studies on the content of polyphenols in some fruits, vegetables, cereals, plants and herbs.

A contemporary emphasis in the work of various scientific groups is the search for solutions to increase the antioxidant capacity of foods, through a number of studies on the antioxidant activity of extracts from various plant species that can be incorporated into them (Ivanov et al., 2014; Petkova et al., 2017; Stoilova et al., 2007).

In the field of grape production and winemaking (as part of the direction of food technology), there was an increased research interest in the role of specific compounds from the chemical composition of grapes and wine on improving human health and preventing diseases. The scientific community's interest in the health benefits of wine had increased after a study known as the "French paradox" (Renaud and Lorgeril, 1992). It found a low mortality rate and incidence of cardiovascular diseases among French men aged between 54 and 65 years (a population at risk for cardiovascular disorders), despite the consumption of significant amounts of animal fat containing high levels of saturated fatty acids and cholesterol. The study found that the main influence on this fact is the moderate intake of red wines.

The factors that influence the growth, development, quantity and quality of the grape harvest are many and varied. The most important are climate and soil, with climatic conditions having a particularly determining importance: temperature during the growing season and during the dormant period, the amount of precipitation and relative humidity of the air, solar radiation, the frequency of adverse effects – low winter temperatures, hail, prolonged droughts, etc. The climatic features of the region depend directly on its geographical location, relief features, exposure, prevailing winds, etc. (Abrasheva et al., 2008).

The region of the city of Pleven is a part of the Northern wine-growing region (Danube Plain), which was characterized by a typical continental climate, early spring with frequent late frosts, hot and relatively dry summer, long and warm autumn with early frosts, cold and frosty winter. The soils include all types of chernozems – typical, carbonate, leached, strongly leached and podzolic, formed on loess. The region was characterized by the following indicators: temperature sum during the growing season 31.30–40.03°C; duration of the growing season 190–210 days; duration of the frost-free period 178 223 days; beginning of the growing season 02.04. to 14.04.; frequency of spring frosts up to 20%; average temperature of the warmest month 20.03–24.02°C; annual precipitation 532–753 mm/dm^3; hydrothermal coefficient for June, July and August 0.7–1.5 (Stoev et al., 1960; Katerov et al., 1990; Pandeliev et al., 2005).

The climate, as the main factor of the terroir and especially the meteorological conditions of the year, influence the content of all components of the chemical composition of grapes, including phenolic substances. This group of compounds was characterized by exceptional diversity. Grapes contain more than 200 phenolic compounds, which are decisive for the color of the wine and to a significant extent for its taste, especially for red wines (Abrasheva et al., 2008).

Ivanova et al. (2015) studied the total polyphenols and antioxidant activity of grape, pear and blackcurrant drinks. In their study, they present the relationship between polyphenols in combination with ascorbic acid and tocopherol and carotenoids, which leads to the protection of body tissues from oxidative stress. They report the presence of a generally proportional relationship between the intake of foods with a high content of phenolic compounds and a number of chronic diseases, as well as mortality caused by diseases of the cardiovascular system. Phenolic compounds also play a significant role in determining the sensory characteristics of grapes and products made from them, as they influence some organoleptic indicators (Ivanova et al., 2015)

A study was conducted to determine biologically active substances in Jerusalem artichoke tubers (*Helianthus tuberosus* L.), where they examined the amount of total phenols and flavonoids in the extracts, as well as their antioxidant activity using the DPPH, ABTS, FRAP and CUPRAC methods. The method for determining the amount of total phenols was Folin-Ciocalteu, and the data obtained were presented as milligrams of gallic acid equivalents. The content of total flavonoids in the extracts was also determined spectrophotometrically. (Petkova et al., 2012)

A study investigating the influence of pre-treatment of blackberries on the content of polyphenols, anthocyanins and antioxidant capacity of the fruit and juices obtained from them, the aim of their study being to monitor the content of total polyphenols, total monomeric anthocyanins and antioxidant activity of blackberries of the varieties "Black Satin", "Dirksen" and "Hul Thornless" and the juices obtained from them. The scientific team has concluded that the highest values of total polyphenols and antioxidant capacity were in the variety "Black Satin" – 439.33 mg GAE/100 g and 2136.11 µmolTE/100g. The raw materials were selected by experts from the Mountain Institute Animal Husbandry and Agriculture – Troyan. The amount of total polyphenols was determined by the "Singleton and Rossi" method, in a modification according to Dinkova et al., 2011. (Petrova et al., 2016a, Petrova et al., 2016b).

Material and Methods

For the purpose of conducting the study, three grape varieties from the 2023 harvest were used. A representative of a typical local variety was Gamza, an introduced variety was Cabernet Sauvignon, and as a hybrid variety, Kailashki Rubin (Pamid x Hybrid IV-2-15 x Gamme Noir x Vitis amurensis) was studied. The three grape varieties were grown in the Middle Danube Plain on an experimental territory at the "Institute of Viticulture and Enology – Pleven", at the Agricultural Academy, and all three grape varieties were previously studied in a period of technological maturity.

Methods

Freeze-drying of the product

The finished products were poured into trays with a layer height of 1.5 cm. and frozen in a freezer at a temperature of -45°C.

Freeze-drying (freeze-drying) is carried out in a lyophilizer, model LYOBETA 6PL. from “Telstar – Spain”. The lyophilized products were presented in Figures 1, 2 and 3.

Determining color characteristics

The color was measured according to the CIEL (Fig. 4) a* b* color system, using the indicators lightness (L), blue spectral color (-b), yellow spectral color (+b), red color (+a), green color (-a), color tone (a/b). Saturation was determined by the values of the index √a2+b2. The color differences between individual samples were represented by the values ΔL, Δa, and Δb, and ΔE served as a generalized indicator. For visual perception of the color difference, the value ΔE = 1 was assumed.

The color characteristics were determined on a Lovibond PFX 880 instrument (Tintometer Limited, UK). A special program calculates the carotene content by processing data obtained from the RYBN color scale, designed to determine the color characteristics of transparent products. The apparatus was calibrated to white (L = 92.8, a = -0.8, b = 0.1) and

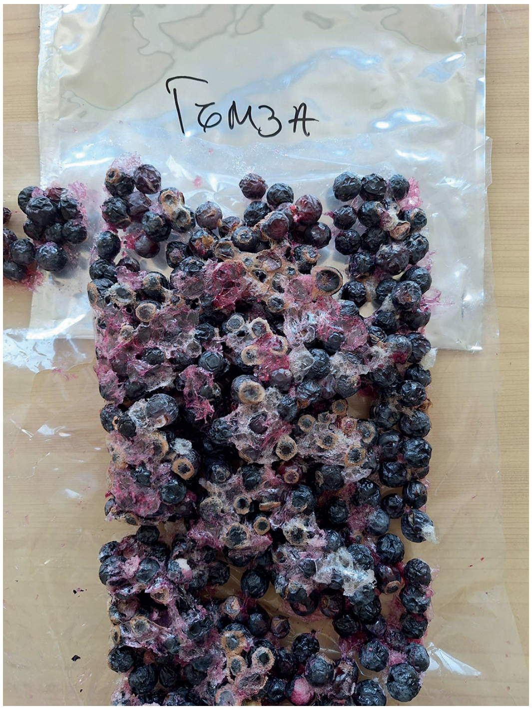

Fig. 1. Freeze-dried grape “Gamza”
Source: Author’ own elaboration

Fig. 2. Freeze-dried grape “Kailashki Rubin”
Source: Author’ own elaboration

Fig. 3. Freeze-dried grape “Cabernet Sauvignon”
Source: Author’ own elaboration

Fig. 4. Colorimeter with serial number 10QC200712
Source: Author' own elaboration

black model tiles. The total color difference, which shows the degree of color change at different temperatures, was calculated by the equation:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

SIEL a* b* colorimetric system

The parameters chromatography and hue were determined by the formulas:

$$C^*_{ab} = \left[\left(a^*\right)^2 + \left(b^*\right)^2\right]^{1/2} \quad \& \quad h_{ab} = \arctan\left(\frac{b^*}{a^*}\right)$$

The values obtained was averages of seven parallel measurements.

Determination of active acidity – pH

A digital pH meter was used – HANNA – SN0111053Nq electrode: HL1230B – Czech Republic. With a range from pH 0.00 to pH 14.00, with an accuracy of reading values up to 0.01 pH; buffer solutions – neutral buffer solution with pH 7.01, acidic buffer solution with pH 4.01 and alkaline buffer solution with pH 10.01.

Determination of water content

The water content (dry matter) was determined by the weight method by drying the samples at a temperature of 100-105 ˚C for 7 h [according to BDS 1109-89]. About 3 g of the sample was weighed into weighing glasses, dried according to the standard method at 105 ºC for four hours. It had been tempered in a desiccator and weighed again. Then it was dried for another two hours. It is weighed to a constant weight. Each value for total moisture was the average of three determinations.

Determination of total phenolic value with Gallic acid standard

Principle of the method: Gallic acid stock solution: 1 mg/ml in 50% CH_3OH

Necessary solutions:

1. Na_2CO3 – 20% solution
2. CH_3OH – 50% solution
3. Folin Chikalto reagent – undiluted, standard

Procedure:

In test tubes (5 pcs.) to 0.5 ml of the corresponding GA dilution (table 2 and table 3) add 3.0 ml distilled water (dH_2O) and 0.25 ml Folin Chikalto reagent. Mix. Let stand for 2 min, then add 0.75 ml of 20% Na_2CO3 solution and add 0.5 ml of dH_2O

Stay in the dark for 2 hours.

Measurement on a spectrophotometer at a wavelength of λ = 765 nm compared to a blank sample. Blank sample: To 0.5 ml of 50% CH_3OH , add 3.0 ml of dH_2O and 0.25 ml of Folin Chikalto reagent. Stir. Let stand for 2 min, then add 0.75 ml of 20% Na_2CO3 solution and 0.5 ml of dH_2O. Stay in the dark for 2 hours.

Sample preparation for lyophilized grapes

1 g of the starting material was ground in a mortar with a small amount of quartz sand. Transfer quantitatively and pour 10 ml of 80% CH_3OH solution. Homogenize with Vortex and leave in the dark for about 12 h. Centrifuge, decant and filter. Make up to 10 ml with 80% CH_3OH solution. The analysis was performed by spectrophotometer.

Sample preparation for natural grapes

3 g of the starting material was ground in a mortar with a small amount of quartz sand. Transfer quantitatively and pour 7 ml of 80% CH_3OH solution. Homogenize with Vortex and leave in the dark for about 12 h. Centrifuge, decant and filter. Make up to 10 ml with 80% CH_3OH solution. The analysis was performed by spectrophotometer.

Determination of antioxidant activity

Determination of radical scavenging ability to DPPH radical (with Trolox standard)

The antioxidant activity of plant extracts was assessed by determining the radical scavenging ability to DPPH.

In this method, solutions of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with different concentrations in methanol were used to prepare a standard. DPPH was one of the most commonly used reagents to determine the ability of antioxidants to scavenge free radicals. DPPH is a relatively stable radical with a dark violet color. When mixing a solution of DPPH with hydrogen atom donors, a color change of the reaction mixture from dark violet to yellow was observed, as the reduced DPPH--H was formed, which in yellow color.

Sample preparation for lyophilized grapes

1 g of the starting material was ground in a mortar with a small amount of quartz sand. Transfer quantitatively and pour 10 ml of 80% CH_3OH solution. It was homogenized with a Vortex and left in the dark for about 12 h. It had centrifuged, decanted and filtered. It was made up to 10 ml with 80% CH_3OH solution. The analysis was performed by spectrophotometer.

Sample preparation for natural grapes

3 g of the starting material was ground in a mortar with a small amount of quartz sand. It is quantitatively transferred and poured with 7 ml of 80% CH_3OH solution. It was homogenized with a Vortex and left in the dark for about 12 h. It has centrifuged, decanted and filtered. It had made up to 10 ml with 80% CH_3OH solution. The analysis was performed by spectrophotometer.

Results and Discussion

Determination of moisture content of natural and freeze-dried grapes of the varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon" (Table 1, Fig. 5).

As can be seen from the results for moisture content of natural and freeze-dried grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon", after freeze-drying the moisture content decreases proportionally to the native product.

Determination of active acidity of natural and freeze-dried grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon" (Fig. 6).

Table 1. Moisture content of grapes, %

	Moisture content, %	
	Natural	Freeze-dried
Gamza	77.60	3.18
Kailashki Rubin	76.23	3.11
Cabernet Sauvignon	64.02	2.93

Source: Author' own elaboration

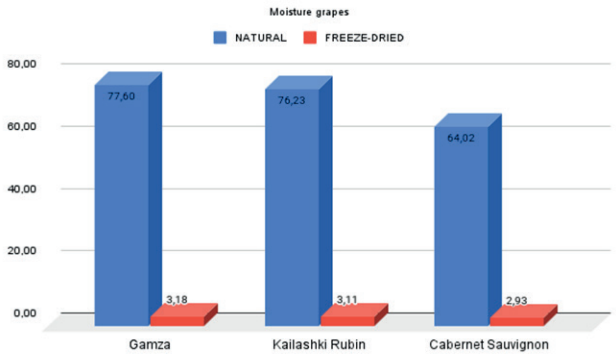

Fig. 5. Moisture content of grapes, %
Source: Author' own elaboration

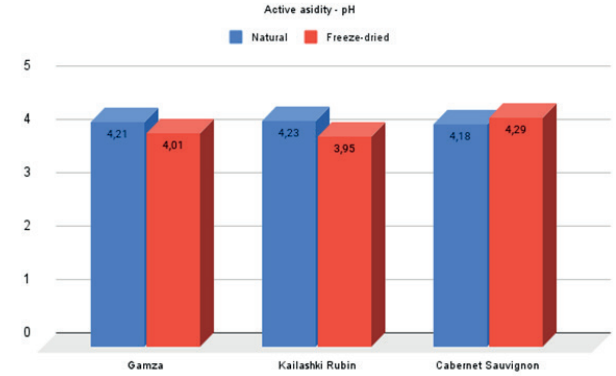

Fig.. 6. Active acidity of natural and freeze-dried grapes of the varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon"
Source: Author' own elaboration

From the analysis conducted to determine the active acidity of natural and freeze-dried grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon", no significant changes were observed in the values of the natural and freeze-dried grapes, that is, the process of freeze-drying grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon" unaffected the active acidity of the product.

Color characteristics of natural and freeze-dried grapes of the varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon".

The color indicators for natural and freeze-dried grapes of the "Gamza" and "Cabernet Sauvignon" varieties maintain a proportional trend, unlike natural and freeze-dried grapes of the "Kailashki Rubin" variety, where the trend observed is inversely proportional (Figures 7, 8 and 9).

Determination of Trolox equivalent antioxidant activity of grapes varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon" were presented on Table 2 and Fig. 10.

Determination of Trolox equivalent antioxidant activity

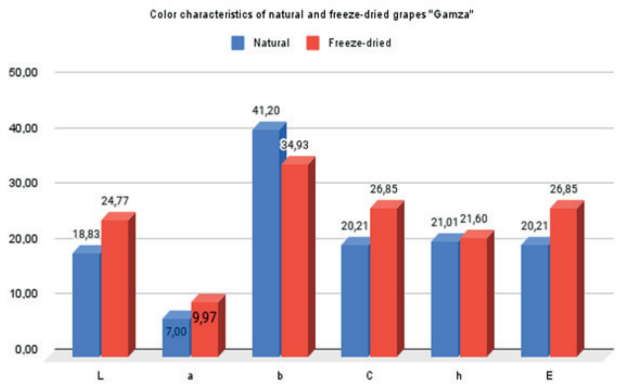

Fig. 7. Color characteristics of grape "Gamza"
Source: Author' own elaboration

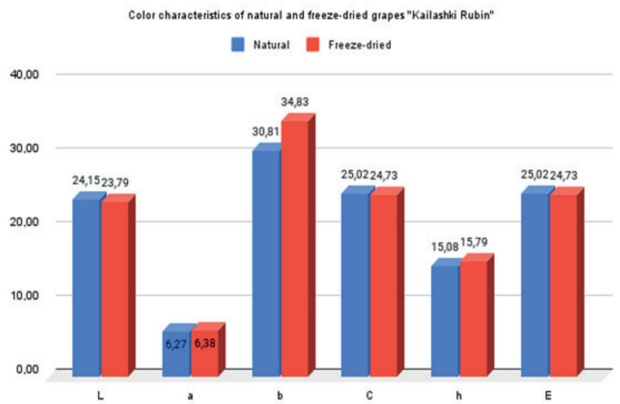

Fig. 8. Color characteristics of grape "Kaylashki Rubin"
Source: Author' own elaboration

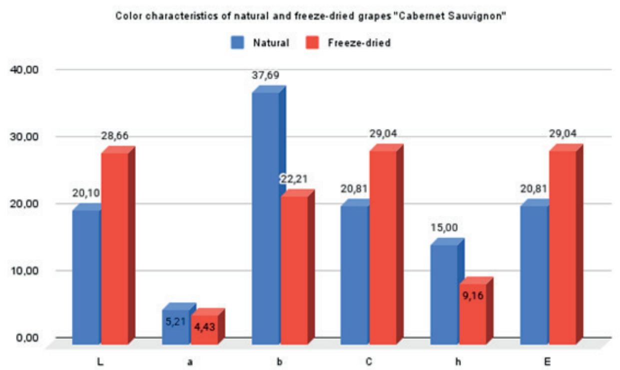

Fig. 9. Color characteristics of grape "Cabernet Sauvignon"
Source: Author' own elaboration

of grapes varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon" were presented on Table 3 and Fig. 11.

The results for Trolox equivalent antioxidant activity of grapes of the varieties "Gamza", "Kaylashki Rubin" and

Table 2. Trolox equivalent antioxidant activity of grapes varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon"

DPPH, TE mg/g		X	SD
Grape variety Gamza	Natural	2.555	0.144
	Freezed	17.055	0.322
Grape variety Kaylashki Rubin	Natural	2.89	0.094
	Freezed	13.003	0.287
Grape variety Cabernet Sauvignon	Natural	3.922	0.115
	Freeze-dried	17.415	0.132

Source: Author' own elaboration

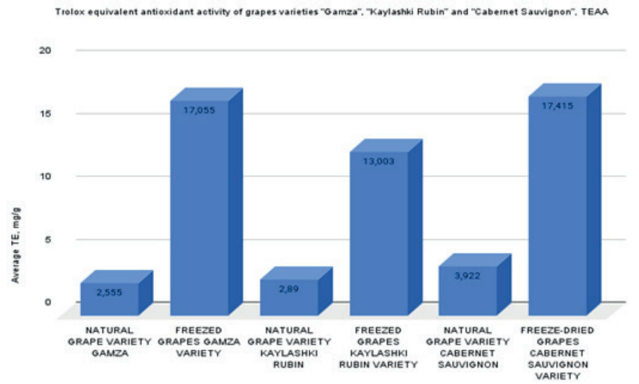

Fig. 10. Trolox equivalent antioxidant activity of grapes varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon"
Source: Author' own elaboration

"Cabernet Sauvignon" and total phenolic value with Gallic acid standard of grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon" show high values in grapes after freeze-drying (Table 3, Fig. 11). At low moisture content of grapes, antioxidant activity and total phenolic value were more concentrated, which leads to the conclusion that the amount of Trolox equivalent antioxidant activity and total phenolic value with Gallic acid standard was not disturbed during the freeze-drying process of grapes of the varieties "Gamza", "Kaylashki Rubin" and "Cabernet Sauvignon" (Dimitrov et al., 2021).

Table 3. Total phenolic value of grapes of the varieties "Gamza", "Kailashki Rubin" and "Cabernet Sauvignon"

Total phenolic value, mg/g		X
Grape variety Gamza	Natural	1.916
	Freezed	11.925
Grape variety Kaylashki Rubin	Natural	2.226
	Freezed	9.618
Grape variety Cabernet Sauvignon	Natural	2.612
	Freeze-dried	14.697

Source: Author' own elaboration

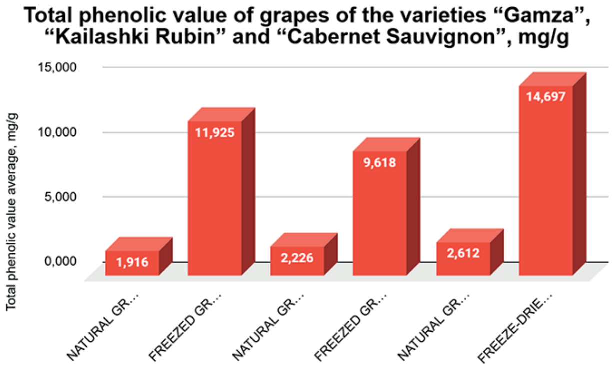

Fig. 11. Total phenolic value of grapes of the varieties “Gamza”, “Kailashki Rubin” and “Cabernet Sauvignon”, mg/g

Source: Author’ own elaboration

Conclusions

In this study, the active acidity of three grape varieties selected and grown in the Central Danube Plain was investigated, with the grapes being compared between natural and freeze-dried. After sublimation, no significant differences in the pH values were observed for the active acidity parameter. When studying and analyzing the data on the moisture content of natural and freeze-dried grapes, the same trend was observed for all three varieties, with the moisture content values of freeze-dried grapes being below 5%, which indicates that the product is suitable for freeze-drying. No significant changes were observed in the parameters determining the color characteristics of grapes in their different states.

The antioxidant activity and total phenolic value in different Bulgarian grape varieties were preserved during the freeze-drying process, which proves that it is gentle for the respective product.

Acknowledgements

This research is supported by the Bulgarian Ministry of Education and Science under the National Program “Young Scientist and Postdoctoral Students – 2”.

References

Abrasheva, P., Bambalov, K. & Georgiev, A. (2008). Vine growing and winemaking. Publ. house Matkom, Sofia (Bg).

Dimitrov, D., Yoncheva, T., Haygarov, V. & Iliev, A. (2021) Phenolic content and antioxidant activity of red grapes grom international, local and hybrid grapevine varieties grown in central northern Bulgaria. *Journal of Faculty of Food Engineering, Stefan cel Mare University of Suceava, Romania*, *XX*(2), 101 – 112.

Dinkova, R., Georgieva, M., Vasilev, K., Mihalev, K., Yoncheva, N. & Dinkov, H. (2011) Antioxidant capacity of blueberry fruits. University of Plovdiv „Paisii Hilendarski“ – Bulgaria. *Scientific papers, 38*(book 5) – chemistry, 205 – 212.

Ivanov, I., Vrancheva, R., Marchev, A., Petkova, N., Aneva, I., Denev, P., Georgiev, V. & Pavlov, A. (2014). Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. *International Journal of Current Microbiology and Applied Sciences, 3*(2), 296 – 306.

Ivanova, P., Petrova, K., Ruskova, M., Petrova, T., Mihalev, K. & Penov, N. (2015). Study of total polyphenols and antioxidant activity of beverages and fruits. *Scientific Works of University of Food Technologies, LXI*, 234 – 239.

Katerov, K., Donchev, A., Kondarev, M., Kurtev, P., Tsankov, B., Zankov, H., Getov, G. & Tsakov, D (1980). Methodology for study and description of vine cultivars and rootstocks. Bulgarian ampelography. *BAS*, 280 (Bg).

Pandeliev, S., Harizanov, A., Botyanski, P., Roychev, V. & Kemilev, S. (2005). Practical Advices on Viticulture and Winemaking. *Zemizdat Publishing*, Sofia, 14 – 18 (Bg).

Petkova, N., Ivanova, L., Filova, G., Ivanov, I. & Denev, P. (2017). Antioxidants and carbohydrate content in infusions and microwave extracts from eight medicinal plants. *Journal of Applied Pharmaceutical Science, 7*(10), 55 – 61.

Petkova, N., Vrancheva, R., Ivanov, I., Denev, P., Pavlov, A. & Aleksieva, Y. (2012). Analysis of biologically active substances in tubers of Jerusalem Artichoke (*Helianthus tuberosus L.).* 50 years FoodRDI International Scientific-Practical Conference “Food, Technologies & Health” At: 50 years FoodRDI International Scientific-Practical Conference “Food, Technologies & Health” Proceedings Book,049-054.

Petrova, K., Ivanova, P., Mihalev, K. & Georgiev, D. (2016 a). Antioxidant capacity, content of polyphenols and anthocyanins in blackberries. *Journal of Mountain Agriculture on the Balkans, 19*(2), 221 – 232.

Petrova, K., Ivanova, P., Mihalev, K. & Georgiev, D. (2016b). Study of the influence of pre-treatment of blackberries on the content of polyphenols, anthocyanins and antioxidant capacity in juices obtained from them. *Journal of Mountain Agriculture on the Balkans, 19*(3), 187 – 196.

Renaud, S. & Lorgeril, M. (1992) Wine, alcohol, plateletes, and the French paradox for coronary heart disease. *The Lancet Epidemiology, 339*, 1523 – 1526.

Stoev, K., Khershkovich, E. & Katerov, K. (1960). Grapevine behaviour to the low winter temperatures. In: K. Stoev (Editor). Repartition of viticultural districts in Bulgaria. *Proceedings of Central Research Institute of Viticulture and Enology, 3*, 45 – 54 (Bg). Zemizdat, Sofia.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. & Gargova, S. (2007). Antioxidant activity of a ginger extract (Zingiber officinale*). Food Chemistry, 102*, 764 – 770.

Received: **December, 10, 2024**; *Approved*: **January, 20, 2025**; *Published*: **April, 2026**