Changes in phenolic complex of table grapes of 'Italia' cultivar during long-term storage

Vladimir Boyko, *Svetlana* Levchenko, *Dmitriy* Belash, *Aleksandr* Romanov*, and *Sofia* Cherviak

Federal State Budget Scientific Institution All-Russian National Research Institute of Viticulture and Winemaking Magarach of the RAS, 31 Kirova Str., 298600 Yalta, Republic of Crimea, Russian Federation

Abstract. This work presents characteristics of qualitative and quantitative composition of phenolic compounds and their changes during long-term storage of 'Italia' table grapevine cultivar. The studies were carried out using method of HPLC on a Shimadzu LC 20 Prominence chromatograph with diode-array detector of ultraviolet and visible range. Grapes were sampled fresh and after long-term storage (60 days). Three groups of phenolic compounds were identified: hydroxycinnamic acids, procyanidins, and flavonols. It is established that major components of phenolic complex of 'Italia' grapes are procyanidins, specifically d-catechin and epicatechin, amounting 33% and 34%, respectively, and also procyanidin B_2 with high proportion of 19%. At the end of long-term storage, a decrease in the mass concentration of phenolic compounds was revealed, the content of hydroxycinnamic acids decreased by 62,3%, procyanidins by 50,1%, and flavonols by 51,0%. A decrease in the activity of polyphenoloxidase enzyme to 60th day of storage by 8,53%, relative to the control, was established. In the process of analysis of variance, statistically significant differences in the components of phenolic profile in grapes of 'Italia' cultivar between the beginning and end of storage were established using Student's t-test, which varied from $4{,}43*10^{-15}$ to $6{,}07*10^{-7}$, being so significantly less than 0,05.

1 Introduction

Nowadays, providing the population with functional food stuff with natural biologically active substances during a long period of time is an urgent task. Grapes for humans are a valuable food product, which contains a large amount of biologically active substances, including phenolic compounds [1].

A characteristic feature of grape plant cell is the formation of phenolic compounds containing in their molecule an aromatic (benzene) ring, which carries one, two or more hydroxyl groups. Phenolic compounds are natural metabolites synthesized in all plant cells with high antioxidant activity; they are widely used in pharmacology, medicine, and food industry [2–4].

* Corresponding author: cod7-4orever@mail.ru

Based on the carbon skeleton, the following classes of compounds are distinguished: hydroxybenzoic acids, flavonols, flavanons, anthocyanidins, catechins, tannins, coumarins, lignans, and others. By structure, phenolic substances are distinguished: monomeric, oligomeric (ellagic acid) and polymeric (tanning substances, lignans and melanins). Polyphenols are secondary metabolites of plants, involved in defense reactions of pathogens. Flavonoids are a large group of low-molecular-weight compounds with high antioxidant properties. Anthocyanins are responsible for the color of grapes. Flavan-3-ols (monomeric catechins and proanthocyanidins) impact flavor astringency. Flavonols (quercentin glucosides and related compounds) are accumulated in the skin during berry growth and protect them from ultraviolet radiation [5,6].

Great interest of scientists is given to antioxidant activity of phenolic compounds. Antioxidant is understood to mean any substance which, when present in low concentrations compared to the concentration of oxidizable substrate, significantly delays or inhibits its free-radical oxidation. Procyanidins, as a part of phenolic complex, have an antioxidant effect and neutralize free, hydroxyl radicals and superoxide anion-radicals. They also promote the synthesis of arachidonic and phosphoric acids, which protect lipids from oxidation. Procyanidins are potent metal chelating agents that chelate metal ions with formation of inert compounds in the organism; promote the absorption of vitamin C [7-11].

The composition of phenolic compounds in grape pomace was studied by Russian scientists. Phenolic compounds were identified in skin and seed extracts. It was found that anthocyanins were present mainly in the skin. In seeds of 'Saperavi' grape cultivar, low concentrations of ten components of anthocyanin complex were revealed. The maximum amount of anthocyanins was found in the skin. The concentration of quercetin in skin-seed mixtures, especially in 'Roesler' grapes, is 1.5-2.0 times higher than in the skin itself. The maximum amount of flavan-3-ols, hydroxycinnamic and hydroxy-benzoic acids and oligomeric procyanidins, as well as the highest antioxidant activity, was registered in skin-seed mixture. The studied samples of grape seeds were dominated by procyanidins of groups B_1, B_2, and B_3, characterized by high antioxidant activity [12].

In Portugal, studies on changes in the composition of phenolic complex of strawberries under long-term storage conditions (90 days) were carried out. Antioxidant activity, number of total phenols, anthocyanins and individual phenolic compounds were evaluated before and after pasteurization under storage conditions. The results showed that pasteurization did not affect concentration of total phenols or total anthocyanins, but significantly reduced the concentrations of quercetin-3-rutinoside, kaempferol and cyanidin-3-glucoside, as well as increased concentrations of (+)-catechin, (-)-epicatechin, epigallocatechin gallate, quercetin-3-galactoside and ellagic acid [13].

One of the main enzymes affecting the content of phenolic compounds in plant cell is a polyphenoloxidase, which catalyzes enzymatic browning reactions in most of fruits and oxidation of o-diphenols and o-quinones, followed by non-enzymatic formation of melanin by polymerization [14].

This enzyme is the most active of those contained in grape berries. It is able to catalyze the oxidation process of not only catechins, but also of other phenolic compounds containing 1-2 and 1-3-hydroxyl groups (OH), pyrocatechin and pyrogallol, as well as the NH_2 group (aromatic amino acids and amines). Polyphenoloxidase is a protein whose prosthetic group contains copper from 0,2 to 0,3%. D. Cartez showed that copper in polyphenoloxidase is in the reduced form both during the enzymatic reaction and quiescence. However, other authors believe that the main action of polyphenoloxidase is the reversible oxidation of monovalent Cu^+ atom to divalent Cu^{2+}. The oxidative action of enzyme consists in dehydration of ortho-diphenols with the formation of ortho-quinone forms. A characteristic feature of polyphenoloxidase is the ability to catalyze oxidation

reactions of diphenols and hydroxylation of monophenols. This enzymatic system is one of the main oxidative systems catalyzing the final stage of oxybiotic process [15, 16].

On the basis of the foregoing, the purpose of our work was to study quantitative and qualitative composition of phenolic compounds and activity of the main redox polyphenoloxidase enzyme during long-term storage using the example of 'Italia' grapevine cultivar.

2 Materials and methods

2.1 Characteristics of the studied grapevine cultivar and research site

The **'**Italia' ('Bicane' x 'Muscat Hamburg') is a table grapevine cultivar of late ripening, by Alberto Pirovano selection. The bunch is large, cylindrical and loose. The berry is large, oval, ovoid, yellowish-amber, matte, covered with a thick purine coating. The skin is strong, thick. The pulp is fleshy with muscat-citron aroma. Bushes are vigorous. Mildew resistance is medium.

Experimental studies were carried out during 2020-2021 on the basis of Morskoye branch of FSUE PJSC Massandra, located in the mountain-valley region of the coastal viticultural zone of the Republic of Crimea and the Grape Storage Laboratory of the FSBSI Institute Magarach of the RAS. Grape culture training system is open-earth. Landing pattern is 3,0 x 1,5 m.

2.2. Scheme and methods for managing experimental studies

Fresh grapes were stored at a temperature of 0±2°C for 60 days. The production technology of storage in industrial freezer with sulfur dioxide regular treatment was used. Sampling for research was carried out on the day of harvest (day 0) and on the 60th day of storage.

Identification of phenolic compounds was carried out on a Shimadzu LC 20 Prominence chromatograph with diode-array detector of ultraviolet and visible range. To separate the components of phenolic complex, a Nucleosil C18 AB column (Macherey-Nagel, Germany), 250 mm long, 2 mm in diameter, and a pore size of 100 Å was used. Elution was carried out in a gradient mode of increasing the proportion of solution B (a mixture of AcCN:MeOH:H_2O in a ratio of 40:40:20, pH 2,5) in a mixture with solution A (an aqueous solution of $HClO_4$, pH 1.8) for 80 min. Sample volume was 2 µl, detection at 280 nm, 310 nm, 330 nm, 525 nm with a scanning frequency of 3 Hz. Identification was carried out by spectral characteristics and void times in accordance with standard samples [17, 18].

Determining of polyphenoloxidase was carried out according to the following method. Experimental must was diluted tenfold, 1 ml of must and 9 ml of buffer solution pH 7,4. Must solution in the amount of 2 cm^3 in buffer solution, 4 cm^3 of distilled water, 2 cm^3 of diethyl paraphenylene diamine sulfate are poured in to the level of 2 cm to a measuring cell. Measuring cell is installed in a KFK-3-01 photoelectric colorimeter, after which 2 cm^3 of pyrocatechin solution is added to the cell, seconds timer is turned on, a cover of measuring cell chamber of the device is closed, and the optical density measurement mode is switched on. The seconds timer is stopped when passing the interval of optical density of 0,150 B.

Enzyme activity is calculated by the formula:

$$A = (\mathbf{E} * \mathbf{a} * \mathbf{b}) / (\mathbf{c} * T) \quad (1)$$

E – optical density change interval = 0,150; a – must dilution; b – degree of constant dilution in the reaction mix (in the measuring cell); c – layer thickness in the measuring cell = 2 cm; T – time in seconds [19].

Analysis of variance was used to determine significant differences in phenolic complex between differentiating groups during long-term storage. The results were analyzed using paired Student's t-test (at $t<0.05$ "significant effect") in the SPSS Statistics 17.0 program.

3 Results and discussion

As a result of analysis of the data obtained (Table 1), it was found that the largest amount of procyanidins and hydroxycinnamic acids was concentrated in seeds of 'Italia' grapes, in fresh berries these parameters amounted to 5255,6 mg/kg and 420,6 mg/kg. The maximum concentration of flavonols was recorded in the skin of grapes and amounted to 122,4 mg/kg.

Table 1. Changes in the component composition of phenolic complex of 'Italia' grapevine cultivar during storage.

Phenolic profile components	Structural particles of the berry	Mass fraction, mg/kg		Student's t-test
		Fresh grapes	End of storage	
Hydroxycinnamic acids	Seed	420,6	137,3	$4,43*10^{-15}$
	Skin	28,8	11,8	$9,19*10^{-7}$
	Flesh	28,3	9,3	$6,07*10^{-7}$
Procyanidins	Seed	5255,6	2527,0	$9,79*10^{-15}$
	Skin	356,3	295,5	$2,83*10^{-11}$
	Flesh	177,9	65,0	$4,48*10^{-11}$
Flavonols	Seed	99,0	75,2	$3,58*10^{-10}$
	Skin	122,4	36,4	$6,17*10^{-12}$
	Flesh	16,2	4,7	$1,12*10^{-8}$

It is noted that 'Italia' grapevine cultivar is characterized by instability in concentrations of phenolic complex components, in which the mass concentration of hydroxycinnamic acids, by the 60th day of storage, decreases by 62.3%, and procyanidins and flavonols - by 50,1% and 51,0%, respectively. This is due to the processes of oxidation and polymerization during long-term storage of grapes.

As a result of the research, it was found that procyanidin group substances in grape berries are mainly represented by epicatechin and d-catechin (Fig. 1).

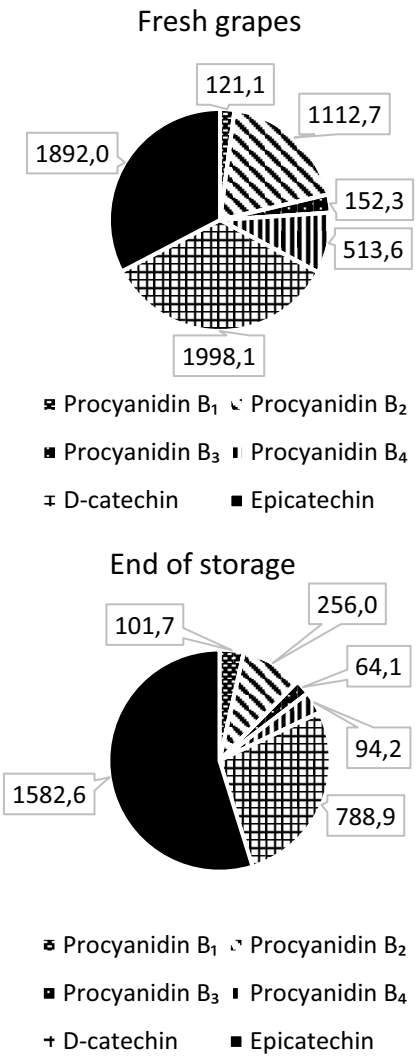

Fig. 1. Dynamics of changes in procyanidins in grape berries of 'Italia' cultivar during long-term storage.

The proportion of epicatechin in fresh berries was 33%, and d-catechin - 34% of the content of all procyanidins in 'Italia' grapes. Also, a high concentration was observed for procyanidin B_2 and amounted to 1112,7 mg/kg or 19%. During long-term storage, a decrease in the concentration of procyanidins was noted. However, at the end of storage, an increase in the concentration of epicatechin in grape berries was registered, and amounted to 55% of the content of all substances.

Among hydroxycinnamic acids, gallic and caftaric acids were identified; among flavonols – quercetin-3-o-glucoside and quercetin (Fig. 2).

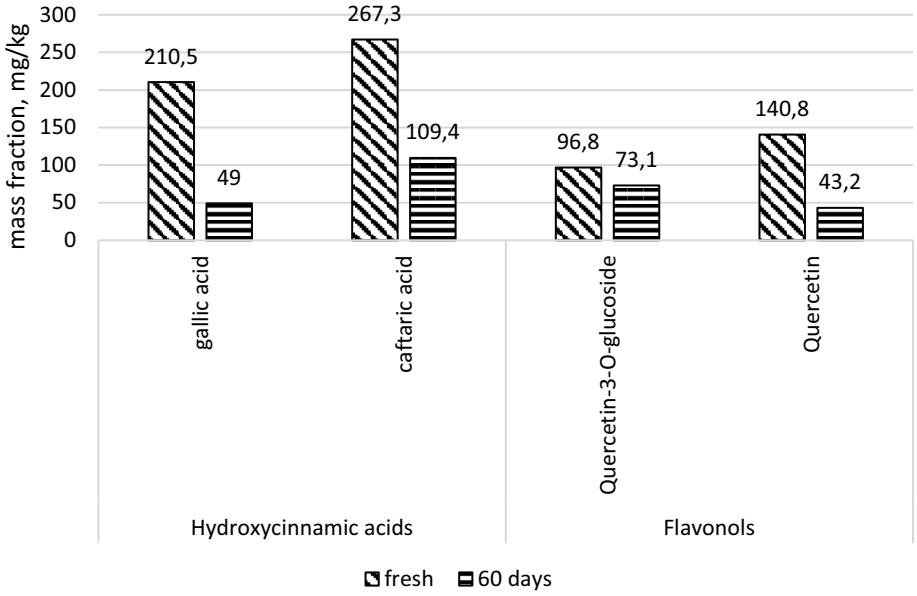

Fig. 2. Dynamics of changes in hydroxycinnamic acids and flavonols in grape berries of 'Italia' cultivar during long-term storage.

The mass concentration of gallic acid to the end of storage decreased by 76,7%, of caftaric acid - by 62,4%. Among flavonols, the mass concentration of quercetin-3-o-glucoside and quercetin decreased by 24,5% and 69,3%, respectively.

In the process of analysis of variance, statistically significant differences in the components of phenolic profile in grape berries of 'Italia' cultivar between the beginning and end of storage were established using Student's t-test, which varied from $4,43*10^{-15}$ to $6,07*10^{-7}$, so that is significantly less than 0,05.

The activity of polyphenoloxidase as the main redox enzyme of grapes was studied. In fresh grapes, the activity of this enzyme was 0,082 c.u./sec. (Fig. 3).

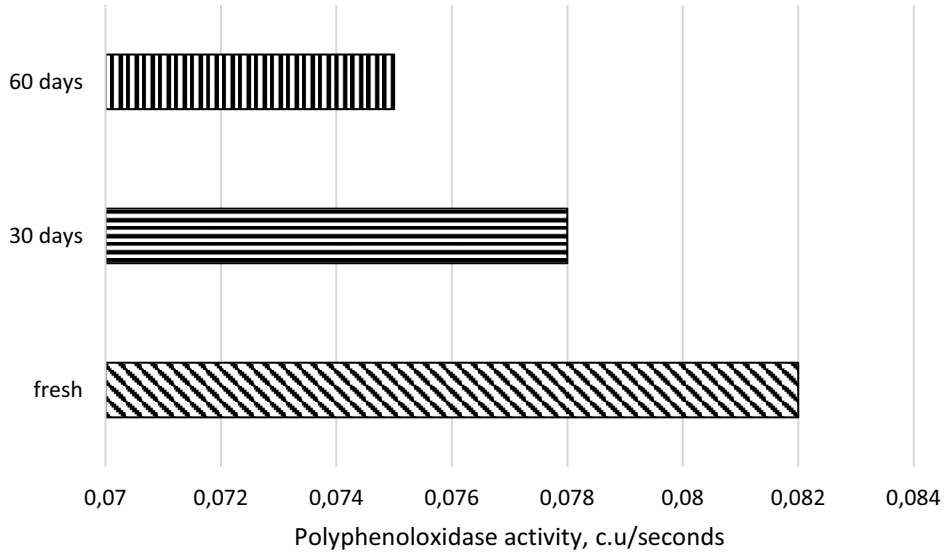

Fig. 3. Dynamics of changes in the activity of polyphenoloxidase in grape berries of 'Italia' cultivar during long-term storage.

After 30 days of storage, the activity of polyphenoloxidase decreased to a value of 0,078 c.u./sec, which is 4,88% lower than the control value. By the end of storage (60 days), the activity of enzyme was 0,075 c.u./sec, reaching an activity decrease by 8,54%.

4 Conclusion

This study made it possible to characterize changes in the composition of phenolic complex of 'Italia' table grapevrine cultivar during long-term storage. Three groups of compounds were identified: hydroxycinnamic acids, procyanidins, and flavonols. It was established that the main components of phenolic complex of 'Italia' grapevine cultivar are procyanidins, namely d-catechin and epicatechin, amounting to 33% and 34%, respectively, and also procyanidin B_2 with high proportion of 19%. General decrease in the mass concentration of phenolic compounds after 60 days of storage was revealed: oxycinnamic compounds by 62,3%, procyanidins by 50,1%, and flavonols by 51,0%. The activity of polyphenoloxidase reached a decrease of 4,88% by 30 days, and 8,54% by 60 days of storage.

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