

# COMPARISON OF THE PHENOLIC COMPLEX OF CRIMEAN AUTOCHTHONOUS AND CLASSIC WHITE-BERRY GRAPE CULTIVARS

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**Abstract.** The profile of the phenolic components and features at the beginning of ripening (12.0 - 14.5 °Bx) and at the end of the observation (20.0-22.0 °Bx) of Crimean autochthonous white-berry grape cultivars are studied. The total content of identified phenolic compounds at the beginning of ripening differed depending on the cultivar from 669 mg kg<sup>-1</sup> (Sauvignon Blanc) to 2411 mg kg<sup>-1</sup> (Kokur Belyi). During the ripening period the content of components in berries of autochthonous grape cultivars decreased on average by 3.5 times of initial values (Kokur Belyi – by 4.1 times). In classic cultivars, this parameter did not change. The lowest concentration of phenolic components was observed in Shabash (256 mg kg<sup>-1</sup>) at 20.0-22.0 °Bx, the highest - in Riesling (1006 mg kg<sup>-1</sup>) and Chardonnay (827 mg kg<sup>-1</sup>). The hierarchical analysis of a cluster on a subject of total phenolic components revealed the similarity of autochthone Kokur Belyi to classic cultivars Chardonnay and Sauvignon Blanc. Autochthonous grape cultivars Sary Pandas and Shabash are closely related and form a separate cluster.

## 1 Introduction

Autochthonous grape cultivars are of a particular interest to winegrowers and winemakers of the world, as they are unique and adapted to certain soil and climatic conditions of a particular locality of production and have a set of valuable features. These cultivars are formed because of natural evolution and fix in the genome the signs and properties necessary for the current generation of industrially cultivated grape cultivars [1]. About 80 autochthonous grape cultivars are differentiated in Crimea [2]. In general, almost all Crimean wine grape cultivars meet the requirements of winemaking, but only 10-12 cultivars are used in the industrial culture. The most famous cultivars are Kokur Belyi, Tashly, Shabash, Kefessia, Ekim Kara, Sary Pandas, Kok Pandas, Kapselski Belyi, Solnechnodolinskii, etc. In Crimea autochthonous grape cultivars are also used in the production of vintage strong dessert wines and “madeira” wines. Currently, most of industrial vineyards with autochthonous cultivars are located in the Sudak region of the East Piedmont zone of Crimea [1]. More than half of the Crimean wine autochthones are white-berry varieties according to their structure [3].

A growing interest in expanding the use of Crimean autochthonous varieties in winemaking of the region has a number of reasons. Firstly, the modern concept of wines quality is based on their authenticity, the uniqueness of the organoleptic characteristics due to the certain terroir and cultivar specificity. Secondly, all Crimean autochthonous grape cultivars are distinguished by their growing and fruit-bearing ability on heavy clay

soils characterized by strong chloride-sulfate salinization. They increased an adaptive resistance to the climatic conditions of the historical habitat (Solnechnaya Dolina village), which is almost a semi-desert zone, with an average annual air temperature of 13.2 °C, the sum of active air temperatures (above 10 °C) - 3635-3820 °C, the number of days with temperatures above 10 °C - 186-202, the annual rainfall - 300-400 mm [4]. Adaptive resistance of grape cultivars to adverse environmental conditions is largely due to biosynthesis and enzymatic transformation of phenolic compounds.

Biosynthesis of specific phenolic components under stressful conditions initiates either stimulation or inhibition of the process. High level of insolation of plants, deficiency of moisture or nitrogen in the soil can lead to an increase in the concentration of flavonoids in berries; the lack of phosphate and low temperatures - to the accumulation of anthocyanins, high temperatures - to the accumulation of flavonols [5-7]. Phenolic compounds of grapes play an important role in shaping of the quality of wines, their adequacy to the physiological needs of humans [8-12]. The phenolic profile of grapes, as well as its variability under the influence of natural and anthropogenic factors, is determined by the species and varietal affiliation of grapes [13-19]. So, the quantitative content and qualitative composition of the phenolic complex of berries of Crimean autochthonous grape cultivars are the important parameters for assessing the effectiveness of their using in winemaking.

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## 2 Materials and methods

### 2.1 Terms of grape growing and the selection of samples

Grape cultivars of ampelographic collection of Institute "Magarach" were analysed in this study (*Vitis vinifera* L.): Crimean autochthonous white-berry cultivars – Kokur Belyi, Shabash, Sary Pandas and classic ones – Chardonnay, Riesling, Sauvignon Blanc. Cultivars Kokur Belyi, Shabash, Sary Pandas belong to the species *Vitis vinifera* and the origin of native grape cultivars of Crimea [2]. The choice of Chardonnay, Riesling, Sauvignon Blanc as control cultivars is explained to their wide distribution in the world; grapes of these cultivars give wine of high quality everywhere.

The ampelographic collection is located in the western piedmont-coastal region of the Crimea (v. Vilino, Bakhchisaray district). Planting scheme of grapevine cultivars is 1.5 x 3 m., forming scheme is 2-branch cordon. Grape plants were grafted to the rootstock Kober 5BB. Load of the bushes was 40-60 buds. Agricultural technology system of the ampelographic collection is in accordance with the technological map adopted for each cultivar in the area. Ten bushes represented each cultivar. Samples of berries (only healthy) were collected at different ripening stages of grapes: August 18-19 and September 10-11, 2016. For each cultivar and for the monitoring point, two samples (from odd and even bushes) weighing about 1000 g each, were selected. The berries were cut off at three levels of bunches, located on the shady, sunny, upper, middle and lower parts of the bush. Samples were transported immediately under refrigeration (2-5°C) to the laboratory for analysis.

**Sample preparation and chemical analysis of grapes.** To determine the content of sugars and titrated acids, 300-350 g of the berries of each sample were crushed in a turbo blender (Moulinex-LM600E, France) during 2 min. The mixture was centrifuged at 5000 rpm (Sigma 4K10 Braun, Germany) for 15 min at room indoor temperature to get a cleansed juice. Then the juice was filtered through 0.45 µm pore size membrane filters and incubated for analysis at 20°C. Total sugars were determined by using areometric method, the titratable acids – by titrimetric method recounted for tartaric acid.

### 2.2 Sample preparation and phenolic complex analysis by HPLC method

To analyze the phenolic complex of berries by HPLC according to the procedure proposed, the berries of each sample were separated manually into skin, pulp and seeds. Each structural component of the berry was weighed and reduced in a mortar to a homogeneous mass. Then, the homogenates of the structural components of the berries were poured into the extract (50% methanol solution to 0.5% aqueous hydrochloric acid) in a ratio of 1:4 and kept in refrigerator at 3-5°C for 5 days. The extracts were centrifuged at 3000 rpm for 5

min; the supernatant was used for the component analysis of the phenol complex by HPLC method.

The study of the phenolic complex of grape berries was carried out on the HPLC system Shimadzu LC20 Prominence (Japan), equipped with a flow degasser, a microplunger pump with a low pressure gradient module, an autosampler, a column thermostat and a spectrophotometric detector with a diode array of the ultraviolet and visible ranges. Nucleosil C18AB (Macherey-Nagel, Germany) column was used to separate the phenolic components by a length of 250 mm, 2.0 mm in diameter, filled with a reversed-phase sorbent with a particle size of 5 µm and a porosity of 100 Å. The injected sample volume was 2 µL. The elution was carried out in a gradient mode of increasing the proportion of solution B (mixture of AcCN: MeOH: HClO<sub>4</sub> in a ratio of 40:40:20, pH 2.5) in a mixture with solution A (aqueous solution of HClO<sub>4</sub>, pH 1.8) for 80 minutes. Chromatograms were recorded with a scanning rate of 3 Hz at the following wavelengths: 280 nm for gallic acid, (+) - D-catechin, (-)-epicatechin and procyanidins; 310 nm - for hydroxycinnamic acids; 330 nm - for derivatives of quercetin; 525 nm - for anthocyanins [20, 21].

### 2.3 Statistical analysis of the data

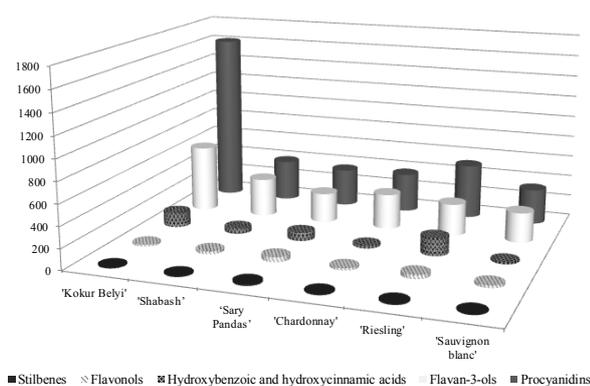
The total number of experimental samples of grape cultivar was 24: 12 samples at each monitoring point. All chemical and HPLC analyses were performed in triplicate.

The experimental material was analyzed using the methods of variation statistics, hierarchical classification. Thus, arithmetic mean value, variance of a single result and standard deviation (SD) were determined. The tables and figures of this article show the arithmetic mean values of the mass concentration of components in berries for each grape cultivar and for each monitoring point; standard deviation was not more than 3% of the mean values of the content of sugars and titrated acids and no more than 8% of the components of the phenolic complex. Statistically significant differences in the phenolic complex of differential cultivars or groups of cultivars were identified based on the Mann-Whitney (U-test) criterion. The predetermined probability of an erroneous result (p) was less than 0.05. The text of the article indicates the values of p for which the U-test results are less critical and differences in the parameters between the samples studied are recognized as significant. In the discussion of the results of the hierarchical cluster analysis of the experimental data Euclidean distances (Ed) are indicated in brackets. The data was processed mathematically with the help of the statistical software package SPSS Statistics.

## 3 Results and discussion

Using the HPLC analysis in the berries of the tested cultivars, monomeric and dimeric phenolic compounds of the flavonoid and non-flavonoid structure were identified.

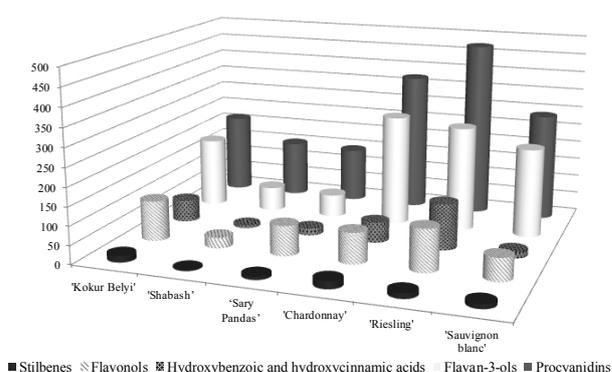
Figures 1-2 show the content of phenolic components at the beginning of ripening (12.0 - 14.5 °Bx) and at the end of the observation (20.0-22.0 °Bx). The total content of identified phenolic compounds at the beginning of ripening ranged depending on the cultivar from 669 mg kg<sup>-1</sup> (Sauvignon Blanc) to 2411 mg kg<sup>-1</sup> (Kokur Belyi). During ripening of grapes, the content of components in the berries of autochthonous cultivars decreased from the initial values on average by 3.5 times (Kokur Belyi – by 4.1 times), in classic ones remained at baseline. At 20.0-22.0 °Bx the lowest concentration of phenolic components was noted in Shabash (256 mg kg<sup>-1</sup>), the largest in Riesling (1006 mg kg<sup>-1</sup>) and Chardonnay (827 mg kg<sup>-1</sup>).



**Fig. 1.** The content of phenolic components at the beginning of ripening (12.0 - 14.5 °Brix).

The monomeric ((+) - D-catechin and (-) - epicatechin) and dimeric (procyanidins B1-B8) forms of flavanols were identified in the berries of the investigated cultivars. The initial concentration of flavan-3-ol monomers in autochthonous grape cultivars varied from 281.0 ('Sary Pandas') to 632.0 ('Kokur Belyi') mg kg<sup>-1</sup>. Their content in classic cultivars was 1.4 times less and ranged from 285.0 to 334.0 mg kg<sup>-1</sup>. During ripening the concentration of flavan-3-ol monomers decreased ( $p < 0.05$ ) by 4.1 times to 60.6-186.8 mg kg<sup>-1</sup>. In classic grape cultivars the concentration of components decreased – only by 11%.

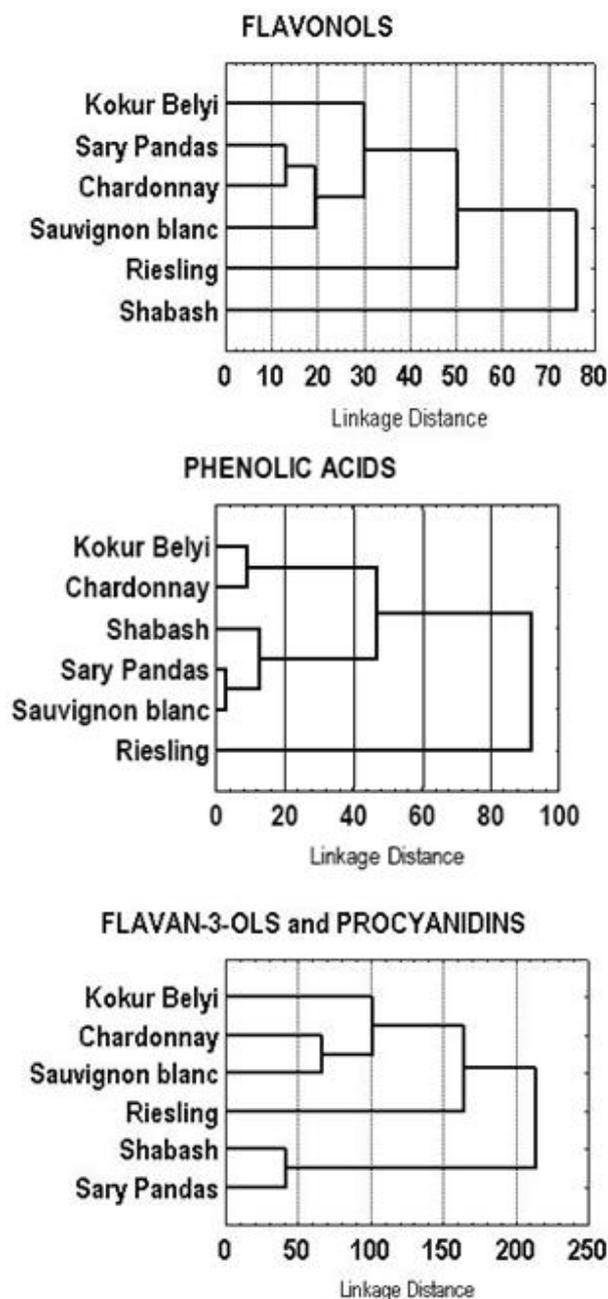
The highest content of procyanidins was distinguished by Kokur Belyi (1647.7 mg kg<sup>-1</sup>), in other cultivars the concentration of components ranged from 335.6 to 518.6 mg kg<sup>-1</sup>. During ripening, the concentration of components decreased ( $p < 0.05$ ) in Kokur Belyi by 7.5 times, in other autochthonous grape cultivars – by 2.5-2.6 times: to 146.4-214.8 mg kg<sup>-1</sup>. In classic grape cultivars the concentration of components decreased only by 7%. It was noted that the percentage of B2 and B3 in the procyanidins complex was the largest and amounted total 40-46% at the beginning of ripening and 35-48% at the end of the period observed.



**Fig. 2.** The content of phenolic components at the end of the observation (20.0-22.0 °Brix).

The ripening of grapes is accompanied by oxidative polymerization of flavan-3-ol monomers and procyanidins, forming high-molecular tannins, localized in seeds [22]. Experimental data indicated more intensive dynamics of flavanols complex in autochthonous grape cultivars in comparison with classic ones. In general, at 20.0-22.0 °Bx cultivars Sary Pandas and Shabash form a separate cluster of the complex of monomeric and dimeric flavanols, as they are similar to each other ( $Ed = 42$ ) and significantly differ from other classic grape cultivars ( $Ed = 148-214$ ). The autochthonous grape cultivar Kokur Belyi is the closest to Sauvignon Blanc at the complex of these components with Euclidean distances - 73 (Figure 3).

The initial summary content of quercetin, kaempferol and their derivatives in autochthonous and classic grape cultivars rated from 17.1 (Kokur Belyi) to 45.7 (Sary Pandas) mg kg<sup>-1</sup>. The flavonols complex was represented by 77-96% of quercetin-3-O-β-D-glucuronide and 1-19 % of quercetin-3-O-β-D-glycoside. During ripening, the content of flavonols and their derivatives increased by 2.3 times in autochthonous grape cultivars (Kokur Belyi – by 6.4 times); by 3.2 times - in classic ones. In general, at 20.0-22.0 °Brix the total content of quercetin, kaempferol and their derivatives in autochthonous grape cultivars reached concentration of 28.9 mg kg<sup>-1</sup> in Shabash, 80.8 mg kg<sup>-1</sup> in Sary Pandas and 107.9 mg kg<sup>-1</sup> in Kokur Belyi and in classic grape cultivars 61.7, 83.2, 113.8 mg kg<sup>-1</sup> in - Sauvignon Blanc, Chardonnay and Riesling respectively. Sary Pandas and Chardonnay are the most similar by the flavonols complex ( $Ed=13.0$ ), and Kokur Belyi is the closest to this group ( $Ed=16.7$ ). Shabash was significantly different from the rest of the classic and autochthonous cultivars with Euclidean distances 32.5-76.0 (Figure 3).



**Fig. 3.** The results of the cluster analysis of different white grape cultivars based on the phenolic complex of berries.

At the beginning of ripening (12.0-14.5 °Bx) in autochthonous grape cultivars the content of gallic acid was 3.5-4.4 mg kg<sup>-1</sup>, hydroxycinnamic acids – 47.8-134.3 mg kg<sup>-1</sup>. In classic cultivars content of gallic acid varied from 0.0 to 5.6 mg kg<sup>-1</sup>, hydroxycinnamic acids – from 11.1 to 170.0 mg kg<sup>-1</sup>. Among hydroxycinnamic acids, the rate of 41-87% consisted of a caftaric acid at the beginning and at the end of the observations. During ripening, the content of phenolic acids in autochthonous cultivars decreased by 3.3 times to 7.7-59.2 mg kg<sup>-1</sup>, in classic ones – by 7%. In plant cell the phenolic acids are the initial materials in the biosynthesis of secondary metabolites and are primarily subjected to enzymatic oxidation [23, 24]. These results indicate a more active participation of hydroxybenzoic and hydroxycinnamic acids in the metabolism of the autochthonous grape

cultivars. The maximum concentration of phenolic acids in autochthonous grape cultivars was noted in Kokur Belyi, amongst classic ones – in Riesling at the beginning and at the end of the observations; the minimum concentration – in Shabash and Chardonnay, respectively. At 20.0-22.0 °Bx content of phenolic acids in Shabash was almost 7 times less than in other cultivars, and amounted to 7.7±0.5 mg kg<sup>-1</sup> [24, 25]. It showed that the caffeic and gallic acids, exhibiting antioxidant properties, are able to inhibit the oxidative transformation of the aroma-forming components of the wines. At the end of the observation, the total concentration of these acids in Riesling was 19.1 mg kg<sup>-1</sup> that exceeded by 4.5 times (p < 0.004) other cultivars.

The hierarchical cluster analysis of the experimental data (Figure 3) revealed the similarity of the complex phenolic acids of cultivars Sary Pandas and Shabash (Ed = 11.6) and was close by the above parameters to Sauvignon Blanc (Ed = 2.8-12.4). Cultivar Kokur Belyi was the closest in the complex of phenolic acids to Chardonnay (Ed = 8.9).

Among the compounds of stilbenoid series in berries, the trans-resveratrol and its glycosylated form, piceid were identified. At the beginning of the ripening the total concentration of stilbenes in autochthonous and classic grape cultivars ranged from 8.9 (Sauvignon Blanc) to 18.0 (Sary Pandas) mg kg<sup>-1</sup>. The percentage of piceid in stilbene complex varied from 77% to 98%. During ripening the concentration of stilbenes in Sary Pandas, Shabash decreased by 2.2-3.7 times. On the contrary, the content of the components increased by 1.9 times in Kokur Belyi and Chardonnay and its values amounted from 17.7 to 20.2 mg kg<sup>-1</sup>. At 20.0-22.0 °Bx the piceid content decreased to 39% in Kokur Belyi. The ratio of trans-resveratrol and piceid did not change in other cultivars.

#### 4 Conclusion

The profile of the phenolic components of the Crimean autochthonous white-berry grape was changing and its features at the beginning of ripening (12.0 - 14.5 °Bx) and at the end of the observation (20.0-22.0 °Bx) were studied. The total content of identified phenolic compounds at the beginning of ripening varied depending on the cultivar from 669 mg kg<sup>-1</sup> (Sauvignon Blanc) to 2411 mg kg<sup>-1</sup> (Kokur Belyi). During ripening of grapes the content of the components in grapes of autochthonous cultivars decreased from the initial values on average by 3.5 times (Kokur Belyi – by 4.1 times), in classic ones it remained at baseline. At 20.0-22.0 °Bx the lowest concentration of phenolic components was observed in Shabash (256 mg kg<sup>-1</sup>), the highest - in Riesling (1006 mg kg<sup>-1</sup>) and Chardonnay (827 mg kg<sup>-1</sup>). The hierarchical cluster analysis of the total phenolic components revealed the similarity of autochthonous cultivar Kokur Belyi to classic ones Chardonnay and Sauvignon Blanc. Autochthonous grapevine cultivars Sary Pandas and Shabash formed a separate cluster of relatively close cultivars.

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