Study of physic-chemical and biochemical parameters of technical varieties of grapes

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Abstract. The authors studied introduced (Aligote, Rkatsiteli, White Muscat, Riesling Rhine), indigenous (Kokur white, Sabbat) and classified varieties of grapes of Magarach Institute (first-born Magarach, Citron Magaracha, Muscat aligote), growing in Crimea, according to physicochemical and biochemical indicators. The feature of grape aromatic varieties is the high content of phenolic substances in the must (404–640 mg/l), the activity of monophenol-monooxygenase, macerating ability. Indigenous grape varieties are characterized by a low content of titratable acids. The activity of monophenol-monooxygenase is interconnected with the content of phenolic substances in the must (r = 0.77). The ratio of tartaric and malic acids increases in the series introduced → indigenous → classified varieties. The values of the glucose-fructose index in the samples are 0.84–1.00. The obtained patterns make it possible to regulate the course of redox processes; to optimize the regimes and parameters of grape processing in the production of different types of wines.

1. Introduction
The assortment of Crimean grapes is represented mainly by introduced varieties of the Western European group (Cabernet Sauvignon, Chardonnay, Merlot, Riesling, etc.) and the Black Sea basin (Rkatsiteli, Saperavi, etc.) [1, 2]. Introduced varieties are varieties introduced into the culture by importing them from different regions of viticulture to regions where they were not previously cultivated, which is a quick and effective method of replenishing and improving the variety of vineyards [3]. White industrial varieties occupy 50.2 % of the area of vineyards of Crimea [1]. However, the introduction method is effective only if the requirements for climatic, soil and other conditions for their growth are met.

Crimean indigenous varieties Kokur white and Shabash occupy a total area of 5.7 %, the proportion of classified varieties is 4 % of the area of vine plantations. A part of the Crimean indigenous (autochthonous) grape varieties is characterized by low resistance to biotic and abiotic environmental factors, which does not ensure the constancy of the quality of unique wines of geographical status taking into the changes in climatic factors [4, 5].

The introduction into production of grape varieties – the analogues of the Crimean indigenous with genetically determined conjugation of qualitative and quantitative traits in combination with resistance to environmental stress factors is an urgent task for the wine industry [6]. The classified department of Magarach Institute selected high-yielding grapes with a nutmeg bouquet and increased resistance to diseases: Citron Magaracha, Riesling muscat, Aligote muscat [7]. The first-born of Magarach and the Gift of Magarach are characterized by consistently high productivity and increased resistance to
adverse environmental factors – low temperatures and the root form of phylloxera [8]. According to a number of indicators, grape varieties of “Magarach” Institute of selection are not inferior, and in some cases superior to traditional ones [7]. The variety of Crimean grape necessitates their technological assessment. In order to determine the direction of the use of grapes, there are complex criteria – gluccacidimetric index (GAI) and operational reliability index (ORI) [9], based on the ratios of the components of the carbohydrate-acid complex and pH. The methodology proposed by E. Ostroukhova et al. [9], takes into account the peculiarities of biochemical processes that occur during the processing of grapes in different directions: table (with natural ethyl alcohol) or liquor wines. In order to characterize grapes, a glucose-fructose index (GFI) is used, which characterizes the ratio of hexoses to grape berry. When fructose predominates in fresh must, there may be risks of the interruption of fermentation process [10].

The purpose of the research is to study the physicochemical and biochemical characteristics of grapes growing in the Crimea.

The object of research is presented by the grapes of introduced white varieties (Aligote, Rkatsiteli, White Muscat), indigenous (Kokur white, Shabash), classified of Magarach Institute (firstborn of Magarach, Citron Magaracha, Aligote muscat) growing in Crimea (Table 1). The authors studied 156 batches of grapes of 9 varieties during 2010–2019.

**Table 1. Characteristics of grape varieties [2, 7, 11]**

| Variety       | Description                                                                 |
|---------------|------------------------------------------------------------------------------|
| Introduced    |                                                                              |
| Aligote       | French mid-ripening variety. It belongs to the ecological-geographical group of West European varieties. It is used to produce table and fizzy wines, brandy. |
| Rkatsiteli    | Georgian variety of medium late ripening. According to morphological characteristics and biological properties, it belongs to the ecological-geographical group of grape varieties of the Black Sea basin. It is used for the production of table and liquor wines, brandy. |
| Nutmeg white  | One of the oldest varieties of the middle ripening period, related to the ecological and geographical group of eastern varieties. It has a high sugar accumulation (30 % and higher) with a slow decrease in acidity. It is used for the production of table, liqueur and fizzy wines. |
| Riesling Rhine | German variety with medium maturity. According to morphological characteristics and biological properties, it belongs to the ecological-geographical group of West European varieties. It is used to produce table, fizzy wine, juice. |
| Indigenous    |                                                                              |
| Kokur white   | According to morphological characteristics and biological properties, it belongs to the ecological-geographical group of grape varieties of the Black Sea basin. Medium ripening. Winter hardness is insufficient. It is used to produce table, fizzy, liquor wines, brandy. |
| Shabash       | According to morphological characteristics and biological properties, it belongs to the group of oriental table and wine varieties. Very late ripening. It is sensitive to winter frosts. It is recommended to produce liquor wines. |
| Classified    |                                                                              |
| Firstborn of  | It is obtained by crossing the variety Rkatsiteli and the hybrid form Magarach No. 2-57-72. Mid-term ripening. It has high frost resistance. It is used for the preparation of table and dessert wines. |
| Magarach      |                                                                              |
| Citron        | It is obtained by crossing Madeleine Anzhevin x “Magarach 124-66-26 (Rkatsiteli x Magarach 2-57-72) + Novoukrainsky early”. Early to mid-term ripening. It has high frost resistance and disease resistance. It is recommended to produce liquor wines. |
| Magaracha Muscat | It is obtained by crossing varieties of Aligote x Muscat white. Early ripening. It has high drought resistance. It is used for the preparation of table and fizzy wines. |

The physic-chemical parameters of grapes were determined in fresh must: the average sample of berries, previously separated from the ridges, was crushed, and the liquid fraction was separated. When determining the qualitative composition and quantitative content of the components of the carbohydrate-acid and phenolic complexes, the sample was pre-centrifuged (centrifuge rotor with the speed of 7000 rpm, 10 minutes). The measurement was carried out for three times.
2. Methods and materials

In grapes, the indicators characterizing its quality were determined [12]: appearance, smell, taste; mass fraction of crushed berries (no more than 20 %); mass fraction of berries damaged by diseases and pests (not more than 10 %); organic impurities (leaves, shoots).

The technological evaluation of grapes was carried out in accordance with the “Methodology for assessing grape varieties by biochemical and physic-chemical indicators” [9]. In must the authors determined: pH, mass concentration of sugars by areometry, titratable acids by potentiometry, phenolic substances by colorimetry [13].

The indicators of ORI and GAI were calculated according to the following formulas:

\[
ORI = \frac{pH^2 \cdot \text{mass concentration of sugars, } g/l}{\text{total sugars, } g/100cm^3}
\]

(1)

\[
GAI = \frac{\text{Mass concentration of titratable acid, } g/l}{\text{total sugars, } g/100cm^3}
\]

(2)

Grape was assessed according to the following indicators:

- Mass concentration of phenolic substances of the must obtained by pressing whole berries (initial content of the component in the must), mg/l;
- The technological stock of phenolic substances (the content of phenolic substances in the must after preheating the pulp to 70 ± 2 °C and holding at this temperature for 30 minutes), mg/l;
- The oxidizing ability of the must, characterizes the tendency of the phenolic complex of the must to oxidize with atmospheric oxygen (the ratio of the content of phenolic substances in the must after oxidation for 1 hour to the original value), %;
- Macerating ability of the must – the tendency of grapes to give off phenolic substances when infusion on the seeds and skins for 4 hours at 20–25 °C (the ratio of the content of phenolic substances in the must after infusion on the seeds and skins to the initial value), %;
- The ability to extract – the potential of grapes to transfer phenolic substances from the solid parts of the berry to the must (the ratio of phenolic substances in the must after infusion for 4 hours at 20–25 °C and the technological stock of phenolic substances), %.

The oxidizing properties of the must were analyzed by the activity of the monophenol-monoxygenase (MPMO) enzyme. The method is based on measuring the rate of formation of a colored oxidation product [14]. The enzyme activity was expressed in relative units per 1 ml of must. The profiles of organic acids (tartaric, malic, citric, amber acids) and sugars (glucose, fructose) were determined by high performance liquid chromatography (Shimadzu LC20 Prominance, Japan). GAI was calculated as the ratio of glucose to fructose [15].

3. Results

All the grapes in appearance, smell and taste, mass fraction of crushed berries (no more than 3 %), mass fraction of berries damaged by diseases and pests (no more than 1 %) met the requirements of regulatory documents; the impurities of leaves and shoots were absent [12]. The analysis of the data (Table 2) shows that the grapes met the requirements for industrial processing – the mass concentration of sugars was 160–254 g/l. The mass concentration of titratable acids in grapes varied in the range of 4.6–9.8 g/l. The lowest indices were characterized by autochthonous grape varieties – Kokur white B and Shabash, as well as Aligote and Citrone of Magarach. The maximum content of titratable acids was observed in Riesling Rhine and Firstborn of Magarach grape samples (7.7 and 8.9 g/l, respectively).

The value of ORI for the grapes of Shabash and Firstborn of Magarach corresponds to the direction of table wines [9]. High values of the indicator (over 260) for the varieties Muscat white, Kokur white, Aligote muscat and Citron Magaracha allow using them for the production of the liquor group of wines. The GFI values of the investigated grapes are at the upper limit of the range of values for the production of table wines, or exceed it by 1.1–2.4 times.
The range of values of the GFI indicator for the studied sample is 0.84–1.00. The variability of the index in the must is explained by varietal characteristics and various stages of the physiological maturity of grapes [15]. A value of more than 0.77 indicates a high potential for complete fermentation of sugars during alcoholic fermentation [10]. The results obtained for Crimean grapes are consistent with the data presented in the world literature on the biochemical characteristics of grapes [16, 17].

The analysis of the physic-chemical composition of the must after pressing whole berries showed that the maximum content of phenolic substances in it is characteristic of aromatic grape varieties: Citron Magaracha, Aligote muscat and Muscat white and averages 640, 455 and 404 mg/l for the samples, respectively (table 3). In grapes of other varieties, the mass concentration of phenolic substances is 1.3–4.5 times lower, amounting to 173–445 mg/l.

The data processing showed that the minimum content of phenolic substances in the must (223–349 mg/l) corresponds to pH 2.85–3.4. With increasing pH, the concentration of phenolic substances increases. The established patterns should be taken into account when choosing a grape processing scheme for the production of different types of wines.

The authors revealed the dependence of the content of phenolic substances in the must on their technological stock in the grape berry (r = 0.83). With a supply of phenolic substances in the range of 600-1000 mg/l, the transition of phenolic compounds to the must is not more than 400 mg/l; the initial value of the indicator more than 1000 mg/l causes the increase in the content of phenols in the must in 1.5–4.0 times.

**Table 2. Indicators of carbohydrate-acid potential of grapes * **

| Variety             | Mass concentration, g/l | pH   | ORI  | GAI  | GFI   |
|---------------------|-------------------------|------|------|------|-------|
|                     | Of sugars               |      |      |      |       |
| Introduced          |                         |      |      |      |       |
| Aligote             | 160–211                 | 4.6–7.0 | 3.0–3.4 | 147–244 | 2.1–4.5 | 0.85–0.96 |
| Rkatsiteli          | 188                     | 5.7   | 3.2  | 192  | 3.3   | 0.91    |
| Nutmeg white        | 205                     | 6.4   | 3.1  | 194  | 3.2   | 0.92    |
| Riesling Rhine      | 184–254                 | 4.7–8.2 | 3.1–3.6 | 188–274 | 2.5–4.5 | 0.93–1.00 |
| Kokur white         | 175–218                 | 3.6–7.6 | 3.4–3.6 | 212–310 | 2.3–6.6 | 0.84–0.98 |
| Shabash             | 165–172                 | 4.9–6.4 | 3.2–3.3 | 143–191 | 2.4–3.5 | 0.85–1.00 |
| Classified          |                         |      |      |      |       |
| Aligote             | 184–224                 | 5.2–7.8 | 3.3–3.6 | 196–284 | 2.4–4.3 | 0.94–0.97 |
| Muscat              | 197                     | 6.7   | 3.4  | 224  | 3.1   | 0.95    |
| Citron              | 215–247                 | 4.6–6.3 | 3.3–3.5 | 246–287 | 3.5–4.7 | 0.89–0.95 |
| Magaracha           | 232                     | 5.6   | 3.4  | 267  | 4.2   | 0.93    |
| Firstborn of        | 170–195                 | 7.4–9.8 | 2.8–3.1 | 136–179 | 1.7–2.6 | 0.94–0.97 |
| Magarach            | 185                     | 8.9   | 2.9  | 159  | 2.1   | 0.95    |

* The numerator represents the range of values, the denominator represents the average value of the indicator.

It is necessary to note the role of varietal characteristics of grapes in the process of saturation of the must with the components of the phenolic complex. The content of phenolic substances in the must of Aligote, Rkatsiteli, Riesling Rhine and Firstborn of Magarach varieties is 12–35 % of their technological stock in the berry. In varieties Muscat white, Kokur white, Shabash, the indicator varies from 27 to 43 %, in varieties Aligote muscat and Citron Magaracha – 34–60 %.
Table 3. Indicators of the phenolic complex and the oxidizing properties of grapes

| Variety          | Characteristics of the phenolic complex of grapes | Activity of MPMO, (x10³) |
|------------------|--------------------------------------------------|--------------------------|
|                  | technological stock mg/l | technological stock % | macerating ability % | oxidizing ability % | extraction ability % |
| Introduced       |                                 |                          |                        |                     |                        |
| Aligote 631–1561 | 631–1561                          | 173–349                  | 71–169                 | 14–48               | 65–125                 | 61–156                 |
| Rkatsiteli 888–1517 | 888–1517                        | 242–396                  | 68–111                 | 12–44               | 73–128                 | 42–139                 |
| Nutmeg white 1253 | 948–1613                          | 374–466                  | 73–114                 | 14–48               | 91–104                 | 87–197                 |
| Riesling Rhine 1250 | 977–1691                        | 223–391                  | 89–151                 | 17–39               | 92–155                 | 36–139                 |
| Kokur white 638–1624 | 638–1624                     | 248–445                  | 75–91                  | 19–43               | 83–116                 | 56–202                 |
| Shabash 942 | 817–1066                          | 298–346                  | 92–93                  | 29–40               | 88–110                 | 221–234                 |
| Indigenous       |                                 |                          |                        |                     |                        |                        |
| Aligote 711–1110 | 711–1110                          | 380–622                  | 80–100                 | 38–58               | 52–137                 | 111–171                 |
| Muscat 821 | 977–1173                          | 569–782                  | 84–101                 | 45–59               | 76–101                 | 61–208                 |
| Citron 933–1300 | 240–260                          | 97–133                   | 20–27                  | 87–108              | 32–114                 |                        |
| Magaracha 1067 | 1067                              | 253                      | 110                    | 23                  | 97                     | 62                      |

The study of the MPMO activity of a single grape variety made it possible to establish its dependence on the content of phenolic substances in the must after pressing whole berries (r = 0.77). Muscat white, Kokur white, Muscat aligote, Citron Magaracha and Shabash were characterized by high MPMO activity and oxidizing ability of the must (75–101 %), which indicates intense oxidation of phenolic substances, their sedimentation and necessitate increased must protection from oxidation.

High ability to release phenolic substances from the solid parts of the berry with short-term infusion of pulp (Aligote muscat, Citron Magaracha), indicate the predominance of extraction processes over oxidative ones.

The revealed features of the oxidation-reduction system of the studied grape varieties must be taken into account when processing them.

The content of organic acids in the berry is reasoned by varietal, soil-climatic and agrotechnical features of the cultivation of grape plants [18]. We have confirmed the specificity of the accumulation of organic acids in berries of different varieties (Table 4).

The minimum concentration of tartaric acid was noted in Shabash and Citron Magaracha grapes (2.0–6.7 g/l). The maximum values of the indicator were found in the varieties Muscat white and Riesling Rhine (7.2, 7.9 g/l, respectively).

The content of malic acid in Muscat white grapes exceeded the values for other varieties by 1.4–2.1 times. The mass concentration of amber acid in the studied samples varied in the range 0.1–1.7 g/l, except for the varieties Aligote and Shabash (1.6 and 2.5 g/l, respectively). The content of citric acid did not exceed 0.5 g/l. Riesling Rhine and Pervenets of Magarach varieties were characterized by the highest concentration of the sum of organic acids. Indigenous and classified varieties have a similar profile of organic acids. The value of the ratio of tartaric and malic acids in the studied varieties increased in the series introduced → indigenous → classified varieties.
Table 4. Grape Organic Acid Profile

| Variety         | Mass concentration of organic acids, g/l | Tartaric acid | Malic acid | Amber acid | Citric acid |
|-----------------|------------------------------------------|---------------|------------|------------|-------------|
| Introduced      |                                          |               |            |            |             |
| Aligote         | 3.5–8.9                                  | 1.3–2.9       | 0.1–3.7    | 0.1–0.2    |             |
| Rkatsiteli      | 4.0–8.5                                  | 1.0–3.2       | 0.1–0.7    | 0.1–0.3    |             |
| Nutmeg white    | 5.3–8.7                                  | 1.2–4.2       | 0.1–0.2    | 0.1–0.3    |             |
| Riesling Rhine  | 5.5–9.0                                  | 1.2–2.3       | 0.1–1.0    | 0.1–0.2    |             |
| Indigenous      |                                          |               |            |            |             |
| Kokur white     | 4.1–9.0                                  | 1.1–2.5       | 0.1–1.2    | 0.1–0.5    |             |
| Shabash         | 4.8–6.3                                  | 1.5–2.8       | 1.3–3.2    | 0.1        |             |
| Classified      |                                          |               |            |            |             |
| Aligote Muscat  | 3.5–8.6                                  | 1.0–2.1       | 0.1–0.8    | 0.1–0.2    |             |
| Citron Magaracha| 2.0–6.7                                  | 0.9–1.7       | 0.1–1.7    | 0.1–0.2    |             |
| Firstborn of Magarach | 5.3–7.9                  | 1.7–2.4       | 0.5–0.9    | 0.2–0.3    |             |

4. Conclusion
A comparative characteristic of the Crimean grape varieties by physical, chemical and biochemical parameters was given. Close relationships between the components of the carbohydrate-acid and phenol-oxidase complexes of grapes were established. Grape varieties with a high activity of oxidases and a tendency to extraction of phenolic substances were determined. The obtained patterns will be used to produce wines with a diverse organoleptic profile, which is due to the ratio of the components of the carbohydrate-acid and phenol-oxidase complexes.

The research work in this direction will be continued.

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