Variation in enzymatic activity and phenolic content in grapes across maturity stages

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Abstract

Introduction. This study is aimed at investigating the changes in the activity of certain oxidoreductase-class enzymes and content of phenolic compounds depending on the level of grape maturity.

Materials and methods. The white technical grape varieties Bayanshira and Rkatsiteli grown in Azerbaijan, as well as the introduced red technical variety Cabernet Sauvignon were used. The activities of ascorbate oxidase, o-diphenol oxidase, peroxidase, and catalase in grapes were determined at different stages of grape maturation. High-performance liquid chromatography was used for the analysis of phenolic compounds.

Results and discussion. Depending on the degree of grape maturity, oxidoreductase-class enzymes remain continuously active. The enzyme activity in the grape varieties Bayanshira, Rkatsiteli, and Cabernet Sauvignon, grown in the Samukh and Goygol regions, varies throughout the ripening stages, with the most pronounced changes observed in unripe and overripe berries. In the Bayanshira variety, enzyme activity in unripe berries increased by 12.5–22.8% and 15.8–38.4%; in ripe berries — by 3.1–5.6% and 3.5–7.9%; and in overripe berries — by 20.4–42.5% and 19.0–33.3%, respectively, which is consistent with the data for the other studied varieties. The lowest enzyme activity was recorded in ripe grapes.

Using chromatographic–mass spectrometric analysis, it was established that the studied grape varieties are rich in phenolic compounds such as flavonoids, phenolcarboxylic acids, and procyanidins. A total of 18 monomeric phenolic compounds were identified in the grape varieties, along with 21 anthocyanin compounds found in the Cabernet Sauvignon variety (a red technical grape). This indicates a higher content of phenolic compounds in the studied grape varieties.

Conclusions. To obtain high-quality brandy wine material, it is recommended to use fully ripened grapes, as they exhibit the lowest enzymatic activity. To enrich the wine material with phenolic and extractive substances, the grape juice should be fermented in contact with the mash for several days.

Introduction

One of the most significant groups of enzymes involved in the biochemical transformations of grapes is the oxidoreductase class of enzymes (Nabiev, 2010). These enzymes play a key role in oxidation–reduction reactions, which underlie processes, such as respiration, the increase in antioxidant content, the degradation of phenolic compounds, and other metabolic changes. Oxidoreductase activity is influenced by the degree of grape ripeness.

Phenolic compounds play an important role in winemaking. The quality of various types of wines, as well as brandy wine material, brandy spirit, and the brandy itself, largely depends on phenolic compounds and their transformation products, or the metabolites formed because of their conversion. It is important to note that the distinctive taste and aroma of brandy are closely associated with phenolic compounds (Fataliyev, 2012). Phenolic compounds have a significant impact on the sensory profile of wines, particularly in terms of color intensity and sensory characteristics such as density, astringency, and bitterness. Most of these compounds originate from the grapes themselves. Therefore, their content in wines is determined by the grape variety, its phenolic potential, and reserves (Ghiselli et al., 1998; Lee and Koh, 2001).

Phenolic compounds are widely distributed in plants, including grapes, and possess important technological properties (Cosme, 2018). The concentration of phenolic compounds is higher in grape skins than in the juice and pulp (Bendaali, et al., 2022). One of the key biological and technological features of phenolic compounds is their antioxidant and antimicrobial activity. Grape varieties and wines rich in phenolic compounds are not only more resistant to diseases but are also rich in extractive substances (Nabiev and Moslemzadeh, 2008).

To produce high-quality brandy, it is essential first to prepare high-quality brandy wine material that meets modern requirements in terms of sensory and chemical characteristics, ensuring a rich flavor and product stability. In the production of brandy wine material, special attention must be paid to the grape variety, its degree of ripeness, its richness in chemical components, and other factors. Brandy wine material should be rich in extractive substances (Dhiman et al., 2022).

Despite the large number of studies devoted to brandy production technology, the impact of the maturity level of technical grape varieties on enzymatic activity and the accumulation of phenolic compounds during the production of brandy wine material remains insufficiently studied, especially with regard to specific grape varieties cultivated in Azerbaijan, such as Bayanshira and Rkatsiteli.

To this end, we examined the changes in the activity of certain oxidoreductase-class enzymes depending on the degree of grape maturity, and identified phenolic compounds, such as flavonoids, phenolcarboxylic acids, stilbenes, and procyanidins in grape varieties used for the production of brandy wine material. Studying the relationship between grape maturity, enzymatic activity, and the chemical composition of the wine material is a relevant and scientifically grounded area of research that contributes to improving the quality of the final product — brandy.

The aim of this study was to determine the influence of the maturity stages of technical grape varieties (Bayanshira, Rkatsiteli, and Cabernet Sauvignon) on the activity of oxidoreductase-class enzymes and the content of phenolic compounds, in order to justify the selection of optimal raw materials for the production of brandy wine material.

Scientific novelty: A comparative study was conducted on the enzymatic activity of oxidoreductases and the content of phenolic compounds in technical grape varieties (Bayanshira, Rkatsiteli, and Cabernet Sauvignon) at different stages of maturity.

Characteristic changes in enzyme activity and the profile of phenolic compounds depending on the maturity level were identified. A correlation between berry maturity and biochemical composition was also established, which is important for optimizing grape processing technologies in the production of high-quality brandy wine material.

Materials and methods

Grape varieties widely cultivated in the Samukh and Goygol regions of Azerbaijan — the white technical varieties Bayanshira and Rkatsiteli, as well as the red variety Cabernet Sauvignon were used in the study (Figure 1). Grapes were collected at different stages of ripening: unripe, ripe, and overripe berries.

a b c

Figure 1. White technical grape variety Bayanshira (a), white technical grape variety Rkatsiteli (b), and red technical grape variety Cabernet Sauvignon (c)

Bayanshira. This white technical grape variety originates from Azerbaijan, where it has become widely cultivated due to its excellent adaptation to local conditions. Bayanshira is also successfully grown in Uzbekistan, Turkmenistan, Kazakhstan, Tajikistan, Ukraine, Moldova, and other countries, where it is widely used in winemaking. The grape is characterized by a light fruity aroma with citrus notes. The sugar content in the berries ranges from 16 to 20%, and the acidity is between 5.5 and 8 g/L. The variety is classified as late-ripening (Fig. 1a).

Rkatsiteli. The homeland of the Rkatsiteli grape variety is Kakheti, Georgia. In Azerbaijan, it is primarily cultivated in the Goygol and Samukh regions. Grapes grown in foothill zones tend to accumulate more sugars compared to those cultivated in lowland areas. Currently, Rkatsiteli is used not only for the production of table and brandy wine materials but also for various types of fortified wines. This variety belongs to the medium-late ripening group. In fully ripened Rkatsiteli berries, the sugar content reaches 18–24%, while the titratable acidity is 5.5–6.5 g/L. The variety is also known for its good resistance to grapevine diseases (Figure 1b).

Cabernet Sauvignon is the most widely cultivated grape variety in the world (Lončarić et al., 2022; Ramos et al., 2018). It is a red technical grape variety that has been

cultivated since the 17th century in the Bordeaux region of France. In Azerbaijan, Cabernet Sauvignon began to be cultivated in the late 19th – early 20th century, initially in collections of research institutions as well as in farms of the Karabakh, Shirvan, and Ganja-Gazakh regions. Today, this variety is most widely grown in the winemaking farms of the Goygol and Samukh districts (Figure 1c).

Methods

Determination of enzymatic activity

Enzyme activity in this study was determined using the method described by Nabiyev et al. (2008). Enzyme activity is defined by the amount of substrate in mg, broken down by the enzyme in 30 minutes in 1 gram of sample mass.

The determination of ortho-diphenol oxidase activity is based on the oxidation of dimethyl-p-phenylenediamine. The resulting compound is colored blue-violet (Nabiyev et al., 2008). In the cuvette, 2 mL of dimethyl-p-phenylenediamine solution, 1 mL of buffer (if necessary), and 0.5 mL of enzyme extract were mixed. The mixture was stirred, and the timing was started immediately. The optical density of the solution was measured after 1, 2, 3, and 5 minutes at a wavelength of approximately 540–550 nm. A control sample was prepared without the enzyme (buffer was added instead) to eliminate the influence of auto-oxidation. This method made it possible to quantitatively determine the activity of ortho-diphenol oxidase by the increase in color intensity of the solution caused by the oxidation of dimethyl-p-phenylenediamine.

Oxidative and reductive processes play a crucial role in the activity of the enzyme peroxidase. This enzyme catalyzes the oxidation of various phenols and aromatic amines. The method for determining peroxidase activity is based on measuring the time it takes for the test solution to reach a specific optical density. Benzidine is used as the substrate, and its oxidation results in the formation of a blue-colored compound. Based on the intensity of this color, the enzyme's activity can be preliminarily assessed using a photoelectric colorimeter (Nabiyev et al., 2008).

The reaction mixture containing 2.0 ml of benzidine solution, 0.5 ml of H_2O_2 solution, and 0.5 ml of buffer was mixed thoroughly with 0.2 ml of the enzyme solution (peroxidase extract). Time measurement was started immediately, and the cuvette was simultaneously placed in the photoelectric colorimeter. The optical density of the solution was measured at 590–610 nm every 10 seconds. The time at which a specific optical density was reached was recorded. A control experiment without the enzyme (with buffer added instead) was carried out in parallel to exclude the possibility of spontaneous benzidine oxidation.

Catalase breaks down hydrogen peroxide, which is formed during tissue respiration, into water and molecular oxygen. A titrimetric method for determining catalase activity was based on measuring the remaining hydrogen peroxide using potassium permanganate, was selected by us as a prototype (Nabiyev et al., 2008).

In the experiment, 5 ml of H_2O_2 solution and 1 ml of enzyme solution (catalase) were added to a test tube. The mixture was then incubated at 37 °C for 1–3 minutes. The reaction was stopped by adding 5 ml of 10% H_2SO_4. Immediately afterward, the solution was titrated with 0.01 N $KMnO_4$ until a persistent pink color appeared. A control sample was prepared following the same procedure, but the enzyme was added after the acid to prevent the decomposition of H_2O_2.

Determining of phenolic compounds

For the analysis, 50 g of grape berries were taken and crushed in a porcelain mortar, then transferred into an Erlenmeyer flask. The sample was then rinsed with methanol in a 1:2 ratio. The flask was sealed and stored in a refrigerator at +4°C for 24 hours. Afterward, the resulting extract was filtered through a Büchner funnel. The residue was extracted again with methanol to remove colored substances. The filtrate was combined with the initial extract and evaporated using a rotary evaporator at 40°C.

The obtained aqueous residue was brought to the original volume by adding deionized water and then passed through a C18 cartridge (Waters Sep-Pak 6 cc tC18). Prior to this, the cartridge is preconditioned by passing through it two volumes (2 ml each) of methanol. The remaining methanol was rinsed off with deionized water. Then, the solution containing phenolic compounds was transferred into the cartridge. To remove non-adsorbed substances (e.g., phenolic acids and others), two volumes of deionized water are added to the cartridge. Adsorbed anthocyanins were eluted from the cartridge with 50 ml of deionized water. The resulting solution was then evaporated in a rotary evaporator at 40°C until a dry residue is obtained. The resulting dry residue was dissolved in deionized water, and the sample is ready for analysis.

High-performance liquid chromatography (HPLC) ProStar-MS 500 (Varian, USA) was used for the analysis of phenolic compounds in the grape sample (Flamini and Traldi, 2010).

Statistical Analysis

The changes in enzyme activities depending on the ripeness stage of the studied grape varieties were determined by the triplicate assay method. Results are presented as mean ± standard deviation. Statistical calculations were performed using software and Microsoft Excel.

Results and discussion

The changes in the enzymatic activities in the grape varieties depending on the ripeness stage

The changes in the activities of the enzymes ascorbate oxidase, o-diphenol oxidase, peroxidase, and catalase in the studied grape varieties were investigated depending on their ripeness stage (unripe, ripe, and overripe).

The changes in enzyme activity at different ripeness stages of grape varieties used for brandy wine production are presented in Tables 1–4 and Figures 2 and 3.

As seen (Tables 1 and 2), the enzyme activity of the grape varieties Bayanshira, Rkatsiteli, and Cabernet Sauvignon, grown in the Samukh district, tends to consistently increase depending on their ripeness stage. The study established that, depending on the ripeness of the grapes, more pronounced changes in enzyme activities were most often observed in unripe and overripe grape berries (Table 1).

In the unripe Bayanshira grape variety, the activity of the enzyme ascorbate oxidase increased by 12.5% before processing; in the ripe grapes, this increase was 5.4%, and in the overripe ones — 42.5% (Table 2).

Analysis of the research results revealed that the increase in enzyme activity in unripe Bayanshira grape berries ranged from 12.5% to 22.8%, in ripe berries — from 3.1% to 5.4%, and in overripe berries — from 20.4% to 42.5%. These values are consistent with those observed in the Rkatsiteli and Cabernet Sauvignon grape varieties.

Table 1

Activities of oxidoreductase class enzymes depending on ripeness stage of grape varieties grown in Samukh district, μmol/min

Enzymes	Unripe			Ripe			Overripe		
	Days of activity measurement								
	1	5	10	1	5	10	1	5	10
.									
Ascorbate oxidase	0.64 ±0.01	0.68 ±0.01	0.72 ±0.01	0.74 ±0.01	0.75 ±0.01	0.78 ±0.02	0.80 ±0.01	0.98 ±0.01	1.14 ±0.02
o-Diphenol oxidase	0.70 ±0.01	0.78 ±0.01	0.86 ±0.01	0.88 ±0.01	0.90 ±0.01	0.92 ±0.01	0.94 ±0.02	1.01 ±0.01	1.28 ±0.02
Peroxidase	2.20 ±0.01	2.42 ±0.02	2.54 ±0.02	2.60 ±0.02	2.62 ±0.01	2.68 ±0.01	2.70 ±0.01	3.02 ±0.02	3.25 ±0.02
Catalase	0.33 ±0.01	0.35 ±0.01	0.39 ±0.01	0.40 ±0.02	0.41 ±0.02	0.42 ±0.01	0.42 ±0.01	0.48 ±0.01	0.54 ±0.02
Rkatsiteli									
Ascorbate oxidase	0.68 ±0.01	0.72 ±0.01	0.78 ±0.01	0.80 ±0.01	0.82 ±0.01	0.86 ±0.02	0.88 ±0.01	1.02 ±0.01	1.20 ±0.02
o-Diphenol oxidase	0.76 ±0.01	0.84 ±0.01	0.92 ±0.02	0.94 ±0.01	0.98 ±0.01	0.01 ±0.01	1.04 ±0.02	1.16 ±0.01	0.38 ±0.01
Peroxidase	2.42 ±0.01	2.54 ±0.01	2.76 ±0.02	2.78 ±0.01	2.82 ±0.01	2.88 ±0.01	2.90 ±0.01	3.10 ±0.01	3.34 ±0.01
Catalase	0.40 ±0.02	0.44 ±0.01	0.48 ±0.01	0.51 ±0.02	0.53 ±0.01	0.54 ±0.01	0.56 ±0.01	0.62 ±0.01	0.71 ±0.01
Cabernet Sauvignon									
Ascorbate oxidase	0.55 ±0.01	0.58 ±0.01	0.62 ±0.01	0.63 ±0.01	0.64 ±0.01	0.66 ±0.01	0.68 ±0.01	0.82 ±0.01	0.98 ±0.01
o-Diphenol oxidase	0.66 ±0.01	0.72 ±0.02	0.78 ±0.01	0.80 ±0.01	0.82 ±0.01	0.84 ±0.01	0.88 ±0.01	0.98 ±0.01	1.20 ±0.01
Peroxidase	1.76 ±0.01	1.98 ±0.01	2.02 ±0.02	2.04 ±0.01	2.08 ±0.01	2.12 ±0.01	2.20 ±0.02	2.42 ±0.01	2.52 ±0.01
Catalase	0.29 ±0.01	0.33 ±0.02	0.35 ±0.01	0.36 ±0.01	0.37 ±0.01	0.39 ±0.01	0.39 ±0.02	0.42 ±0.01	0.48 ±0.01

According to Tables 1 and 2, depending on the ripeness stage of the grapes, the highest activity was exhibited by the enzyme peroxidase, followed by o-diphenol oxidase, then ascorbate oxidase, while the lowest enzymatic activity was observed for catalase.In the Bayanshira grape variety grown in the Samukh district, the activity of ascorbate oxidase in unripe berries increased by 12.5%, in ripe berries by 5.4%, and in overripe berries by 42.5%. This pattern remained consistent up to the stage of grape processing.

Analysis of the literature indicates that the enzyme ascorbate oxidase catalyzes the conversion of ascorbic acid (vitamin C) into D-dehydro-L-ascorbic acid, while the enzyme o-diphenol oxidase catalyzes the oxidation of ortho- and para-diphenols into o-quinones (Nabiev et al., 2008). The change or darkening in the color of grape and other plant juices, as well as certain food products, is associated with increased activity of o-diphenol oxidase (Gonzalez et al., 2023).

It is known that enzyme activity increases with the ripening stage of grapes, contributing to the breakdown of nutritional components in the fruit for use in respiratory processes (García-Martínez et al., 2021). This once again confirms that metabolic processes in the studied grape varieties occur constantly.

Table 2

Activity of oxidoreductase class enzymes depending on ripeness stage of grape varieties, grown in Samukh district, μmol/min

Enzymes	Unripe				Ripe				Overripe			
	In	F	Δ	%	In	F	Δ	%	In	F	Δ	%
Bayanshira												
Ascorbate oxidase	0.64	0.72	0.08	12.5	0.74	0.78	0.04	5.4	0.80	1.14	0.34	42.5
o-Diphenol oxidase	0.70	0.86	0.16	22.8	0.88	0.93	0.05	5.6	0.94	1.28	0.34	36.2
Peroxidase	2.20	2.54	0.34	15.4	2.60	2.68	0.08	3.1	2.70	3.25	0.55	20.4
Catalase	0.33	0.39	0.06	18.2	0.40	0.42	0.02	5.0	0.42	0.54	0.12	28.6
Rkatsiteli												
Ascorbate oxidase	0.68	0.78	0.10	14.7	0.80	0.86	0.06	7.5	0.88	1.20	0.32	36.4
o-Diphenol oxidase	0.76	0.92	0.16	21.0	0.94	1.01	0.07	7.4	1.04	1.38	0.34	32.7
Peroxidase	2.42	2.76	0.34	14.0	2.78	2.88	0.10	3.6	2.90	3.34	0.44	15.2
Catalase	0.40	0.48	0.08	0.20	0.51	0.54	0.03	5.9	0.56	0.71	0.15	26.8
Cabernet Sauvignon												
Ascorbate oxidase	0.55	0.62	0.07	12.7	0.63	0.66	0.03	4.8	0.68	0.98	0.30	44.1
o-Diphenol oxidase	0.66	0.78	0.12	18.2	0.80	0.84	0.04	5.0	0.88	1.20	0.32	36.4
Peroxidase	1.76	2.02	0.26	14.8	2.04	2.12	0.08	3.9	2.20	2.52	0.32	14.5
Catalase	0.29	0.35	0.06	20.7	0.36	0.39	0.03	8.3	0.39	0.46	0.07	17.9

Note: In- initial, F – final, Δ – difference.

The changes in enzyme activity depending on the ripeness stage of the Bayanshira grape variety grown under the conditions of the Samukh district are shown in Figure 2.

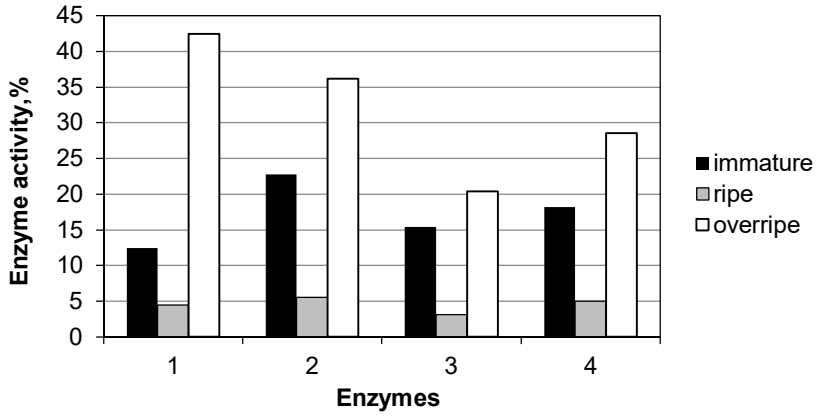

Figure 2. Enzyme activity, depending on the ripeness stage of the Bayanshira grape variety grown under the conditions of the Samukh district:
1 – ascorbate oxidase, 2 – o-diphenol oxidase, 3 – peroxidase, 4 – catalase

As seen in the figure, the activity of the studied oxidoreductases varies depending on the ripeness stage of the Bayanshira grape variety. Enzyme activity was high in both unripe and overripe grape berries. A similar pattern was also observed in the other grape varieties.

Tables 3 and 4 show the changes in the activity of the studied enzymes during grape storage prior to processing, depending on the ripeness stage of the technical grape varieties Bayanshira, Rkatsiteli, and Cabernet Sauvignon, grown in the Goygol district.

It became evident that the activity of these enzymes changes differently depending on the degree of ripening of the grapes. For each grape variety, the dynamics of enzyme activity during ripening were determined through triplicate measurements. For example, in unripe Bayanshira grapes, the activity of ascorbate oxidase was 0.58 on the first day, 0.62 on the fifth day, and 0.68 on the tenth day. As shown in the table, a consistent activation of enzymes was observed across all the studied grape varieties.

Table 3

Activity of oxidoreductase class enzymes depending on the ripeness stage of grape varieties grown in the Goygol district, μmol/min

Enzymes	Unripe			Ripe			Overripe		
	Days of activity measurement								
	1	5	10	1	5	10	1	5	10
Bayanshira									
Ascorbate oxidase	0.58 ±0.01	0.62 ±0.01	0.68 ±0.01	0.70 ±0.01	0.72 ±0.01	0.73 ±0.02	0.75 ±0.01	0.88 ±0.01	1.10 ±0.02
o-Diphenol oxidase	0.68 ±0.01	0.76 ±0.01	0.84 ±0.01	0.85 ±0.01	0.86 ±0.01	0.88 ±0.01	0.90 ±0.02	1.05 ±0.01	1.20 ±0.02
Peroxidase	1.90 ±0.01	2.00 ±0.02	2.20 ±0.02	2.30 ±0.02	2.35 ±0.01	2.40 ±0.01	2.40 ±0.02	2.60 ±0.02	2.95 ±0.01
Catalase	0.26 ±0.01	0.32 ±0.01	0.36 ±0.01	0.38 ±0.02	0.39 ±0.02	0.41 ±0.01	0.42 ±0.01	0.46 ±0.01	0.50 ±0.02
Rkatsiteli									
Ascorbate oxidase	0.64 ±0.01	0.68 ±0.01	0.75 ±0.01	0.76 ±0.01	0.78 ±0.01	0.81 ±0.02	0.82 ±0.01	0.96 ±0.01	1.05 ±0.02
o-Diphenol oxidase	0.72 ±0.01	0.80 ±0.02	0.88 ±0.01	0.90 ±0.02	0.92 ±0.01	0.94 ±0.01	0.94 ±0.01	1.10 ±0.02	1.35 ±0.01
Peroxidase	2.32 ±0.01	2.54 ±0.02	2.74 ±0.01	2.76 ±0.02	2.86 ±0.01	2.90 ±0.02	2.90 ±0.02	3.10 ±0.02	3.26 ±0.01
Catalase	0.36 ±0.01	0.44 ±0.01	0.44 ±0.01	0.46 ±0.01	0.48 ±0.01	0.49 ±0.01	0.50 ±0.02	0.58 ±0.01	0.64 ±0.01
Cabernet Sauvignon									
Ascorbate oxidase	0.51 ±0.01	0.55 ±0.02	0.59 ±0.01	0.60 ±0.03	0.62 ±0.02	0.64 ±0.01	0.65 ±0.01	0.74 ±0.01	0.96 ±0.01
o-Diphenol oxidase	0.62 ±0.01	0.70 ±0.02	0.76 ±0.01	0.78 ±0.01	0.80 ±0.02	0.82 ±0.01	0.84 ±0.01	1.01 ±0.02	1.10 ±0.01
Peroxidase	1.70 ±0.02	1.84 ±0.01	1.96 ±0.01	1.98 ±0.01	2.05 ±0.02	2.10 ±0.02	2.10 ±0.02	2.32 ±0.01	2.48 ±0.01
Catalase	0.28 ±0.02	0.31 ±0.01	0.34 ±0.01	0.36 ±0.01	0.38 ±0.01	0.40 ±0.02	0.40 ±0.02	0.44 ±0.01	0.48 ±0.01

Changes in enzyme activity depending on the ripeness stage of the studied grape varieties are expressed as percentages (Table 4). Among the studied grape varieties, relatively small changes in enzyme activity were recorded in ripe berries compared to the other ripeness stages.

The study of ripe Rkatsiteli grapes prior to processing revealed a 4.4% increase in o-diphenol oxidase activity. In unripe grapes, this increase was 22.2%, while in overripe grapes it reached 43.6%. Among unripe grapes grown in the Goygol district, o-diphenol oxidase activity increased by 23.5% in the Bayanshira variety, 22.2% in Rkatsiteli, and 22.6% in Cabernet Sauvignon. In ripe grapes, the enzyme activity increase was 3.5% for Bayanshira, 4.4% for Rkatsiteli, and 5.1% for Cabernet Sauvignon.

Table 4

Activity of enzymes depending on the ripeness stage of grape grown in the Goygol district

Enzymes	Unripe				Ripe				Overripe			
	In	F	Δ	%	In	F	Δ	%	In	F	Δ	%
Bayanshira												
Ascorbate oxidase	0.58	0.68	0.10	17.2	0.70	0.73	0.03	4.2	0.75	1.10	0.35	26.2
o-Diphenol oxidase	0.68	0.84	0.16	23.5	0.85	0.88	0.03	3.5	0.90	1.20	0.30	33.3
Peroxidase	1.90	2.20	0.30	15.8	2.30	2.40	0.10	4.3	2.40	2.95	0.55	22.9
Catalase	0.26	0.36	0.10	38.4	0.38	0.41	0.03	7.9	0.42	0.50	0.08	19.0
Rkatsiteli												
Ascorbate oxidase	0.64	0.75	0.11	17.2	0.76	0.81	0.05	6.6	0.82	1.05	0.23	28.1
o-Diphenol oxidase	0.72	0.88	0.16	22.2	0.90	0.94	0.04	4.4	0.94	1.35	0.41	43.6
Peroxidase	2.32	2.74	0.42	18.1	2.76	2.90	0.14	5.1	2.90	3.26	0.36	12.4
Catalase	0.36	0.44	0.08	22.2	0.46	0.49	0.03	6.5	0.50	0.64	0.14	28.0
Cabernet Sauvignon												
Ascorbate oxidase	0.51	0.59	0.08	15.7	0.60	0.65	0.05	8.3	0.65	0.96	0.31	47.7
o-Diphenol oxidase	0.62	0.76	0.14	22.6	0.78	0.82	0.04	5.1	0.84	1.10	0.26	30.9
Peroxidase	1.70	1.96	0.26	15.3	1.98	2.10	0.12	6.1	2.10	2.48	0.38	18.1
Catalase	0.28	0.34	0.06	21.4	0.37	0.40	0.04	7.5	0.40	0.48	0.08	20.0

Note: In – initial, F – final, Δ – difference.

As shown in Table 4, o-diphenol oxidase activity increased by 33.3% in overripe Bayanshira grapes, 43.6% in Rkatsiteli, and 30.9% in Cabernet Sauvignon. The data also indicate that the activity of other enzymes changed only slightly in the ripe grape varieties. These findings are consistent with the results obtained for the other enzymes studied.

During grape processing, as well as throughout fermentation and even during the formation and maturation of wine material, the activity of o-diphenol oxidase must be continuously regulated. Otherwise, increased activity of this enzyme may lead to changes in the color of the final products and the degradation of nutritional components in their

composition (Nabiev et al., 2008).Therefore, when producing cognac wine material from various grape varieties, special attention must be paid to the ripeness stage of the grapes.

This is because the production of high-quality cognac wine material largely depends on the ripeness stage of the grapes. A significant increase in enzyme activity creates conditions for the breakdown of the grape's nutritional components for respiration processes. Therefore, in unripe and overripe grape varieties, the lower content of extractive substances makes it impossible to produce high-quality cognac wine material. These grape varieties also exhibit a low content of aromatic compounds.

The dynamics of changes in enzyme activity depending on the ripeness stage of the Cabernet Sauvignon grape variety grown under the conditions of the Goygol district are shown in Figure 4. Enzymatic activity was expressed in percentage as relative activity, i.e. demonstrating how much the enzyme activity increased comparative to the initial value.

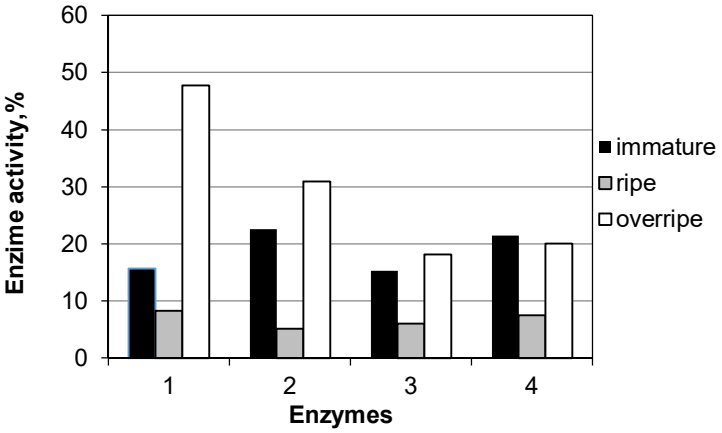

Figure 3. Enzyme activity, depending on the ripening stage of Cabernet Sauvignon grapes grown in the Goygol region:
1 – Ascorbate oxidase, 2 – o-Diphenol oxidase, 3 – Peroxidase, 4 – Catalase

The biochemical processes occurring in grapes at different stages of ripening determine the quality and chemical composition of the berries, which ultimately affects the organoleptic and technological properties of the final product (Fataliyev, 2012). The quality of the raw materials used in processing, as well as the final product, largely depends on enzymatic processes. Therefore, it is essential to regulate enzymatic processes that occur both during the ripening of raw materials, including grapes, and during their processing.

The enzyme peroxidase catalyzes the oxidation of polyphenols and a number of aromatic amines in the presence of hydrogen peroxide. The enzyme catalase, by breaking down hydrogen peroxide, formed during tissue respiration, into water and molecular oxygen, protects the product from toxic effects (Nabiev et al., 2008).

As seen in Figure 2, the activity of all enzymes tends to increase depending on the ripening stage of Cabernet Sauvignon grapes. A relatively slight increase in enzyme activity was observed in fully ripened grape berries (Figure 2). In unripe and overripe grapes, higher activity of the enzyme ascorbate oxidase contributes to a more intensive breakdown of vitamin C, exceeding normal levels. This pattern is also observed in the other enzymes under study. Based on the data in Figures 1 and 2, it becomes evident that the smallest changes in enzyme activity were detected in ripened grape varieties.

Thus, the results of the study showed that enzymes of the oxidoreductase class remain in an active form throughout the entire ripening period of the grapes.

The slightest increase in enzyme activity was observed in fully ripened grape varieties. In these ripened grapes, the minimal changes in enzyme activity reduce the breakdown of nutrients during respiration. Therefore, for the production of high-quality cognac wine material, it is advisable to use ripened technical grape varieties, with careful attention paid to their ripening stage.

The presence of phenolic compounds in grapes and wine contributes to their enrichment with aromatic substances (Dutra et al., 2021). As a result of the hydrolysis of dimeric, oligomeric, and polymeric phenolic compounds present in wine, aromatic substances (vanillin, syringaldehyde, coniferyl, sinapic, ferulic compounds, and their corresponding aldehydes, alcohols, etc.) are formed, which positively influence the organoleptic properties and flavor quality of the wine (Kazimova et al., 2022).

In the course of the study, the content of phenolic compounds in grape varieties intended for the production of cognac wine material was determined using chromate-mass spectrometry.

Table 5 presents selected phenolic compounds identified in the studied grape varieties. Chromatography–mass spectrometry analysis revealed the presence of 18 distinct phenolic compounds.

Table 5

Phenolic compound content in grape varieties

Phenolic compounds	M.m.	Grape varieties					
		Bayanshira		Rkatsiteli		Cabernet Sauvignon	
		DT, min	mg/kg	DT, min	mg/kg	DT, min	mg/kg
(-)epicatechin	289	20.344	253.98	20.130	670.9	20.124	54.76
(+)catechin	289	16.570	164.37	16.376	840.1	16.206	88.58
Chlorogenic acid	352	15.286	324.64	15.375	632.2	14.990	54.53
Caffeic acid	179	17.880	103.8	18.162	474.54	17.806	23.93
Vanillin	151	22.095	3.98	21.908	81.0	22.212	27.95
Trans-p-coumaroylvinyl acid	295	traces	-	traces	-	-	-
ProcyanidinB1,B3,B4,B5	577	traces	-	-	-	-	-
ProcyanidinC1,T2,T3	865	-	-	-	-	-	-
Quercetin-3-o-glucuronide	477	traces	-	traces	-	traces	-
Quercetin-3-o-galactoside	463	-	-	traces	-	-	-
Myricetin-3-o-glucuronide	493	traces	-	-	-	-	-
Trans-caffeoylvinyl acid	311	-	-	traces	-	-	-
Cis-trans-resveratrol-3-o-glucoside	389	-	-	traces	-	-	-
Myricetin-3-o-glucoside	479	-	-	traces	-	-	-
Trimeric procyanidin	865	-	-	traces	-	-	-
Gallic acid	301	traces	-	-	-	traces	
Astilbin	449	-	-	-	-	-	-
Epicatechin-3-o-gallate	441	-	-	-	-	-	-

Note: M. m. – molecular mass; DT – determination time, min.

Data present in Table 5 show that the content of phenolic compounds is higher in red grape varieties compared to white varieties. For example, the red grape variety Cabernet Sauvignon contained 54.76 mg/kg of epicatechin, while the white grape variety Rkatsiteli contained 670.9 mg/kg, and the Bayanshira variety contained 253.98 mg/kg.

A comparison of the varieties shows that vanillin is present in the greatest amount in the Rkatsiteli variety. While Rkatsiteli contained 81.0 mg/kg of vanillin, this value was 3.98 mg/kg in Bayanshira and 27.95 mg/kg in Cabernet Sauvignon. The other phenolic compounds studied in red grape varieties, particularly in Cabernet Sauvignon, are present in higher amounts compared to white grape varieties (Table 5).

The study revealed that the grape variety Bayanshira contains seven different phenolic compounds, among which quercetin-3-o-glucuronide and gallic acid are present in trace amounts. In the Rkatsiteli variety, 14 phenolic compounds were identified, five of which were found in higher concentrations, while nine were present in trace amounts. In the Cabernet Sauvignon variety, seven phenolic compounds were detected (Table 5).

The molecular weights of the phenolic compounds also vary (Jimenez-Lopez et al., 2021). The lowest molecular weight was observed in vanillin, while the highest molecular weight was found in trimeric procyanidin and procyanidins C1, C2, and C3 (molecular weight 865) (Capanoglu et al., 2013). It was established that the studied grape varieties contain a significant amount of phenolic compounds. The results of the analysis showed that the Rkatsiteli grape variety surpasses the other varieties in terms of phenolic content.

Phenolic compounds are considered to be the main indicators of the composition of wines obtained from other fruits, as well as in grapes (Chiusano et al., 2015). Phenolic compounds determine the quality indicators of the product, such as its appearance, taste and aroma. Phenolic compounds play an important role in the maturation of wines, giving them physiological and colloidal properties (Fataliyev et al., 2025).

Another group of phenolic compounds—anthocyanins—are widespread in plants, including grapes (Moldovan, 2020). Anthocyanins are the most common flavonoids found in grapes and wine (Baydar et al., 2010). They are dyes that primarily accumulate in the skins of red and pink grape varieties (Baiano et al., 2009). However, there are also grape varieties, such as Saperavi, in which anthocyanins have been detected in the pulp (Garcha et al., 2003).

Recent studies have shown that plants, including grapes, contain more than 10 anthocyanin aglycones (Kulcan et al., 2015). Newly identified anthocyanins are typically named after the plant in which they are found. In plants, anthocyanin aglycones are usually present in the form of glycosides (Kanakis et al., 2005). Anthocyanins are also referred to as anthocyanidins and anthocyanins (Woźniak et al., 2014). The distribution of anthocyanins in grapes depends on the characteristics of the variety, climatic conditions, and other factors (Yılmaz et al., 2015).

Since anthocyanins possess antioxidant and antimicrobial properties, they play an important role in the processing of table wines and other types of wine (Lorrain et al., 2015). Anthocyanin monoglucosides inhibit the growth of fungi such as *Botrytis cinerea* (Rodriguez-Saona and Wrotstad, 2001). One of the main biological properties of anthocyanins is their ability to reduce the levels of complex fats, including cholesterol, in the body, as well as their beneficial effect on the elimination of radionuclides from the body (Lima, 2014). Therefore, in this study, changes in anthocyanin content in the red grape variety Cabernet Sauvignon were examined.

Quantitative and qualitative changes in anthocyanins in the Cabernet Sauvignon grape variety observed during the study are presented in Table 6.

Table 6

Anthocyanin content in Cabernet Sauvignon grape (% of total anthocyanins)

Anthocyanins	M.m.	Content,%	Determination time, min
delphinidin-3,5-o-diglucoside	627	traces	-
petunidin-3,5-o-diglucoside	641	0.0875	14.299
delphinidin-3-o-monoglucoside	465	2.9745	14.796
malvidin-3,5-o-diglucoside	655	-	-
cyanidin-3-o-monoglucoside	449	1.5789	15.163
petunidin-3-o-monoglucoside	479	5.1786	-
Peonidin-3-o-monoglucoside	463	10.6781	-
malvidin-3-o-monoglucoside	493	45.6857	16.467
delphinidin-3-o-acetylmonoglucoside	507	0.1534	-
petunidin-3-(6-o-p-coumaroyl), 5-o-diglucoside	787	traces	-
petunidin-3-o-acetylmonoglucoside	521	0.7645	-
malvidin-3-(6-o-p-coumaroyl),5-o-diglucoside	801	traces	-
peonidin-3-o-acetylmonoglucoside	505	0.0085	-
malvidin-3-o-acetylmonoglucoside	535	8.7632	19.624
delphinidin-3-(6-o-p-coumaroyl)monoglucoside	611	traces	-
malvidin-3-(6-o caffeoyl)monoglucoside	655	traces	-
cyanidin-3-(6-o-p-coumaroyl)monoglucoside	595	traces	20.705
petunidin-3-(6-o-p-coumaroyl)monoglucoside	625	2.8756	-
peonidin-3-(6-o-p-coumaroyl)monoglucoside	609	traces	21.998
malvidin-3-(6-O-p-coumaroyl)monoglucoside	639	18.0546	21.305
delphinidin-3-o-(6-o-p-coumaroyl)-pyruvic acid-monoglucoside	679	traces	-

As seen in Table 6, under local growing conditions, 21 mono- and diglucosideanthocyanins were identified in the technical grape variety Cabernet Sauvignon. According to a study by the world-renowned winemaker, who used chromatographic methods to identify between 6 and 17 anthocyanins in European grape varieties, including those grown in France, only monoglucosideanthocyaninswere found in red grape varieties cultivated in Europe (Spacil et al., 2008), with diglucosides absent from their composition. It should be noted that the presence of diglucosides in grapes—especially malvidindiglucoside—indicates a hybrid or genetically modified origin (Urpi-Sarda et al., 2009). The Cabernet Sauvignon variety, however, is natural and environmentally pure (Xia et al., 2010). It is known that Cabernet Sauvignon is an introduced grape variety (Dani et al., 2007). The diversity of the chemical characteristics of Cabernet Sauvignon grown in the western region of Azerbaijan is associated with the specific local soil and climatic conditions. As seen in Table 5, the molecular weights of anthocyanins vary. The highest molecular weights were recorded for malvidin-3-(6-o-p-coumaroyl), 5-o-diglucoside (801) and petunidin-3-(6-o-p-coumaroyl), 5-o-diglucoside (787). The lowest molecular weight was found in cyanidin-3-o-monoglucoside (449).

In the Cabernet Sauvignon grape variety, malvidin-3-o-monoglucoside accounted for 45.7% of the total anthocyanin content, malvidin-3-(6-o-p-coumaroyl) monoglucoside for 18.05%, and peonidin-3-o-monoglucoside for 10.7%. Other anthocyanin compounds present in Cabernet Sauvignon were found in smaller amounts. The studied white technical grape varieties (Bayanshira and Rkatsiteli), as well as the red variety Cabernet Sauvignon, are rich in phenolic compounds, including anthocyanins (Spranger et al., 2008). Phenolic compounds in grapes are mainly concentrated in the skin (Cosme et al., 2008). Therefore, in order to enrich the brandy wine material with extractive substances, it is advisable to ferment the grape juice in contact with the mash for several days (Ribeiro, 2018).

The conducted study confirms the feasibility of using the studied grape varieties for the production of cognac wine materials. Let's consider it promising to study in-depth the aspects covered in the work. The conducted study allowed us to establish the influence of the degree of grape maturity on the enzymatic activity and the content of phenolic substances in technical varieties grown in various agroclimatic conditions of Azerbaijan. However, like any scientific study, it has both advantages and certain limitations that should be taken into account when interpreting and practically applying the results obtained. The study of three stages of grape maturity (unripe, ripe, and overripe) allowed us to comprehensively evaluate the biochemical processes occurring in the berries during their ripening. This gives a more complete picture of the dynamics of enzymatic activity and the accumulation of phenolic compounds.

Conclusions

1. During the study, it has been established that enzymatic activity in the grape varieties Bayanshira, Rkatsiteli, and Cabernet Sauvignon, grown in the Samukh and Goygol regions of Azerbaijan, varies depending on the degree of berry ripeness. The most significant fluctuations in enzyme activity were observed in unripe and overripe samples. For example, in the Bayanshira variety, enzyme activity in unripe berries increased by 12.5–22.8% and 15.8–38.4%, in ripe berries by 3.1–5.6% and 3.5–7.9%, and in overripe berries by 20.4–42.5% and 19.0–33.3%, respectively. Similar trends were observed in the other grape varieties studied. The lowest enzymatic activity values were recorded in ripe grapes. In the ripe varieties, the smallest increase in enzyme activity was noted, indicating the completion of metabolic processes, including the breakdown of extractive substances.
2. Chromatography–mass spectrometry analysis revealed that the studied grape varieties are rich in phenolic compounds such as flavonoids, phenolic carboxylic acids, and procyanidins. Across the studied grape varieties, 18 monomeric phenolic compounds were identified, while 21 anthocyanin compounds were detected exclusively in the Cabernet Sauvignon variety, classified as a red technical grape. This indicates a higher content of phenolic substances in the grape varieties under study.
3. Studies have shown that the content of phenolic compounds, including anthocyanins, varies significantly among the grape varieties Cabernet Sauvignon, Rkatsiteli, and Bayanshira. Notably, Cabernet Sauvignon exhibits a high level of mono- and diglucoside anthocyanins, making it particularly suitable for producing high-quality wine materials – especially for brandy, where extractive substances play an important role. A key aspect is the variation in the molecular weights of anthocyanins and other phenolic compounds, which reflects the uniqueness of each variety depending on the region of cultivation.

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