Changes in the carbohydrate-acid and phenolic complexes of grapes in the dynamics of ripening

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Abstract. The primary challenge in red wine production is optimizing conditions for the accumulation of phenolic compounds in grape berries, crucial for the wine's aroma, color, and flavor. With climate change impacting these components, it's vital to study the transformation of carbohydrate-acid and phenolic complexes as grapes reach ripeness. This study focuses on the 'Bastardo Magarachskiy' grape variety and the effects of foliar mineral fertilization (NTP-Sintez LLC, Belarus) during ripening. Our findings show that experimental processing schemes enhance sugar accumulation in the berries by 1-4 g/100 cm³. The total anthocyanin potential ranged from 1187 to 1684 mg/dm³, with maximum extraction efficiency (61-65%) occurring at sugar concentrations of 21-23 g/100 cm³. Higher sugar levels led to reduced extraction efficiency. The phenolic profile was dominated by flavan-3-ols and anthocyanins, comprising 82.7% to 96.3% of total phenolics. Throughout ripening, there was a significant increase in the mass concentration of primary phenolic compounds in the berries, highlighting the importance of monitoring these changes for optimal wine production.

1 Introduction

The primary technological challenge in red wine production is to create optimal conditions for the maximal extraction of phenolic compounds, which contribute to the aroma, color, and flavor profile of the wine, as well as to their preservation during various stages of wine development and maturation [1]. The efficiency of transferring aromatic and color compounds from pomace is influenced by several factors, including the degree of grape ripeness, methods of physical treatment applied to the berries, levels of mechanical or enzymatic cell disruption, the quantitative content of these compounds, temperature, and more [2]. The extractable grape phenols are predominantly found in seeds (60–70%), skins (28–35%), and pulp (approximately 10% or less) [3].

Understanding the relationship between a wine's quality and its phenolic composition is a central focus of enological research. For instance, anthocyanin profiles of varietal wines have been proposed as an analytical tool for certifying authenticity. The distribution of

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certain classes of flavonoids is subject to strict genetic verification and varies significantly depending on grape variety [4].

The phenolic composition of grapes is influenced by various factors, including terroir, grape variety, ripeness at harvest, irrigation practices, and more [5-8]. One method to regulate the composition of the grape phenolic complex is through foliar treatments during the growing season [9].

In China, researchers investigated the effects of foliar spraying with various iron-containing additives during the color development stage on the quality, phenolic composition, and proanthocyanidin content of 'Cabernet Sauvignon' grapes. The experimental treatment schemes improved the carbohydrate-acid balance of the grapes and increased the weight of 100 berries. An increase in proanthocyanidin and phenolic acid monomer content positively influenced the flavor of the resulting wine [10]

Additionally, studies examined the effects of calcium (Ca), silicon (Si), and combined Ca+Si treatments on the composition and quality of *'Tempranillo'* grapes. All treatments resulted in an increased content of aromatic compounds and amino acids. Specifically, calcium treatment enhanced malvidin levels; however, like other treatments, it did not significantly affect anthocyanin content [11]. Previous research has also demonstrated the positive impact of foliar treatments with nitrogen-containing preparations on crop yield and achieving phenolic ripeness earlier than in control groups [12].

In light of climate change, it is crucial to investigate the transformation of carbohydrate-acid and phenolic complexes in grapes as they reach technical and phenolic ripeness. This imbalance is becoming increasingly pronounced against the backdrop of ecological changes [13]. The aim of this study is to examine changes in the carbohydrate-acid and phenolic complexes of the *'Bastardo Magarachskiy'* grape variety throughout its ripening process.

2 Materials and Methods

The object of the study was *'Bastardo Magarachskiy'* grape variety, cultivated in the Mountain-Valley-Coastal viticulture and winemaking region of Crimea. The research was carried out during winemaking season of 2023 in the dynamics of grape ripening. The plot is irrigated, it has a southern exposure. Culture is open earth. The planting pattern is 3.0 x 1.5 m. Bush shaping is AZOS-1, vertical trellis training system.

A set of actions providing foliar treatment by tractor spraying of a mineral fertilizing scheme (NTP-Sintez LLC, Belarus), consisting of liquid complex fertilizers with microelements in chelated form, was carried out at the experimental plot. Spraying was provided in accordance with two schemes as per Table 1; control was the system of agricultural actions adopted in the farm.

During the period of industrial harvesting, an average sample of grapes in the amount of at least 10 kg was taken from each analyzed plot. In the sample prepared must, the following indicators were determined: mass concentration of sugars by the areometry method, pH value, mass concentration of titratable acids by the potentiometric method, phenolic substances and monomeric anthocyanins by the colorimetric method [14]. The degree of grape ripeness was assessed using the glucoacidimetric indicator (GAI), calculated as a ratio of mass concentrations of sugars and titratable acids, and technical ripeness indicator – TRI (the product of mass concentration of sugars and pH^2).

Table 1. Treatment schemes for the NTP-Sintez fertilizing complex

Growth stage	Preparations	Preparation consumption, l/ha	Working in liquid consumption, l/ha
Experiment 1			
Before flowering	KompleMet Boron (B, N)	1	300
	KompleMet Bud (N, P, Ca, B)	1	
	KompleMet Iron + Zinc (Fe, Zn, P, K, S)	1	
After flowering	KompleMet Boron (B, N)	1	300
	KompleMet CO (N, P, K, S, Mn, Cu, Zn, B, Mo, Co)	2	
Fruit set	KompleMet PK (P, K)	2	300
	KompleMet Iron + Zinc (Fe, Zn, P, K, S)	1	
Beginning of ripening	KompleMet PK (P, K)	2	300
	KompleMet CO (N, P, K, S, Mn, Cu, Zn, B, Mo, Co)	2	
Experiment 2			
Before flowering	KompleMet Iron + Zinc (Fe, Zn, P, K, S)	1	300
	KompleMet Bud (N, P, Ca, B)	2	
After flowering	KompleMet CO Impuls (N, P, K, S, Mn, Cu, Zn, B, Mo, Co)	2	300
	KompleMet Boron (B, N)	1	
Fruit set	KompleMet CO (N, P, K, S, Mn, Cu, Zn, B, Mo, Co)	2	300
	KompleMet Boron (B, N)	1	
	KompleMet Iron + Zinc (Fe, Zn, P, K, S)	1	
Beginning of ripening	KompleMet PK (P, K)	2	300
	KompleMet CO (N, P, K, S, Mn, Cu, Zn, B, Mo, Co)	2	

Phenolic ripeness of grapes was assessed using the Glories method, based on determining quantity content of anthocyanins in grape berries ($A_{ph1.0}$), potential number of extractable anthocyanins under winemaking conditions ($A_{pH3.2}$), extractable anthocyanins percentage (A), seed tannins proportion (Mp), total content of phenols (A_{280}) [15]. All measurements were performed in triplicate.

Homogenization of grape berries was carried out after seeds removal in a planetary micro-mill PULVERISETTE 7 (premium line) in milling pots with agate surface and balls. Afterwards, 1 g of homogenized sample was placed into a glass vial with 3 ml of 1% alcohol solution HCL added.

The analysis of phenolic complex was carried out on LC 20 Prominence (Shimadzu, Japan) HPLC system with EC 250/2 NUCLEOSIL 100-5 C18 AB (Macherey-Nagel, Germany) column on SPD-M20A (Shimadzu, Japan) photodiode array detector [16]. All

analytical measurements were carried out in triplicate; a discrepancy between determinations in parallel did not exceed method errors.

Determination of physicochemical parameters of grapes was carried out in fresh must after pressing berries. To do this, an average sample of berries, previously separated from ridges, was taken and crushed, the liquid fraction was separated. When determining the quality composition and quantity content of carbohydrate-acid complex components, and the content of phenolic substances, a sample of must after preliminary centrifugation was used (centrifuge rotor speed 7000 rpm, duration - 10 minutes). The experimental data were processed of mathematical statistics using the IBM SPSS Statistics (v 17.0) and Microsoft Excel software package.

3 Results and Discussion

The first stage of the study involved the assessment of carbohydrate-acid complex of 'Bastardo Magarachskiy' grape variety in the dynamics of ripening (Table 2, 3 and 4). Harvested grapes after reaching the conditions recommended for industrial processing were analytically assessed (mass concentration of sugars was 17 g/100 cm^3 or more).

Table 2. Indicators of carbohydrate-acid complex of grapes

Date of sampling	Experimental variant	Mass concentration		pH	GAI	TRI
		sugars, g/100 cm^3	titratable acids, g/dm^3			
15.09.2023	Control	17.8	5.3	3.6	3.4	228
	Experiment 1	18.8	5.7	3.6	3.3	240
	Experiment 2	18.8	5.9	3.6	3.2	243
22.09.2023	Control	21.5	5.4	3.7	4.0	297
	Experiment 1	22.6	5.4	3.7	4.2	307
	Experiment 2	22.8	5.8	3.8	3.9	324
10.10.2023	Control	21.0	5.1	3.7	4.1	284
	Experiment 1	25.0	5.5	3.9	3.9	375
	Experiment 2	26.0	5.0	3.9	4.3	393

Table 3. Indicators of phenolic ripeness of grapes

Date of sampling	Experimental variant	$A_{pH1,0}$, mg/dm^3	$A_{pH3,2}$, mg/dm^3	A, %	Mp, %
15.09.2023	Control	1216	653	53,7	13,7
	Experiment 1	1241	717	57,8	14,2
	Experiment 2	1575	856	54,3	17,2
22.09.2023	Control	1287	785	61,0	11,6
	Experiment 1	1187	772	65,0	15,5
	Experiment 2	1684	1075	63,9	22,0
10.10.2023	Control	1724	930	53,9	15,0
	Experiment 1	1342	728	54,2	21,0
	Experiment 2	1463	681	46,5	17,5

The analysis of the data obtained indicates that experimental grape processing schemes provide more intense sugar accumulation in grape berries (by 1-4 g/100 cm^3). In addition, experimental grape samples are characterized by a higher content of titratable acids compared to the control.

As grape sugar content increases, the value of mass concentration of titratable acids decreases by 0.2-0.9 g/dm^3 due to physiological processes in grape plants, and the use of organic acids as food sources. The value of technical ripeness indicator and glucoacidimetric

indicator varied in the range of 3.2-4.3 and 228-393, respectively. Some grape batches under study exceeded the recommended values for raw materials used in the production of dry red wines: TRI = 155–270, GAI = 2.5–3.9 [17]. The use of mineral fertilizers allows for higher sugar accumulation and achieves the required quality indicators for producing high-quality dry wines earlier. These conclusions are also supported by other authors [12].

According to the literature references, total anthocyanin potential of grapes is assessed as excellent at the value of $A_{pH1.0}$ more than 1200 mg/dm^3, and very good at 1000-1200 mg/dm^3 [18, 19]. The analysis of the data obtained indicates that indicator value in the studied batches of grapes varies in the range of 1187-1684 mg/dm^3, which allows us to characterize the anthocyanin potential as very good and excellent (Table 2).

During the ripening process of berries, the permeability of the cell walls in the berry skin gradually increases, leading to enhanced extractability of anthocyanins and other components within the grape skin. The maximal degree of extraction of the anthocyanin complex (61-65%) during maceration was observed at a sugar concentration of 21-23 g/100 cm³ [20]. A further increase in the sugar content of grape berry is accompanied by a decrease in extraction processes. The results obtained indicate the necessity in additional agricultural actions at the vegetative stage in order to increase the phenolic ripeness of grapes, as well as the use of technological methods to intensify extraction processes in wine production with anthocyanin extraction degree of less than 60%.

An important indicator of phenolic ripeness of grapes is also the proportion of seed tannins, since the high content of tannins extracted from grape seeds increases the risk of a negative effect on the base wine flavor. The indicator value ranged from 11.6 to 22.0%. The studies carried out did not reveal the dependence of the proportion of seed tannins on the degree of grape ripeness.

Table 4. Profile of grape phenolic complex

Phenology Class compounds	**Phenolic compounds**	**15.09.2023**	**22.09.2023**	**10.10.2023**
Anthocyanins	Delphinidin-3-O-glycoside	11.7	16.5	70.1
	Cyanidin-3-O-glucoside	1.4	6.8	11.5
	Petunidin-3-O-glucoside	18.1	26.0	82.6
	Peonidin-3-O-glucoside	33.6	103.6	161.6
	Malvidin-3-O-glucoside	453.2	495.7	932.8
	Delphinidin-3-O-acetylglucoside	0.2	1.4	2.9
	Cyanidin-3-O-acetylglucoside	0.6	2.4	3.0
	Petunidin -3-O-acetylglucoside	3.1	9.3	11.3
	Peonidin -3-O- acetylglucoside	0.4	0.6	1.1
	Malvidin -3-O- acetylglucoside	42.8	138.6	165.8
	Petunidin -3-O- coumaroyl glucoside	3.0	5.8	24.5
	Malvidin -3-O- coumaroyl glucoside	190.7	239.1	487.7
Hydroxybenzoic and hydroxycinnamic acids	Gallic acid	19.2	25.7	30.1
	Caftaric acid	0.8	1.0	12.5
Procyanidins	B1	5.8	7.9	13.0
	B2	3.7	12.1	19.8
	B3	3.6	5.5	17.9
	B4	16.2	30.9	107.4
Flavan-3-ols	(+)-D-Catechine	15.2	30.5	110.3
	(-)-Epicatechine	599.4	702.6	1220.0

The analysis of the data obtained indicates that the main phenolic substances of grapes are flavan-3-ols and anthocyanins, which amount from 82.7 to 96.3% of all phenolic substances (Table 4). The main representatives of flavan-3-ols in berries are epicatechine and d-catechine, the value of mass concentration of which varies in the range of 777-1910 and 45-195 mg/kg, respectively. Flavan-3-ols account for an average of 36-42% of all phenolic complex components.

The prevailing anthocyanins in grapes are malvidin-3-O-glucoside and malvidin-3-O-coumaroyl glucoside. The amount of mass concentration of malvidin-3-O-glucoside ranges from 580 to 1224 mg/kg or 47.4-81.3% of all grape anthocyanins. Malvidin-3-O-coumaroyl glucoside accounts for 9.3-23.8% of the components of anthocyanin complex.

The content of delphinidin-3-O-acetylglucoside, cyanidin-3-O-acetylglucoside, petunidin-3-O-acetylglucoside, peonidin-3-O-acetylglucoside and coftaric acid was in trace amounts and did not exceed 13 mg/kg.

The study of changes in the profile of grape phenolic complex during ripening process showed an increase in the mass concentration of basic classes of phenolic substances present in grape berry. The main components of phenolic complex undergoing quantity changes are: malvidin-3-O-glucoside, malvidin-3-O-coumaroyl glucoside and epicatechine, the content of which increased by 2.1, 2.6 and 2 times, respectively. The mass concentration of delphinidin-3-O-glycoside increased by 6 times, petunidin-3-O-glucoside by 4.6 times, petunidin-3-O-coumaroyl glucoside by 8.2 times, while the content of the components did not exceed 100 mg/kg.

4 Conclusion

The studies conducted demonstrate variations in the concentrations of carbohydrate-acid and phenolic complexes in grapes during the ripening process, particularly in relation to the application of foliar mineral fertilization systems. It was found that the experimental grape processing methods resulted in a more significant accumulation of sugars in the berries, increasing by 1-4 g/100 cm^3. The total anthocyanin potential of the grapes ranged from 1187 to 1684 mg/dm^3, indicating a quality classification of very good to excellent. The maximum extraction rate of anthocyanin components during maceration was observed to be between 61% and 65% when the sugar concentration in the grapes was between 21 and 23 g/100 cm^3. However, the percentage of extracted anthocyanins did not exceed 65%. Further increases in sugar content were associated with a decline in extraction efficiency.

Based on the data presented, we can conclude that for the ‘Bastardo Magarachskiy’ grape variety, achieving phenolic and technical ripeness corresponds to a sugar concentration in the range of 21-23 g/100 cm^3. The phenolic complex of these grapes primarily consisted of flavan-3-ols and anthocyanins, which accounted for 82.7% to 96.3% of all phenolic substances present. Throughout the ripening process, an increase in the mass concentration of the main classes of phenolic compounds in the grape berries was observed.

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