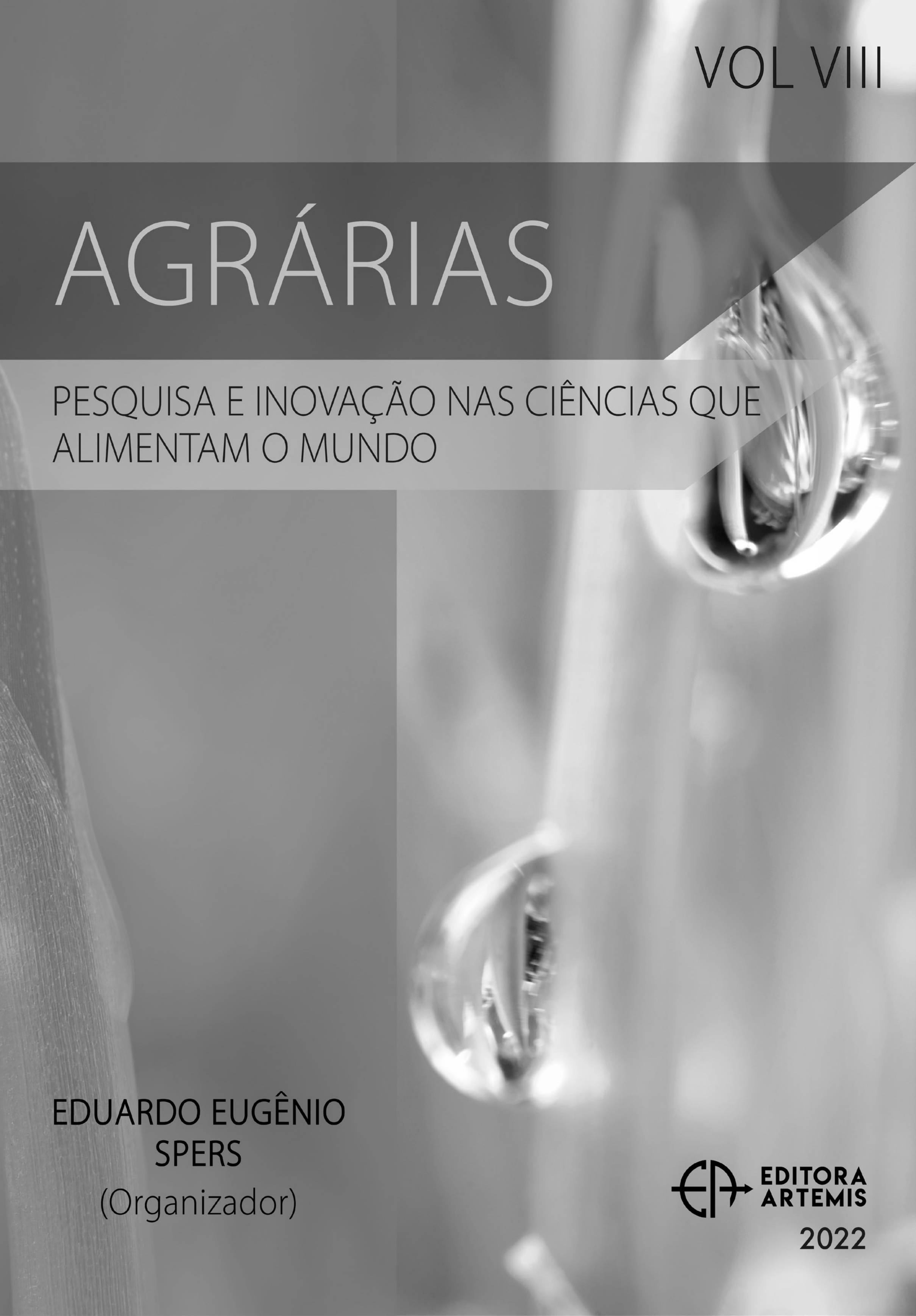
VOL VIII

AGRÁRIAS

PESQUISA E INOVAÇÃO NAS CIÊNCIAS QUE ALIMENTAM O MUNDO

EDUARDO EUGÊNIO SPERS
(Organizador)

EDITORA ARTEMIS
2022



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APRESENTAÇÃO

As Ciências Agrárias são um campo de estudo multidisciplinar por excelência, e um dos mais profícuos em termos de pesquisas e aprimoramento técnico. A demanda mundial por alimentos e a crescente degradação ambiental impulsionam a busca constante por soluções sustentáveis de produção e por medidas visando à preservação e recuperação dos recursos naturais.

A obra **Agrárias: Pesquisa e Inovação nas Ciências que Alimentam o Mundo** compila pesquisas atuais e extremamente relevantes, apresentadas em linguagem científica de fácil entendimento. Na coletânea, o leitor encontrará textos que tratam dos sistemas produtivos em seus diversos aspectos, além de estudos que exploram diferentes perspectivas ou abordagens sobre a planta, o meio ambiente, o animal, o homem e a sociedade no ambiente rural.

É uma obra que fornece dados, informações e resultados de pesquisas tanto para pesquisadores e atuantes nas diversas áreas das Ciências Agrárias, como para o leitor que tenha a curiosidade de entender e expandir seus conhecimentos.

Este Volume VIII traz 26 artigos de estudiosos de diversos países, divididos em quatro eixos temáticos: *Cultura e Sociedade no Contexto Rural; Produção Sustentável; Produção Vegetal e Solos e Aquacultura, Produção Animal e Veterinária*.

Desejo a todos uma proveitosa leitura!

Eduardo Eugênio Spers

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CAPÍTULO 12

THE EFFECT OF VEGETATIVE TREATMENT OF GRAPES WITH A PREPARATION BASED ON AMINO ACIDS ON THE PHENOLIC COMPLEX OF BERRIES

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ABSTRACT: One of the modern problems of winemaking is the lag in the formation of the phenolic complex of berries in relation to the accumulation of sugars during the ripening of grapes. This leads to a distortion of the established organoleptic styles of red wines. It seems promising to influence the metabolism of grapes in the direction of increasing the synthesis of secondary metabolites through the use of foliar dressings. The precursors of the formation of phenolic compounds in the plant cell amino acids. We studied the effect of foliar fertilizers based on a complex of amino acids ("Siamino Pro 500), introduced at the beginning of grape ripening, on the phenolic compounds in grape berries of 4 cultivars from the South Coast zone of the Crimea. Using HPLC, it was revealed that at a similar level of sugar accumulation (in the range 183-218 g L^{-1}), the content of anthocyanins complex in the skins of the experimental grapes of 'Merlot' and 'Shiraz' was 71% and 12% higher than in the control ($\alpha < 0.05$); in 'Cabernet Sauvignon' and 'Sangiovese' it was 17% and 45% less. Experimental processing of 'Merlot' and 'Shiraz' grapes led to an increase in the content of monoglucosides of delphinidin, cyanidin, formed from predecessors, by an average of 1.7 times. In 'Cabernet Sauvignon' and 'Sangiovese', the treatment stimulated the conversion of anthocyanins, increasing the proportion of monoglucosides of malvidin by 1.2 and 1.4 times.
KEYWORDS: Phenolic compounds. Phenolic maturity. Complex of amino acids. Berry skin. Seeds.

O EFEITO DO TRATAMENTO VEGETATIVO DE UVAS COM PREPARAÇÃO À BASE DE AMINOÁCIDOS NO COMPLEXO FENÓLICO DE BAGAS

RESUMO: Um dos problemas modernos da vinificação é o atraso na formação do complexo fenólico das bagas em relação ao acúmulo de açúcares durante o amadurecimento das uvas. Isto leva a uma distorção dos estilos organolépticos estabelecidos de vinhos tintos. Parece promissor influenciar o metabolismo das uvas no sentido de aumentar a síntese de metabólitos secundários através do uso de coberturas foliares. Os precursores da formação de compostos fenólicos nos aminoácidos das células vegetais. Estudamos o efeito de fertilizantes foliares à base de um complexo de aminoácidos ("Siamino Pro 500), introduzido no início do amadurecimento da uva, sobre os compostos fenólicos em bagas de uva de 4 cultivares da zona da costa sul da Crimeia. Usando HPLC , foi revelado que em um nível semelhante de acúmulo de açúcar (na faixa de 183-218 g L-1), o teor de complexo antocianinas nas cascas das uvas

experimentais ‘Merlot’ e ‘Shiraz’ foi de 71% e 12 % maior que no controle (α < 0,05), em ‘Cabernet Sauvignon’ e ‘Sangiovese’ foi 17% e 45% menor O processamento experimental das uvas ‘Merlot’ e ‘Shiraz’ levou a um aumento no teor de monoglicosídeos de delfinidina, cianidina, formada a partir de antecessores, em média 1.7 vezes Em ‘Cabernet Sauvignon’ e ‘Sangiovese’, o tratamento estimulou a conversão de antocianinas, aumentando a proporção de monoglicosídeos de malvidina em 1.2 e 1.4 vezes.
PALAVRAS-CHAVE: Compostos fenólicos. Maturidade fenólica. Complexo de aminoácidos. Casca de baga. Sementes.

1 INTRODUCTION

One of the modern problems of winemaking associated with climate change in the direction of increasing ambient temperature is the lag in formation of berry phenolic complex relative to accumulation of sugars during grape ripening. Consequently, research on grapevine genetic diversity has intensified toward finding varieties characterized by early synthesis of anthocyanins in ontogeny under various abiotic environmental factors (Teixeira et al., 2013; Pastore et al, 2017; Garcia et al. 2018); and cross breeding and genetic studies based on creating new genotypes with high climatic plasticity and berry component composition potential for high quality wines (Risovannaya et al., 2017; Ostroukhova et al., 2019., Davis et al, 2012; Adjeva et al, 2015). Studies are underway to modify the anthocyanin pathway using regulatory and structural genes (Boss et al. 1996, Ananga, et al. 2013). The impact on grape metabolism in the direction of increasing the synthesis of secondary metabolites through agronomic practices seems promising (Zhao et al, 2005). It is known that the biosynthesis of phenolic compounds is based on a combination of acetate-malonate and shikimate reactions, with the bulk of phenolic compounds coming from hydroxycinnamic acids, which are formed from phenylalanine and tyrosine (Heldt and Piechulla, 2011). Current studies indicate a positive effect of biostimulants on the intensification of biosynthesis processes, increased resistance to stress and increased pigment content in berries due to stimulation of the phenylalanine pathway. The positive effect of the combined application of nitrogen and potassium fertilizers on the synthesis of polyphenols and the improvement of berry color characteristics in grapes was established (Delgado, 2006). Considering that most of the amino acids in the plant are capable of transformation from one form to another through amination and reamination reactions, treatment of plants with preparations containing amino acids can intensify the synthesis of secondary metabolites, in particular phenolic substances.

The research is devoted to evaluation of the influence of amino acid preparation application at the phenological phase of vegetation “the beginning of berry ripening” the final of ripening stages on formation of phenolic complex of berries and phenolic maturity of the crop.

2 MATERIALS AND METHODS

2.1 PROCEDURE AND CONDITIONS OF THE EXPERIMENT

Experimental studies were carried out during 2020 on grape 'Cabernet-Sauvignon', 'Merlot', 'Shiraz' and 'Sangiovese' from mountain-valley zone of viticulture of Crimea. Planting scheme was 3.0 × 1.5 m. Bushes shape was a cordon on a middle trunk. Vertical shoot positioned trellising system was used. Single foliar treatment with a preparation based on a complex of amino acids (600 g L-1) "Seamino Siamino Pro 500" at the rate of 1 kg ha-1 was carried out on the experimental plot (1 ha for each cultivar). The preparation was applied in at the phenological phase of vegetation "the beginning of berry ripening". System of agricultural actions without "Sieamino Pro 500" application served as a control.

2.2 SAMPLE PREPARATION AND CHEMICAL ANALYSIS OF GRAPES

Grape samples from control and experimental plots were taken during the commercial harvesting period (September 10, 2020). Healthy bunches were sampled diagonally of plots, from the shady, sunny, upper, middle and lower parts of bushes. The total weight of each sample was 20 kg. Technological assessment of grapes was carried out according to parameters: of mass concentration of sugars, (Compendium, 2017) and phenolic maturity.

The phenolic maturity of grapes was assessed using the Glories method, based on the determining the potential amount of anthocyanins in the grape berry (ApH1,0), concentration of easily extractable anthocyanins in the winemaking conditions (ApH3,2), proportion of easily extractable anthocyanins from their potential amount (Ea= ApH3,2 x 100/ApH1,0), the proportion of seed tannins (Mp) (Glories, 1998; Rajha, 2017;). The method makes it possible to assess the potential of the present proanthocyanidins and anthocyanins, as well as their ability to be extracted from grapes during processing and further technological operations (Rajha, 2017).

2.3 PHENOLIC COMPLEX ANALYSIS BY HPLC METHOD

The quantitative and qualitative composition of the polyphenol complex in the studied samples was determined by HPLC using the AgilentTechnologies system (model 1100) with a diode-matrix detector. A Zorbax SBC18 chromatographic column was used for the separation of substances; chromatograms were recorded at the following wavelengths: 280 nm for gallic acid, (+)-D-catechin, (-)-epicatechin and procyanidins, 313 nm for hydroxycinnamic acids derivatives, 371 nm for quercetin and 525 nm for anthocyanins. The substances were identified by comparing their spectral characteristics in terms of retention time with similar characteristics of the standards. Gallic acid, caffeic acid, (+)-D-catechin, malvidin 3-O-glucoside chloride, quercetin dihydrate, isoquercitrin (FlukaChemie AG, Switzerland), (-)-epicatechin, syringic acid (SigmaAldrich, Switzerland) were used as standards.

2.4 STATISTICAL ANALYSIS OF THE DATA

All chemical analyses were performed in triplicate. The entries were mathematically processed with the help of statistical software package SPSS Statistics 17.0. Arithmetic mean value, variance of a single result and standard deviation (SD) were determined. Significance of the difference of indicators in grapes of control and experimental batches was estimated by U-test. The predetermined probability of an erroneous result (α) was less than 0.05.

3 RESULTS AND DISCUSSION

The application of dressing under experimental conditions had no significant effect on the accumulation of sugars in berries, which allowed us to evaluate the effect of the studied treatment on the phenolic complex of grapes at parity content of sugars in the experimental and control batches of grapes.

From the perspective of wine quality, an important characteristic of berry phenolic complex is, on the one hand, the level of accumulation and extraction of skin anthocyanins; on the other hand, the degree of polymerization and extraction of seed polyphenols (Ribereau-Gayon, et al., 2006; Ren et al., 2017).

Monomeric and dimeric phenolic components of flavonoid and non-flavonoid structure were identified in the skin of the experimental and control lots of grapes using HPLC analysis (Table 1). The content of the components in the control lots of grapes ranged from 1630.6 ± 97.8 mg kg-1 of skin ('Merlot') to 6868.0 ± 549.4 mg kg-1 ('Cabernet Sauvignon'). The vegetative treatment of grapes with amino acid based preparation had ambiguous effect on the components content in berry skin: in 'Merlot' and 'Shiraz' grapes their concentration was increased (by 67% and 3% respectively), in 'Cabernet Sauvignon' and 'Sangiovese' - it decreased by 23% and 50%.

The proportion of anthocyanins among identified components in the skins of control lots of grapes ranged from 54% ('Cabernet Sauvignon') to 83% ('Merlot') and increased by 2%-6% under the influence of vegetative treatment of grapes. At the same time, the mass concentration of anthocyanins complex in the skin of the experimental batches of 'Cabernet Sauvignon' and 'Sangiovese' grapes was 17% and 45% lower than in the control lots, amounting to 3065.8 ± 122.6 and 1206.3 ± 78.4 mg kg-1. In contrast, the content of anthocyanins complex in 'Merlot' and 'Shiraz' grapes under the influence of its treatment with 'Siamino Pro 500' increased by 71% and 12%, reaching 2306.0 ± 161.4 and 2598.5 ± 90.9 mg kg-1, respectively.

Table 1. The content (arithmetic mean value[1], mg kg^{-1}) of phenolic compounds in the skin of berries.

Phenolic compounds	**'Cabernet Sauvignon'**		**'Merlot'**		**'Sangiovese'**		**'Shiraz'**	
	control	**experiment**	**control**	**experiment**	**control**	**experiment**	**Control**	**experiment**
Hydroxybenzoic and hydroxycinnamic acids	87.1	40.5	19.8	25.1	20.6	18.1	49.9	68.6
Flavonols	91.2	119.3	26.9	42.9	9.8	3.0	49.3	52.3
Flavan-3-ols	2045.9	1462.9	165.5	298.4	481.5	166.3	1095.6	935.4
Procyanidins	941.1	510.2	74.0	48.4	178.7	22.7	308.5	266.2
Anthocyanins:								
delphinidin-3-O-β-D-glucoside	358.2	172.9	106.8	199.3	380.2	116.3	173.8	126.0
cyanidin-3-O-β-D-glucoside	79.3	18.2	15.4	22.3	300.3	99.3	29.7	81.2
petunidin-3-O-β-D-glucoside	293.2	131.1	96.6	171.7	377.6	167.2	193.4	375.7
peonidin-3-O-β-D-glucoside	188.9	71.8	53.3	89.7	205.6	124.1	154.8	155.8
malvidin-3-O-β-D-glucoside	1501.3	1567.4	605.7	1211.8	919.8	683.3	994.9	916.9
delphinidin-3-O-β-D-glucoside-6'-O-acetate	75.8	42.6	16.8	34.0	0.6	1.4	32.5	147.7
cyanidin-3-O-β-D-glucoside-6'-O-acetate	22.9	10.3	4.7	7.1	1.1	0.6	12.6	52.1
petunidin-3-O-β-D-glucoside-6'-O-acetate	105.4	50.9	24.3	46.0	1.3	3.7	53.1	196.9
peonidin-3-O-β-D-glucoside-6'-O-acetate	3.4	1.1	0.0	3.4	1.1	1.7	6.6	2.7
malvidin-3-O-β-D-glucoside-6'-O-acetate	601.2	716.8	204.4	331.7	6.1	2.3	297.1	363.0
petunidin-3-O-β-D-glucoside-6'-O-p-coumarate	52.3	15.9	17.0	14.1	6.2	0.4	35.4	41.1
malvidin-3-O-β-D- glucoside-6'-O-p-coumarate	420.9	266.9	199.4	175.0	12.4	6.1	331.5	139.4
Total anthocyanins:	3702.8	3065.8	1344.4	2306.1	2212.3	1206.3	2315.4	2598.5
Total phenolic components	6868.0	5198.8	1630.6	2720.9	2902.9	1416.5	3818.7	3921.0

[1]SD-values were lower than 9% for all assays.

Siamino Pro 500 treatment in ‘Merlot’ grapes stimulated biosynthesis of anthocyanins from precursors quercetin and myricetin (Teixeira et al., 2013; Mattioli et al, 2020), as evidenced by 1.9 and 1.5-fold increases ($\alpha < 0.05$) in monoglucosides of delphinidin, cyanidin, and their derivatives acylated by acetic acid or by p-coumaric acid, and by interconversion of anthocyanins, as indicated by an average 1.7-fold increase in monoglucosides of malvidin, peonidin, petunidin and their derivatives. The increase in the content of the anthocyanin complex in the experimental lots of ‘Shiraz’ grapes was due to the increased formation of monoglucosides of delphinidin, cyanidin and their derivatives (1.6-fold) and monoglucosides of petunidin (2.2-fold), at the same time there was a 13% decrease in the content of monoglucosides of malvidin relative to control. Considering that the final metabolites of the chain of transformation of anthocyanin compounds of Vitis vinifera use cursives for scientific name are malvidin-3-O-β-D-glucoside and its derivatives (Narduzzi et al., 2015), the presented data suggest that the anthocyanin complex accumulated in the experimental batches of ‘Shiraz’ grapes is in the stage of active interconversion of components. In ‘Cabernet Sauvignon’ grapes the vegetative treatment did not lead to the intensification of biosynthesis of anthocyanins from precursors: on the contrary, the content of monoglucosides of delphinidin, cyanidin and their derivatives in experimental lots was 2 and 3.6 times lower than in control. At the same time, the use of top dressing stimulated mutual transformation of anthocyans, as evidenced by the increased proportion of monoglucosides of malvidin (from 68% in the control batches to 83% - in the experimental), while the proportion of all other components of the anthocyanin complex decreased. Similar effect was exerted by the treatment on the formation of anthocyanin complex of ‘Sangiovese’ grape skins. At the same time, in both control and experimental batches, a low proportion of monoglucosides of malvidin (42% and 57%, respectively) with relatively high proportions of monoglucosides of cyanidin, peonidin, petunidin, which indicates incomplete processes of mutual transformation of components and/or variety-specific features of the ‘Sangiovese’ anthocyanin complex.

The observed effect of vegetative grape treatment on the content and component composition of anthocyanins complex of berry skin was reflected in a significant ($\alpha < 0.05$) increase in the concentration of easily extractable anthocyanins in ‘Sangiovese’ and ‘Shiraz’ by 24 % and 47 % relative to control (Table 2).

Table 2. Indicators of phenolic maturity of grapes from experimental and control lots (1).

Cultivar	Option	$A_{pH1.0}$, mg L^{-1}	$A_{pH3.2}$, mg L^{-1}	Ea, %	Mp, %
‘Cabernet Sauvignon’	control	1150±67	602±24	52±1	23±1
‘Cabernet Sauvignon’	experiment	1104±55	604±24	55±2	21±1
‘Merlot’	control	1293±71	651±20	50±1	24±2
‘Merlot’	experiment	1031±41	618±21	60±3	20±1
‘Sangiovese’	control	1190±36	343±14	29±2	21±2
‘Sangiovese’	experiment	841±42	425±17	50±3	14±2
‘Shiraz’	control	1468±66	528±26	36±2	23±2
‘Shiraz’	experiment	1377±69	776±29	56±2	30±3

[1] arithmetic mean value ±SD; $A_{pH1,0}$ – potential amount of anthocyanins in the grape berry; $A_{pH3,2}$ – concentration of easily extractable anthocyanins in the winemaking conditions; Ea – proportion of easily extractable anthocyanins from their potential amount; Mp – the proportion of seed tannins

The grapes of the experimental batches of all cultivars were characterized by a higher proportion of easily extractable anthocyanins from their potential amount (Ea), reflecting the proportion of easily extractable anthocyanins in the anthocyanin complex. In the experimental grapes ‘Sangiovese’ and ‘Shiraz’ the increase of Ea index was 20-21%, in the grapes ‘Cabernet Sauvignon’ and ‘Merlot’ - 3-10%. Thus, treatment of grapes with “Siamino Pro 500” promoted formation of anthocyanin complex, and at the same level of accumulation of sugars the grapes of experimental batches were characterized by better indicators of “phenolic maturity”.

Regarding the other phenolic components of grape skins, it should be noted (Table 1) that in the experimental batches of grapes of all varieties the mass concentration of flavan-3-ols and procyanidins decreased by 2% (‘Sangiovese’) - 93% (‘Shiraz’) and 14% (‘Shiraz’) - 72% (‘Sangiovese’) respectively.

Phenolic complex of seeds changed under the influence of vegetative treatment of grapes (Table 3). The content of phenolic components in the seeds of the control lots varied from 513.3 ± 40.1 mg kg-1 (‘Sangiovese’) to 2299.1±137.9 mg kg-1 (‘Shiraz’ in the seeds of experimental lots - less by 12% (‘Cabernet Sauvignon’) to 38% (‘Sangiovese’) relative to the control values. The phenolic complex of the seeds of the control and experimental lots of ‘Sangiovese’ grapes differed by a high proportion of hydroxybenzoic and hydroxycinnamic acids - 21%, and procyanidins and flavan-3-ols accounted for 74% in the experimental lots and 76% in the control. In all other cases, the proportion of procyanidins and flavan-3-ols in the phenolic complex of seeds of control and experimental lots of grapes was 91-96%. A simultaneous reduction in the content of flavan-3-ols (by 3 - 27%) and procyanidins (by 15 - 45%) in the seeds of ‘Merlot’, ‘Sangiovese’ and ‘Shiraz’ grapes was observed under the effect of vegetative treatment. In the seeds of 'Cabernet Sauvignon' grapes of the experimental lots, the content of flavan-3-ols decreased by 50% relative to control, while the concentration of procyanidins increased by 60%.

Table 3. The content (arithmetic mean value[1], mg kg^{-1}) of phenolic compounds in the seeds.

Phenolic compounds	'Cabernet Sauvignon'		'Merlot'		'Sangiovese'		'Shiraz'	
	control	experiment	control	experiment	control	experiment	control	experiment
Hydroxybenzoic and hydroxycinnamic acids	42.1	77.9	73.7	37.5	110.0	66.7	79.8	39.0
Flavonols	4.6	9.0	1.7	2.4	11.9	2.5	18.5	36.5
Flavan-3-ols	754.1	373.8	282.1	207.1	69.5	58.0	732.7	709.1
Procyanidins	354.7	568.5	479.0	405.7	321.7	77.4	1358.8	1092.6
Anthocyanins:	11.5	2.0	1.4	2.7	0.37	11.4	9.2	7.1
Total phenolic compounds	116.,0	1031.2	837.9	655.4	513.4	216.0	2199.0	1884.2

[1]SD-values were lower than 9% for all assays.

The data presented, on the one side indicate that the applied treatment did not stimulate the biosynthesis of procyanidins and flavan-3-ols in grape seeds and grape skins. On the other side, vegetative treatment of grapes promoted oxidative polymerization of components with the formation of high-molecular-weight tannins, which led to a decrease in easily extractable monomeric and dimeric flavonoids.

The analysis of grapes according to the Glories method (Glories and Vivas, 1998). Table 2 showed a decrease ($\alpha<0.05$) in the extractable polyphenols of seeds in the experimental lots of 'Cabernet Sauvignon', 'Merlot', 'Sangiovese' by 2-7%, which indicates a higher degree of technological maturity of their phenolic complex compared to control. In 'Shiraz' grapes the share of extractable seed polyphenols in the experimental lots was 7% higher than in the control. This indicates incomplete processes of oxidative polymerization and condensation of seed polyphenols.

4 CONCLUSIONS

Thus, the treatment of grapes with the preparation "Siamino Pro 500" in the cvs. 'Cabernet Sauvignon', 'Merlot', 'Sangiovese', 'Shiraz' at the beginning of berry ripening stimulated the formation of monoglucosides of delphinidin, cyanidin from precursors (1.7 times on average) and/or transformation of anthocyanins, increasing the proportion of monoglucosides of malvidin in anthocyanins complex by 1.2-1.4 times. At the same time, application of amino-acid-based fertilizer under the experimental conditions had no effect on the biosynthesis of procyanidins and flavan-3-ols in berry seeds and skin, but it apparently promoted their oxidative polymerization, which reduced the content of procyanidins by 14-72%, flavan-3-ols - by 2-93%. The effect of vegetative treatment of grapes phenolic complex of berries was the improvement of yield phenolic maturity indicators at a close level of sugars accumulation: the share of extractable seed polyphenols decreased, and easily extractable anthocyans - increased by 3-21%.

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ÍNDICE REMISSIVO

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