Study of Phenolic Compounds of Grape Pomace

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ABSTRACT

The article presents experimental data on the component composition of the phenolic complex of aqueousalcoholic extracts of sweet and fermented pomace of Cabernet Sauvignon, Merlot, Saperavi, Krasnostop Anapskiy, Dostoyny and Alkor red grape varieties. In extracts of sweet pomace, the presence of various concentrations of flavones, anthocyanins, procyanidins, oxycinnamic and hydroxybenzoic acids has been revealed. Flavonoid monomers are represented by anthocyanins, quercetin, (+)-D-catechin, (-)-epicatechin. Monomers of non-flavonoids are represented by hydroxy acids - caftaric, cautaric, and gallic. As for oligomeric polyphenols, the presence of bB₁, B₂, B₃, and B₅ procyanidins has been revealed. The presence of resveratrol stilbene has been registered as well. The greatest amount of most of the components of the phenolic complex, including resveratrol, was found in extracts from the Krasnostop Anapsky and Saperavi grape varieties, while the smallest amount was found in Merlot. In fermented pomace, the concentration of all groups of phenolic compounds, especially procyanidins and anthocyanins, was less than in sweet pomace, while the presence of myricetin, myricetin-3-glycoside, peonidin-3-O-(6'-acetyl-glycoside), and cyanidin-3.5-O-diglycoside malvidin- 3-O- (6'-p-coumaroyl glycoside) has not been registered. All extracts had antioxidant activity, the value of which depended on the grape variety and the technology for obtaining pomace. The highest value of antioxidant activity was registered in the extract of sweet pomace of KrasnostopAnapsky and Saperavi grape varieties.

KEYWORDS

Sweet and Fermented Grape Pomace, Extracts, Phenolic Compounds, Antioxidant Activity.

Introduction

In the southern regions of the Russian Federation, much attention is paid to the improvement of the range of grapes for technical processing in order to produce high-quality wines. Particular attention is paid to red wines and the grape varieties used for their production. The wholesomeness of red wines is well-known [1, 2, 3]. Almost all researchers associate the positive effect of wine on the human body with the presence of natural bioflavonoids (phenolic compounds) that have antioxidant, antiviral and antiradical properties. At present, about 70% of all vineyard areas in Krasnodar Krai are occupied by red grape varieties. With an increase in the production of technical red grape varieties, the amount of waste from their processing increases, first of all, the amount of grape pomace, which makes up about 20% of the mass of processed grapes. Currently, grape pomace is practically not processed and is a natural waste product. Meanwhile, the pomace of red grape varieties contains a large amount of phenolic antioxidants and can become a raw material for the production of extracts, concentrates, soft drinks, and coloring components instead of synthetic dyes. It is known that the concentration of phenolic compounds in red grapes and wines varies within wide limits [3, 4, 5, 6, 7] depending on the grape variety. In grapes, phenolic compounds are concentrated mainly in the skin (28-35%) and seeds (up to 70%). This suggests that grape pomace can be a good source of natural antioxidants and coloring agents. The pomace of red grape varieties is subdivided into sweet, obtained after pressing the grapes and separating the must, and fermented, obtained after pressing the fermented pulp.

The purpose of the present study is to identify the concentration of phenolic compounds in grape pomace of red grape varieties depending on the technology of their processing for further use in the production of extracts and polyphenol concentrates.

Objects and methods of research. As objects of research, the following materials were used: aqueous-alcoholic extracts of sweet and fermented grape pomace of the classic grape varieties Vitis vinifera Cabernet Sauvignon, Merlot, Saperavi, intraspecific and interspecific hybrids - Krasnostop Anapsky (clone selection of the KrasnostopZolotovsky variety), Dostoyny (phylloxerous-resistant Dzhemete x Hamburg Muscat), and Alcor(Serexiax Cabernet Sauvignon). To prepare a water-alcohol extract, sweet pomace was used, obtained by the following technology: grapes were crushed, the pulp was infused at a temperature of $40-45^{\circ}$ C for 6 hours, after which it was pressed with the separation of pulp and must. The fermented pomace was obtained by pressing the pulp after its fermentation and separation of the wine material. Aqueous-alcoholic extracts of sweet and fermented pomace were obtained by infusing them for 10 days. The volume fraction of ethyl alcohol in the extracts was 60%. The total concentration of phenolic compounds was determined by the colorimetric method using the Folin–Ciocâlteu reagent (FCR) [8].

Antioxidant activity (further referred to as AOA) of water-alcohol extracts was determined by the amperometric method using a "Tsvet - Yauza - 01AA" device produced by the research and manufacturing association NPO Khimavtomatika [9] in terms of TROLOX, a synthetic analogue of gallic acid.

Qualitative and quantitative estimation of polyphenols was carried out by HPLC (high performance liquid chromatography) using Agilent Technologies chromatographic system (model 1100) with a diode array detector. For separation, a Zorbax SB-18 chromatographic column with a size of 2.1-150 mm was used, filled with silica gel with a grafted octadecylsilyl phase with a sorbent particle size of 3.5 μ m. Elution was carried out in gradient mode. The flow rate of the eluent was 0.25 ml/min. To form the gradient, the following was used: solution A - methanol; solution B - 0.6% aqueous solution of trifluoroacetic acid. The volume of the injected sample was 2 μ L. Chromatograms were recorded by the optical absorption of the eluate at the following wavelengths: 280 nm for gallic acid, (+)-D-catechin, (-)-epicatechin, and procyanidins; 313 nm for hydroxycinnamic acid derivatives; 371 nm for quercetin; 350 nm for quercetin glycosides and 525 nm for anthocyanins. To register trans-resveratrol, a fluorometric detector was used at absorption wavelengths of 280 and 320 nm of radiation. The components were identified by comparing the spectral characteristics and retention times of the peaks in the studied samples and in standard calibration solutions. The calculation of the quantitative content of individual components was performed using calibration curves of the dependence of the peak area on the concentration of the substance, plotted for solutions of individual substances. Statistical data processing was carried out using the following software: Microsoft Exel.

Discussion

Table 1 presents experimental data on the ranges of variation of the total concentrations of phenolic compounds and the antioxidant activity of extracts of sweet and fermented pomace, obtained during 5 years of observation (more than 400 samples of pomace were analyzed). Studies have shown that the total concentration of phenolic compounds varied in a wide range depending on the type of pomace and grape variety. A general trend has been revealed: the concentration of phenolic compounds and the value of antioxidant activity in the extracts of sweet pomace was higher than in the extracts of fermented pomace.

As a result of Pearson's correlation analysis between the amount of catechins, the mass concentration of anthocyanins, the amount of procyanidins, and AOA (Table 2), a high level of connection was revealed between all indicators of phenolic compounds of the grape pomace. With a change in gallic acid, a tendency was revealed for the total of catechins to change; the linear correlation coefficient was r=0.97; the boundaries of the 95% confidence interval (hereinafter CI) varied from 0.90 to 0.99.

The significant correlation between the total content of catechins and the mass concentration of procyanidins in the studied grape pomace (r=1, CI = 0.99 - 1.00) is explained by the chemical nature of these substances: procyanidins are di- and trimers of catechins.

Table 1. Ranges of variation of total concentrations of phenolic compounds for 5 years of observation

Component	Component concentration, mg/dm ³ , in the pomace of varieties							
	Cabernet	Merlot	Saperavi	Krasnostop	Dostoyny	Alkor		
	Sauvignon			Anapsky				
Sweet pomace								
Gallic acid	257-274	187-203	312-356	322-365	264-292	288-318		
Catechins, total	846-868	764-793	908-931	988-1022	845-878	918		
Anthocyanins	127-139	92-118	182-213	218-254	145-192	187-208		
Procyanidins, total	436-510	322-400	478-520	528-620	388-425	412-478		
AOA, g/dm^3 , in terms of	14.8–16.7	10.2-	21.7 -	24.0-28.6	14.5-17.8	18.7–		
TROLOX		12.8	24.6			21.3		
Total of phenolic	20.8-22.4	16.7–	29.4-	38.3-44.2	21.7-25.7	23.8-		
compounds, g/dm ³		19.8	33.7			26.5		
Fermented pomace								
Gallic acid	54-68	17-22	97-122	108-123	50-56	50-62		
Catechins, total	144-162	110-122	160-185	180-194	148-164	166-178		
Anthocyanins	7.4–7.8	3.0-4.2	10.3–	14.7–18.6	4.2-5.8	5.4-6.8		
			11.8					
Procyanidins, total	80-87	60-66	98-112	106-121	68-78	80-85		
AOA, g/dm^3 , in terms of	10.0-10.7	7.0–7.6	10.1-	10.0–13.4	7.8-8.3	8.6–9.0		
TROLOX			11.2					
Total of phenolic	8.7–9.4	4.1–4.5	10.1-	11.8–14.1	7.0–7.7	7.6-8.4		
compounds, g/dm ³			12.7					

Table 2. Results of Pearson's multiple correlation analysis

Component	r/CI	Catechins, total	Anthocyanins	Procyanidins, total	AOA, g/dm ³ , in terms of TROLOX	Total of phenolic compounds, g/dm ³
Gallic acid	r	0.97	0.98	0.98	0.94	0.97
Game actu	CI	0.90-0.99	0.92-0.99	0.92-0.99	0.79–0.98	0.88–0.99
Cataching total	r		0.97	1.00	0.89	0.93
Catechins, total	CI		0.90-0.99	0.99-1.00	0.65-0.97	0.78–0.98
Anthoayaning	r			0.97	0.95	0.96
Anthocyanins	CI			0.91-0.99	0.82-0.99	0.87–0.99
Procyanidins,	r				0.91	0.95
total	CI				0.72–0.98	0.83-0.99
AOA, g/dm^3 , in	r					0.98
terms of	CI]				0.94–1.00
TROLOX						

Tables 3 and 4 show the component composition of water-ethanol extracts of sweet and fermented pomace obtained during the processing of grapes of various varieties. The identity of the qualitative composition of the phenolic complex of red table wine and the water-ethanol extract of sweet pomace was established, which is consistent with [10, 11, 12]: the presence of various concentrations of flavones, anthocyanins, procyanidins, oxycinnamic and hydroxybenzoic acids was revealed. Flavonoid monomers are represented by anthocyanins, quercetin, (+)-D-catechin, (-)-epicatechin. Monomers of non-flavonoids are represented by hydroxy acids - caftaric, cautaric, and gallic. Among oligomeric polyphenols, procyanidins B_1 , B_2 , B_3 , and B_5 , which are condensed derivatives of catechin, have been found. It should be noted that the mass concentration of most phenolic compounds in aqueous-alcoholic extracts was higher than in red table wine material, especially the material produced without the use of thermovinification and/or pulp fermentation [3,13,14]. This is due to the presence in the pomace of grape seeds containing various groups of polyphenols, especially catechins, gallic acid, B_1 procyanidin, and their transfer from seeds to the extract.

varieties (average data for 5 years)								
Component	Component concentration, mg/dm ³ , in the pomace of varieties							
	Cabernet Sauvignon	Merlot	Saperavi	KrasnostopAn apsky	Dostoyny	Alkor		
Gallic acid	257	187	312	322	264	288		
(+)-D-Catechin	537	486	583	612	527	550		
(-)-Epicatechin	317	288	343	410	324	368		
Syringic acid	224	188	233	248	218	231		
Caftaric acid	47	45	54	66	48	49		
Cautaric acid	21	12	43	56	18	16		
p-Coumaric acid	12	7	16	10	6	12		
Quercetin-3-O-glycoside	4.2	1,8	6.7	11	0.8	3.8		
Quercetin	42	18	45	51	27	40		
Myricetin	1.4	-	3.2	2.7	-	1.7		
Myricetin-3-glycoside	0.8	0.3	0.8	1.1	-	_		
Delphinidin-3.5-O-diglycoside	12	7	12	14	8	12		
Cyanidin-3.5-O-diglycoside	3.2	-	4.6	4.6	-	1.1		
Petunidin-3.5-O-diglycoside	12	10	14	21	12	14		
Delphinidin-3-O-glycoside	12	8	14	17	10	14		
Peonidin-3.5-O-diglycoside	4	-	6	8	-	6		
Malvidin-3.5-O-diglycoside	56	40	58	67	75	58		
Cyanidin-3-O-glycoside	1.6	1,8	3.3	2.2	1.4	2.0		
Petunidin-3-O-glycoside	1.6	1.2	2.6	3.5	2.0	2.2		
Peonidin-3-O-glycoside	1.3	-	2.7	4.4	0.9	1.5		
Malvidin-3-O-glycoside	3.9	3.3	4.6	4.0	3.2	3.2		
Delphinidin-3-O-(6'-acetyl-glycoside)	13	10	16	17	10	15		
Cyanidin-3-O-(6'-acetyl-glycoside)	6.8	3.4	7.2	8.0	4.4	5.7		
Delphinidin-3-O-(6'-p-coumaroyl glycoside)	1.3	-	1.2	2.7	I	3.0		
Peonidin-3-O-(6'-acetyl-glycoside)	5.2	3.7	6.4	6.0	3.9	3.5		
Malvidin-3-O-(6'-acetyl-glycoside)	4.1	3.7	3.3	4.4	3.8	4.4		
Cyanidin-3-O-(6'-p-coumaroyl-glycoside)	2.5	2.2	2.7	3.4	2.7	3.3		
Petunidin-3-O-(6'-p-coumaroyl-glycoside)	1.1	-	1.7	1,8	1.2	1.4		
Peonidin-3-O-(6'-p-coumaroyl-glycoside)	1.1	0.4	1.7	1.5	0.8	1.0		
Malvidin-3-O-(6'-p-coumaroyl-glycoside)	1.2	0.4	1.2	1.4	0.8	1.1		
trans-resveratrol	4.8	4.2	8.6	8.6	4.4	4.0		
Procyanidins			•					
B ₁	238	212	256	315	227	244		
B ₂	128	97	134	156	112	126		
B ₃	65	48	72	70	52	70		
B5	29	24	32	34	18	21		
AOA, g/dm ³ , in terms of TROLOX	16.8	13.2	23.7	27.0	15.5	19.7		
Total of phenolic compounds, g/dm ³	24.8	18.7	31.6	40.4	23.7	25.8		

 Table 3. Component composition of phenolic compounds in water-ethanol extract of sweet pomace of red grape varieties (average data for 5 years)

A significant influence of the varietal characteristics of grapes on the concentration of phenolic compounds in the aqueous-alcoholic extract of sweet pomace was established. The mass concentration of gallic acid, one of the most powerful antioxidants, varied from 187 (Merlot) to 322 mg/dm³ (Krasnostop Anapsky). The greatest amount of most of the components of the phenolic complex, including resveratrol, was found in extracts from the Krasnostop Anapsky and Saperavi grape varieties. In the aqueous-alcoholic extract of Merlot, the concentration of phenolic compounds had the lowest values. The authors of the present article believe that the results obtained can be explained by the fact that Merlot, in comparison with other grape varieties under study, has a lower technological reserve of phenolic compounds in grapes. This makes it possible to state that the same pattern is characteristic of the grape pomace.

The different degree of extraction of phenolic compounds with a water-alcohol solution can also be explained on the basis of data on the physical and mechanical properties of grape skins [15, 16, 17, 18]. Using the dynamometric method according to the method [17], a variation in the strength of the skin was established varying from 0.793-830 (Saperavi, Krasnostop Anapsky, Alkor) to 1.164 N (Dostoyny, Cabernet Sauvignon).

The presence of high concentrations of catechins and procyanidins caused the antioxidant activity of aqueousalcoholic extracts of sweet pomace, the value of which correlates well with the concentrations of the sum of catechins, procyanidin B_1 and the sum of phenolic compounds. Among the procyanidins, procyanidin B_1 stood out in terms of concentration, which, according to [19, 20], shows the greatest antioxidant activity and is able to suppress the activity of free radicals [21].

Table 3 shows experimental data on the component composition of the phenolic complex of the fermented pomace. The general tendencies revealed in the analysis of sweet pomace are also characteristic of fermented pomace: the highest total concentration of the sum of phenolic compounds, catechins, and antioxidants was found in the fermented pomace of Saperavi and Krasnostop Anapsky varieties, followed by Alcor and Cabernet-Sauvignon (close values of indicators).

Comparative analysis of the experimental data in Tables 3 and 4 showed that in the extracts of fermented pomace (Table 4), many components are absent, primarily anthocyanins. For example, myricetin, myricetin-3-glycoside, peonidin-3-O-(6'-acetyl glycoside), cyanidin-3,5-O-diglycoside, or malvidin-3-O-(6'-p-coumaroyl glycoside) have not been found in the extracts of fermented pomace of any of the grape varieties. However, the extracts of the fermented pomace were intensely colored. Almost all components of the phenolic complex were found in the fermented pomace of Saperavi and Krasnostop Anapskiy varieties.

Due to the presence of catechins, anthocyanins and procyanidins, as well as grape seeds, the extract of fermented pomace has antioxidant activity, but its value is 1.5-2 times less than in the extracts of sweet pomace. This indicates the existence of a direct correlation between catechins, procyanidins, gallic acid and antioxidant activity (Table 2).

Thus, the presented experimental data indicate the high biological value and antioxidant properties of sweet and fermented grape pomace of red grape varieties and the appropriateness of their use for the production of extracts.

Component Component concentration, mg/dm ³ , in the pomace of y						
	Cabernet Sauvignon	Merlot	Saperavi	Krasnostop Anapsky	Dostoyny	Alkor
Gallic acid	64	17	97	112	52	56
(+)-D-Catechin	123	97	132	144	125	137
(-)-Epicatechin	36	24	34	42	31	37
Syringic acid	14	19	13	21	18	16
Caftaric acid	1.2	-	3.4	3.2	1.3	1.4
Cautaric acid	1.2	-	1.4	3.6	-	1.0
p-Coumaric acid	0.8	-	1.2	10	-	0.8
Quercetin-3-O-glycoside	4.2	-	6.7	11	-	3.8
Quercetin	0.2	-	45	51	27	0.2
Myricetin	-	-	-	-	-	-
Myricetin-3-glycoside	-	-	-	-	-	-
Delphinidin-3.5-O-diglycoside	0.12	-	0.12	0.14	-	0.04
Cyanidin-3.5-O-diglycoside	-	-	-	-	_	-
Petunidin-3.5-O-diglycoside	1.0	-	1.1	1.1	-	-
Delphinidin-3-O-glycoside	-	_	1.1	1.3	_	-
Peonidin-3.5-O-diglycoside	-	-	-	1.0	_	-
Malvidin-3.5-O-diglycoside	1.3	2.4	3.0	3.2	3.7	3.8
Cyanidin-3-O-glycoside	-	-	-	0.4	_	0.2
Petunidin-3-O-glycoside	0.3	_	0.3	0.4	_	-
Peonidin-3-O-glycoside	-	_	0.5	0.6	_	-
Malvidin-3-O-glycoside	0.7	0.2	0.5	0.5	0.2	0.2
Delphinidin-3-O-(6'-acetyl-glycoside)	0.2	-	0.6	0.5	0.2	0.3
Cyanidin-3-O-(6'-acetyl-glycoside)	0.5	_	0.8	0.8	-	0.4
Delphinidin-3-O-(6'-p-coumaroyl glycoside)	-	_	0.2	0.7	_	-
Peonidin-3-O-(6'-acetyl-glycoside)	-	-	_	_	_	-
Malvidin-3-O-(6'-acetyl-glycoside)	0.8	0.4	3.3	3.8	0.4	0.4
Cyanidin-3-O-(6'-p-coumaroyl-glycoside)	0.6	_	0.7	1.0	0.3	0.5
Petunidin-3-O-(6'-p-coumaroyl-glycoside)	-	-	0.5	0.5	_	0.3
Peonidin-3-O-(6'-p-coumaroyl-glycoside)	0.2	_	0.4	0.5	_	0.2
Malvidin-3-O-(6'-p-coumaroyl-glycoside)	-	-	_	_	-	_
trans-resveratrol	1.9	0.4	1.6	2.0	0.4	0.40
Procyanidins	•					
B ₁	42	37	46	52	44	46
B ₂	27	19	38	42	19	23
B ₃	8	4	10	12	8	10
B ₅	5	2	6	6	_	3
AOA, g/dm ³ , in terms of TROLOX	10.3	7.6	10.8	10.6	8.3	9.0
Total of phenolic compounds, g/dm ³	9.1	4.3	11.4	12.0	7.3	8.2

 Table 4. Component composition of phenolic compounds in water-ethanol extract of fermented pomace of red grape varieties (average data for 5 years)

Conclusion

A difference in the qualitative composition and concentration of phenolic compounds in sweet and fermented pomace of red grape varieties has been established. The study has shown the dependence of the concentrations of phenolic compounds on the characteristics of the grape variety. Sweet and fermented pomace have high antioxidant activity mainly due to the presence of catechins, procyanidins, hydroxybenzoic and hydroxycinnamic acids. The study has revealed a high level of connection between all indicators of phenolic compounds of the grape pomace. The largest amounts of antioxidants were found in water-ethanol extracts of pomace from Saperavi and KrasnostopAnapsky grape varieties.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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