





COMPARISON OF PHENOLIC CONTENT IN CABERNET SAUVIGNON AND SAPERAVI WINES

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ABSTRACT

Several studies reveal that the phenolic compounds present in the wine and their concentrations determine physiological activities of the red wine. In this study, the main polyphenol components, including hydroxycinnamic acids, flavones, flavan-3-ols and stilbenoids, were investigated via HLPC-UV in the "Cabernet Sauvignon" and "Saperavi" wines selected from different regions and different years. In assistance of a meta-analysis, we found that there are no fundamental differences in phenolic compounds between the wines Cabernet Sauvignon and Saperavi. However, the amounts of several important phenolic materials such as catechin, caffeic acid, p-coumaric acid, chlorogenic acid and myricetin significantly higher in Saperavi wine as compared to Cabernet Sauvignon. Moreover, on the basis of the correlation analysis, we assume that flavones synthesis and regulation of stilbenoids coordinated to a greater extent in "Saperavi" than in "Cabernet Sauvignon".

Keywords: phenolic compounds, red wine, Cabernet Sauvignon, Saperavi, HPLC-UV

INTRODUCTION

Recently, there is a decreasing tendency of alcohol consumption. According to International Agency for Research on Cancer and World Health Organization (IARC-WHO), alcohol and acetaldehyde have a carcinogenic effect (IARC, 2010, 2012). Therefore in 2010, the WHO adopted a Global Strategy to reduce the harmful use of alcohol (WHO, 2010). They aimed to decrease the alcohol consumption by 10% in 2025, and some progress has been achieved by now (WHO, 2010). Grape wine is an attribute of the traditional feast in some countries and it is a rich source of biologically active substances. Hundreds of articles have been published, showing the multiple potentially beneficial properties of polyphenols of wine (Chiva-Blanch at al., 2013; Friedman, 2014; Biasi at al., 2014). Despite this, there is no evidence, fully meet all the criteria of evidence-based medicine that red wine reduces oxidative stress in humans (Covas at al., 2010). It is possible that alcohol, a natural component of wine, is the cause of conflict in the experimental data and the results of epidemiological studies. That is the conclusion led the study of the relationship between the content of polyphenols in the diet and the risk of breast cancer (Touvier at al., 2013). High levels of dietary hydroxybenzoic acids, flavones, flavonols, catechins, theaflavins and pro anti-cyanide are associated with a low risk of developing the disease, but only for those women who consumed less than 6.5 g of alcohol per day. In contrary, women with higher consumption of alcohol, hydroxybenzoic acids, flavones, anthocyanins, catechins and pro anti-cyanide are associated with the risk of breast cancer. Thus, it seems that alcohol is not only carcinogenic by itself, but also contributes to the modulation anticarcinogenic properties of polyphenols in procarcinogens. The most obvious challenge facing the oenology is to produce from traditional wine a new dietary supplement - a soft drink with an optimal composition for the prevention of malignant tumors, cardiovascular diseases, diabetes, intestinal and neurodegenerative diseases. One of the key ways to address these problems is to know variations in the composition of polyphenols of grape wines and their impact on human health. In this regard, the question about fundamental differences in the composition of the polyphenols of red wines produced from grapes of different species has to be investigated. In the present study, we compared the two age-old technical grape varieties, the Cabernet Sauvignon, one of the most famous in the world of grapes, and the Saperavi, another well-known species among the world assortment of red grape (Ketskhoveli at al., 2012). The analysis could be carried out by directly comparing the composition of the extracts from the berries or the composition of the wine of the harvest, but bearing in mind significant variability in the content of polyphenols, depending on the conditions of growth and maturation, we anazed several samples of wines from different years and harvest different regions to provide a balanced assessment of the potential of these varieties.

MATERIALS AND METHODS

Wine samples

Wines from different manufacturers harvested in 2009-2012 have been obtained from the market stocks (Table 1). Samples were opened, protected against sunlight and stored at 4° C. Analyses were carried out within 3 days. Each wine was analyzed 1 time.

Reagents

Formic acid, acetonitrile, ethanol and chromatographic standard set ((+)-catechin, (-)-epicatechin, gallic acid, trans-caffeic acid, trans-p-coumaric acid, transferulic acid, chlorogenic acid, quercetindehydrate, myricetin, kaempferol, (±)-naringenin, resveratrol, polydatin) was from Sigma-Aldrich.

HPLC analysis

Analysis was conducted polyphenols content by HPLC using the Agilent 1290 chromatograph Infinity. The separation was carried out in a gradient mode on ZORBAXRRHDSB-C18 column 2.1×100 mm, 1.8 mm. The mobile phase consisted of A: 0.1% aqueous solution of formic acid B: acetonitrile containing formic acid at a concentration of 0.1%. The gradient was carried out in the following sequence:

t, min	0	5	10	25	26	30	31	35
% B	0	10	10	35	100	100	0	0

The flow rate is 0.3° ml / min at 30° C (samples stored at 4° S in bottles of dark glass). Registration was carried out diode array detector at 280 nm and 325 nm. For the analysis was used 31 of the sample. Calibration solutions

Resveratrol, myricetin, quercetin and kaempferol were dissolved in 96% ethanol; the rest was dissolved in a mixture of standards of ethanol / water 50/50 to a

concentration of 5 mg / ml and stored at -20°C. Working calibration mixtures were prepared with concentrations of 0.01 mg / 1 to 500 mg / 1.

Table 1 The wine samples tested

Wine samples	Grape variety	Year	pН	Alcohol content	Location		
1	Cabernet Sauvignon	2011 (n=3)	3.2-3.8	11-13.5%	Chili (n=8)		
	_	2012 (n=3)					
		2013 (n=2)					
2	Cabernet Sauvignon	2006	3.2-3.8	11-13.5%	France (n=8)		
	_	2009 (n=2)					
		2012 (n=3) 2011 (n=2)					
3	Cabernet Sauvignon	2011 (n=3)	3.2-3.8	11-13.5%	Moldova (n=8)		
	_	2009 (n=3)					
		2012 (n=2)					
4	Cabernet Sauvignon	2011	3.2-3.8	11-13.5%	Argentine (n=1)		
5	Cabernet Sauvignon	2011	3.2-3.8	11-13.5%	South Africa (n=1)		
6	Cabernet Sauvignon	2011	3.2-3.8	11-13.5%	Italy (n=1)		
7	Cabernet Sauvignon	2010	3.2-3.8	11-13.5%	Kazakhstan (n=1)		
8	Saperavi	2007-2013	3.2-3.8	11-13.5%	Georgia (n=29)		
	Saperavi	2009 (n=3)	3.2-3.8	11-13.5%	Kazakhstan (n=3)		

Statistical methods

Test the hypothesis of normal distribution of data sets obtained and samples belong to the same population, and correlation analysis was performed using STATISTICA 8.0 StatSoft program. Composition of polyphenols between two types of wine samples was analyzed using nonparametric Mann-Whitney U-test (Ledermann at al., 1984).

Table 1 Contents of certain polyphenol (mg / L) in samples of wine.

RESULTS AND DISCUSSION

The concentration of polyphenols

Table 1 shows the minimum and maximum concentration of polyphenol found in the experimental wine samples. The resulting set of most of the polyphenols concentrations are not normally distributed, so the table shows the median values and the results of testing the hypothesis about the accessories one set of samples using the nonparametric Mann-Whitney U-test.

Wine	Caberne S	Sauvignon (n=28	8)	Saperavi	p _{U-test Mann-Whitney}		
Polyphenols	min	max	median	min	max	median	
(+)-Catechin	9,7	68,8	32,8	24,6	57,0	43,9	0,002
(-)-Epicatechin	0,8	29,6	13,6	4,1	21,7	12,9	0,273
Gallic acid	23,0	62,0	40,4	17,3	102,2	38,0	0,177
trans-Caffeic acid	3,73	10,07	6,94	4,44	17,46	8,66	0,0002
trans-p-Coumaric acid	1,16	7,69	3,27	1,04	11,12	4,61	0,047
trans-Ferulic acid	0,22	3.81	0,67	0,00	3,19	0,83	0,993
Chlorogenic acid	0,00	1,51	0,19	0,07	8,93	0,29	0,004
Quercetin	0,46	9,95	2,09	1,13	7,34	2,42	0,313
Myricetin	0,02	5,60	0,95	0,46	8,55	2,69	0,012
Kaempferol	0,00	0,85	0,03	0,01	0,64	0,05	0,330
(±)-Naringenin	0,01	0,09	0,04	0,01	0,06	0,04	0,976
Resveratrol	0,20	2,66	0,82	0,38	4,96	1,07	0,103
Polydatin	0.75	9.18	3.51	1.08	6.41	3.99	0.293

pU-test - the probability belong to the same set of concentrations of polyphenols in wines Cabernet Sauvignon and Saperavi by U-Mann-Whitney test.

As can be seen, significant differences were found in concentrations of catechin, caffeic, p-coumaric and chlorogenic acid, and myricetin. The most pronounced of these differences for caffeic acid and catechin. To what extent these differences are due to grape variety, and not, for example, the technology of preparation of wine? It is possible that our number of samples is not representative enough. The representativeness of our data on samples of Cabernet Sauvignon wines can be estimated using similar research results available in the literature. We used the information from the site Phenol-Explorer and add more information from several articles. The results obtained by the base analysis are presented in Table 2.

First of all attention should be paid to the fact that our values of the concentrations of almost all materials are within ranges known from the literature. In two cases, namely, the minimum concentration of epicatechin and a pair of coumaric acid were lower than literature values, but those were the only observation. Next in order of importance were already above the minimum concentration of the border. In parentheses we note that the formal reason to remove these small values of our variational series we did not have. Thus, our estimates generally do not conflict with the combined data in the literature. The values of the probabilities given in the table correspond to the results of testing the hypothesis of a single set of accessories, we have obtained from the literature and well-known wine analysis Cabernet Sauvignon by U-Mann-Whitney (/ CS pU test). The significance of the differences on a number of parameters likely to be of natural differences of local climatic conditions prevailing in different years, as well as differences in the production technology. It must be admitted that we have not analyzed the composition of the best examples of Cabernet Sauvignon

wine. However, it is now important that we estimate the concentration of catechin and caffeic acid in Cabernet Sauvignon not differ from the cumulative data of other research groups. In our view, this means that the application for a higher concentration of caffeic acid in wines Saperavi than Cabernet Sauvignon can be taken with confidence, even when compared with the published data (/ S pU test = 0.0315). Regarding the concentration of catechins such cannot be stated unconditionally. Note that a slight excess of the average concentration of caffeic acid in the order of magnitude smaller Saperavi range where there may be fluctuations in Cabernet Sauvignon. In addition, the assessment regarding the published data, the content of steam coumaric acid and myricetin in Cabernet Sauvignon and Saperavi from our sample wines go from low to be acceptable. In other words, in general, we can assume that potential, we investigated the composition of polyphenols, the wine produced from grapes Saperavi and Cabernet Sauvignon, are virtually indistinguishable. This conclusion may not be consistent with the opinion of some Georgian wine-makers who are confident of the advantages of traditional Kakheti wine production technologies (Shalashvili at al., 2011). We are aware that the community of our findings is limited by sample specimens Saperavi wine.

Spearman's correlation coefficients

The concentrations of phenolic compounds is obviously dependent. Gallic acid metabolites formed from shikimatnogo path leading to the synthesis of phenylalanine and tyrosine, which is formed from p-coumaric acid - a precursor of all other investigated here phenolic compounds (see Figure.).

Polyphenols	min	max	median	n	/CS p _{U-test}	$/S p_{U-test}$	References
(+)-Catechin	5,80	535	40,0	55	0,225	0,585	Frankel at al., 1995; Goldberg at al., 1998; Burns at al., 2000; Teissedre at al., 2000; Landrault at al., 2001; Rossouw at al., 2004; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Ertan Anli at al., 2009; Di Paola-Naranjo at al., 2011; Li at al., 2011; Cáceres at al., 2012; Jiang at al., 2012; Bai at al., 2013; Muccillo at al., 2014
(-)-Epicatechin	3,15	186	31,5	49	<0,0001	<0,0001	Frankel at al., 1995; Goldberg at al., 1998; Burns at al., 2000; Teissedre at al., 2000; Landrault at al., 2001; Rossouw at al., 2004; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Ertan Anli at al., 2009; Di Paola-Naranjo at al., 2011; Li at al., 2011; Jiang at al., 2012; Bai at al., 2013; Muccillo at al., 2014
Gallic acid	15	126	35,6	40	0,737	0,468	Frankel at al., 1995; Burns at al., 2000; Teissedre at al., 2000; Landrault at al., 2001; Rossouw at al., 2004; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Ertan Anli at al., 2009; Di Paola-Naranjo at al., 2011; Li at al., 2011; Bai at al., 2013; Kilinc at al., 2003; Gambelli at al., 2004
Caffeic acid	2,02	30,0	6,95	36	0,588	0,031	Frankel at al., 1995; Burns at al., 2000; Teissedre at al., 2000; Landrault at al., 2001; Rossouw at al., 2004; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Di Paola-Naranjo at al., 2011; Li at al., 2011; Bai at al., 2013; Gambelli at al., 2004
p-Coumaric acid	1,30	21,6	4,80	24	0,0008	0,112	Burns <i>at al.</i> , 2000; Teissedre <i>at al.</i> , 2000; Rossouw <i>at al.</i> , 2004; de Villiers <i>at al.</i> , 2005; Pour Nikfardjama <i>at al.</i> , 2006; Di Paola-Naranjo <i>at al.</i> , 2011; Bai <i>at al.</i> , 2013; Kilinc <i>at al.</i> , 2003; Gambelli <i>at al.</i> , 2004; Goldberg <i>at al.</i> , 1998
Ferulic acid	0,00	4,00	0,30	6	0,288	0,297	de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Di Paola-Naranjo at al., 2011; Kilinc at al., 2003
Quercetin	0,00	23,0	3,20	63	0,439	0,644	Frankel at al., 1995; Burns at al., 2000; Rossouw at al., 2004; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Di Paola-Naranjo at al., 2011; Li at al., 2011; Bai at al., 2013; Muccillo at al., 2014; Goldberg at al., 1998; McDonald at al., 1998; Tsanova-Savova at al., 2002; Fang at al., 2007
Myricetin	0,00	17,9	3,20	53	0,0002	0,155	Frankel at al., 1995; Burns at al., 2000; Rossouw at al., 2004; de Villiers at al., 2005; Di Paola-Naranjo at al., 2011; Li at al., 2011; Bai at al., 2013; McDonald at al., 1998; Tsanova-Savova at al., 2002; Fang at al., 2007
Kaempferol	0,00	3,53	0,20	27	0,006	0,003	Burns <i>at al.</i> , 2000; Rossouw <i>at al.</i> , 2004; de Villiers <i>at al.</i> , 2005; Di Paola- Naranjo <i>at al.</i> , 2011; Li <i>at al.</i> , 2011; Bai <i>at al.</i> , 2013; Tsanova-Savova <i>at al.</i> , 2002; Fang <i>at al.</i> , 2007
Resveratrol	0,00	15,2	1,30	48	0,042	0,492	Frankel at al., 1995; de Villiers at al., 2005; Pour Nikfardjama at al., 2006; Di Paola-Naranjo at al., 2011; Li at al., 2011; Bai at al., 2013; Muccillo at al., 2014; Gambelli at al., 2004; Lamuela-Raventos at al., 1995; Goldberg at al., 1996; Lamikanra at al., 1996; Mozzon at al., 1996; Sato at al., 1997; Gu at al., 1999; Abril at al., 2005; Vitrac at al., 2005
Polydatin	0,00	11,6	0,72	22	<0,0001	<0,0001	de Villiers at al., 2005; Li at al., 2011; Jiang at al., 2012; Bai at al., 2013; Lamuela-Raventos at al., 1995; Goldberg at al., 1996; Sato at al., 1997

CS pU-test - the probability belong to the same set of concentrations of polyphenols in wines Cabernet Sauvignon from the literature and our results on the U-Mann-Whitney test. /S pU-test - the probability belong to the same set of concentrations of polyphenols in wines Cabernet Sauvignon from the literature and the results of our analysis of the wine Saperavi by U-Mann-Whitney test.

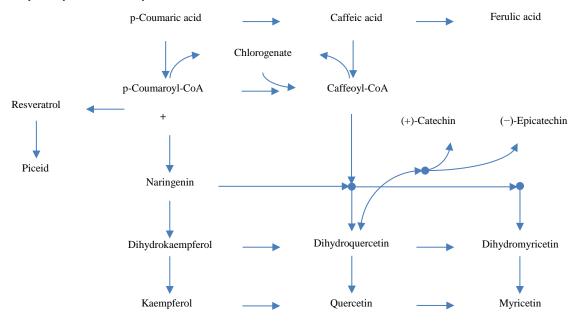


Figure 1 Scheme of metabolic relationships between investigated (poly) phenols (For simplicity, many intermediary metabolites omitted)

Communications between the concentrations of polyphenols indicators are presented in Table 3 above are arranged diagonally Spearman's correlation coefficients obtained in the analysis of wines Cabernet Sauvignon, below the diagonal - Saperavi. It can be seen that there are the same type as the connection between the concentrations of polyphenols, and different in magnitude and even direction. Table 4 (above the diagonal) are united by the correlation coefficients of variation series, which clearly identifies the common features of the two types

of wine. Grade-specific linkages are represented as differences of correlation coefficients (diagonally below in Table 4). Noteworthy positive correlation in pairs of p-coumaric acid - resveratrol and p-coumaric acid - naringenin and negative correlation of p-coumaric acid and ferulic acid occurring Saperavi wines but not in Cabernet Sauvignon. For Saperavi are also characterized by a close relationship between the concentrations of flavonols and catechins.

Table 3 Spearman's correlation coefficients for wines Cabernet Sauvignon (rS1, above the diagonal) and Saperavi (rS2, below the diagonal). Statistically significant (p <0.05) coefficients in bold

	(+)-Catechin	(-)-Epicatechin	Gallic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Chlorogenic acid	Quercetin	Myricetin	Kaempferol	(±)-Naringenin	Resveratrol	Polydatin
(+)-Catechin		,88	,60	-,29	,16	-,02	,45	-,003	,13	-,07	,27	,29	,13
(-)-Epicatechin	,53		,46	-,10	,30	,21	,24	,17	,36	,25	,43	,34	-,05
Gallic acid	,04	,19		-,32	,14	-,36	,36	-,13	-,22	,11	,15	,10	,28
Caffeic acid	,10	-,04	,07		,33	,42	-,62	,49	,35	,19	,27	,28	-,12
p-Coumaric acid	,15	,31	,03	,19		,24	,02	,07	,13	-,08	,07	,01	-,20
Ferulic acid	-,22	-,05	-,15	-,27	-,49		-,24	,65	,50	,72	,19	,19	-,21
Chlorogenic acid	,06	,49	,19	-,08	,15	,31		-,33	-,24	-,33	-,18	-,11	,26
Quercetin	,54	,30	-,21	,29	,38	-,38	,07		,67	,75	,55	,58	,22
Myricetin	,55	,43	-,12	,35	,38	-,36	,17	,92		,44	,72	,78	,05
Kaempferol	,64	,41	-,04	,27	,37	-,36	,01	,91	,86		,47	,28	-,24
(±)-Naringenin	,31	,43	-,01	-,06	,56	-,36	-,13	,42	,31	,46		,84	,17
Resveratrol	,37	,41	-,13	,26	,58	-,35	,17	,84	,84	,74	,45		,33
Polydatin	,25	,12	,09	-,05	-,10	-,36	-,18	,18	,19	,09	,13	,17	

These differences are the links between concentrations of phenolic compounds in wines suggest that the regulation of metabolism in grapes of different varieties has its own characteristics. This is confirmed, for example, direct studies of the composition of extracts of peel and expression of relevant genes grape varieties Cabernet Sauvignon and Shiraz in the course of maturation [43]. Furthermore, in the production of wine and its aging processes occur which lead to a change in its composition. Thus, the structure of the various relations of phenolic compounds

in the final product is the result of various factors set action. Nevertheless, the relationship between phenolic compounds in some of the key points of metabolism observed in wines Saperavi, you can probably be regarded as a reflection of the relatively more rigid coordination of metabolic pathways from the Saperavi grape variety than that of Cabernet Sauvignon.

Table 4 Spearman correlation coefficients for common wines Cabernet Sauvignon and Saperavi (above the diagonal) and the difference rS1-rS2 (below the diagonal, represented only a statistically significant (p <0,05) difference).

	(+)-Catechin	(-)-Epicatechin	Gallic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Chlorogenic acid	Quercetin	Myricetin	Kaempferol	(±)-Naringenin	Resveratrol	Polydatin
(+)-Catechin		,59	,21	,12	,25	-,07	,42	,24	,38	,36	,26	,39	,30
(-)-Epicatechin	,35		,37	-,12	,27	,12	,29	,20	,31	,33	,42	,35	,03
Gallic acid	,57			-,18	,07	-,24	,14	-,21	-,21	,00	,08	-,05	,12
Caffeic acid					,34	,07	-,09	,40	,45	,32	,10	,33	-,04
p-Coumaric acid						-,13	,24	,19	,35	,24	,25	,31	-,14
Ferulic acid				,69	,73		-,01	,27	,16	,06	-,06	-,03	-,26
Chlorogenic acid				-,54				-,01	,17	-,05	-,15	,15	,07
Quercetin	-,54					1,02			,77	,79	,54	,70	,16
Myricetin						,86		-,25		,68	,53	,81	,13
Kaempferol	-,71					1,08			-,42		,49	,56	-,03
(±)-Naringenin					-,49				,41			,64	,16
Resveratrol					-,57	,54		-,25		-,46	,39		,27
Polydatin													

CONCLUSION

In the present work, we report the investigation and comparison on major phenolic compounds and concentrations of two types of wine samples, the "Cabernet Sauvignon" and the "Saperavi" wines.

The study of the phenolic compounds of Cabernet Sauvignon and Saperavi wines has shown that there are no fundamental differences in the variety of phenolic compounds between the two types of wines. However, the concentrations of several important phenolic materials such as catechin, caffeic acid, p-coumaric acid, chlorogenic acid and myricetin significantly higher in Saperavi wine as compared to Cabernet Sauvignon, whereas, non-significant differences were

noticed in the concentration of hydroxycinnamic acids, flavonols, flavan-3-ols and stilbenoids. Correlation analysis of Cabernet Sauvignon and Saperavi wines between the studied components presumably reflect a more rigid coordination of metabolism in the synthesis of flavonols and stilbenoids from Saperavi grape variety

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