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THE EFFECT OF SUPPLEMENTARY QUANTITIES OF SEEDS DURING FERMENTATION ON THE PHENOLIC COMPOUNDS OF WINES

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ABSTRACT
The evidence of compounds that increase the nutritive value of red wines has been presented in a number of papers. These compounds include catechins and proanthocyanidins among others. Their protective effect of cardiovascular system and the anticarcinogenic properties have been proved. The proanthocyanidins increase the resistency and elasticity of blood vessels, with vitamin C they prevent the cholesterol deposit in blood vessels and have scavenging activity of radicals proved to provoke cancer and ageing of the human organism. The chemical investigations showed that proanthocyanidins are mostly represented in solid parts of the grape, seeds in the first place. The influence of increased ratio of solid parts of the grape, first of all of seeds, on phenols content in wine was investgated. Several microtrials with Merlot grape were planned, with highly increased content of seeds in certain variants during alcohol fermentation. This resulted in increased phenols content in wines, especially proanthocyanidins. The sensory evaluation of produced wines is also very interesting.
KEYWORDS
Compounds, chemical investigations, protective effect, grape, seeds

1. INTRODUCTION

Phenols are a large group of compounds of particular importance for the quality of red wines. They occur at much lower concentrations in white wines, compared to red ones, due to the way of obtaining. Numerous investigations have proved that the content and composition of this big group of compounds affect significantly the sensory quality and nutritive value of wine. The presence of some matters in wine that decrease the toxic action of alcohol has been known for a long time (1).

Two distinct phenol groups occur in grapes and wine, the flavonoids and the nonflavonoids. Flavonoids are characterized by two phenol molecules joined by a pyran carbon ring. The most common flavonoids in wine are flavonols, flavan-3-ols and in red wines anthocyanins. Small amount of free leukoanthocyanidins (flavan-3,4-diols) also occur. Flavonoids may exist in wines as free or polymerized to other flavonoids, sugars (glycosides), simple phenols or a combination of these (2).

Proanthocyanidins are the result of oligoisomerization of flavan-3-ols (2, 3). The pharmacological characteristics (activity) of proanthocyanidins can be expressed in several ways: more economical use of C-vitamin in the organism, protection of protein structure of blood vessels, decrease of blood cholesterol, radical scavenging activity, antiviral activity (3). The investigations of chemical composition showed that catechins and proanthocyanidins are mostly found in the solid parts of the grape, first of all in seeds; this explains the low content of these matters in white wines, since their production includes fermentation of must, without the solid parts of the grape (4). However, the content of these compounds in red wines is the result both of grape origin and of technological conditions of wine production (5, 6). The ratio of phenolic compounds in

wine, as well as the color and color stability of red wines, depend on way of winification and of keeping (storage) and ageing conditions (7).

It has been noticed that the liberation of catechins and (-)-epicatechins is more intensive than of dimers and trimers of proanthocyanidins under given conditions of maceration, and that the extraction of higher amount of these compounds affects the quality and color stability of red wines (6).

2. MATERIALS AND METHODS

Wine was made in laboratory conditions from Merlot sort, grown in the vineyard of Sremski Karlovci Experimental Institute and harvested in 2003. The control was made by classical winification process. 5 kg of grapes were used per variant. The mass of separated seeds was about 50 g/kg of grape. The whole quantity of stems was separated after the crushing, while the amount of seeds was not changed. In three varieties the crushed grapes were enriched with +100%, +200% and +300% of seeds, e.g. the amount of seeds was decreased by addition of seeds separated from grapes of the same sort and from the same vineyard, by 50 g/kg; 100 g/kg and 150 g/kg. The pomace was treated with 100 mg of $K_2S_2O_5$/kg of grape inoculated with 25 g/hl of dry wine yeast *Saccharomyces cerevisiae*. The fermentation started soon after the inoculation. The alcoholic fermentation of pomace was conducted at 30°C. After 6 e.g. 9 days of fermentation the pomace was filtered. SO_2 was corrected after the first decanting by adding 100 mg of $K_2S_2O_5$/kg. Wine was poured into 500 ml bottles, closed with crown caps and kept at 12-13°C. The analysis and sensory evaluation of samples were carried out after 2 months.

Analyses: The total acids content was determined by the official method OIV (8), the total phenols were determined with Folin-Ciocalteau reagent and expressed as g/l gallic acid. Total tannins were determined measuring the absorbance at 280 nm (9). Color intensity was obtained as the sum of absorbencies at 420 nm, 520 nm and 620 nm, measured in 1 mm cuvette, and hue was determined according to (10).

The amount of anthocyanins was evaluated by decoloration with $K_2S_2O_5$ (11). The amount of catechins was determined using the acidified vanillin method (12).

Table 1. Trial scheme

Grape	Total acidity g/l*	Reducing sugars, %	Maceration (days)	Addition of seeds (g/kg)
Merlot	5,62	25,20	6	-
				+50
				+100
				+150
			9	-
				+50
				+100
				+150

*expressed as tartaric acid

The change of color intensity in the 6 day maceration trial is significant. The color intensity increases with the increase of supplement quantities of seeds; in variant with the addition of 150 g/kg of seeds the color intensity was two times higher compared to the control. The variation of color intensity with the amount of added seed is less pronounced in the trial with 9 days of maceration. No significant differences in color intensity values of control wine and wine samples with the addition of seeds was found. The amount of added seeds had no influence on color intensity value reached during 9 days of maceration. No evident influence of time of maceration and amount of added seeds on hue was observed. The content of anthocyanins slightly increased over the 6-day maceration when 50 g/kg of seed were added. However, the increased addition of seeds resulted in decreased anthocyanins content; even in wine with addition of 150 g of seed per kg the anthocyanins content was lower than in the control sample. In the trial where the maceration was 9 days, the decrease of anthocyanins compared to control wine is even more intensive. The presented results are in accordance with the available

literature data. Lower amounts of seed affect positively the color stability. The extraction of higher amounts of phenolic compounds, as the result of addition of bigger amounts of seed and longer time of maceration result in decrease of the mentioned parameters.

Table 2. Influence of seed addition on color intensity, hue, total phenols and catechins content

maceration (days)	seeds	colour intensity ($A_{420}+A_{520}+A_{620}$)	hue (A_{420}/A_{520})	anhocyanin (mg/l)	FC* (g/l)	A 280 (· 100)	(+)-catechin (g/l)
6	-	0,657	0,648	309,02	1,42	307	0,59
	+50	1,033	0,635	341,11	2,04	405	1,13
	+100	1,295	0,628	338,42	2,55	516	2,27
	+150	1,364	0,640	303,83	3,29	585	2,58
9	-	1,006	0,697	312,07	2,01	402	0,89
	+50	1,140	0,676	299,34	2,63	436	1,70
	+100	1,027	0,692	226,75	2,69	444	2,02
	+150	1,085	0,690	258,12	3,07	510	2,71

* expressed as gallic acid

The change of total phenols is more pronounced depending on the amount of seeds added. So, in case of 6-day maceration, the content of total phenols is 2,3 times higher in wine with the addition of 150 g seeds/kg compared to control wine. In the trial where the maceration was performed for 9 days, this difference was not so expressed. Regarding the time of maceration, on the basis of obtained results, a significant difference was observed only between the control wines, while there were no deviations between other variants. So, the addition of seeds shortens the time of maceration regarding the concentration of phenolic compounds. The change of relative absorbances A_{280} are practically the same as of total phenols, and in this case more expressed in the variant of 9 days of maceration.

The content of catechins was exceptionally increased in all variants with increased seed content compared to the control. The increase of catechins was not proportional to the prolongation of maceration from 6 to 9 days, and this is evident in samples where 100 g and 150 g/kg of seeds were added per kg of crushed grapes.

All obtained wine samples were evaluated sensorily. Wines containing increased amounts of seed compared to control samples obtained by process including 6 days og maceration, were graded better. The evaluated wines were described as ones of somewhat rougher taste, however, having in mind that these are young wines, this finding was not considered as a shortcoming. In case of 9 days of maceration, the wines were graded very high and rather uniform. These wines were also charaterized as somewhat rougher, but also as wines with high ageing potential.

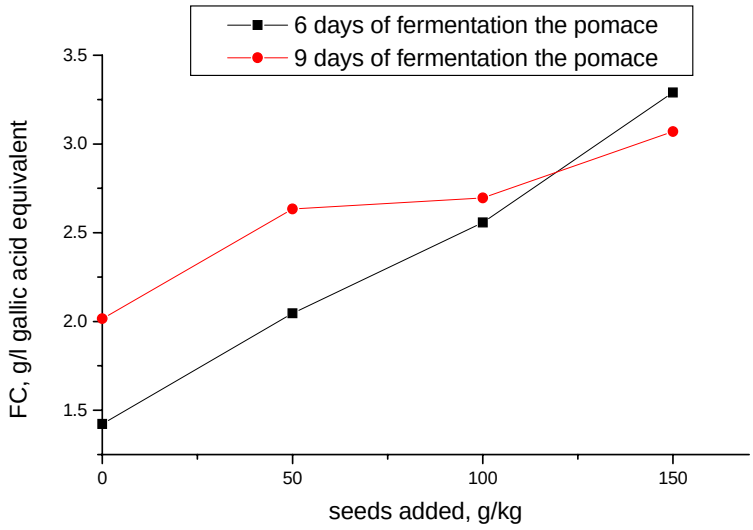

Fig. 1. Total phenol content, Folin-Ciocalteau expressed as g/l gallic acid

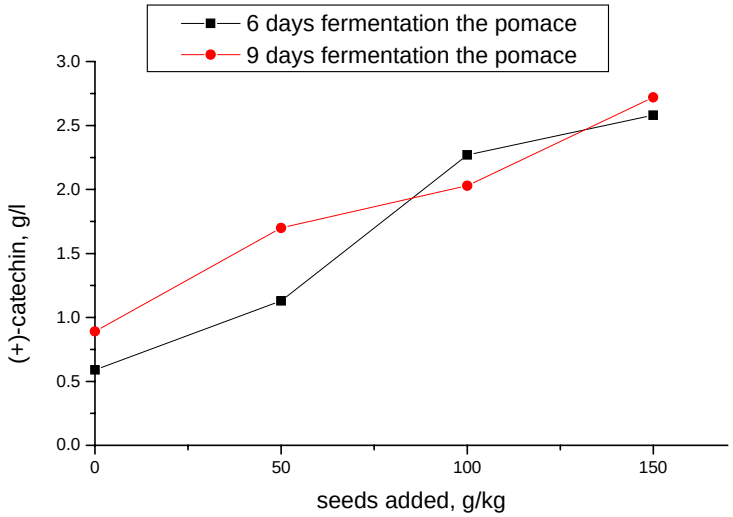

Fig.2. Content of (+)-catechin, g/l

3. CONCLUSION

Supplementary quantities of seeds during maceration result in increase of total phenols and catechins. A significant influence on color of wines was also observed, especially in wines obtained applying shorter maceration. The increase of phenols content is not allways proportional to the time of maceration. The preliminary sensory evaluation of produced wines showed that the addition of grape seeds resulted in wines evaluated as of somewhat rougher taste, however, with great ageing potential.

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