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Influence of Different Maceration Techniques and Ageing on Proanthocyanidins and Anthocyanins of Red Wine cv. Babić (*Vitis vinifera*, L.)

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Summary

Effects of winemaking techniques on the polyphenolic composition of specific Croatian wines made from Babić (*Vitis vinifera*, L.), from the Primošten vine-growing region, were subjected to examination. Winemaking processes and reactions that take place during maturation significantly influence the content of anthocyanins and proanthocyanidins in wine. Prolonged maceration duration caused an increase in the content of total phenols, vanillin index and proanthocyanidins, as well as a decrease in the content of anthocyanins in young wine. Cold maceration brought about a decrease in anthocyanins, total phenols and proanthocyanidins in Babić wine. The effects of winemaking techniques on the index of vanillin and proanthocyanidins in wines maturated for 6 and 14 months were significant.

Key words: anthocyanins, low-molecular proanthocyanidins, high-molecular proanthocyanidins, vinification, maturation and ageing of wine, Babić

Introduction

The study of proanthocyanidins and anthocyanins as compounds responsible for bitterness and astringency (*1*), as well as factors important in colour stability of red wine (*2,3*), has aroused great interest. They are involved in creation of major organoleptic sensations of red wine. Moreover, these compounds give wine its distinct personality. The studied aspects of polyphenols have become more numerous, based on reports that a large number of polyphenolic compounds, isolated from red grapes and wines, considerably influence human health due to their strong antioxidant action including radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reduction capacity. It has been suggested that proanthocyanidins and anthocyanins are responsible for beneficial effects deterring cardiovascular diseases, which brings them into the group of bioflavonoids (*4,5*).

The properties of proanthocyanidins, also called condensed tannins, depend on their structure, which is determined by the polymerization number of flavan-3-ols units (*6*), such as (+)-catechin and (–)-epicatechin, built by successive addition of catechin extension units through C-4 to C-8 interflavan linkages (*7*). In grapes and wine these compounds range from dimeric and trimeric to oligomeric and polymeric proanthocyanidins. Oligomers are low-molecular weight proanthocyanidins,

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containing two to five or six catechin units. They are primarily responsible for bitter sensations, whereas polymeric proanthocyanidins provide red wine with astringency (*8*). It has been reported that grape-seed proanthocyanidins are present in polymeric forms. Some authors have argued that seeds are the principal source of these compounds in red wine (*9*), but recently it has been shown that seeds are important sources of catechins and oligomeric proanthocyanidins, while higher amounts of polymeric proanthocyanidins may be extracted from skins and stems (*10*).

Free anthocyanins are responsible for the red colour of young wine (*11*); however, colour stability of aged wine is considered to be closely related to reactions between anthocyanins and other polyphenols. Upon completion of the maceration, anthocyanins react with proanthocyanidins and generate more stable pigmented proanthocyanidin products that stabilize wine colour (*3*).

Extraction of polyphenols from grapes occurs during maceration depending on vinification conditions, mainly the maceration time and maceration temperature, intensity of cap punching, alcohol and SO_2 levels. Nonetheless, the skin fermentation time has the largest impact on the content of anthocyanins and proanthocyanidins of all the processing factors (*12*,*13*). It has been shown that the extraction of catechins and proanthocyanidins increased progressively with the length of maceration (*12*). Extraction of anthocyanins is quite different from other polyphenols, showing decrease after few days of maceration (*12*,*14*).

Dalmatia has a long and established tradition in wine-making, dating from ancient times, and is nowadays the main area of the production of red wine in Croatia. The famous red wines in the Karst relief of central Dalmatian littoral are made from Babić (*V. vinifera*, L.), a variety included in the official specific (autochthonous) Croatian cultivar register (*15*).

The measurement of total phenol levels is the most widely used analysis for evaluation of the entire content of polyphenols in red wine, but in enology it is important to quantify different classes of polyphenols in wine. Spectrophotometric methods (*i.e.* indirect methods) for determination of oligomeric and polymeric proanthocyanidins (*16*,*5*) used in this work are well-correlated (*17*) to the normal-phase HPLC method (*i.e.* direct method) for separation of proanthocyanidins (*18*), and are generally more practical in terms of vinification control processes.

In order to determine the potential of the specific Croatian red wine cv. Babić for production of young wines and those for ageing, investigations of the composition of polyphenols were carried out. The objective of the task was to determine the effects of different maceration techniques (maceration duration and temperature) on the content of anthocyanins, oligomeric and polymeric proanthocyans, as well as to estimate the changes in their content during maturation and ageing of Babić wine.

Material and Methods

Samples and winemaking protocol

The grapes of the Babić cultivar (*Vitis vinifera*, L.) were harvested at the technological stage of ripening in September 2001 on the Primošten vine-growing location (Dalmatia). At harvest, the grapes had the total must sugar content of 21.1 % and 7.45 g/L of total acidity. Experiments were carried out with 600 kg of grapes. They were crushed and destemed immediately after the harvest and SO_2 (100 mg/kg of grapes) was added. Each fermentation tank was loaded with 50 kg of must and fermentation was carried out without adding selected yeast strains (*i.e.* spontaneously) and with punching 3 times a day. Each vinification was carried out in 3 replicates according to the maceration protocol shown in Table 1.

Table 1. Maceration protocol scheme for Babić cv.

Vinification	Maceration duration/day	Maceration temperature/°C	Fermented sugar in wine at pressing/%
V-1	4	19–29	60
V-2	6	19–29	100
V-3	13	19–29	100
V-4	7	19–23	100

Upon completion of the maceration, the must was pressed in 25-litre glass containers. In December the wine was first racked and SO_2 (50 mg/L) was added. After 3 months the wine was racked again and then stored at room temperature (12–16 °C in winter; 22–28 °C in summer) in 0.75-litre glass bottles with cork closures.

Analyses were performed at the end of maceration, *i.e.* after 6 months (following the second racking) and after 14 months of wine ageing (*i.e.* 8 months of storage in glass bottles).

Wine analysis

Basic wine parameters: relative density, alcohol concentration, total dry extract, reducing sugars, pH value, total acidity, volatile acidity, ash content, free and total SO_2 were all determined according to EU regulations (*19*). Total phenols were determined by the official AOAC spectrophotometric method with Folin-Ciocalteu reagent (*20*).

Proanthocyanidins

Proanthocyanidins were determined by already established methods (*16*) under optimized conditions by their transformation into cyanidin in acid media, as previously described (*5*). This reaction is more sensitive to high molecular weight proanthocyanidins than to monomers and oligomers of flavan-3-ols.

Index of vanillin

The vanillin index was used to determine catechins and proanthocyanidins reacting with vanillin under a controlled vanillin-HCl method (*21*) following the conditions described by Di Stefano *et al.* (*16*), calculated as (+)-catechin (mg/L). The vanillin index is more sensitive to catechins (monomers) and oligomeric proanthocyanidins than to high-molecular weight proanthocyanidins.

Total anthocyanins

Total anthocyanins were determined according to the method of Di Stefano *et al.* (*16*) on the basis of maximum absorbance in the visible range (536–540 nm).

Colour intensity and hue

Colour intensity and hue were estimated by measuring absorbance at 420, 520 and 620 nm according to EU regulations (*19).* The absorbance at 280 nm of wine samples diluted with water at 1:50 ratio was multiplied by 50 to obtain the relative ultraviolet absorption.

Data analysis

All data-processing, analyses of variances and comparasion of vinification treatments (LSD test) were performed using Statistica 6.0. (STSC, Inc., USA).

Results and Discussion

Basic analytical data for Babić wine aged for 6 months are shown in Table 2. Total phenols, total anthocyanins, proanthocyanidins, vanillin index, colour intensity and hue in Babić wine were determined as shown in Tables 3, 4 and 5.

Table 2. Basic analytical data for Babić wine produced according to different vinification protocols

Basic wine parametres	Vinification			
	V-1	V-2	V-3	V-4
ρ(relative density)/20 °C	0.9962	0.9965	0.9969	0.9961
φ(alcohol)/%	14.74	14.92	14.87	14.74
γ(total extract)/(g/L)	39.1	40.0	41.25	38.8
γ(reducing sugar)/(g/L)	1.55	1.72	1.96	1.8
γ(extract without sugar)/(g/L)	38.55	39.28	40.29	38.0
γ(total acidity)/(g/L)	7.29	6.94	6.99	7.41
γ(volatile acidity)/(g/L)	0.38	0.36	0.38	0.36
γ(free SO_2)/(mg/L)	6.0	2.5	4.0	2.5
γ(bound SO_2)/(mg/L)	56.3	54.5	44.5	63.0
pH	3.49	3.53	3.53	3.48
m(ash)/(g/L)	2.80	3.02	3.01	2.81

During the wine ageing, the increase in the amount of high molecular weight proanthocyanidins and decrease of vanillin index show that the polymerization of flavan-3-ol units from oligomers to high molecular polymers occurs.

The decreasing values of the vanillin index/proanthocyanidins ratio, as wines aged for 6 to 14 months (0.45→0.18 in V-1; 0.55→0.26 in V-2, 0.56→0.29 in V-3 and 0.54→0.22 in V-4), indicated an increase of polymerization units (molecular size) of proanthocyanidins. A strong decrease of the vanillin index values in aged wines had already been reported (*17*).

Table 3. Polyphenols and colour characteristics in new Babić wine

	Vinification			
	V-1	V-2	V-3	V-4
γ(total phenols)/(mg/L)	2316^{a}	2793^{b}	3103^{c}	2580^{d}
A(rative UV absorption)	63.1	71.1	64.7	57.9
γ(total anthocyanins)/(mg/L)	960^{a}	1023^{b}	670^{c}	896^{d}
γ(proanthocyanidins)/(mg/L)	1362^{a}	1793^{b}	2130^{c}	1218^{a}
γ(vanillin index)/(mg/L)	845^{a}	1152^{b}	1669^{c}	1119^{b}
A(colour intensity)	13.51^{a}	13.29^{a}	10.8^{b}	11.2^{b}
A(hue, tint)	4.977^{a}	5.263^{a}	5.857^{a}	5.048^{a}

^{a,b,c,d}statistically significant differences (LSD, p<0.001). There is no statistically significant difference between the treatments denoted by the same letter

Table 4. Polyphenols and colour characteristics in Babić wine aged for 6 months

	Vinification			
	V-1	V-2	V-3	V-4
γ(total phenols)/(mg/L)	2380^{a}	2575^{b}	2875^{c}	2250^{a}
A(relative UV absorption)	54.4	56	62.55	53.65
γ(total anthocyanins)/(mg/L)	601^{a}	638^{a}	516^{b}	516^{b}
γ(proanthocyanidins)/(mg/L)	1534^{a}	1951^{b}	2301^{c}	$1568^{a,b}$
γ(vanillin index)/(mg/L)	695^{a}	1090^{b}	1294^{c}	841^{a}
A(colour intensity)	12.01^{a}	11.01^{a}	8.58^{b}	10.21^{a}
A(hue, tint)	8.581^{a}	6.509^{b}	6.759^{b}	6.660^{b}

^{a,b,c}statistically significant differences (LSD, p<0.001). There is no statistically significant difference between the treatments denoted by the same letter

Table 5. Polyphenols and colour characteristics in Babić wine aged for 14 months

	Vinification			
	V-1	V-2	V-3	V-4
γ(total phenols)/(mg/L)	2277^{a}	2383^{a}	2997^{b}	2190^{a}
A(relative UV absorption)	55.3	60.6	64.13	56.4
γ(total anthocyanins)/(mg/L)	291^{a}	314^{a}	249^{a}	245^{a}
γ(proanthocyanidins)/(mg/L)	1601^{a}	2008^{b}	2353^{c}	1654^{a}
γ(vanillin index)/(mg/L)	295^{a}	532^{b}	691^{c}	364^{a}
A(colour intensity)	11.49^{a}	11.80^{a}	9.73^{b}	11.23^{a}
A(hue, tint)	8.442^{a}	8.317^{a}	8.771^{a}	8.361^{a}

^{a,b,c}statistically significant differences (LSD, p<0.001). There is no statistically significant difference between the treatments denoted by the same letter

Effects of maceration duration

Analyses of variances revealed statistically significant differences in total phenols (p<0.001), total anthocyanins (p<0.001), proanthocyanidins (p<0.001) and vanillin index (p<0.001) in new wine with regard to maceration duration (Table 3). High molecular proanthocyanidins were higher than low molecular proanthocyanidins (vanillin index) by 62 % after 4 days (V-1), by 64 % after 6 days (V-2), with the peak variance of 78 % after 13 days (V-4).

The obtained results show that, in contrast to the increase of total phenols, proanthocyanidins and vanillin index, the content of anthocyanins tends to be the lowest in young wine produced with a 13-day maceration, and the highest when produced with a 6-day maceration. The decrease of anthocyanin concentration might be ascribed to the forming of copolymerization products with other polyphenols, mainly proanthocyanidins (*22*). The decrease of anthocyanins in prolonged maceration is in agreement with the results obtained by Kovac *et al.* (*12*).

It was observed that the level of proanthocyanidins increased significantly in wines produced with 4-day maceration and 6-day maceration after ageing for 6 months in contrast to wine produced by 13-day maceration, in which significant increase was achieved in wine aged for 14 months (Tables 4 and 5). However, statistically significant differences (p<0.001) still existed in their levels in Babić wine in respect to maceration duration. Although lower amounts of vanillin index were reported in wine during its ageing, which is possibly due to the existence of low-molecular catechins and small oligomers in reactions of proanthocyanidin polymerization, the ratio between their levels in different winemaking techniques remained invariable. Statistically significant differences (p<0.001) in their levels were also established.

According to the results obtained in this research shown in Table 3, the new wine produced with prolonged maceration (13 days) had a significantly lower colour intensity (p<0.001) than the wines produced by maceration of 4 and 6 days. The colour intensity significantly dropped during the ageing only in the wine produced with the prolonged maceration, which significantly differed from the others (Tables 4 and 5). On the other hand, the duration of maceration had no effect on the hue (tint) of Babić wine. As indicated in Table 5, the value of the hue was more intense in aged wines than in the new ones.

Effects of temperature

In vinification V-4, the maceration started to cool when temperature reached about 21 °C (Fig. 2) and fermentation was performed for 7 days, with the aim to find out to which extent maceration temperature influences the extraction of polyphenols of Babić cultivar. The maceration resulted in a significantly lower concentration of anthocyanins (12 %), total phenols (8 %) and proanthocyanidins (32 %), compared to vinification V-2, which was accomplished without cooling (temperature from 19–29 °C, see Fig. 1) and pressing after 6 days (p<0.001). There were no significant effects on the vanillin index.

Temperature did not have the effect on the amount of anthocyanins and total phenols in the wine aged for 14 months. The content of proanthocyanidins and

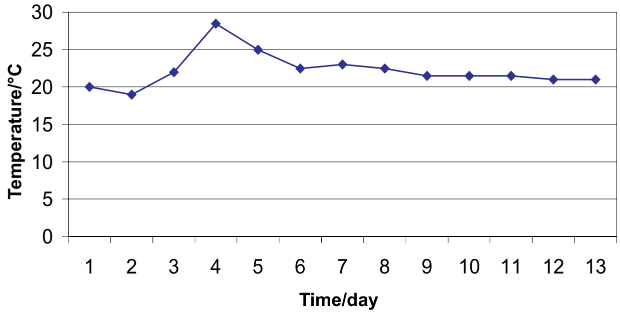

Fig. 1. Time/maceration temperature curve in vinifications V1–V3

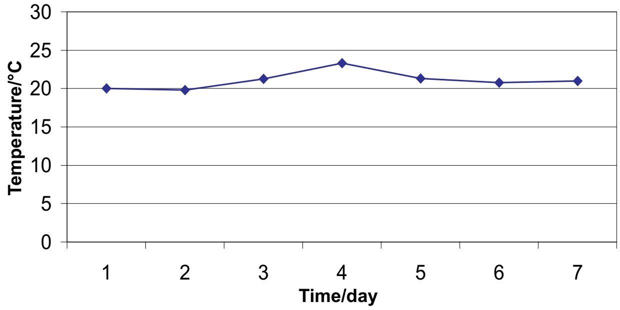

Fig. 2. Time/maceration temperature curve in vinification V4

vanillin index (in this case) remained significantly different from the wine produced in vinification V-2 (p<0.001). It was determined that lower maceration temperature affected the colour intensity but not the hue of new wine. No significant variations in colour intensity were observed during the ageing of wine; however, a significant increase in the wine hues was noted.

Conclusions

The results obtained suggest that maceration duration and temperature affect significantly the content of total phenols, low molecular (catechins and small oligomers) and high molecular proanthocyanidins (polymers) as well as anthocyanins of new Babić wine. Wine ageing contributes to polymerization of wine polyphenols. Wines produced by maceration of various duration and temperature demonstrated different concentrations of low molecular and high molecular proanthocyanidins, while their partition remained constant during the maturing and ageing.

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Utjecaj različitih maceracijskih tehnika i dozrijevanja na proantocijanidine i antocijanine crnoga vina sorte Babić (*Vitis vinifera*, L.)

Sažetak

Predmet istraživanja bio je utjecaj tehnološkog postupka proizvodnje vina na polifenolni sastav vina autohtone hrvatske sorte Babić (*Vitis vinifera*, L.) iz primoštenskoga vinogorja. Proces proizvodnje vina i reakcije koje se odvijaju tijekom dozrijevanja bitno utječu na koncentraciju antocijanina i proantocijanidina u vinu. Produžena maceracija uzrokovala je povećanu koncentraciju ukupnih fenola, vanilinskog indeksa i proantocijanidina, te smanjenje koncentracije antocijanina u mladom vinu. Hladnom maceracijom smanjena je koncentracija antocijanina, ukupnih fenola i proantocijanidina. Prerada vina koje je dozrijevalo šest i četrnaest mjeseci bitno je utjecala na vanilinski indeks i udjel proantocijanidina.