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Effects of maceration conditions on the polyphenolic composition of red wine 'Plavac mali'

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Summary

The influence of the maceration treatments on the polyphenolic composition of red wines produced from Croatian autochthonous cultivar 'Plavac mali' (*Vitis vinifera* L.) has been investigated. The results showed that in general different skin contact times influenced the content of anthocyanins, total phenols and vanillin index in young wines. Prolonged skin contact time significantly increased the content of low molecular weight proanthocyanidins and decreased the content of anthocyanins. Young wine produced with maceration at lower temperature demonstrated the lower content of anthocyanins and proanthocyanins. No significant differences were observed between the levels of anthocyanins, total phenols and proanthocyanidins in wines aged for 14 months. Weaker colour intensity of 14-month-aged wines was detected in the wine made with prolonged skin maceration time in which the ratio of proanthocyanidins/anthocyanidins was the highest.

K e y w o r d s : 'Plavac mali', autochthonous, polyphenols, proanthocyanidins, maceration, ageing.

Introduction

Polyphenols are a large and diverse group of very important molecules of red wines, which are responsible for wine quality since they determine its colour, flavour, body and structural characteristics. Wine polyphenols are divided in two main groups: flavonoids (anthocyanins, flavan-3-ols or catechins, proanthocyanidins or condensed tannins/tannins and flavanols) and non-flavonoids (hydroxybenzoic acids, hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols). Anthocyanins and pigments derived from them are mainly responsible for the colour of the wines. The colour of the young red wines is mainly due to free anthocyanins extracted from the grapes during maceration process (Glories 1999, Zimman *et al*. 2002). The astrigency and bitterness of the wine are mainly attributed to proanthocyanidins. Oligomers and low molecular weight proanthocyanidins are responsible for the bitterness, while the high molecular weight proanthocyanidins give wine the sensation of astrigency. During wine ageing, both anthocyanins and proanthocyanidins are involved in the creation of organoleptic perception due to complex phenomena of copigmentation and tannin interaction, which are still under investigation. Namely, anthocyanins are progressively transformed into more stable oligomeric and polymeric pigment-tannin derivatives, which leads to changes from bright-red to brick-red colour and simultaneous decrease in astrigency (Remy *et al.* 2000). The polyphenolic composition of wines depends on the polyphenolic compounds in the grapes from which the wines are made, the extraction from the grapes during winemaking and the reactions that take place during ageing of wine. Winemaking techniques greatly influence the polyphenols since they can give very different final polyphenolic contents of the wine. Nonetheless, the skin contact time and temperature have the greatest influence on the extraction of polyphenols during winemaking (Kovac *et al.* 1992, Vrhovsek *et al.* 2002, Zimman *et al.* 2002, Zou *et al.* 2002, Spranger *et al.* 2004, Budić-Leto *et al.* 2005). The polyphenolic composition of the wines produced in several regions has been studied by many researchers (Auw *et al.* 1996, Gil-Muñoz *et al.* 1999, Mazza *et al.* 1999, Fischer *et al.* 2000, Zimman *et al.* 2002, Kelebek *et al.* 2006). However, there are no data on the influence of maceration conditions on the content and profile of different groups of polyphenols of 'Plavac mali' wine. 'Plavac mali' is an autochthonous Croatian cultivar of *Vitis vinifera*, L. grown in Dalmatia, a winegrowing region near the Adriatic Sea with ancient tradition in production of red wines. This is the main grape variety that gives strong, full body red wine and it has been shown that it has got a close genetic relationship with the origin of the most famous American grape cultivar 'Zinfandel' (Maletić *et al.* 2004). As a young wine, its highly astrigent character has a negative impact on the taste and the mouthfeel, and needs longer time for maturation and formation of desirable flavour for consumer. Therefore, the main objective of this paper was to study the influence of maceration time and maceration temperature on the polyphenolic composition of the most important autochthonous Croatian red wine 'Plavac mali'.

Material and Methods

G r a p e s a n d w i n e m a k i n g : The grapes of the cultivar 'Plavac mali' (*Vitis vinifera* L.) were harvested at technological maturity (242 $g \cdot l^{-1}$ of reducing sugars and 6.2 $g \cdot l^{-1}$ of titratable acidity) in 2001 at the vine-growing location Dingač at the Pelješac peninsula. Experiments were

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carried out with 700 kg of grapes. Grapes were destemmed and crushed and then homogenously transferred into tanks for maceration with the addition of 50 mg·l^{-1} of sulphur dioxide ($K_2S_2O_5$). Each vinification was performed in three replicates, in 70-kg capacity cubes. Four skin maceration periods were applied, for 5, 8 or 17 d at the average temperature of 25 °C and for 7 d at the average temperature of 20 °C. After maceration, pomace was pressed gently in a vertical press. Alcoholic fermentation was spontaneous. During the fermentation, temperature and must density were monitored twice a day. After the fermentations (alcoholic and malolactic), the wines were racked for the first time in December 2001. Second racking was done in March 2002. The third racking was carried out in June 2002 after which the wines were bottled into 750 ml bottles and stored until analysis.

Grape seed and skin extract: Three representative samples (9 kg) were selected at the technological stage of ripening during the harvest of 2001. From the clusters, 200 g of berries of each sample were randomly selected. Grape extracts were prepared according to Mattivi *et al.* (2002). The seeds and skins were manually separated from the berries and put into hydro alcoholic solution (ethanol/water, 12:88 v/v) which comprised 50 mg·l^{-1} of SO_2 and 5 mg·l^{-1} of tartaric acid, neutralized with 1/3 M NaOH to pH = 3.2. Extraction lasted for five days at 30 °C. After extraction, the solution was separated from the solid parts and then used for the spectrophotometric analysis.

Reagents and standards: All chemicals used in this study were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MQ, USA), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia).

Standard chemical analysis: Total acidity and reducing sugar analysis were performed in the must and wines. Additionally, the wines were analysed for basic physicochemical parameters according to EU methods (1990).

Total phenols: Total phenol concentration in wine samples was determined spectrophotometrically by reaction with Folin-Ciocalteu reagent according to the official AOAC Folin-Ciocalteu method (Singleton and Rossi 1965, Rigo *et al.* 2000). Gallic acid was used as a chemical standard for calibration. The total phenolic content was expressed as mg of gallic acid equivalents per litre of sample (mg·GAE·l^{-1}).

Total anthocyanins: The total anthocyanin content in wines was determined using the method of Rigo *et al.* (2000) on the basis of maximum absorbance in the visible range (536-540 nm) in acid medium.

Vanillin index: The vanillin index was determined by the controlled reaction of flavonoids with vanillin reagent in acid medium under conditions described by Rigo *et al.* (2000) using a previously designed method by Broadhurst and Jones (1987). This method is based on the fact that low-molecular weight flavonoids (catechins and low molecular weight proanthocyanidins) react with vanillin and produce a red coloured product with a maximum absorbance at 500 nm. The vanillin index was calculated using the calibration curve with the (+)-catechin and expressed in mg·l^{-1}.

Proanthocyanidins: Proanthocyanidins were determined by the method of Rigo *et al.* (2000) under the optimized conditions leading to their transformations into cyanidin in acidic medium. This method is applied to determine high-molecular weight proanthocyanidins. The proanthocyanidins content was expressed as mg·l^{-1} of (+)-cyanidin.

Colour intensity and hue: Colour intensity and hue were estimated by measuring absorbance at 420, 520 and 620 nm on the basis of the method reported by EU regulations (1990).

Spectrophotometric measurements: Spectrophotometric measurements were done by UV-VIS spectrophotometer (double-beam) Varian, DMS 200.

Statistical analysis: Three replicates of each maceration were done. Each replicate was chemically analyzed in duplicate samples. The data were processed using one-way analysis of variance (ANOVA) to test the influence of different maceration conditions on the polyphenolic composition of the wines. Furthermore, Fischer's least significant difference test (LSD) was used to compare the mean values. Statistical analyses were performed using statistical software Statistica 6.0 (STSC, Inc., USA).

Results and Discussion

Tab. 1 shows the general chemical composition of wines obtained from 'Plavac mali' at different maceration times and temperatures. All wines present a high level of ethanol. Maceration time had no uniform effect on relative density, alcohol, total extract, reducing sugars, total acidity, volatile acidity, pH and ashes of 'Plavac mali' wine samples and no statistically significant differences were found among these wines regarding the general chemical composition. These results are in agreement with those reported by others (Kelebek *et al.* 2006). However, temperature had an influence on the basic chemical data. Relative density, volatile acidity, pH and ashes were higher, while the alcohol content and total acidity were lower in the wine obtained by the maceration at lower temperature (20 °C) compared to wines made at higher temperature (25 °C).

The effect of maceration time on the polyphenolic composition of the wines: Tab. 2 summarizes the results of the amount of total phenols, total anthocyanins, vanillin index, proanthocyanidins, colour intensity and hue expressed as the average value of three analytical repetitions as well as the results relative to the LSD test, showing the statistically significant differences resulting from maceration conditions for each studied parameter of the 'Plavac mali' wines.

The total phenolic content of young and aged 'Plavac mali' wines examined (Tab. 2) varied from 2467 to 2723 GAE·l^{-1} and 2287 to 2580 GAE·l^{-1}, respectively. The content of the phenolic compounds was similar to those presented in the literature (Kallithraka *et al.* 2006) and fell within the range presented by Woraratpohoka *et al.* (2007), who reported the levels of total phenolic content of Zinfandel wine from China ranging from 1856 to 2750 mg·l^{-1} in two different vintages. Different maceration conditions result-

Table 1

Basic chemical analysis for the Plavac mali wines produced under different maceration conditions

	Days of maceration, average temperature 25 °C			Days of maceration, average temperature 20 °C
	5	8	17	7
Relative density (20/20 ºC)	0.9929[a]	0.9933[a]	0.9929[a]	0.9947[b]
Alcohol (vol %)	15.42[a]	15.39[a]	15.45[a]	15.06[b]
Total extract ($g \cdot l^{-1}$)	32.37[a]	33.43[a]	32.63[a]	33.77[a]
Reducing sugars ($g \cdot l^{-1}$)	2.81[a]	2.52[a]	2.59[a]	2.61[a]
Total acidity[1] ($g \cdot l^{-1}$)	5.2[a]	5.1[a,b]	5.1[a,b]	4.9[b]
Volatile acidity[2] ($g \cdot l^{-1}$)	0.34[a]	0.38[a]	0.36[a]	0.53[b]
Free SO_2 ($mg \cdot l^{-1}$)	3[a]	3[a]	4[a]	3[a]
Bound SO_2 ($mg \cdot l^{-1}$)	57[a]	53[a]	54[a]	50[a]
pH	3.79[a]	3.83[a]	3.83[a]	3.88[a]
Ash ($g \cdot l^{-1}$)	3.33[a,b]	3.26[a]	3.26[a]	3.47[b]

Results are the means of three repetitions of each maceration from which the wine was made.
[1]As tartaric acid. [2]As acetic acid. [a,b]Values with the different letter for each parameter were significantly different according to the LSD test ($p < 0.05$).

ed in differences in phenolic content. In general, the total phenolic content of young wine increased significantly ($p > 0.001$) with the length of maceration time. These results are in agreement with those reported by researchers (Ricardo da Silva *et al.* 1993, Auw *et al.* 1996). During ageing, the three wines (skin contact of 5, 8 and 17 d) showed very similar trend: A no significant decrease from 6-month-aged wine to 14-month-aged wines made by 8 and 17 d of skin maceration times as well as no significant increase in wine obtained by maceration with 5-d-contact time. Changes in the concentration of total phenols in wine obtained maceration with 5-d-contact time during ageing are in agreement with the results of Monagas *et al.* (2006). These changes could have occurred during polymerization of phenolic compounds and formation of condensed forms with fewer hydroxyl groups that Folin-Ciocalteu reagent can oxidise. Namely, in the method used for determination of total phenolics, Folin-Ciocalteu reagent oxidises hydroxyl groups of the phenols forming blue colouring with a maximum intensity between 725 and 760 nm.

The total content of anthocyanins in 'Plavac mali' wines reached a maximum of 649 $mg \cdot l^{-1}$ at the maceration time of 8 d (Tab. 2). Anthocyanin content of young wine obtained after 5-d and 8-d skin contact was not significantly different ($p > 0.05$). It has been reported that the decrease of anthocyanins in Babić wine occurred after the fifth day of maceration (Budić-Leto *et al.* 2005). Kelebek *et al.* (2006) and Ribéreau-Gayon *et al.* (2000) reported maximum colour extraction for *Vitis vinifera* grapes on the maceration of 6 d. Anthocyanin content in young wines obtained by skin contact of 17 d was significantly lower ($p < 0.001$) compared to all other 'Plavac mali' wines. Similary, the decrease of anthocyanins during the prolonged maceration has been reported (Kovac *et al*. 1993, Spranger *et al.* 2004). The decrease of the anthocyanin level could be due to the reactions of anthocyanins with proanthocyanins that form copolymerization products or anthocyanin adsorption by yeast lees (Vasserot *et al.* 1997). Anthocyanins can be attacked by nucleophilic reagents, such as (+)-catechin forming condensed anthocyanins. These substances can react with oligomeric proanthocyanins yielding pigmented polymerized forms. Anthocyanin-derived pigment molecules have been found to arise from the reaction between anthocyanin-pyruvic acid adducts and vinyl-flavanol adducts (Mateus *et al.* 2003).

A progressive decrease in total anthocyanin content of wines was observed during the ageing of wines. This could be due to the numerous reactions of anthocyanins with flavanol molecules (Fulcrand *et al.* 1998, Mateus *et al.* 2003). The difference in total anthocyanin content between the wines from different maceration times decreased during ageing and was no more significant after 14 months ($p > 0,05$), which is in agreement with the results found in the previous study (Budić-Leto *et al.* 2003).

Vanillin index in young wines significantly increased with longer skin contact from day 5 to day 17. Also, it was observed that the vanillin index was significantly higher ($p < 0.001$) in 6-month aged wine produced after 17 d of maceration in contrast with the wines produced after 5 and 8 d of maceration. During 14 months of ageing, the vanillin index slowly decreased. This indicated that the levels of low molecular weight proanthocyanidins decreased in wines, which is in agreement with the research of other authors (Pérez-Magariño and González-San Jose, 2006). In this assay, monomeric and oligomeric proanthocyanidins reacted with vanillin in acidic medium to yield chromophores absorbing at 500 nm. This reaction is more sensitive for free A-rings of monomeric flavanols and upper flavanol units of proanthocyanidin oligomers and polymers, so the vanillin index indicated the content of low molecular weight proanthocyanidins (catechins and oligomeric proanthocyanidins). It has been shown that the spectrophotometric method for determination of vanillin index used in this study correlated well with the normal phase HPLC method for determination of low-molecular weight proanthocyanidins (2-4 units) (Vrhovsek *et al.* 2001).

Table 2

Polyphenolic content of the Plavac mali wines produced under different maceration contitions at different times of maturity

Parameter	Young wines				6-month-aged wines				14-month-aged wines			
	Maceration (day), 25 °C			Maceration (day), 20 °C	Maceartion (day), 25 °C		Maceration (day), 20 °C	Maceartion (day), 25 °C			Maceration (day), 20 °C	
	5	8	17	7	5	8	17	7	5	8	17	7
TP (mg·l^{-1})	2560 ± 25 ^{a}	2723 ± 26 ^{b}	2677 ± 27 ^{a}	2467 ± 17 ^{a}	2383 ± 12 ^{a}	2560 ± 30 ^{b}	2657 ± 37 ^{b}	2340 ± 5 ^{a}	2427 ± 55 ^{a}	2526 ± 32 ^{a}	2580 ± 44 ^{a,c}	2287 ± 3 ^{a,b}
TA (mg·l^{-1})	635 ± 7 ^{a}	649 ± 4 ^{a}	467± 6 ^{b}	567 ± 10 ^{c}	448 ± 2 ^{a}	458 ± 3 ^{a}	370 ± 2 ^{b}	425± 5 ^{a}	294 ± 6 ^{a}	300± 13 ^{a}	259± 11 ^{a}	286± 1 ^{a}
VAN (mg·l^{-1})	1305 ± 100 ^{a}	1345± 64 ^{a}	1512 ± 11 ^{b}	1324 ± 39 ^{a}	1309± 9 ^{a}	1384± 7 ^{a}	1499± 5 ^{b}	1319 ^{a}± 19 ^{a}	1094± 20 ^{a}	1192± 13 ^{a}	1330 ± 36 ^{c}	904 ± 48 ^{b}
PROC (mg·l^{-1})	2402 ± 47 ^{a}	2292 ± 103 ^{a}	2491 ± 65 ^{a}	2024 ± 73 ^{b}	2426± 23 ^{a}	2599 ± 53 ^{a}	2512± 27 ^{a}	2273± 58 ^{a}	2642± 42 ^{a}	2704± 86 ^{a}	2739± 83 ^{a}	2530± 126 ^{a}
CI	11.18 ± 0.34 ^{a}	9.59 ± 0.16 ^{b}	6.12 ± 0.08 ^{c}	5.89 ± 0.17 ^{c}	6.09± 0.07 ^{a}	6.80 ± 0.10 ^{a,b}	5.54 ± 0.71 ^{a,c}	6.06± 0.07 ^{a}	6.91 ± 0.08 ^{a}	7.53 ± 0.22 ^{a,b}	6.45 ± 0.12 ^{c}	6.65 ± 0.02 ^{a}
H	6.77 ± 0.08 ^{a}	6.82 ± 0.03 ^{a}	6.91 ± 0.01 ^{a}	6.14 ± 0.14 ^{a}	7.55 ± 0.01 ^{a}	7.71 ± 0.06 ^{a}	8.37 ± 0.02 ^{a}	7.63± 0.04 ^{a}	8.97 ± 0.04 ^{a}	9.02 ± 0.19 ^{a}	9.62 ± 0.02 ^{a}	8.84 ± 0.05 ^{a}

^{a,b,c} Values with the different letter for each parameter at the same time of wine maturity were significantly different according to the LSD test ($p<0.001$).
TP-total phenols, TA-total anthocyanins, VAN-vanillin index, PROC-proanthocyanidins, CI-colour intensity, H-hue, tint.

Proanthocyanidins were present in all wines at high concentrations (Tab. 2). According to the results obtained in this investigation, 'Plavac mali' is rich in proanthocyanidins, which was not affected by the duration of maceration. Statistically significant differences have been reported in 'Babić' wine during ageing (Budić-Leto *et al.* 2003), which has not been shown for 'Plavac mali' wine. The reason for this may be due to the effect of grape variety, and also because of different ethanol levels reached. Proanthocyanidins have been reported as the ones that are more readily extracted from the skins than from the seeds (Lee *et al.* 2008), and it was already reported that the large majority of skin polyphenols are extracted in the first 5 d (Mattivi *et al.* 2002).

Since in 'Plavac mali' most proanthocyanidins are localised in the skin (Tab. 3), this could be the main reason why wines produced with different maceration time did not show significant differences in their concentrations. It is obvious that in comparison with Babić wine, 'Plavac mali' has quite a different polyphenolic profile, which could be seen from the changes of vanillin/proanthocyanidins ratio during wine maturation. Little difference in decreasing value of vanillin/proanthocyanidins ratio shown in Tab. 4 indicates that the increase of polymerization of wine polyphenols occurs very slowly. These results show that this change in the value of the ratio was slightly smaller than in that reported for 'Babić' wine (Budić-Leto *et al.* 2003).

The higher colour intensity of the young wines made after 5 and 8 d of maceration is probably due to a better condensation of anthocyanins and proanthocyanidins or catechins giving rise to newly formed anthocyanins which stabilise the violet tinge of the wine. It was observed that colour intensity decreased with the decrease in anthocyanin content. However, wines macerated for 8 and 17 d after 6 and 14 months of ageing revealed a significant difference in colour intensity. In summary, lowest colour intensity was observed in wines made with maceration for 17 d. The proportions of phenolic compounds could explain these results. It can be seen from the data that 'Plavac mali' is a wine rich in high molecular weight proanthocyanidins and low molecular weight proanthocyanidins, which seems to be a characteristic of the cultivar.

Thus, the higher colour intensity was observed in wines aged for 6 months, which is in connection with the higher vanillin/proanthocyanidin ratio. On the contrary, the lower colour intensity was observed in wines aged for 14 months, in which the ratio of proanthocyanidins/anthocyanidins was the lowest.

The effect of maceration temperature on the polyphenolic composition of the wines: In attempt to determine the influence of maceration time on the polyphenolic content of 'Plavac mali' wine, maceration was conducted at the average temperature of 20 °C, which was 5 °C lower than the other maceration processes. It can be seen that the maceration temperature affected significantly the content of total anthocyanins ($p < 0.001$) and proanthocyanidins in young wine. The ageing process induced modifications

Table 3

Content of extractable polyphenols in skin and seed extracts of Plavac mali, vintage 2001

	Skins	Seeds	Totals	% Skins	% Seeds
Total phenols ($mg \cdot kg^{-1}$)[a]	1999	612	2611	77	23
Vanillin index ($mg \cdot kg^{-1}$)[b]	1208	414	1622	74	26
Proanthocyanidins ($mg \cdot kg^{-1}$)[c]	2342	476	2818	83	17
Anthocyanins	879	-	879	100	-

[a]equivalent of GAE; [b]equivalent of (+)-catechin; [c]equivalent of (+)-cyanidin

Table 4

Mean values of the ratio vanillin index (VAN)/proanthocyanidins (PROC) and proanthocyanidins (PROC)/total anthocyanins (TA) of the Plavac mali wine

	Days of maceration, average temperature 25 °C			Days of maceration, average temperature 20 °C
	5	8	17	9
Young wine				
VAN/PROC	0.54	0.59	0.61	0.65
PROC/TA	3.78	3.53	5.33	3.57
6-month aged wines				
VAN/PROC	0.54	0.53	0.59	0.58
PROC/TA	5.42	5.67	6.79	5.35
14-month aged wines				
VAN/PROC	0.41	0.44	0.49	0.36
PROC/TA	8.99	9.01	10.57	8.85

in the phenolic content, and it could be seen in the analysis of variance that temperature did not have an influence on the amount of anthocyanins and proanthocyanidins in wine aged for 6 and 14 months. Regarding the data, lower colour intensity was determined in young wines produced at lower temperature, but no significant variations in colour intensity were observed during the ageing of wines.

The results of this research demonstrate the influence of different maceration conditions on the content of the polyphenolic compounds in the most important native Croatian red wine 'Plavac mali'. The results reveal that the wine contains high concentrations of low and high molecular weight proanthocyanidins. In the wines produced with different maceration time the difference in the concentration of high molecular weight proanthocyanidins during the production and ageing was not determined. It was determined that prolonged maceration of 17 d results in a significant increase in the concentration of low molecular weight proanthocyanidins and decrease in the concentration of anthocyanins, while the maceration at lower temperature reduces the concentration of high molecular weight proanthocyanidins and anthocyanins in young wine. The results of the research show a significant difference in the concentration of low molecular weight proanthocyanidins in wines aged for 14 months, regarding the different time of maceration (5, 8, 17 d) and temperature (25 and 20 °C). A small change in the vanillin/proanthocyanidins ratio during wine ageing indicates a slow polymerization of phenols.

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