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THE EVALUATION OF COLOUR COMPONENTS AND ANTHOCYANINS IN *Babica* AND *Crljenak kaštelanski* WINES

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ABSTRACT

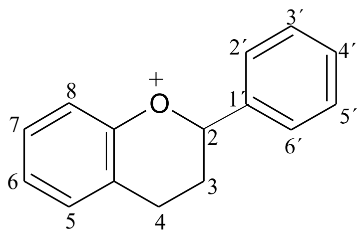
Babica and *Crljenak kaštelanski* are historic and autochthonous red grape cultivars of the Kaštela wine region (Central Dalmatia, Croatia). In the last few years, *C. kaštelanski* has been getting much moreattention, since it was discovered that it is a parent of the popular American variety *Zinfandel* and the Italian variety *Primitivo*. Therefore, *C. kaštelanski* has become economically important and it has been increasingly cultivated in Croatia, while other cultivars, like *Babica*, have almost vanished from the Dalmatian vineyards. As the colour of red wines is an important element of wine quality and it is the first feature that influences its commercial acceptance, this study was conducted with the aim of investigating the evaluation of the colour components and anthocyanins in *Babica* and *C. Kaštelanski* wines. The anthocyanin profile was determined using HPLC, while monomeric anthocyanins and basic colour characteristics of wine (density, hue, chromatic structure, and brilliance) were detected spectrophotometrically. The total monomeric anthocyanins in *Babica* were higher than in *C. kaštelanski* and the dominant anthocyanin in both wines was malvidin-3-glucoside, with over 71 % of all detected anthocyanins in *Babica* and with over 52 % in *C. kaštelanski*. The other colour parameters were mainly higher for *C. kaštelanski*, probably due to the almost six-fold higher content of cyanidin derivatives in *C. kaštelanski* wine.

Keywords: red wine, vinification, colour, anthocyanins, HPLC

INTRODUCTION

Colour is one of the most important attributes of red wine and it is well known that it depends on the phenolic composition of wine, primarily on the level and the profile of anthocyanins and other compounds that are formed during the vinification process (Bautista-Ortín et al., 2006; Jensen et al., 2008; He et al., 2012).
Anthocyanins are natural, water-soluble pigments from the group of flavonoids, responsible for the red, blue and purple colour of different fruits, vegetables, flowers, leaves, etc. (Heim et al., 2002). Anthocyanidins are the basic chemical structures of

anthocyanins (glycoside form, bonded to a sugar). They consist of an aromatic ring (A) bonded to a heterocyclic ring that contains oxygen and is bonded by a carbon-carbon bond to a second aromatic ring (B). Anthocyanins have diverse chemical structures, with differences related to the number and position of hydroxyl and methoxyl groups in the aromatic ring B, as well as sugar molecules bounded to a heterocyclic ring. The most common anthocyanins are usually conjugated to sugars (usually glucose), hydroxycinnamates and organic acids (malic or acetic acid). Almost 200 different anthocyanins have been identified in plants. (Belitz et al., 2004; Shahidi and Weerasinghl, 2004; Jackson, 2008; Jensen et al., 2008; Katalinić et al., 2010; He et al., 2012; Ma et al., 2012; Ristovski et al., 2014; Generalić Mekinić et al., 2016). The most common anthocyanidins in nature are pelargonidine, cyanidine, delfinidine, peonidine, petunidin and malvidin (Fig. 1).



Anthocyanidins	3	5	7	3´	4´	5´
Cyanidin	OH	OH	OH	OH	OH	-
Cyanin	O-Glu*	OH	OH	OH	OH	-
Peonidin	OH	OH	OH	OCH_3	OH	-
Delphinidin	-	OH	OH	OH	-	OH
Pelargonidin	OH	OH	OH	-	OH	-
Malvidin	OH	OH	OH	OCH_3	OH	OCH_3

** Glu - glucose*

Fig. 1. Structures of common anthocyanidins (Stalikas, 2007).

The anthocyanin composition of red wines depends on the anthocyanin profile of the grape variety and also on the applied winemaking technique (Bautista-Ortín et al., 2006; He et al., 2012). The red wine pigments are absorbed into the wine from red grape berry skin during its soaking in the must (maceration). In the fermentation and during the maturation, the present monomeric anthocyanins undergo a wide variety of reactions and form new compounds (anthocyanin-derived new pigments), which are crucial for the colour of wine, its intensity and stability (Bautista-Ortín et al., 2006; Jackson, 2008; Ivanova et al., 2011; He et al., 2012). In the red wines made from *Vitis vinifera* grapes the main monomeric anthocyanins are the 3-*O*-monoglucosides of the six anthocyanidins, including pelargonidin-3-*O*-glucoside (callistephin), cyanidin-3-*O*-glucoside (kuromanin), delphinidin-3-*O*-glucoside (myrtillin), peonidin-3-*O*-glucoside (peonin), petunidin-3-*O*-glucoside (petunin) and malvidin-3-*O*-glucoside (oenin) (Gao et al., 1997; Bautista-Ortín et al., 2006; Jackson, 2008; Jensen et al., 2008; He et al., 2012). Different scientific papers point out that the anthocyanins that possess more hydroxyl groups in the B ring can contribute more blueness, while the degree of methylation of the B ring in anthocyanins molecules, like in malvidin-3-*O*-glucoside, increases wine redness

(Jackson, 2008; He et al., 2012). Malvidin glycosides are the major anthocyanins in grapes, and among the most stable, due to the dimethoxylation of the molecule (Generalić Mekinić et al., 2016).
Anthocyanins also differ in their susceptibility to oxidation, so these changes could be more significant to wine-colour stability than the total anthocyanin content in wine. The initial purplish colour of young red wines is generated by the residual level of anthocyanins in copigment associations (Jackson, 2008) and it fades as the copigment complexes dissociate and the free anthocyanins react with other compounds from the group of flavonoids and non-flavonoids (such as flavonols, flavan-3-ols, oligomeric proanthocyanidins, and cinnamic acids and its derivatives) (Liao et al., 1992; Boulton, 2001; Jackson, 2008; Busse-Valverde, 2011; He et al., 2012). Among these compounds, flavan-3-ols, such as (+)-catechin or (-)-epicatechin, are recognized as powerful cofactors which can most easily and intensely form coloured complexes (Liao et al., 1992; González-Manzano, 2009; He et al., 2012). They can increase colour density, affect colour tint, and give a more purple hue to young red wines by provoking displacement of anthocyanin equilibria towards their coloured forms (He et al., 2012). Furthermore, better understanding of self-association and copigmentation can help us in predicting young wine colour attributes from the phenolic profiles of raw material - red grapes (Liao et al., 1992; Boulton, 2001; He et al., 2012).
As the colour of red wine is an important element of its quality and the first feature that influences its commercial acceptance, the objective of this study was to investigate and compare the anthocyanin profile and colour attributes of two young wines, produced using the same winemaking procedure, from two historic red grape cultivars from the Kaštela wine region; *Babica* and *C. kaštelanski*.

MATERIALS AND METHODS

All used chemicals were of adequate analytical grade. The measurements were performed on a Specord 200 spectrometer (Analytik Jena GmbH, Germany) while the individual anthocyanins were separated and identified using the Perkin Elmer HPLC-UV/Vis system (all from the 200 Series, Perkin Elmer, Walthamn, Massachusetts, USA).

Wine samples

In this study, young red wines produced from two autochthonous grape cultivars from the Kaštela wine region (Central Dalmatia, Croatia): *Babica* and *Crljenak kaštelanski* were analysed. In both vinifications 100 kg of grapes were used and thetraditional winemaking procedure was applied. The destemmed and crushed grapes were distributed into fermentation tanks and treated with potassium metabisulphite (10 g/100 L). After sulfiting, 10 g/100 L of active dry Burgundy Yeast (E. Begerrow GmbH & Co., Langenlonsheim, Germany) was added to initiate the alcoholic fermentation. The cap of grape solid was kept soaked using a mechanical barrier. The fermentation temperature was in the range from 25 to 27°C and the fermentation was accomplished within six days. After the fermentation has been completed, the must was devoted and the rest was pressed. The wine was then sealed using the tank's floating lid and paraffin oil.

Spectrophotometric analysis of monomeric anthocyanins and wine colour parameters

The amount of total anthocyanins in samples was determined using the bisulphite bleaching method (Amerine and Ough, 1980; Katalinić et al., 2010) and the monomeric anthocyanins content was calculated using the molar absorption coefficient for malvidin 3-glucoside. The results are expressed as mg of malvidin-3-glucoside equivalents per litre (mg M-3-gl/L). The measurements were performed in triplicate, and the results are expressed as means ± SD.

Wine colour intensity (CI), hue (T) and chromatic structure (optical density (OD) at 420, 520 and 620 nm) were determined by measuring the absorbance of the samples at 420, 520 and 620 nm (Glories 1984), and the colour parameters were calculated according to the equations described in Babincev et al. (2016). Colour intensity (CI) represents the amount of wine colour and it was calculated as the sum of absorbance at 620 nm, 520 nm and 420 nm, and the hue was calculated as the ratio between absorbance at 420 nm and absorbance at 520 nm (Glories, 1984; Bautista-Ortín et al., 2006; Babincev et al., 2016). Optical density represents the contribution of red (OD 520), yellow (OD 420) and blue (OD 620) colour to the colour of the red wines as described in Glories (1984).

HPLC analysis of anthocyanins

The HPLC analysis of anthocyanins was performed according to the previously published protocol with minor modifications (Fredotović et al., 2017). The separation, quantification, and identification of anthocyanins were carried out using a Kinetex C18 core-shell column (150×4.60 mm, 5 µm, Phenomenex, Torrance, CA, USA). The elution solvents were 0.3% perchloric acid in water (solvent A) and 0.3% perchloric acid in methanol (solvent B). The applied flow rate was 0.6 mL/min and the injected volume was 10 µL. The detection was carried out at 520 nm and the peaks were identified according to their retention times. The quantification was determined using a standard curve for malvidin 3-*O*-glucoside. The analysis was performed in duplicate, and the results are expressed as means ± SD.

RESULTS AND DISCUSSION

Red wine is characterized by deep purple to pale red colour, though most young red wines initially have a purplish-red hue (Jackson, 2008). The red pigment of wine increases with aging, while the share of the yellow pigment decreases. The colour intensity also decreases during wine aging, while the colour hue increases (Poiana et al., 2007; Babincev et al., 2016). All of these changes in colour parameters can be explained by the transition of monomeric anthocyanins (which contribute to the red colour of wine) into polymeric forms (Pasku, 2005; Badicev et al, 2016).

The data presented in Fig. 2 show the colour parameters of investigated wines: colour intensity, hue and the contribution of yellow (OD 420), red (OD 520), and blue (OD 620) pigments to the overall wine colour.

The highest absorption of the samples was detected at the wavelength of 520 nm, as expected, since red wines have a maximum spectrum at this wavelength due to the presence of anthocyanins. The contribution of the red pigment was slightly higher in *C. kaštelanski* wine (49.59 %), in relation to *Babica* (45.38 %). In contrast, the share of the yellow pigment was higher in *Babica* wine than in *C. kaštelanski* (43.78 % vs 37.64 %). The participation of the blue pigment in wine colour was 10.84 % for *Babica* and 12.78 % for *C. kaštelanski*. This parameter is one of the major characteristics of young red wines (Poiana et al., 2007; Babincev et al., 2016).

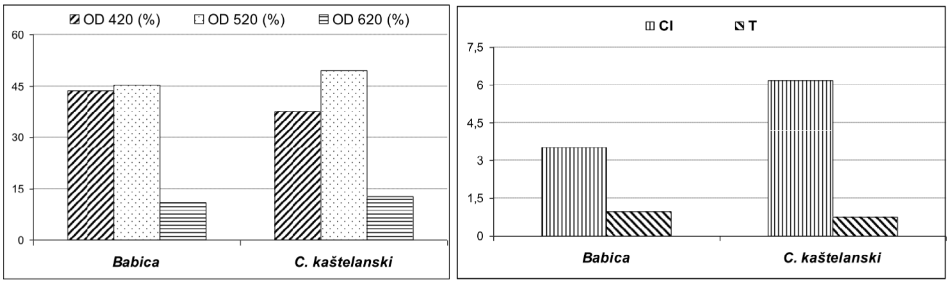


Fig. 2. Colour parameters (CI- colour intensity; T- hue; OD- optical density) of *Babica* and *C. kaštelanski* young wines

Colour intensity is defined as the amount of colour, determined by the content and structure of the anthocyanins present in wine (Glories, 1984; Ivanova et al., 2011). This parameter is usually defined as the sum of the absorbances recorded at 420, 520 and 620 nm (Glories, 1984) and in our case it was considerably higher for *C. kaštelanski* wine. Since the effect of red and blue colour in *Babica* wine was lower, it was expected that the colour intensity will be lower, since the ratio between the yellow and red colour in the wine *Babica* was lower. The wine hue is defined as the ratio of A_{420}/A_{520}, and gives a measure of the wine's redness (Glories, 1984). Wine hue indicates the development of a colour towards orange and it increases through wine aging. In this study, the detected hue was higher for *Babica* (0.97) in relation to the wine *C. kaštelanski* (0.76). While young wines have the value of 0.5-0.7, the upper limit is around 1.2-1.3 (Fig.2) (Poiana et al., 2007; Babincev et al., 2016).
The determination of total monomeric anthocyanins was performed using the spectrophotometric bisulphite bleaching method and the results are shown in Fig 3. The sum of anthocyanidin derivatives detected by HPLC is presented in Table 1 and the content of the individual compounds detected by HPLC in Table 2.

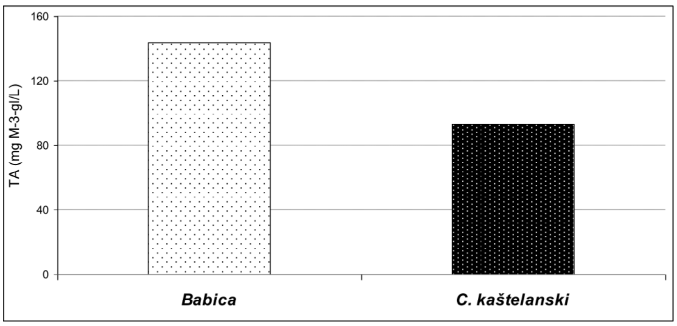


Fig. 3. The content of total anthocyanins (TA) in *Babica* and *C. kaštelanski* wines

Table 1. The content of anthocyanidin derivatives in *Babica* and *C. kaštelanski* wines

	Babica	*C. kaštelanski*
Total delphynidins (mg/L)	4.10	10.41
Total cyanidins (mg/L)	0.45	2.69
Total petunidins (mg/L)	6.43	10.39
Total peonidins (mg/L)	4.87	13.68
Total malvidins (mg/L)	67.95	58.43

The content of total monomeric anthocyanins in *Babica* was higher (143.73 mg M-3-gl/L) than in the *C. kaštelanski* (92.91 mg M-3-gl/L) wine. The dominant anthocyanin in both wines was malvidin-3-glucoside, with over 71 % of all detected anthocyanins in *Babica* and with over 52 % in *C. kaštelanski*. In our previous study on red grape anthocyanidins, we also investigated the profile of anthocyanidins in *Babica* and *C. kaštelanski* grape skin extracts. The results of that study also confirmed the higher content of malvidin derivatives in Babica (60.1 %) than in C. kaštelanski (52.1 %), which is in accordance with these results (Generalić Mekinić et al., 2016). Aside fromthe content of total malvidins, the *C. kaštelanski* wine contained higher proportions of delphynidins, cyanidins, petunidins and peonidins (Table 2).

The results for individual compounds are presented in Table 2. Generally, the dominant anthocyanin in wines is malvidin-3-O-glucoside (Liao et al., 1992; Gao et al., 1997; He et al., 2012), which was also confirmed by our results as expected. The anthocyanins identified in *Vitis vinifera* L. varieties are 3-O-monoglucosides and 3-O-acylated monoglucosides of the main anthocyanidins, and the acylated forms are the esters of the coumaric and caffeic acid (Dimitrovska et al., 2011).

In *Babica*, among other detected anthocyanins, there were petunidin-3-*O*-glucoside (7.33 %), malvidin-3-(6-*O*-coumaroyl) glucoside (5.13 %), peonidin-3-*O*-glucoside (4.83 %), delphinidin-3-O-glucoside (3.90 %), malvidin-3-*O*-acetylglucoside (3.09 %) and malvidin-(6-*O*-caffeoyl) glucoside (1.39 %). Furthermore, in *C. kaštelanski* wine the presence of peonidin-3-*O*-glucoside (12.86 %), petunidin-3-*O*-glucoside (8.95 %), delphinidin-3-O-glucoside (7.84 %), malvidin-3-(6-*O*-coumaroyl) glucoside (4.16 %), malvidin-3-*O*-acetylglucoside (2.65 %) and malvidin-(6-*O*-caffeoyl) glucoside (1.50 %) was confirmed. In comparison to *Babica*, slightly higher amounts of delfinidin-3-O-acetylglucoside (3-fold higher) and cyanidin-3-O-glucoside (10-fold higher) were detected in *C. kaštelanski*.

Studies showed that acylated anthocyanins contribute to the red colour of young red wine and that their concentrations vary during aging, while monomeric forms disappear within a few months after fermentation (He et al., 2012). Therefore, small amounts of acylated anthocyanins present in the investigated samples are not surprising. Most of these free anthocyanins will react, combine or condense with other present phenolics and form more complex and stable pigments, while a relatively small fraction will disappear by degradation, oxidation, precipitation, or formation of other colourless compounds (Jackson, 2008; He et al., 2012).

Table 2. Anthocyanin composition (%) of *Babica* and *C. kaštelanski* young

Anthocyanins	**Babica**	**Crljenak**
Delphinidin-3-*O*-glucoside	3.90 ± 0.02	7.84 ± 0.33
Cyanidin-3-*O*-glucoside	0.17 ± 0.01	1.82 ± 0.37
Petunidin-3-*O*-glucoside	7.33 ± 0.14	8.95 ± 0.48
Peonidin-3-*O*-glucoside	4.83 ± 0.42	12.86 ± 0.58
Malvidin-3-*O*-glucoside	71.49 ± 0.52	52.81 ±0.24
Delphinidin-3-*O*-acetylglucoside	0.98 ± 0.05	3.05 ± 0.16
Cyanidin-3-*O*-acetylglucoside	0.04 ± 0.00	0.49 ± 0.02
Petunidin-3-*O*-acetylglucoside	0.15 ± 0.00	0.95 ± 0.06
Peonidin-3-*O*-acetylglucoside	0.30 ± 0.01	0.60 ± 0.05
Petunidin-(6-*O*-caffeoyl)glucoside	0.13 ± 0.00	0.60 ± 0.01
Malvidin-3-*O*-acetylglucoside	3.09 ± 0.02	2.65 ± 0.03
Malvidin-(6-*O*-caffeoyl)glucoside	1.39 ± 0.02	1.50 ± 0.01
Cyanidin-(6-*O*-coumaryoyl)glucoside	0.32 ± 0.00	0.50 ± 0.01
Petunidin-(6-*O*-coumaryoyl)glucoside	0.05 ± 0.00	0.37 ± 0.01
Peonidin-3-(6-*O*-coumaroyl)glucoside	0.69 ± 0.00	0.85 ± 0.03
Malvidin-3-(6-*O*-coumaroyl)glucoside	5.13 ± 0.02	4.16 ± 0.04

CONCLUSION

According to the obtained results, notable differences in content of total anthocyanins, their chemical profile, and ratios of individual compounds in young wine from *Babica* and *C. kaštelanski* grapes were detected. Since the winemaking procedure was the same, those differences could be attributed to the grape variety and the chemical potential of the used raw material. In addition, anthocyanins in these red grapes/wines can be used as chemical markers for the differentiation of the grape cultivars. Although the content of total monomeric anthocyanins in *Babica* was significantly higher, other investigated parameters imply that the wine obtained from *C. kaštelanski* is highly coloured. Therefore, it can be concluded that the presence and the amount of individual anthocyanins have a considerable effect on the colour parameters of wine.

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