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ORIGINAL PAPER

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Anthocyanin composition of the red wine Babić affected by maceration treatment

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Abstract Identification of anthocyanins in the wine made of the Croatian autochthonous grape variety of Babić (*Vitis vinifera* L.) was carried out and their profile was determined by means of high-performance liquid chromatography with diode array and mass spectrometric detection. Dependence of anthocyanins content and profile on maceration treatment conditions was investigated. Statistically significant differences of anthocyanins concentration in wines Babić produced by various maceration treatments were confirmed by the use of multivariate analysis of variances. The investigation results indicated that the maceration temperature exerts higher influence on anthocyanins concentration than the duration of maceration. In addition, on the basis of anthocyanin composition and using different multivariate statistical analyses, differentiation of wines Babić according to maceration treatments was procured.

Keywords Anthocyanins · High-performance liquid chromatography · Maceration treatment · Multivariate analysis · Wine Babić

Introduction

Anthocyanins are pigments of red, blue and purple colours, mainly occurring in cellular vacuoles of grape skin [1, 2]. Anthocyanins are important compounds for characterization of red grape varieties, which signifies them as chemical markers in distinguishing varietal red wines [3, 4]. It was determined that the mutual relations of anthocyanins (the anthocyanin profile) is a property of the vine variety, even though their absolute content in ripe grapes is variable [5, 6] and dependent on factors that concern the environmental factors, such as intensity of light and temperature [7].

Although the wine anthocyanin composition is primarily determined by the genetic factor of the grape variety, the vinification parameters also have quite a significant impact. It was shown that the maceration parameters, primarily the temperature and maceration duration, have a significant influence on extraction of anthocyanins from grape skins [8–11]. The conditions of maceration, fermentation and maturation of wine influence the anthocyanin composition, which is very significant, because the total concentration and composition of anthocyanins determine the colour of red wines [10, 12]. A proficient extraction of anthocyanins had been effected at the Pinot Noir variety grapes when maceration was carried out at a higher fermentation temperature [13]. There have been reports on a rise of anthocyanin concentrations in proportion to maceration duration only for the first several days, while a decrease occurred with an increase of maceration period [13–15]. Monomeric or free anthocyanins extracted from grapes are reactive components, which gradually turn into polymeric anthocyanins, depending on the conditions of wine maturing and age. Such condensing reactions occur in the presence of other flavonoid components, primarily flavan-3-ols, such as catechins and proanthocyanidins (condensed tannins) [16, 17], or in the presence of other non-phenolic compounds, such as acetaldehyde or pyruvic acid [18].

Babić is a Croatian autochthonous [19] red variety of *Vitis vinifera*, which acquires its superior quality in the Primošten vineyards where wine with a protected geographic origin are produced. This grape variety is very important in production of high-quality red wines in the region of North Dalmatia. It attains its high quality in an extremely rocky area in the vicinity of Primošten, under specific soil conditions.

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The objective of this investigation was to determine the impact of maceration conditions (temperature and duration) on the concentration of anthocyanins in the Babić wine. Identification and quantitative determination of anthocyanins were performed by the reverse-phase HPLC method with diode array (DAD) detection. Also, detection and identification of anthocyanins were carried out by the HPLC-diode array detection-mass spectrometry analysis.

Materials and methods

Vinification was carried out on 600 kg of the Babić grape variety by random sampling in the Primošten vineyards. The grapes were harvested at the technological ripeness stage (21.1% of soluble solids and 7.45 g/l of total acidity). After desteming and crushing, which had been done instantly upon harvesting, an aqueous solution of SO_2 (5%, w/v) was added to the must to give 100 mg SO_2/kg, homogenized and divided into 70 l fermentation tanks, so that each tank contained about 50 kg of must. Fermentation was spontaneous (without addition of selected yeast). Maceration was performed in the traditional fashion, with punching the must 3 times a day (punching lasted 4 days in treatment 1, 6 days in treatment 2, 13 days in treatment 3 and 7 days in treatment 4). Four variants of maceration treatment, in three repetitions, were performed.

Maceration treatment 1

Maceration lasted until 60% total fermented sugars was attained. The average temperature during maceration was 23 °C (maximal temperature was 29 °C), while the maceration procedure lasted for 4 days.

Maceration treatment 2

Maceration lasted until complete fermentation of sugar, which was achieved in 6 days. The maceration process proceeded at average temperature of 23 °C (maximal temperature was 29 °C).

Maceration treatment 3

Young wine was left on the pomace for another 7 days after the fermentation of sugar was completed with daily punching, while the overall period of maceration was 13 days. The temperature during maceration was the same as in the previous two maceration treatments (average temperature was 23 °C, with the maximum of 29 °C).

Maceration treatment 4

The maceration process proceeded at an average temperature of 20 °C (maximum of 23 °C). Maceration, i.e. the period needed for the whole sugar to ferment lasted for 7 days.

After the maceration treatment was completed, the wine was decanted from the pomace, transferred into 25-l glass vessels and left at room temperature (21 °C). During maturation of the wine, the total SO_2 was controlled and kept at the level of 50–60 mg/l, while free SO_2 amounted to 10–15 mg/l. The wine was decanted for the first time through open racking in December 2001 into 25-l glass vessels. The second time the wine was decanted in March 2002 through closed racking into 25-l vessels. The third decanting of the wine was carried out in June 2002, after which the wine was racked into 0.75-l bottles and closed with cork stoppers. Analyses of anthocyanins were done in August 2002.

Purification of anthocyanins prior to HPLC analysis

Solid-phase extraction (SPE) was the method used for isolation of anthocyanins. Conditioning and cleansing of wine sample was carried out by their passing through the Sep-Pak C18 cartidge of 1 g (Waters, Milford, USA) previously conditioned with 2 ml of methanol and 5 ml of 0.0050 mol/l H_2SO_4. After the wine sample was passed through, the cartidge was washed first with 6 ml of 0.3% $HClO_4$, and then with 4 ml of water. Then the cartridge was dried in the steam of nitrogen. Anthocyanins were eluted with 5–10 ml of methanol. The methanol was evaporated to dryness under vacuum on a rotary evaporator at 37–40 °C. The dry residue was then dissolved in the 0.5 ml of 27% (v/v) methanol and 73% of 0.3% (v/v) $HClO_4$ in water solution. Prior to the analysis, the sample was filtered through 0.22 μm polytetrafluoroethylene (PTFE) syringe tip filter (Gelman Science, Ann Arbor, MI) into flint glass HPLC vial equipped with PTFE-lined crimp cap.

HPLC diode array analysis

Analysis of anthocyanins were performed in a Hewlett-Packard Model 1090 high-performance liquid chromatograph equipped with a Photodiode Array Ultraviolet (UV)-Visible Detector and the HPChemstation software, according to the method of Iacono et al. [20]. The injected sample volume was 10 μm. Separation of anthocyanins was carried out at the column Purospher RP C_{18}-LiChroCART (250 mm × 4 mm, 5 μm particle size) with the Purospher RP 18 precolumn (particle size of 5 μm) kept at 40 °C. The chromatographic method conditions were as follows: mobile phase flow rate: 0.45 ml/min; DAD detection in the visible at 520 nm; mobile phase A: 0.3% (v/v) perchloric acid in water; mobile phase B: methanol, with the elution program being a gradient one starting from mobile phase A: 71.5% and mobile phase B: from 28.5 to 45.5% B in 32 min, then from 45.5 to 68.5% B in 32–45 min, and then from 68.5 to 100% B in 45–47 min and finally 100% B in 47–50 min.

Anthocyanins were identified in correlation to the retention time, elution sequence, and UV-VIS spectral properties. Quantitative determination of compounds was

carried out by the standard method with malvidin-3-glucoside (Extrasynthese, France). All anthocyanins were expressed as malvidin-3-glucoside.

HPLC-diode array detection-mass spectrometry analysis

Confirmation of peak identity was performed on Waters 2690 HPLC equipped with Waters 996 Diode Array Detector (Waters Corp., Milford, MA), Micromass ZQ ESI-MS system (Micromass, Manchester, UK), and Empower Software (Waters Corp.). Separation was performed using a column XTerra MS C_{18} (Waters Corp.) 2.1 mm × 150 mm, 3 μm, with a guard cartridge.

An amount of 10 μl of the sample was injected into the column, kept at 40 °C. The mobile phase consisted of 5% formic acid in H_2O (A) and 5% formic acid in methanol (B) and flowed at 0.2 ml/min. The linear gradients started from 10 to 30% B in 10 min, to 40% B in 7 min, to 51.2% B in 4 min, to 90% B in 9 min. The column was equilibrated for 7 min before each injection. The UV-VIS spectra were recorded from 230 to 600 nm, with detection at 520 nm. The MS detector operated at the capillary voltage of 3000 V, the extractor voltage of 6 V, the source temperature of 105 °C, the desolvation temperature of 200 °C, the cone gas flow (N_2) of 30 l/h, the desolvation gas flow (N_2) of 450 l/h. The outlet of the HPLC system was split (9:1) to the ESI interface of the mass analyzer. Electrospray mass spectra ranging from *m/z* 200 to 700 were taken in positive mode with a dwell time of 0.1 s. At the end of a 30-min run with mass spectra taken in positive mode, a 1-min run in negative mode was added. The cone voltage (CV) was set in a scan mode at the values of 65 V for the identification based on the aglycone peak, and of 30 V for the identification based on both the fragment aglycone and molecular ion.

Each compound was identified based on the following parameters: (1) retention time, (2) UV-VIS spectra, (3) base fragment corresponding to the aglycone, and (4) molecular ion.

Statistical analysis

The data were processed through the analysis of variance (ANOVA), multivariate analysis (MANOVA) and linear discriminant analysis (LSD) test by the use of the Statistica 6.0 Software [21]. The analysis of variance (ANOVA) was performed by grouping wines according to their maceration treatment by building a data matrix of 4 rows (different maceration treatmens) and 16 columns (anthocyanin concentrations) [22, 23]. Moreover, the principal component analysis (PCA) and linear discriminant analysis were further used to describe the data set satisfactory.

Results and discussion

The following 16 anthocyanins were identified and determined quantitatively in the Babić wine produced by different maceration treatments: five 3-monoglucosides: delphinidin (Dp-3-gl), cyanidin (Cy-3-gl), petunidin (Pt-3-gl), peonidin (Pn-3-gl) and malvidin (Mv-3-gl); five acetic-acid-acylated 3-monoglucosides: delphinidin (Dp-3-gl-ac), cyanidin (Cy-3-gl-ac), petunidin (Pt-3-gl-ac), peonidin (Pn-3-gl-ac) and malvidin (Mv-3-gl-ac), five *p*-coumaric acid esters of the 3-monoglucosides: 3-monoglucoside *p*-coumarats of delphinidin (Dp-3-gl-*p*cum), cyanidin (Cy-3-gl-*p*cum), petunidin (Pt-3-gl-*p*cum), peonidin (Pn-3-gl-*p*cum) and malvidin (Mv-3-gl-*p*cum) and malvidin-3-monoglucoside-caffeoate (Mv-3-gl-caf). Table 1 shows the concentrations of the above mentioned anthocyanins, together with standard deviations. The measured differences between the maceration treatments proved to be statistically significant when analyzed by the MANOVA (Table 1) and ANOVA (Table 2) for *p* level lower then 0.05. The statistically significant influences (LSD, $p<0.001$) of maceration parameters on concentrations of respective anthocyanins are marked with various letters (a, b, c and d) in Table 1. The values followed by the same letter indicates that they are not significantly different at 99% confidence level.

The dendrogram of cluster analysis (CA) performed for four different maceration treatments (Fig. 1) included a classification by Euclidean distances from group centroids. It was employed to characterize and classify four maceration treatments, based the results of anthocyanin concentrations. The first multivariate approach to data was carried out by means of the CA (Fig. 1) resulting in clustering of variables in two classes that corresponded to different maceration treatments.

The analysis of variance (ANOVA) was used to monitor the impact of treatments on concentrations of respective anthocyanins. Statistically significant difference

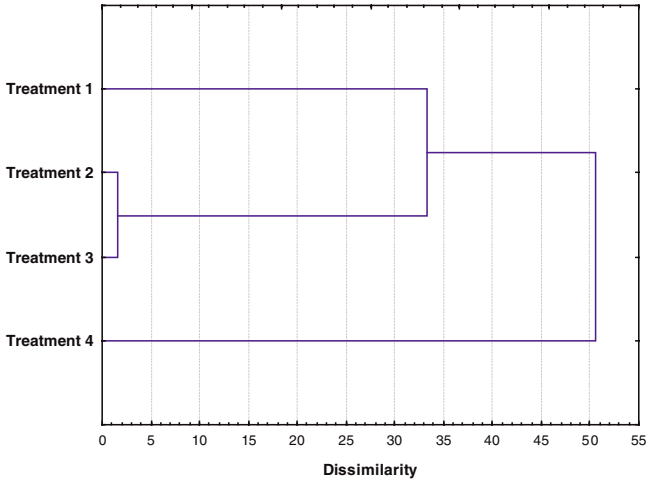

Fig. 1 Dendrogram of cluster analysis performed for different maceration treatments. *Treatment 1*: Maceration: until attained 60% total fermented sugars; temperature range: 19–29 °C, procedure lasted 4 days. *Treatment 2*: Maceration: until complete fermentation of sugar; temperature range: 19–29 °C, procedure lasted 6 days. *Treatment 3*: Maceration: wine left on the pomace for 7 days after the fermentation of sugar was completed, with daily punching; temperature range: 19–29 °C, procedure lasted 13 days. *Treatment 4*: Maceration: pomace cooled after 2 days to 23 °C and lasted till the whole sugars fermented; temperature range: 19–23 °C, procedure lasted 7 days

Table 1 Anthocyanin concentrations (mg L^{-1} ± standard deviations) in the Babić wine produced by various maceration treatments

Anthocyanins	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Delphinidin-3-monoglucoside	16.98±0.19 a	17.09±1.28 a	13.94±1.18 a,c	11.06±1.58 b,c
Cyanidin-3-monoglucoside	1.72±0.00 a	1.96±0.07 b	1.92±0.11 b	0.74±0.05 c
Petunidin-3-monoglucoside	26.09±1.52 a	24.34±1.84 a	22.94±0.62 a,b	19.29 ±2.69 b
Peonidin-3-monoglucoside	13.60±0.52 a	14.13±1.10 a	14.41±0.16 a	9.43±1.55 b
Malvidin-3-monoglucoside	166.82±11.38 a	148.05±9.81 a,b	151.67±3.06 a,b	129.86±18.54 b
Delphinidin-3-monoglucoside acetate	1.42 ±0.06 a	1.28 ±0.59 a	1.70±0.10 a,b	2.11±0.11 b
Cyanidin-3-monoglucoside acetate	0.56±0.09 a	1.15±0.09 b,c	0.81±0.00 a	1.38±0.15 b,c
Petunidin-3-monoglucoside acetate	0.98±0.04 ns	1.05 ± 0.06 ns	0.74±0.06 ns	0.84±0.07 ns
Peonidin-3-monoglucoside acetate	1.08±0.04 ns	0.96±0.12 ns	0.90±0.01 ns	0.78±0.08 ns
Malvidin-3-monoglucoside acetate	7.76±0.09 ns	8.22±1.61 ns	8.13±0.11 ns	6.75±0.41 ns
Malvidin-3-monoglucoside-caffeoate	0.79± 0.12 ns	0.75±0.21 ns	1.01±0.04 ns	0.63±0.05 ns
Delphinidin-3-monoglucoside *p*-coumarat	0.28±0.64 a	0.64±0.73 b	0.40±0.54 c	0.57±0.64 d
Cyanidin-3-monoglucoside *p*-coumarat	0.64 ± 0.04 a	0.74 ± 0.13 b	0.54±0.07 c	0.64±0.16 d
Petunidin-3-monoglucoside *p*-coumarat	3.21±0.27 ns	2.83 ±0.51 ns	2.69±0.49 ns	2.53±0.37 ns
Peonidin-3-monoglucoside *p*-coumarat	3.96±0.24 a	3.17±0.45 b	3.08±0.10 b	2.96±0.37 b
Malvidin-3-monoglucoside *p*-coumarat	22.24±2.39 a	16.37±2.08 b	17.15±0.98 b	15.84±2.28 b
Total monoglucosides	225.21±13.62 a	205.58±14.10 a,b	204.89±4.82 a,b	170.396±24.31 b
Total monoglucoside acetates	11.82±0.05 ns	12.66±1.11 ns	12.29±0.25 ns	11.861±0.29 ns
Total monoglucoside *p*-coumarats	30.05±2.86 a	23.12±3.07 b	23.47±1.25 b	21.987±3.19 b
Sum total	270.46±16.84 a	244.56±18.95 a,b	243.28±6.65 a,b	206.397±28.25 b

Note. ns, no statistically significant difference determined. The values followed by the same letter (a, b, c and d) indicate that they are not significantly different ($p<0.001$)

of the following anthocyanins was determined: Cy-3-gl ($F=61.74$; $p<0.001$), Pn-3-gl ($F=6.74$, $p<0.01$), Cy-3-gl-ac ($F=13.65$; $p<0.05$), Mv-3-gl-caf ($F=15.86$; $p<0.05$), and total 3-monoglucosides ($F=13.20$; $p<0.01$) (Table 2). The multivariate analysis of variances (MANOVA) of anthocyanins in wines, tested with regard to maceration conditions, indicated a higher statistically significant difference of monitored anthocyanins. Additionally, it was determined by the multiple analysis of variances which anthocyanins were affected by the temperature, and which ones were impacted by the duration of maceration. In such a way, it was possible to separate respective influences of maceration parameters.

Effects of temperature

The wine produced at higher maceration temperatures under treatment 2 (average 23 °C, 6 days) contained a significantly higher ($p<0.001$) concentration of total monoglucosides and total monoglucoside *p*-coumarats than the wine produced at lower temperatures under treatment 4 (average 20 °C, 7 days). As indicated in Table 1, the concentrations of 3-monoglucosides of delphinidin, cyanidin, petunidin and peonidin were significantly higher ($p<0.001$) in the wine produced under treatment 2 (average 23 °C, 6 days) than under treatment 4 (average 20 °C, 7 days) and in both cases the maceration lasted until complete fermentation of sugar. Also, the concentration of Dp-3-gl-ac was significantly lower ($p<0.001$), whereas the concentration of Dp-3-gl-*p*-cum and Cy-3-gl-*p*-cum was significantly higher ($p<0.001$) under treatment 2 than under treatment 4. Temperature had no effect on malvidin-3-monoglucoside ($p>0.05$), which is the major anthocyanin in the Babić wine (61.9%).

This difference was presumably due to higher temperature (maximum 29 °C) that was used in treatment 2, as opposed to the temperature in treatment 4 (maximum 23 °C), which rendered easier the release of anthocyanins from the grape skin cellular vacuoles. These results show that the maceration temperature is a very important factor that affects the anthocyanin concentration in wine as it was also confirmed by using CA presented in Fig. 1. Extraction of anthocyanins was increased with higher fermentation temperature.The results obtained are in accordance with those [24] reported on changes in anthocyanin composition during fermentation of the Pinot Noir wine, where was determined that the maximum concentration of the major anthocyanin (Mv-3-gl) in traditional maceration is reached earlier at 30 °C (3 days), than at 20 °C (4 days). Vrhovšek [11] reported the effect of high maceration temperature (25 °C) for the significant increase in the total amount of anthocyanins in wine, cv. Blaufränkish, in maceration variant 1 compared to maceration variant 2, which was macerated for the same time, but without raising temperature in the last 48 h (from 25 °to 35 °C).

Effects of maceration duration

In comparison with other monoglucosides, Cy-3-gl was a minor component (0.4–0.8%), and, even though it was determined that its concentration (0.744–1.959 mg/l) was significantly dependent ($p<0.001$) on the maceration

Table 2 One-way analysis of variance

	F-maceration treatment
Dp-3-gl	5.87
Cy-3-gl	61.74***
Pt-3-gl	2.5
Pn-3-gl	6.74**
Mv-3-gl	1.59
Dp-3-gl-ac	1.45
Cy-3-gl-ac	13.65*
Pt-3-gl-ac	5.77
Pn-3-gl-ac	2.92
Mv-3-gl-ac	0.65
Mv-3-gl-caf	15.86*
Dp-3-gl-*p*cum	3.31
Cy-3-gl- *p*cum	0.54
Pt-3-gl-*p*cum	0.89
Pn-3-gl-*p*cum	1.94
Mv-3-gl-*p*cum	2.13
Total 3-gl	13.20**
Total 3-gl-ac	2.16
Total 3-gl-*p*cum	3.30

Note. The Fisher coefficient and significant levels for maceration treatments. Dp-3-gl, delphinidin-3-monoglucoside; Cy-3-gl, cyanidin-3-monoglucoside; Pt-3-gl, petunidin-3-monoglucoside; Pn-3-gl, peonidin-3-monoglucoside; Mv-3-gl, malvidin-3-monoglucoside; Dp-3-gl-ac, delphinidin-3-acetylmonoglucoside; Cy-3-gl-ac, cyanidin-3-acetylmonoglucoside; Pt-3-gl-ac, petunidin-3-acetylmonoglucoside; Pn-3-gl-ac, peonidin-3-acetylmonoglucoside; Mv-3-gl-ac, malvidin-3-acetylmonoglucoside; Dp-3-gl-*p*cum, delphinidin-3-*p*-coumarylmonoglucoside; Cy-3-gl-*p*cum, cyanidin-3-*p*-coumarylmonoglucoside; Pt-3-gl-*p*cum, petunidin-3-*p*-coumarylmonoglucoside; Pn-3-gl-*p*cum, peonidin-3-*p*-coumarylmonoglucoside; Mv-3-gl-*p*cum, malvidin-3-*p*-coumarylmonoglucoside; Mv-3-gl-caf, malvidin-3-caffeoylmonoglucoside; otal-3-gl, sum of monoglucoside; Total-3-gl-ac, sum of acetylmonoglucoside; Total-3-*p*cum, sum of *p*-coumarylmonoglucoside

$*p<0.05$

$**p<0.01$

$***p<0.001$

duration, its contribution to the overall concentration of anthocyanins was rather minor. The concentration of Mv-3-gl, Pn-3-gl, Pt-3-gl and Dp-3-gl under treatments 1, 2 and 3 was not affected by maceration duration ($p>0.05$), which can be linked to an increased extraction of flavan-3-ols (catechins and proanthocyanidins) under prolonged maceration [10]. It has been reported that mutual interactions of anthocyanins and flavan-3-ols occurs in wine. As a consequence of such reactions, an entry of anthocyanins into the structures of polymeric anthocyanin-tannin pigments probably occured, as had been determined at an earlier date [17, 25]. Earlier report had shown that young wine comprises most free anthocyanins, and that their concentration tends to decrease as wine ages [26], partly due to anthocyanin oxidation, and partly to condensation of anthocyanins with other polyphenols. The above mentioned monoglucosides (delphinidin, petunidin, peonidin and malvidin) prevail in the Babić variety, forming 82.9% of the total content of anthocyanins; therefore it is important that the maceration duration did not impact their concentrations. In addition, it was determined that the concentrations of Dp-3-gl-ac, Pt-3-gl-ac, Pn-3-gl-ac, Mv-3-gl-ac, Mv-3-gl-caf and Pt-3-gl-pcum (total relative content: 5.9%) were not influenced by the maceration duration ($p>0.05$). Therefore, all of the above indicates that the maceration duration impacted 11.9% of the total anthocyanin content. All these data suggest that the changes in anthocyanin composition (profile) of wine during winemaking are related to the influence of maceration temperature and duration.

The PCA analysis helped separate the anthocyanins that manifested independence from wine-production conditions. Figure 2 confirms significant differences depending on the vinification technique for the nine identified anthocyanins whose concentrations did not depend on the maceration treatment. These nine anthocyanins form the group in the right section of the figure, and are separately enlarged and isolated within the figure in the left corner. The remaining seven anthocyanins are not parts of the set, showing through their distancing from it that the impact of the maceration procedure on their concentration in the wine is extensive. It has to be taken into consideration that in this case PC1 explains 93% of total variances, and it can be inferred that the most significant distance regards Mv-3-gl, for which it has been determined that its concentrations was not significantly affected by the maceration technique.

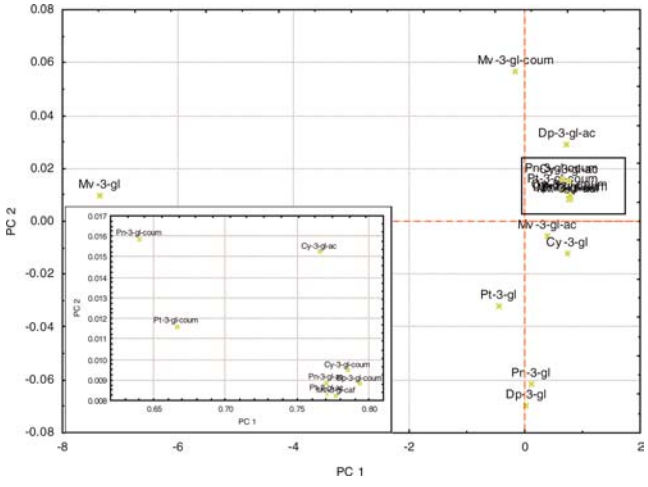

Fig. 2 PCA plot (two-dimensional) from HPLC data for the Babić wine. Dp-3-gl, delphinidin-3-monoglucoside; Cy-3-gl, cyanidin-3-monoglucoside; Pt-3-gl, petunidin-3-monoglucoside; Pn-3-gl, peonidin-3-monoglucoside; Mv-3-gl, malvidin-3-monoglucoside; Dp-3-gl-ac, delphinidin-3-acetylmonoglucoside; Cy-3-gl-ac, cyanidin-3-acetylmonoglucoside; Pt-3-gl-ac, petunidin-3-acetylmonoglucoside; Pn-3-gl-ac, peonidin-3-acetylmonoglucoside; Mv-3-gl-ac, malvidin-3-acetylmonoglucoside; Dp-3-gl-pcum, delphinidin-3-*p*-coumarylmonoglucoside; Cy-3-gl-pcum, cyanidin-3-*p*-coumarylmonoglucoside; Pt-3-gl-pcum, petunidin-3-*p*-coumarylmonoglucoside; Pn-3-gl-pcum, peonidin-3-*p*-coumarylmonoglucoside; Mv-3-gl-pcum, malvidin-3-*p*-coumarylmonoglucoside; Mv-3-gl-caf, malvidin-3-caffeoylmonoglucoside

Conclusion

The results obtained indicate that the concentrations of particular anthocyanins in wine Babić are significantly affected by the maceration treatment. The concentrations of Dp-3-gl, Cy-3-gl, Pt-3-gl, Pn-3-gl, Dp-3-gl-ac, Dp-3-gl-pcum, Cy-3-gl-pcum, forming 23.5% of total anthocyanins of the Babić wine, were under the influence of maceration temperature. On the other hand, the concentrations of Cy-3-gl, Cy-3-gl-ac, Dp-3-gl-pcum, Cy-3-gl-*p*cum, Pn-3-gl-*p*cum and Mv-3-gl-pcum, forming 11.2% of total anthocyanins of the Babić wine, were significantly dependent on the maceration duration. The use of the cluster analysis and principal component analysis facilitated separation of wines into two categories, according to their anthocyanin profile, produced by different maceration techniques. The application of multivariate analyses is essential for treatment of such an extensive data set because the combination of chemical analyses and statistical treatments is a suitable tool for differentiating maceration treatments.

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