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Differences in chemical composition of 'Plavac mali' grape berries

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Summary

Evaluation of differences in yield and quality of grapevine is often based on the random samples of berries harvested on the same date. Due to differences in ripeness of berries from the same cluster, and among berries from different clusters of the same vine, the determined differences among examined treatments (clones, agricultural practices etc.) based on random berry samples may be over- or underestimated. The aims of this study were to determine: (1) differences among three 'Plavac mali' clones in the proportion of berries of different density classes (sugar concentration level) using the flotation method; (2) differences in berry weight and chemical composition among density classes of the same clone, and (3) differences between clones of the same density class. Significant differences were determined for all observed characteristics, among different berries density classes and clones. The results obtained indicate that berry samples used to compare different clones should be, to the greatest possible extent, at the same level of ripeness, as this significantly effects chemical composition. This can be achieved using the density separation of berries with the simple flotation method presented in this study. The presented results could be useful in the future improvement of clonal selection methodology as well as in improvement of sampling strategy for other types of researches.

K e y w o r d s : asynchronous berries development; 'Plavac mali'; intravarietal variability; berries floatation.

Introduction

Asynchronous berry development (Jackson and Lombard 1993) leads to differences in the ripeness of berries on the same vine. Cluster position on the vine, berry position in the cluster, and even berry size (Letaief *et al.* 2008, Chorti *et al.* 2010, Dai *et al.* 2011) are causes for differences in the ripeness of berries among clusters of the same vine, and between berries of the same cluster. For that reason, estimation of differences based on random berry samples may lead to incorrect conclusions.

Using the simple flotation method, berries from a single cluster can be separated based on their ripeness stage. Using different salt or sucrose solutions (Flora and Lane 1979, Rolle *et al.* 2009, 2012), berries can be sorted into several density classes containing berries of a similar sugar concentration, *i.e.* ripeness stage.

Clonal selection represents one of the most important breeding methods for grapevines. However, despite many improvements in recent decades, this continues to be a time and energy consuming process. To confirm the intravarietal variability caused by mutations, it is necessary to eliminate the influence of environmental conditions and pathogens. To achieve this, virus-tested vines of all clone candidates are planted in an experimental vineyard with homogenous environmental conditions. To compare clone candidates and to confirm clonal differences, they are monitored for several years. During this process, a variety of aspects of yield and quality are assessed.

In different studies of intravarietal variability of grapevine varieties, the main sampling strategy is to harvest all clone candidates from one location on the same date, and not when each of them reaches full maturity. In this way, the full quality potential of later ripening clone candidates is often not recognised.

The aim of this study was to determine differences between three divergent clones of Croatian 'Plavac mali' in the portion of berry fractions sorted by flotation, *i.e.* presumed to be of a similar level of ripeness, and to compare the basic chemical composition, organic acid and phenolic composition of berries of different density classes of the same clone and among the same density classes of different clones. This study was carried out on the most important Croatian autochthonous grapevine variety 'Plavac mali', which is used in the production of famous red wines from the southern Dalmatia wine region and which shows a high level of asynchronous berries development within clusters (Fig. 1) (Zdunić *et al.* 2012).

Material and Methods

G r a p e s a m p l e s : The study was conducted in 2012 with three clones of 'Plavac mali' (PMC002, PMC099 and PMC108) grafted on 'Kober 5BB' rootstock. All clones

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Fig. 1: 'Plavac mali' cluster with high level of asynchronous berries development.

were grown in the experimental vineyard Baštica (located near Zadar in Dalmatia) planted in 2008. Plants were grown on a vertical trellis with 2.2 m between rows and 1.1 m between plants and pruned according to the Cordon training system, leaving cca 10 buds per vine (5 spurs with 2 buds each). After verasion, grape maturation was monitored and based on this, the harvest date was determined and performed on the same day (October 14, 2012) for all three clones. On the 15 vines of each clone, the number of clusters and their weight was determined separately, and a sample of minimum 15 kg of grapes (whole clusters) was taken from each clone, taking basal clusters from basal shoot on each spur. Berries were removed from the clusters leaving attached pedicels in the proximity of the receptacle using small scissors. All berries from three clones were then sorted according by density, which was estimated by flotation in a variety of sucrose solutions (from 160 to 215 $g \cdot L^{-1}$) (Flora and Lane 1979). Densities of the four applied solutions ranged between 1071 and 1092 $kg \cdot m^{-3}$, and for each clone, 5 L of fresh solution was used. A total of 500 g of berries were floated at the same time and were initially introduced in the solution with the highest density. Floating berries were removed from the denser solution and transferred to the next less dense solution. This process was repeated for all solutions. The sunken berries in each solution were considered to be a separate fraction which had a density higher than the solution used, and lower than the solution in which they floated. The same solution densities were used for all three clones. For each fraction of berries, a different letter was assigned according to density: A < 1071 $kg \cdot m^{-3}$, B = 1071-1081 $kg \cdot m^{-3}$, C = 1081-1086 $kg \cdot m^{-3}$, D = 1086-1092 $kg \cdot m^{-3}$, and E >1092 $kg \cdot m^{-3}$. For each clone, berries belonging to a different fraction were weighed and their distribution percentages calculated. To determine the average weight of a single berry from each fraction, 30 berries were weighed in triplicate for every clone. To determine the average sugar content, titrable acidity, and pH value of must for the three clones used in study, three 200 g berry samples per clone were prepared from all fractions, according to their distribution percentages. Must sugar content, titrable acidity and pH value were determined in the juice obtained from a manually crushed and centrifuged sample of 100 berries from each fraction and clone in triplicate. These analyses were performed according to the methods of the International Organization of Vine and Wine (OIV 2009).

Samples of 100 berries from fractions A, B, C and a combined sample of D+E of each clone were taken and analysed for organic acid and phenol content. In two clones, a low yield of berries of the fractions D and E was obtained and therefore the berries of these two fractions were pooled. Samples for organic acids analyses were immediately crushed manually and centrifuged at 5000 rpm for 10 min and used for analyses.

Organic acid analysis: Concentration of tartaric, malic and citric acids ($g \cdot L^{-1}$) were determined by HPLC in samples of fresh juice obtained from 100 g of berries as described above. Analyses were performed isocratically at 0.6 $mL \cdot min^{-1}$ flow and 65 °C column temperature with a 300 × 7.8 mm i.d. Aminex HPX-87H cation exchange column and a Cation H+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA), using 0.065 % H3PO4 as the mobile phase and a Agilent Diode Array Detector (Series 1100; Agilent, Palo Alto, CA) set to 210 nm. Data analysis was carried out using the Chem Station chromatography data system (Agilent, Palo Alto, CA).

Phenolic composition of berry skins: Extraction: Berry skins were manually removed from the pulp and air dried. Dry skins were ground and the obtained powder (500 mg) was extracted with 10 mL 70 % aqueous ethanol containing 1 % formic acid in the dark for one day (Tomaz and Maslov 2016). The extract was centrifuged in a LC-321 centrifuge (Tehtnica, Železnik, Slovenia) for 20 min at 5000 rpm at room temperature. The supernatant was collected, concentrated under vacuum to remove ethanol (40 °C) on a Hei-VapAdventage G3 rotary evaporator (Heidolph, Schwabach, Germany) and brought to a final volume of 10 mL with mobile phase A (water:phosphoric acid, 99.5:0.5, v/v). The extract was filtered with a Phenex-PTFE 0.20 mm syringe filter (Phenomenex, Torrance, USA) and analyzed by HPLC.

HPLC method: The separation, identification and quantification of flavonoids from grape skin extracts were performed according the method described by Tomaz and Maslov (2016) on an Agilent 1100 Series system (Agilent, Germany). The separation was performed with a reversed-phase column Luna Phenyl-Hexyl (4.6 × 250 mm; 5 m particle (Phenomenex, Torrance, USA)). The solvents were water:phosphoric acid (99.5:0.5, v/v, eluent A) and acetonitrile:water:phosphoric acid; 50:49.5:0.5, v/v/v, eluent B). Using DAD, flavonol glycosides were detected at 360 nm, anthocyanins at 518 nm, hydroxycinnamic acids at 320 nm, stilbens at 308 nm and hydroxybenzoic acids at 280 nm. Using FLD, flavan-3-ols were detected at ex =

225 nm and em = 320 nm. Quantification of individual flavonoid peaks was completed by using a calibration curve of the corresponding standard compound. The results are expressed in mg·kg^{-1} of dry weight (d.w.) of grape skin.

Statistical analysis: All variables were examined separately by analysis of variance (ANOVA). Means separation by Duncan's multiple range tests was used to establish whether there were significant differences among the clones, fractions within clones, and among same fractions of different clones ($p \leq 0.05$). The average of the clone was calculated for each variable determined in fractions by taking the portion of these fractions into account. Correlations were determined between the sugar content of different fractions and other analysed parameters. The results were analyzed using SAS statistical software, version 9.3 (SAS Institute, Cary, NC).

Results and Discussion

The average yield and basic quality parameters of the three 'Plavac mali' clones are shown in Tab. 1. Significant differences were determined in all parameters among the three clones. Clone PMC-099 had the highest yield, number of clusters and average cluster weight. PMC-002 had the highest must sugar content but yield, cluster weight and titrable acidity were lower than in other two clones. The differences in yield and yield quality determined among the three 'Plavac mali' clones in this study are relatively high but comparable to other studies of intravarietal variability of other cultivars, such as 'Merlot' (Bettiga 2003), 'Albarino' (Alonso *et al.* 2004), 'Pinot noir' (Mercado-Martín *et al.* 2006, Anderson *et al.* 2008b), 'Chardonnay' (Anderson *et al.* 2008a). The differences among the selected clones of 'Plavac mali' are the result of high intravarietal variability determined within this cultivar (Preiner 2012, Zdunić *et al.* 2012), and are comparable to other Croatian native grapevine cultivars (Preiner *et al.* 2012).

Table 1

Yield, cluster number and weight of three 'Plavac mali' clones used in this research

	Clone		
	PMC002	PMC099	PMC108
Yield (kg·vine^{-1})	3.24 b	6.42 a	4.05 b
Number of clusters/vine	17 b	23 a	16 b
Average cluster weight (g)	200 b	266 a	247 ab

[a]Means separation by Duncan's multiple range test at $p \leq 0.05$. Means with the same letter are not significantly different within the same year.

The distribution of grape berries of the three 'Plavac mali' clones in different fractions are presented in Fig. 2. Clear differences in distribution are present among the clones. Clones PMC002 and PMC099 showed a somewhat similar distribution, with the highest percentage of berries in fraction C, but with differences in other fractions. While PMC002 had a higher percentage of berries in fractions D and E, the opposite was found in PMC099, with a higher percentage of berries in fractions A and B. The distribution of berries of clone PMC108 showed the highest percentage in fraction A, which decreased toward the denser classes.

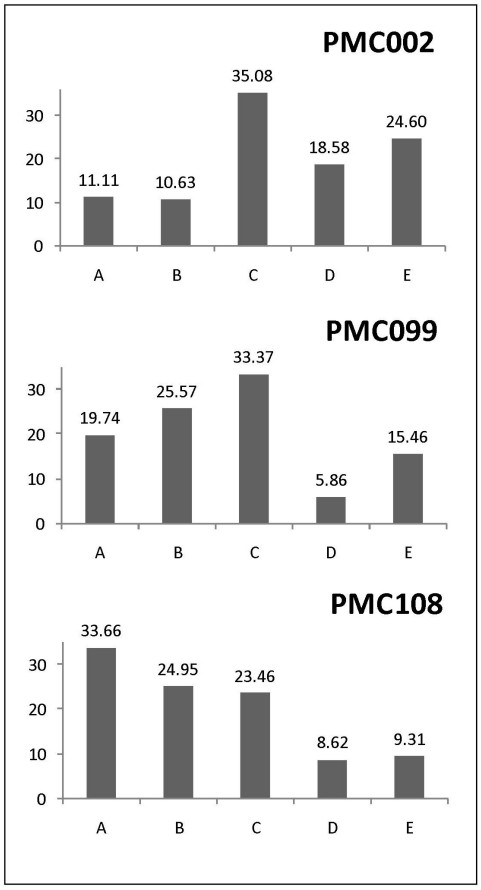

Fig. 2: Distribution of 'Plavac mali' grape berries (%) in different density classes at harvest in three clones studied. Density classes: **A** < 1071 kg·m^{-3}, **B** = 1071-1081 kg·m^{-3}, **C** =1081-1086 kg·m^{-3}, **D** = 1086-1092 kg·m^{-3}.

The clones used in this study did not show a clear Gaussian bell-shaped distribution of fractions as reported in similar studies of different cultivars using the flotation method (Singleton *et al.* 1966, Flora and Lane 1979, Rolle *et al.* 2012). This can be partially explained by the differences in sampling method berries used for flotation. While all of the berries from clusters were used in this study, other studies randomly selected berries (Singleton *et al.* 1966), or used a random sample of three berries per cluster (Rolle *et al.* 2012). There are no similar studies on clonal differences in berry ripeness distribution and homogeneity within clusters.

Three clones studied had significant differences in average berry weight of different fractions, though differences among clones were also present within the same fraction. Clone PMC099 had the lowest, but also the most uniform, berry weight in all fractions comparing to other clones. Only berries from fraction B of PMC099 clone were significantly larger than berries from other fractions. The highest average berry size was determined for all fractions of the clone PMC108 in comparison to the other clones, except for fractions A and D of the PMC002

clone. For all clone candidates, fraction A berries had the smallest berries, though differences were detected in berry weight of other density fractions among clones. This can be seen in the correlation of the average berry weight and must sugar content. While the correlation was high for clones PMC002 ($r = 0.82$; $p < 0.01$) and PMC108 ($r = 0.79$; $p < 0.01$), PMC099 did not show a correlation of these two parameters ($r = -0.05$). Opposite to these results, Rolle *et al.* (2012) determined a negative correlation between average berry weight and their density in the case of 'Nebbiolo', which can be explained by varietal differences and by the different procedure of sampling berries used for flotation.

For each clone, a significant difference was observed in the must sugar content between fractions. The same fractions of the selected clones also differed, though a significant difference was determined only for fraction A, where the must sugar content was significantly lower in clones PMC099 and PMC108 than in clone PMC002 (Tab. 2). A significant difference was determined in the titrable acidity both among clones, where PMC108 had the significantly highest level in all density classes except in fraction D, and among fractions within the clones. In general, titrable acidity was lower and pH value higher in fractions with higher density within all three clones, which corresponds to the changes in berry composition that occur during berry maturation (Dai *et al.* 2011), where the acidity of grapes depends not only on the organic acid concentration, but also on the ratio of the concentration between free organic acids and their potassium salt forms that increases throughout ripening (Kliewer 1965, Ribéreau-Gayon 2006). Titrable acidity was in a high negative correlation with sugar concentration for PMC099 ($r = -0.84$; $p < 0.01$) and PMC108 ($r = -0.81$; $p < 0.01$) while for PMC002 it was also negative but lower ($r = -0.41$; $p > 0.05$).

Differences among the clones for the same fractions can be partly explained by the differences found in the titrable acidity, and in the organic acid composition. This is in accordance with the results of Rolle *et al.* (2012) in the case of dissimilar floating behaviour of 'Nebbiolo' grape berries from different production zones.

Anthocyanin content: The five most common anthocyanins in -3-*O*-glucoside form were analysed (Tab. 3). Significant differences were detected in the level of anthocyanins among the three 'Plavac mali' clones used in this study. Malvidin (malvidin-3-*O*-glucoside) was the most abundant anthocyanin in all fractions of all clones, while other anthocyanins were present in much lower concentrations or were not detected in some fractions, while cyanidin-3-glycoside was not detected in any sample. A similar anthocyanin profile was determined for 'Plavac mali' for skin extracts (Budić-Leto *et al.* 2009) and wine (Maletić *et al.* 2009). Significant differences in the malvidin content of berry skin were detected among all fractions

Table 2

Berry weight and basic juice composition, and organic acids content of three 'Plavac mali' clones and berries from different density classes separated by floatation

Clone/ Density class[a]	Average berry weigth (g)	Sugar content ($g \cdot L^{-1}$)	Titrable acidity ($g \cdot L^{-1}$)	pH value	Tartaric acid ($g \cdot L^{-1}$)	Malic acid ($g \cdot L^{-1}$)	Citric acid ($mg \cdot L^{-1}$)
PMC002							
A	1.54 d A[b]	141.3 e A	4.43 a B	3.66 d A	4.60 a C	0.48 a A	116 a C
B	1.85 c B	156.9 d A	2.71 d C	3.74 bc A	4.22 b A	0.41 b A	87 b B
C	2.25 a B	183.3 c A	3.23 c B	3.73 c B	4.27 b C	0.34 c A	80 b C
D	2.34 a A	200.9 b A	3.72 b A	3.79 b B	4.59 a B	0.18 d B	73 b B
E	2.06 b B	211.4 a A	2.91 d C	3.85 a A			
Average[c]	2.10	186.01	3.32	3.76	4.44	0.29	81.72
PMC099							
A	1.07 b B	135.3 e B	4.92 a B	3.60 c A	4.97 b B	0.26 b C	150 a A
B	1.45 a C	164.1 d A	4.03 b B	3.77 b A	4.28 c A	0.26 b C	117 b A
C	1.21 b C	178.8 c A	3.35 c AB	3.82 b A	4.82 b B	0.27 b B	97 b B
D	1.24 b B	191.9 b A	3.46 c A	3.91 a A	5.83 a A	0.31 a A	113 b A
E	1.07 b C	206.1 a A	3.44 c B	3.87 a A			
Average	1.21	169.02	3.81	3.73	4.86	0.27	114.45
PMC108							
A	1.62 c A	130.3 e B	5.47 a A	3.51 b B	5.49 b A	0.37 a B	130 a B
B	2.07 b A	163.1 d A	5.26 a A	3.60 a B	4.32 c A	0.32 b B	73 b B
C	2.56 a A	173.5 c A	3.49 bc A	3.60 a C	5.51 b A	0.19 c C	113 a A
D	2.23 b A	197.2 b A	3.40 c A	3.63 a C	6.08 a A	0.34 ab A	110 a A
E	2.45 a A	209.6 a A	3.75 b A	3.63 a B			
Average	2.08	161.77	4.61	3.58	5.31	0.31	108.20

[a] Density classes: A < 1071 $kg \cdot m^{-3}$, B = 1071-1081 $kg \cdot m^{-3}$, C =1081-1086 $kg \cdot m^{-3}$, D = 1086-1092 $kg \cdot m^{-3}$.

[b] Means separation by Duncan's multiple range test at $p \leq 0.05$. Means with the same letter are not significantly different. Lowercase letters indicate significant differences for density classes within clones; uppercase letters indicate significant differences for same density class among different clones.

[c] Average of the clone was calculated by taking into account the portion of different density classes.

Table 3

Concentration of anthocyanins[a] in berry skin of three different clones of 'Plavac mali' and from berries from different density classes separated by floatation

mg·kg^{-1} of d.w. berries skin				
Clone/ Density class[b]	Delphinidin	Petunidin	Peonidin	Malvidin
PMC002				
A	0	0	0	182.08 d B
B	0	0	0	261.05 c B
C	0	0	4.61 b B	294.28 b B
D	0	26.42 B	12.45 a B	509.37 a B
Average[d]		11.40	6.99	371.16
PMC099				
A	0	0	0	195.49 d A
B	0	23.55 c	14.70 b	926.00 c A
C	17.53 b[c]	93.17 b	32.11 a A	1765.47 a A
D	29.61 a	106.36 a A	27.57 a A	1557.46 b A
Average	11.87	58.73	20.08	1180.45
PMC108				
A	0	0	0	37.27 c C
B	0	0	0	69.76 c C
C	0	0	0	163.83 b C
D	0	4.34 C	10.87 B	368.53 a C
Average		0.78	1.95	134.46

[a] In form of -3-*O*-glucoside, cyanidin was not detected in any of the samples analyzed.
[b] Density classes: A < 1071 kg·m^{-3}, B = 1071-1081 kg·m^{-3}, C = 1081-1086 kg·m^{-3}, D = 1086-1092 kg·m^{-3}.
[c] Means separation by Duncan's multiple range test at $p \leq 0.05$. Means with the same letter are not significantly different. Lowercase letters indicate significant differences for density classes within clones; uppercase letters indicate significant differences for same density class among different clones.
[d] Average of the clone was calculated by taking into account the portion of different density classes.

of PMC099 and PMC002 clones, while the difference was not significant only between fractions A and B of PMC108. Based on this and the strong positive correlation between sugar and malvidin content (r ranging from 0.87 to 0.93), it is evident that the maldivin content increased significantly during maturation of 'Plavac mali'. Somewhat different results were obtained in a study of 'Nebbiolo' (Rolle *et al.* 2012), where no significant differences were determined in the malvidin derivate contents in berry skins among berries from different fractions. This could be due to varietal differences and also the different berry sampling method applied in this study.

Differences were also significant among fractions of all three clones, where PMC099 had highest and PMC108 lowest concentration of this dominant anthocyanin. The differences in malvidin content found among the clones were greater than in similar studies (Revilla *et al.* 2009, Burin *et al.* 2011).

Phenolic acid content: Differences among clones and fractions of clones in berry skin phenolic acid composition are shown in Tab. 4. Gallic acid was the most abundant in all three clones, but with a significantly higher content in all fractions of the PMC002 clone. Berries from higher density fractions had a higher content of gallic acid, with a significant correlation ($p < 0.05$) detected for all three clones. This is evident in the significantly highest content of gallic acid in fraction D in all three clones. Other phenolic acid contents depended also on fractions within clones, and differences among clones in same fraction, though in the case of some clones or fractions they were not detected. In this way, coumaric acid was detected in all fractions of the PMC099 clone and only in fraction A of PMC002, which did not differ significantly from the same fraction of PMC099. In the case of PMC108, none of the fractions had a coumaric acid content above the level of quantification.

Syringic acid was determined only in fraction A of PMC099, and was significantly higher than in fraction A of other two clones, though it was below the quantification level in the remaining fractions of this clone. The two other clones had similar concentrations of syringic acid in all fractions, and only fraction B differed significantly between them. Caftaric acid content was highest in all fractions of clone PMC002 compared to the same fractions of other two clones, except in case of fraction D. Differences among the fractions within clones were smaller.

Similar differences were also detected for caffeic acid, with a significant correlation ($p < 0.01$) with density for

Table 4

Phenolic acids content in berry skin of three different clones of 'Plavac mali' and from berries from different density classes separated by flotation

	mg·kg^{-1} of d.w. berries skin					
Clone/ Density class[a]	Coumaric acid	Sinapic acid	Caffeic acid	Syringic acid	Caftaric acid	Galic acid
PMC002						
A	11.72 a A[b]	0	3.99 d A	7.63 a B	3.68 b A	32.05 d A
B	0	9.35 b AB	6.60 b A	7.37 a A	4.51 b A	106.41 c A
C	0	11.24 a A	15.34 a A	7.15 a A	6.74 a A	142.29 b A
D	0	9.87 b A	5.18 c B	6.02 b A	3.33 b A	174.48 a A
Average[c]	1.30	9.20	8.76	6.74	4.69	138,7
PMC099						
A	13.29 a A	10.57 a	2.10 b B	14.49 a A	1.41 b B	0
B	14.96 a	11.08 a A	6.86 a A	0	2.48 a B	0
C	12.57 a	10.56 a A	8.09 a B	0	2.76 a B	0
D	12.52 a	9.43 a A	8.52 a A	0	2.39 ab A	23.47 B
Average	13.15	10.33	6.59	2.82	2.32	4,63
PMC108						
A	0	0	1.52 b B	6.56 a B	2.25 a B	0
B	0	7.44 b B	4.15 a A	6.15 a B	3.21 a B	0
C	0	9.91 a A	3.51 a C	6.13 a A	3.22 a B	0
D	0	0	4.37 a B	6.27 a A	2.19 a A	13.94 B
Average		4.18	3,15	6.30	2.71	1,29

[a] Density classes: A < 1071 kg·m^{-3}, B = 1071-1081 kg·m^{-3}, C =1081-1086 kg·m^{-3}, D = 1086-1092 kg·m^{-3}.

[b] Means separation by Duncan's multiple range test at $p \leq 0.05$. Means with the same letter are not significantly different. Lowercase letters indicate significant differences for density classes within clones; uppercase letters indicate significant differences for same density class among different clones.

[c] Average of the clone was calculated by taking into account the portion of different density classes.

PMC099 and PMC108. Due to the low concentration of fraction D of PMC002, this correlation was not significant for this clone. Differences among clones were also significant in comparing the same fractions, and in most cases PMC108 showed the lowest content of caffeic acid. Sinapic acid content showed small but in some cases significant differences among the fractions and among the same fraction of different clones. PMC099 had the highest content of synapic acid in all fractions, and was the only clone with a content of this compound above the quantification level in fraction A. There are few studies on the clonal differences in phenolic acid composition, mainly in wines produced from different clones (Burin *et al.* 2011); however, they suggested that clones can be differentiated based on phenolic acid composition during ripening. Difference among clones in phenolic acid content is important because of the copigmentation phenomenon, where anthocyanins display far greater colour than would be expected from their concentration (Boulton 2001). The very high concentration of gallic acid detected in PMC002 clone, which was more than eight times greater than in fraction D of the other two clones, was higher than reported elsewhere (Obreque-Slier *et al.* 2010, Fanzone *et al.* 2011).

Flavonol and flavan-3-ol content: Content of kampferol, quercentin and myricetin (in the form of -3-*O*-glycosides) in berry skins from different fractions of three clones of 'Plavac mali' are shown in Tab. 5. Significant differences were determined in all three flavonols among fractions of all three clones, and also between the same fraction of different clones. The content was typically higher in higher density fractions, which is in accordance with the results of a similar study comparing berries at different stages of maturity (Fanzone *et al.* 2011), though some studies have presented opposite results (Obreque-Slier *et al.* 2010).

The flavan-3-ols contents of berries from three clones and four density fractions is presented in Tab. 5. Significant differences in the flavan-3-ol content of berry skin were determined among density fractions of all three clones used in this study, and the PMC099 clone had a significantly higher content of monomeric flavan-3-ols in all four fractions, compared to the other two clones, except for epicatechin in fraction A. Procyanidins B1, B2 and B4 were also highest in all fractions of PMC099 clone, with significant differences among the fractions within all three clones. A lower content of flavan-3-ols in higher density fractions corresponds to the literature data, where a decrease of their content was determined with the progression of berry maturation (Obreque-Slier *et al.* 2010, Fanzone *et al.* 2011, Teixeira *et al.* 2013).

Conclusions

This study showed that different density classes of berries, separated using flotation, represent berries at different

Table 5

Flavonol-3-*O*-glycosides and flavan-3-ols content in berry skin of three different clones of 'Plavac mali' and from berries from different density classes separated by floatation

	mg·kg^{-1} of d.w. berries skin									
Clone/ Density class[a]	Kaempferol-3-*O*-glucoside	Quercetin-3-*O*-glucoside	Myricetin 3-*O*-glucoside	Epigallo-catechin	Epi-catechin	Gallo-catechin	Catechin	Procyanidin B1	Procyanidin B2	Procyanidin B4
PMC002										
A	5.55 c A[b]	21.20 d B	0	37.75 a B	20.41 C	21.75 a B	25.33 a C	21.26 b C	20.79 B	21.03 B
B	8.86 b B	31.55 c B	0	19.05 b B	0	12.19 b B	11.69 b B	19.37 bc C	0	0
C	10.03 a B	43.71 b B	0	18.29 b B	0	10.33 c B	10.34 b B	25.91 a B	0	0
D	10.33 a AB	59.32 a B	31.70 B	14.59 b B	0	6.88 d B	5.73 c B	15.47 c C	0	0
Average[c]	9.54	46.66	13.69	18.94	2.27	10.31	10.16	20.19	2.31	2.34
PMC099										
A	6.92 b A	62.29 b A	0	89.05 a A	36.91 a B	48.02 a A	56.37 a A	113.92 a A	39.76 a A	40.86 a A
B	10.73 a A	95.54 a A	37.10 b A	43.03 c A	17.56 c	32.26 b A	22.08 b A	68.13 c A	43.53 a	29.20 ab
C	12.91 a A	70.96 b A	48.84 a A	57.08 b A	27.98 b	34.44 b A	22.18 b A	86.25 b A	41.10 a	35.89 ab
D	11.99 a A	108.66 a A	44.77 a A	41.85 c A	14.25 c	22.38 c A	14.80 c A	58.97 d A	0	26.84 b
Average	10.84	82.32	34.88	55.89	23.91	33.64	27.03	80.37	32.59	32.85
PMC108										
A	0	13.76 c B	0	42.98 a B	55.04 A	18.05 a B	34.23 a B	56.96 a B	17.65 B	19.53 B
B	0	24.70 bc B	0	20.44 b B	0	10.31 b B	9.48 b B	37.38 b B	0	0
C	0	35.94 ab C	0	15.52 c B	0	9.10 b B	5.52 b C	30.65 c B	0	0
D	9.43 B	45.62 a B	31.75 B	15.56 c B	0	8.52 b B	5.64 b B	31.61 c B	0	0
Average	1.69	27.41	5.69	26.00	18.53	12.31	16.19	41.36	5.94	6.57

[a] Density classes: A < 1071 kg·m^{-3}, B = 1071-1081 kg·m^{-3}, C =1081-1086 kg·m^{-3}, D = 1086-1092 kg·m^{-3}.

[b] Means separation by Duncan's multiple range test at $p \leq 0.05$. Means with the same letter are not significantly different. Lowercase letters indicate significant differences for density classes within clones; uppercase letters indicate significant differences for same density class among different clones.

[c] Average of the clone was calculated by taking into account the portion of different density classes.

levels of maturity that can be found within the total berry sample of all three clones used. Considering that maturity level has a significant effect on all the qualitative indicators, sampling all the clone candidates at the same time does not reveal their full potential. Consequently, there is a need to implement berry flotation during the preparation of samples for different analyses to evaluate clone candidates. For this purpose, one uniform fraction of berries with similar density for all the clone candidates could be used to compare them, though more accurate results can be obtained by analysing all the fractions. Including this method in the clonal selection process does not reduce the need for several years of evaluation of clone candidates in experimental plantations, as different environmental factors, in addition to maturity level, can have a significant influence on the same quality parameters. Including berry floatation in berries samples preparation can give more precise and accurate results in the comparison of clone candidates but also in other researches related to grapevine.

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