Varietal differences among berry composition of three Croatian red grape cultivars during ripening

Ana Mucalo[1], Goran Zdunić[1], Ivan Pezo[1], Irena Budić Leto[1], Iva Tomić Potrebuješ[1], Edi Maletić[2]

[1]*Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21 000 Split, Croatia;*
[2]*Faculty of Agriculture University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia*

Abstract

The quality of red wines depends on the precise harvest date that is linked with the ripeness of a pulp, and skin and seeds. The concentration of phenolic compounds from grape skin and seeds and their extractability during maceration is directly linked to the color and the flavor of the wine. The content of phenolics varies greatly depending on the grape variety, ripening stage and the other environmental effects. This study has been done to evaluate the dynamics of physiochemical variables of berries during the last stage of ripening from the major red Croatian variety 'Plavac Mali' and two less known and almost neglected varieties, 'Dobričić', and 'Ljutun'. The chemical composition of berries and the surface color of the skin (CIELab parameters) have been analyzed on four different sampling dates. A significant varietal variability has been observed for the most common maturity indices (total soluble solids, total acidity, pH, single berry weight), whereas some parameters such as total colorimetric value (CIRG) derived from the color evaluation showed linear progression over the sampling dates. Total anthocyanins and total phenols concentrations increased during ripening, while significant differences among varieties were observed. 'Ljutun' showed significantly the highest content of total acidity and the smallest content of total soluble solids in comparison to the other two varieties suggesting its good potential to improve 'Plavac Mali' and 'Dobričić' wines by blending. The results of berry composition during the four different ripening stages for 'Plavac Mali', 'Dobričić' and 'Ljutun' indicated that choosing the optimal harvest date is important for wine quality although large heterogeneity of grapes has been noticed within sampling dates.

Key words: Maturity index, Phenolic maturity, CIELab, Ripening stages

Introduction

Berry ripening is a genetically programmed process during which accumulation, respiration and biochemical transformation occur in a berry parallelly. The transition from berry growth to berry ripening is known as veraison. Beginning of this transition is characterized by softening (Coombe & Bishop, 1980), sugar accumulation, berry coloring and post-veraison berry growth (Coombe, 2000). The composition of grape berries during the harvest is the key factor that influences the quality of wine. During maturity, the chemical composition of the berries becomes more balanced (Kennedy, 2008). Phenolic compounds are very important for the red wine quality. They contribute to the color, taste-bitter, feeling-astringency of wine, microbiological stability of the wine, and present the basis for wine maturation (Ribereau-Gayon *et al*., 2006). Wines from unripe grapes are bitter, astrigent, without harmony, because of the lower final alcohol content, pH, concentration of anthocyanins, total phenolic index and increased total acidity. Grapes that reached phenolic ripeness have a higher concentration of anthocyanins, are less astrigent and bitter because of the declining share of proanthocyanidins seeds that are galloylated in total tannins (Kennedy *et al.,* 2006, Meléndez *et al.,* 2013).

Today, due to the climate change and other viticultural practices, the grapes that reach phenolic maturity often come to be highly alcoholic wines with high pH, low total acidity and are often judged as unbalanced, 'flabby' wines (Jones *et al.,* 2005). Grape ripening is not a homogenous process. Heterogeneity of grapes at the harvest time affects the composition and quality of the future wine (Kontoudakis *et al.,* 2011). Therefore it is highly important to monitor both the technological (ripeness of pulp), and phenolic (anthocyanin, flavan 3-ols) ripeness.

During the past few years in Croatia we have focused on the improvement of the red wine production. There are a few native varieties that have a potential to improve the red wine production in the coastal region of Croatia. ‘Plavac mali’ is the major red grape variety in Croatia especially highly regarded on the steep slopes next to the Adriatic Sea. The high degree of the heterogeneity of ‘Plavac mali’ grapes can be observed at the harvest time (Zdunić *et al.*, 2007). Besides the major grape variety, there are ‘Ljutun’ and ‘Dobričić’, which are old neglected varieties traditionally grown in the Central Dalmatia region (Bulić, 1949). Although these three varieties have a great potential to be used for planting the new vineyards, there is very little information about the fruit composition of these varieties. The objective of the present study was to evaluate the physiochemical parameters and the basic phenolic composition of the grape berries of ‘Plavac mali’, ‘Dobričić’ and ‘Ljutun’ in order to evaluate heterogeneity of their grapes during the ripening period and to find the precise harvest date.

Material and Methods

Plant material

In 2012, three native red grape varieties ‘Plavac mali’, ‘Dobričić’ and ‘Ljutun’ (*Vitis vinifera* L.) from the grape germplasm repository of the Institute for Adriatic Crops and Karst Reclamation in Split were

evaluated at four ripening stages starting after the veraison. The 8 year old vines are grafted onto the 1103-Paulsen rootstocks and trained on the bilateral spur cordon system with spacing of 1.0 m among vines in a row and 2.0 m between the rows, eight buds were pruned on each vine. 100 berries were collected from different parts of the cluster from each of the six vines/replicates for 'Plavac mali', 'Dobričić' and 'Ljutun' at four different sampling dates starting after veraison on August 20, following dates were September 05, September 23, and October 11.

Standard grape analysis

Total berry weight (g) was measured, and 50 berries were used immediately for measuring the physiochemical parameters: the total soluble solids (TSS) using the temperature-compensating refractometer (Atago, model WM_7), total acidity (TA) was measured using 1M NaOH with Bromothymol blue as the indicator, juice pH was measured with a pH meter (Methrom, model 1719 S).

Berry skin color analysis

The skin color variables were measured of the 50 berries, immediately after sampling. The berries were carefully cleaned with a cotton cloth to avoid irregularities in the measurement due to dust and/or remains of pesticides. The color of berries was measured on the cheek area with a colorimeter (CR-400, Konica Minolta, Japan) in the combination with a computer program SpectraMagic NX Lite, ver. 2.0. The colorimeter was calibrated with a standard calibration plate before usage. The differences between the 'Plavac mali', 'Dobričić'and 'Ljutun' phenotype were quantified using the CIELab technique (Carreno *et al.,* 1995). The CIELab uniform color space include the lightness coordinate (L*), the red/green coordinate, with +a* indicating red, and -a* indicating green, while b* defines the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue. C* is the chroma coordinate, the distance from the lightness axis while h* is the hue angle, expressed in degrees, with 0° being a location on the +a* axis, then continuing to 90° for the +b* axis, 180° for -a*, 270° for -b*, and back to 360° = 0°. The chroma value (C) and the hue (h) were calculated as $C = [(a^{*2} + b^{*2})]\ 0.5$ and the hue (h) value as h = arctang b*/a*. Based on these variables, the color index for red grapes (CIRG) was calculated according to Carreno *et al.* (1995).

Spectrophotometric analysis

Total anthocyanins and total phenolics of grape were measured based on the method described by Iland *et al.* (1996). Briefly, fresh samples were stored cool and analyzed within 24 hours of collection. Whole berries without pedicel were homogenized and 1 g of representative sample of the combination of the pulp, the skin and seed components was weighed. Total anthocyanins and total phenolics were extracted with 50% ethanol (pH 2), diluted with HCl and the absorbance value was recorded at 520 nm and 280 nm in 10 mm quartz cuvette using a spectrophotometer (UV-VIS spectrophotometer, Varian DM 200).

Statistical analysis

Statistical analysis was conducted with the programme Statistica 8.0 (StatSoft, Inc., USA). One-way analysis of Variance (ANOVA) tests was used for the analysis of the physiochemical variables and color parameters. Differences between varieties were estimated with Fisher LSD test ($p<0.05$).

Results and discussion

Data assembled by the general compositional analysis of grape juice and solids of the three different *Vitis vinifera* L. varieties, 'Plavac mali', 'Dobričić' and 'Ljutun' on four different sampling dates in 2012 vintage are shown in Table 1.

Contrary to the expectations that grape total soluble solids will increase continually during ripening, no significant difference was found between all three varieties in the first and the last ripening stage, although these stages were 53 days apart. This could be explained by the large heterogeneity on sampling dates demonstrating the variation of berry ripening stages in the vine, from underripe to overripe berries.

Significant differences between 'Ljutun' and the other two varieties according to the total soluble solids content were found in all the ripening stages. The highest total soluble solids (24.84 °Brix) and the lowest total acidity (3.47 g/L) were found in berries of 'Plavac mali' at the fourth ripening stage. The lowest total soluble solids (20.36 °Brix) and the highest total acidity (4.79 g/L) were found in berries of 'Ljutun' at the fourth ripening stage. Grape juice total acidity decreased on the sampling dates up to the harvest with 'active acidity', pH, values increasing, in agreement with previous reports (Coombe & McCarthy, 2000, Boido *et al.,* 2011, Bindon *et al.,* 2013). There were no significant differences observed in total acidity content in the second and the third ripening stage for 'Dobričić' and 'Ljutun' indicating that these could be earlier ripening varieties than 'Plavac mali'. Significant differences in pH value were observed between 'Ljutun' and 'Dobričić' in all the ripening stages. The phenolic composition of grape berries was determined in terms of total anthocyanins and total phenolics, both expressed on a berry mass basis (concentration) or per berry basis (content).

Significant differences between the three analyzed varieties according to the concentration of total anthocyanins (mg/g) were observed at the second and the fourth ripening stage, while there were no differences between 'Ljutun' and 'Plavac mali', early during the ripening and also no differences found among 'Dobričić' and 'Ljutun' at the third ripening stage. Total anthocyanin concentration of 'Ljutun' constantly increases from (1.49 mg/g) to (3.20 mg/g) and was the highest on the fourth sampling date, indicating that this is a late maturing variety. The highest content of total anthocyanins (mg/berry) was observed for 'Dobričić' in all the ripening stages. The highest concentration of total phenolics was observed for 'Ljutun' in all the ripening stages and varied from (5.39 mg/g) to (6.22 mg/g). There was no significant difference in the concentration of total phenolics of 'Plavac mali' and 'Dobričić' in the third and the fourth ripening stage.

Grape CIELab parameters and the calculated colorimetric index CIRG showed effective potential to differentiate the studied varieties and give the fast information about the grape maturity (Table 2).

As the maturity of the three cultivars progressed, the significant differences between the cultivars became evident at the various grape ripening stages. Based on this to the CIRG index, the grapes,

following the criterion of Carreño *et al*. (1996) can be classified into two groups starting from the first-dark red (5<CIRG<6) to the last ripening stage-blue-black (CIRG>6).

At the fourth ripening stage, CIRG of the 'Plavac mali' and 'Ljutun' showed significant difference from what CIRG measured at the earlier stages; on the contrary, CIRG of the 'Dobričić' at the fourth stage was not significantly different from CIRG at the third stage. The greatest value of CIRG index was found at 'Plavac mali' variety at fourth ripening stage (7.36), and the smallest (6.70) was found in 'Dobričić'.

CIRG index showed constant increase during the four ripening stages, respectively for 'Dobričić' from (5.82) to (6.70), for 'Ljutun from' (6.62) to (7.01) and for 'Plavac mali' from (6.34) to (7.36).

Conclusion

The investigated varieties showed significant differences in most of the observed physiochemical variables on the four sampling dates during the ripening. Significant heterogeneity of grapes was observed (from under ripe to overripe berries) within each of the observed sampling date suggesting the difficulty in monitoring of the grape ripening and justifying the classification of grapes according to their ripeness level at the time of grape crushing. However, to obtain more precise information on the fruit composition during the grape ripening it will be necessary to analyze more individual phenolic compounds (e.g. proanthocyanidins) in grapes that will provide more information for the decision on the optimal time for the grape harvest.

Reference

1. Bindon, K., Varela, C., Kennedy, J., Holt, H., Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. *Cabernet Sauvignon* Grape and wine chemistry, Food Chem. 138:1696-1705
2. Bulić, S., (1949). Dalmatinska ampelografija, Poljoprivredni nakladni zavod, Zagreb.
3. Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., (1995). Proposal of an index for the objective evaluation of the colour of the red table grapes, Food Res. Int. 28:373-377
4. Carreño, J., Martínez, A., Almela, L., Fernández-López, A.J., (1996). Measuring the color of table grapes, Color Research and Application, 21:50–54
5. Coombe, BG., McCarthy MG., (2000). Dynamics of berry growth and physiology of ripening, Australian Journal of Grape and Wine Research 6**:**131–135.
6. Coombe, BG., Bishop, GR., (1980). Development of the grape berry. II. Changes in diameter and deformability during veraison, Australian Journal of Agricultural Research 31:499-509**.**
7. Iland, P.G., Cynkar, W., Francis, I.L., Williams, P.J., Coombe, B.G., (1996). Optimisation of methods for the determination of total and red-free glycosyl glucose in black grape berries of *Vitis vinifera***,** Australian Journal of Grape and Wine Research, 2 (3):171–178
8. Joanes, V.G., (2005). Climate change and the global wine industry, Proceedings, 13th Australian wine industry technical conference 1-8
9. Kennedy, J.A., Saucier, C., Glories, Y. (2006). Grape and wine phenolics: History and perspective. American Journal of Enology and Viticulture, 57, (3), 239-248

10. Kennedy, J., (2008). Grape and wine phenolics: Observations and recent findings, Cien. Inv. Agr. 35 (2): 107-120.
11. Kontoudakis, N., Esteruelas, M., Fort, F., Canals, M.J., De Freitas, V., Zamora, F. (2011). Influence of the heterogeneity of grape phenolic maturity on wine composition and quality, Food Chemistry 124:767–774
12. Meléndez, E.,Ortiz C.M., Sarabia, L.A., Í ˜niguez, M., Puras, P., (2013). Modelling phenolic and technological maturities of grapes by means of the multivariate relation between organoleptic and physicochemical properties, Analytica Chimica Acta 761:53-61
13. Ribereau-Gayon P., Glories Y., Maujean A., Dubourdieu D., Handbook of Enology: The Chemistry of Wine Stabilization and Treatments 2:2
14. Rolle, L., Guidoni, S., (2007). Color and anthocyanin evaluation of red winegrapes by CIEL*, a*, b* parameters. J. Int. Sci. Vigne vin. 41, 193-201.
15. Zdunić, G., Maletić, E., Vokura, A., Kontić, K.J., Pezo, I., Pejić, I., (2007). Phenotypical, Sanitary and Ampelometric Variability within the Population of cv. Plavac Mali (Vitis vinifera L.), Agric. conspec. sci. 72 (2):117-128

197 Table 1 General compositional analysis of grape juice and solids of the three different Vitis vinifera L.
198 varieties, Plavac mali, Dobričić and Ljutun from different sampling points in 2012

Juice composition	Variety	Ripening stage											
		20-Aug-12			05-Sep-12			23-Sep-12			11-Oct-12		
		Mean±sd			Mean±sd			Mean±sd			Mean±sd		
Total soluble solids (°Brix)	**Dobričić**	22.21±1.11	a	A	24.17±1.28	a	B	23.20±1.60	a	AB	23.50±1.80	a	AB
	Ljutun	18.48±1.01	b	A	20.88±1.11	b	C	19.30±1.03	b	AB	20.36±1.15	b	BC
	Plavac mali	23.42±2.12	a	AB	22.46±1.63	c	A	23.24±0.79	a	AB	24.84±1.56	a	B
Total acids (tartiaric) (g/L)	**Dobričić**	5.62±0.29	a	C	5.07±0.41	a	A	4.72±0.33	a	A	4.25±0.38	a	B
	Ljutun	8.78±1.46	b	C	6.04±0.66	b	A	6.34±0.90	b	A	4.79±0.65	a	B
	Plavac mali	5.25±0.22	a	C	4.02±0.29	c	B	3.68±0.40	c	AB	3.47±0.36	b	A
pH	**Dobričić**	3.91±0.05	a	C	4.07±0.07	a	AB	4.00±0.05	a	A	4.14±0.07	a	B
	Ljutun	3.39±0.16	b	B	3.68±0.23	b	A	3.78±0.24	b	A	3.89±0.30	b	A
	Plavac mali	3.84±0.04	a	C	4.02±0.06	a	B	3.93±0.09	ab	A	3.98±0.06	ab	AB
Single berry weight (g)	**Dobričić**	2.04±0.33	a	A	1.01±0.25	a	B	2.06±0.30	a	A	1.53±0.39	a	C
	Ljutun	1.42±0.17	b	B	1.00±0.19	a	A	1.18±0.28	b	AB	1.19±0.26	b	AB
	Plavac mali	1.85±0.26	a	B	2.14±0.13	b	C	1.53±0.21	c	A	1.58±0.12	a	A
Grape solid composition													
Total anthocyanins (mg/g)	**Dobričić**	2.75±0.47	a	A	3.22±0.55	a	A	2.96±0.38	a	A	2.70±0.46	a	A
	Ljutun	1.49±0.18	b	B	2.59±0.41	b	C	3.03±0.63	a	A	3.20±0.39	b	A
	Plavac mali	1.54±0.17	b	A	1.72±0.25	c	AB	2.19±0.17	b	C	1.81±0.21	c	B
Total anthocyanins (mg/berry)	**Dobričić**	5.66±1.57	a	B	3.21±0.72	ab	A	6.04±0.67	a	B	4.06±0.98	a	A
	Ljutun	2.11±0.40	b	A	2.57±0.59	a	A	3.53±1.03	b	B	3.81±1.02	ab	B
	Plavac mali	2.85±0.49	b	A	3.67±0.53	b	B	3.35±0.58	b	AB	2.84±0.21	b	A
Total phenolics (mg/g)	**Dobričić**	5.24±0.54	a	A	5.18±0.29	a	A	5.22±0.43	a	A	4.51±0.63	a	B
	Ljutun	5.39±0.62	b	B	6.22±0.57	b	A	5.95±0.73	b	A	6.13±0.43	b	A
	Plavac mali	4.25±0.56	b	A	4.28±0.48	c	A	4.55±0.43	a	A	4.06±0.47	a	A
Total phenolics (mg/berry)	**Dobričić**	10.81±2.73	a	B	5.24±1.38	a	A	10.73±1.90	a	B	6.89±2.00	a	A
	Ljutun	7.63±1.15	b	A	6.20±1.39	a	A	7.04±2.02	b	A	7.29±1.80	a	A
	Plavac mali	7.89±1.59	b	BC	9.10±0.76	b	C	6.96±1.18	b	AB	6.38±0.50	a	A

199

200 Values as mean± standard deviation, significant differences ($p \leq 0.05$) are indicated by different Latin letters determined by
201 One way analysis of Variance ANOVA. Different lower-case Latin letters indicate significant differences among the three
202 varieties at the same ripening stage. Different capital Latin letters indicate significant differences among the grapes of the
203 four different ripening stages for the same variety.

204

Table 2 Color parameters: the lightness coordinate (L*), the red/green coordinate (a*), the yellow/blue coordinate (b*), Hue (h), Chroma (C) and the color index (CIRG) of skin tissue of the Dobričić, Ljutun and Plavac mali during four ripening stages.

Color parameters	Variety	Ripening stage											
		20-Aug-12			05-Sep-12			23-Sep-12			11-Oct-12		
		Mean±sd			Mean±sd			Mean±sd			Mean±sd		
L*	Dobričić	25.81±0.41	a	A	25.02±0.26	a	B	26.38±0.46	a	C	23.97±0.53	a	D
	Ljutun	24.97±0.73	b	A	23.17±0.20	b	B	26.93±0.79	a	C	25.02±0.35	b	A
	Plavac mali	25.78±0.55	a	B	24.30±0.34	c	A	28.10±1.44	b	C	24.42±0.37	a	A
a*	Dobričić	1.50±0.38	a	A	1.21±0.16	a	A	0.78±0.07	a	B	1.32±0.33	ab	A
	Ljutun	2.30±0.61	b	C	1.49±0.54	ab	B	0.68±0.39	a	A	0.91±0.40	a	A
	Plavac mali	2.40±0.39	b	C	1.90±0.22	b	A	1.19±0.38	b	B	1.67±0.32	b	A
b*	Dobričić	0.48±0.10	a	AB	0.57±0.13	a	B	(-0.03)±0.10	a	C	0.27±0.33	a	A
	Ljutun	0.09±0.10	b	B	(-0.2)±0.14	b	A	(-0.74)±0.31	b	C	(-0.06)±0.10	b	AB
	Plavac mali	0.16±0.20	b	B	0.05±0.12	c	AB	(-1.10)±0.73	b	C	(-0.35)±0.12	c	A
C	Dobričić	1.65±0.36	a	A	1.4±0.17	a	A	0.96±0.12	a	B	1.47±0.34	a	A
	Ljutun	2.38±0.60	b	C	1.6±0.54	ab	B	1.35±0.31	b	AB	1.05±0.38	b	A
	Plavac mali	2.47±0.38	b	B	1.94±0.23	b	A	1.99±0.27	c	A	1.82±0.31	a	A
H	Dobričić	68.23±18.00	a	AB	57.95±18.60	a	A	140.30±28.62	a	C	105.31±49.70	a	BC
	Ljutun	161.22±39.27	b	A	206.15±46.29	b	B	248.71±33.81	b	C	167.60±30.02	b	AB
	Plavac mali	122.57±69.09	ab	A	121.16±42.87	c	A	243.65±96.31	b	B	243.23±31.77	c	B
CIRG	Dobričić	5.82±0.18	a	A	5.86±0.30	a	A	6.55±0.27	a	B	6.70±0.28	a	B
	Ljutun	6.62±0.19	b	B	7.60±0.22	b	A	7.65±0.35	b	A	7.01±0.16	b	C
	Plavac mali	6.34±0.25	c	C	6.82±0.10	c	A	7.12±0.52	c	AB	7.36±0.20	c	B

Values as mean± standard deviation, significant differences ($p \leq 0.05$) are indicated by different Latin letters determined by One way analysis of Variance ANOVA. Different lower-case Latin letters indicate significant differences among the three varieties at the same ripening stage. Different capital Latin letters indicate significant differences among the grapes of the four different ripening stages for the same variety.