PAPER

COMPARATIVE STUDY OF THE PHENOLIC COMPOSITION OF SEEDS FROM GRAPES *CV* CARDINAL AND ALPHONSE LAVALLEE DURING LAST MONTH OF RIPENING

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ABSTRACT

During the last month of ripening, the phenolic composition of seeds from two widely distributed table grapes, *cv* Cardinal and Alphonse Lavallee, was determined by HPLC/DAD/ESI/MS. Besides, the concentrations of nutrients in leaf blades of grapevine were measured. Generally, the most abundant phenolic in grape seed was gallic acid, followed by methyl gallate and monomeric flavan-3-ols. In comparison to Alphonse Lavallee, the amounts of phenolics were higher in grape seed of Cardinal, in which gallic acid glucoside was not detected. Among analyzed phenolics, the increase of gallic acid was evidenced in grape seed of Cardinal. The most of phenolics decreased during the last month of grape ripening, and some of them had no significantly different content. Results of bivariate correlations showed that the amounts of some phenolics in grape seed of Cardinal increased with increasing of the content of potassium and phosphorus in leaves.

- Keywords: correlation, grape, leaf, nutrient, phenolic, seed -

INTRODUCTION

The investigation of the changes of phytochemicals during ripening of fruit contributes to the understanding of the biochemical and physiological processes in the developing fruit (USENIK *et al.*, 2008; VEBERIC, 2010). The phenolic compounds, as secondary metabolites, in grapes have attracted much interest recently, because of potential beneficial effects of phenolics on health and their strong contribution to the organoleptic characteristics of grapes. Numerous studies showed that the contents of phenolic compounds in grape berries are genetically determined and also depends on climatic and geographical factors, agricultural practices, stage of ripeness, and vegetative vigor of the plant (CANTOS *et al.*, 2002; VIAN *et al.*, 2006; PEREIRA *et al.*, 2006; LOVINO *et al.*, 2006; GODJEVAC *et al.*, 2010; OBREQUE-SLIER *et al.*, 2010). During the first period of berry growth, phenolics are accumulated, while colouring occurring during the second period (ripening) is characterized by increasing of the content of anthocyanins. Changes in the phenolic composition can also be observed in the seeds during grape maturation (FERRER-GALLEGO *et al.*, 2010; KENNEDY *et al.*, 2000; BRAIDOT *et al.*, 2008; OBREQUE-SLIER *et al.*, 2010).

In grape berries, phenolic compounds are present mainly in skins and seeds (OBREQUE-SLIER *et al.*, 2010). Grape seeds are rich in flavan-3-ols: monomers such as (+)-catechin, (-)-epicatechin and (-)-epicatechin 3-*0*-gallate as well as proanthocyanidins. Proanthocyanidins (condensed tannins) are oligomers and polymers composed of flavan-3-ol units (including 3-*0*-gallates) linked mainly through C4→C8 bond, but the C4→C6 linkage also exits (both are called B-type) (FULEKI and RICARDO DA SILVA, 1997).

Almost all flavonoids of the seed are contained in the outer integument, between the cuticle and the hard seed coat, whereas tannins localize in the epidermis and in the last layers of the inner integument (ADAMS, 2006; CADOT *et al.*, 2006). The flavonoid composition changes throughout the overall process of seed maturation, together with macroscopic changes in the tissues, such as the color and hardness (BRAIDOT *et al.*, 2008).

These compounds affect the taste; produce a sensation of astringency arising from the precipitation of oral proteins and mucopolysaccharides. Beside flavan-3-ol monomers (BELL *et al.*, 2000), there is an evidence that proanthocyanidins are also absorbed into the bloodstream (HOLT *et al.*, 2002). The flavan-3-ols are powerful antioxidants that demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and vasodilatory activities (KALIN *et al.*, 2002; ENG *et al.*, 2003; JAYAPRAKASHA *et al.*, 2003; STANKOVIC *et al.*, 2008). Recognition of the health benefits of flavan-3-ols initiated the manufacture of grape seed extracts as dietary supplements. In addition, seed-containing grapes could be useful to make juice because during the crushing the juice is enriched with flavan-3-ols coming from the seeds (CANTOS *et al.*, 2002).

On the contrary to wine grape varieties, which were widely investigated, very little information is available on identification and quantification of phenolics in table grape varieties.

The objective of the present study was to determine and to compare the phenolic composition of grape seeds of two widely distributed table grape varieties, Cardinal and Alphonse Lavallee, during the last month of ripening. Besides, considering the important role of plant nutrients in photosynthesis (SALISBURY and ROSS, 1992) and consequently their potential effect on accumulation of phenolics, the relationship between macro- and microelements in grapevine leaf and phenolics in grape seed was investigated.

MATERIALS AND METHODS

Sampling site characteristics

The commercial vineyard (13 Jul - Plantaže a.d.) is located about 10 km southeast from the town Podgorica (N 42° 27', E 19° 28', 10-50 m AMSL), Montenegro, in the area with Mediterranean climate. In Podgorica, for period from the beginning of April to the end of August 2008, the sum of rainfall was 325.3 mm, the sum of the sunlight hours 1406.7 h and mean temperature 23.5°C. The soil type is euthric brown on a fluvioglacial deposit consisting of carbonates. *Vitis vinifera* L. *cv* Cardinal on SO4 rootstock (Selection Oppenheim Nr. 4) and Alphonse Lavallee on Paulsen rootstock were planted in 1997/98. Vine spacing was 1.2 m, with a row spacing of 2.6 m. Cardinal is a table grape cultivar with an early ripening period, and Alphonse Lavallee with medium to late ripening period. The studied vines of one cultivar were within the same row, whereas the distance between rows with vines of *cv* Cardinal and Alphonse Lavallee was about 200 m. All applied agrotechnical measures were the same (pruning, fertilization through soil, irrigation, plant protection, etc.).

Sampling of grapevine leaves

The leaves of grapevine were taken from the opposite grapes near the middle of the shoot, just before the first sampling of grapes (on 16th of July for Cardinal and 6th of August for Alphonse Lavallee). The leaf blades were immediately separated from petioles. Twenty leaf blades from five vines (within one replicate) represented one sample.

Leaf blade analyses

N and S were determined on CHNS/O elemental analyzer (Vario EL III, Elementar, Germany). For determination of the other nutrients, dried (for 24 h at 65°-70°C) and grinded (in porcelain mortar with pestle) plant material was digested by HNO_3 and $HClO_4$ (RYAN *et al.*, 2002). P was spectrophotometrically determined (Cary 100, Varian, Australia); K and Ca flame photometrically (PFP 7, Jenway, United Kingdom); Mg, Fe, Mn, Cu and Zn by flame atomic absorption spectrophotometry (AA – 6800, Shimadzu, Japan). Boron was determined after dissolving of ashed material in 20% HCl (MUNTER and GRANDE, 1984) by inductively coupled plasma optical emission spectrometry (iCAP 6500 Duo ICP, Thermo Fisher Scientific, United Kingdom). These results are given on dry basis at 105°C.

Sampling of grapes

The samples of grape were collected 3 times during last month of ripening (for Cardinal I: 16th of July; II: 30th of July and III: 13th of August; whereas for Alphonse Lavallee I: 6th of August, II: 19th of August and III: 1st of September). For each sampling date, about 2.5 kg of grape were taken on five vines (in average about 0.5 kg per vine). There were 8 replications (in total 40 vines per cultivar) with 3 buffer vines between replications.

Determination of general parameters

Total soluble solids – TSS (by manual refractometer) and titratable acidity – TA (with 0.1M NaOH) were determined in the grape juice (obtained by crushing and pressing of grape berries by hand through two layers of gauze).

Preparation of grape seed extracts

Grape seeds were manually separated from pulp and dried on filter paper. Air dried grape seeds (about 2.4 g) were extracted in 30 mL of 50% methanol. The mixtures were sonicated on ultrasonic bath (12 h), filtered over 0.45 μm syringe cellulose filter and transferred into HPLC vials.

HPLC/DAD and LC/MS analyses

Were described by GODJEVAC *et al.* (2010). HPLC analysis of extracts was performed using an Agilent 1200 equipped with DAD model G1315B, Bin pump model G1312A, autosampler model G1313A, and RR Zorbax Eclipse Plus C18 column (1.8 μm, 150 x 4.6 mm). Mobile phase A was 0.2% formic acid in water and mobile phase B was acetonitrile. The injection volume was 5 μL, and elution at 0.95 mL/min with gradient program (0-20 min 5-16% B, 20-28 min 16-40% B, 28-32 min 40-70% B, 32-36 min 70-99% B, 36-45 min 99% B, 45-46 min 99-5% B).

UV-VIS detection was carried out at 230, 280, and 320 nm. Quantification was based on the measured integration area applying the calibration equation of the corresponding standard. The concentrations used for the calibration were 0.1-1.0 and 0.02-0.2 mg/mL for catechin and gallic acid (Sigma, St. Louis, MO, Usa), respectively. Some compounds were quantified as equivalents of the most similar chemical structures: gallic acid for methyl gallate and gallic acid glucoside; but catechin for all other compounds.

LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC, using the same column and gradient program as those for HPLC–DAD analysis. Mass spectra were acquired using an Agilent ESI-MSD TOF. Drying gas (N_2) flow was 12 L/min; nebulizer pressure was 45 psig; drying gas temperature was 350°C. For ESI analysis, the parameters were: capillary voltage, 4,000 V; fragmentor, 140 V; skimmer, 60 V; Oct RF V 250 V, for positive and negative modes. The mass range was from 100 to 2,000 m/z. Processing of data was done with the software Molecular Feature Extractor. Characteristic ions were used to assign procyanidin oligomers, but in the absence of authentic standards, oligomers differing in combinations of epimeric catechin and epicatechin units cannot be distinguished.

Statistical analysis

Descriptive statistics (mean and standard error), Pearson correlation (2-tailed), one way ANOVA and multiple range test using Duncan's test at $p < 0.05$ were performed by SPSS 10.0 Program.

RESULTS AND DISCUSSION

Sugars are among the most important ingredients determining fruit quality, which are responsible for the sweet taste of fruit. The acidity affects not only the sour taste, but also sweetness by masking the taste of sugars (NELSON *et al.*, 1973). Towards common practice for determination of harvest time, the total soluble solids and titratable acidity in grape juice were measured during the last month of ripening. As expected, TSS increased and TA decreased during the last month of ripening (Fig. 1). A significant difference in these parameters was noticed between Cardinal and Alphonse Lavallee at the first sampling date. On the contrary to expectations, due to the relatively high variability of the data for second date, the means of total soluble solids were not statistically different (p = 0.192). The same was for titratable acidity (p = 0.080). At the end of sampling i.e. at the state of full maturity, the grapes of both cultivars were

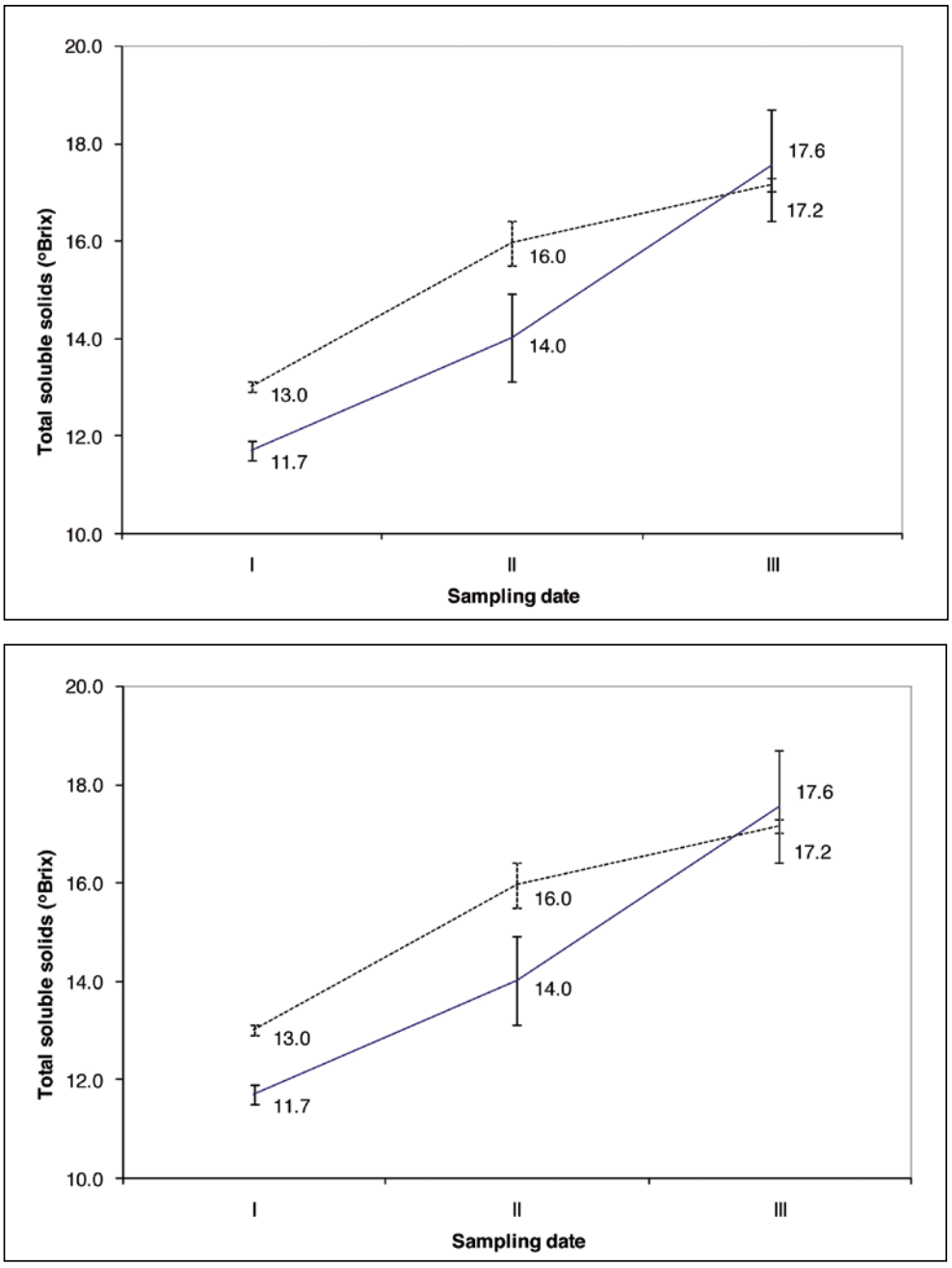

Fig. 1 - Total soluble solids (°Brix) and titratable acidity (g TAE/L) of grape juice (solid line: Cardinal; dashed line: Alphonse Lavallee) at three sampling dates (n = 8).

not different in the average of TSS and TA. The ratio between °Brix and titratable acids ranged from 16.9 at the first sampling date through 34.2 at second date to 53.2 at the end of ripening for Cardinal, and from 27.4 through 49.8 to 67.1 for Alphonse Lavallee, respectively. In study of the relation between Brix/acid ratio and favour preference, NELSON *et al.* (1973) stated that the rate of increase in consumer acceptability decreased at the higher Brix/acid ratios, which had been demonstrated with samples of Cardinal grapes with ratios as high as 45:1.

The amounts of analyzed phenolic compounds are presented in Table 1. The most abundant phenolic in grape seed of Cardinal and Alphonse Lavallee was gallic acid, followed by methyl gallate and monomeric flavan-3-ols. There were differences in the content of phenolics in grape seeds of both cultivars during the last month of ripening. The exception was gallic acid at the first sampling date as well as methyl gallate which amount was not significantly different at the first and second sampling date. Having in mind already mentioned difference in general parameters at the beginning, the difference in phenolic composition of seed was expected. However, at the end of ripening, when TSS and TA were similar for both cultivars, the evidenced difference

was mainly influenced by genetic factors. Gallic acid glucoside was not found in grape seed of Cardinal. For Alphonse Lavallee gallic acid glucoside was not detected at the first sampling date and was at almost constant value thereafter.

During the last month of grape ripening for both cultivars, the contents of catechin, proanthocyanidin trimer, epicatechin, proanthocyanidin dimer monogallate and epicatechin gallate decreased from the first to second sampling date and were not significantly different thereafter, while proanthocyanidin dimer (t_R = 13.2) was at similar level. In grape seed of Alphonse Lavallee the same trend as for epicatechin gallate was found for gallic acid and methyl gallate, while proanthocyanidin dimer (t_R = 8.9) was significantly lower at the end in comparison with the first sampling date. Differently to Alphonse Lavallee, in grape seed of Cardinal there were no significant differences in the content of methyl gallate and proanthocyanidin dimer (t_R = 8.9), the concentration of proanthocyanidin dimer (t_R = 9.8) decreased from the first to second sampling date and was similar thereafter, while the content of gallic acid significantly increased from the beginning to the end of the last month of ripening. The increasing of gallic acid in seeds of Cardinal could be partly caused by hydrolysis of some compounds such as methyl gal-

Table 1 - The content (mean ± standard error, n = 8) of the analysed compounds in grape seeds in mg/kg DW. Values with asterisk in the row are not different at $p > 0.05$ and without asterisk are different at $p < 0.05$. Values followed by different letters within column for each phenolic compound are significantly different according to Duncan's multiple range test at $p < 0.05$.

Compound	Molecular formula/Mass/Ion species	t_R (min)	Cardinal		Alphonse Lavallee	
Gallic acid	$C_7H_6O_5$ 170.0215 M-H	3.3	Jul 16 Jul 30 Aug 13	1532.5±90.8^{a*} 1842.5±180.2^{ab} 2080.0±139.6^{b}	Aug 6 Aug 19 Sep 1	1371.3±121.9^{b*} 1070.0±54.3^{a} 813.8±76.7^{a}
Gallic acid glucoside	$C_{13}H_{16}O_{10}$ 332.0744 M-H	4.8	Jul 16 Jul 30 Aug 13	nd nd nd	Aug 6 Aug 19 Sep 1	nd 240.0±97.5^{a} 236.3±51.0^{a}
Proantho-cyanidin dimer	$C_{30}H_{26}O_{12}$ 578.1424 M-H, 2M-H	8.9	Jul 16 Jul 30 Aug 13	203.8±13.5^{a} 153.8±19.5^{a} 138.8±32.3^{a}	Aug 6 Aug 19 Sep 1	81.3±14.4^{b} 53.8±6.8^{ab} 47.5±7.5^{a}
Proantho-cyanidin dimer	$C_{30}H_{26}O_{12}$ 578.1424 M-H, 2M-H	9.8	Jul 16 Jul 30 Aug 13	197.4±21.0^{b} 126.1±14.9^{a} 151.8±7.8^{ab}	Aug 6 Aug 19 Sep 1	53.8±15.5^{a} 37.5±4.5^{a} 30.0±3.8^{a}
Methyl gallate	$C_8H_8O_5$ 184.0372 M-H	10.2	Jul 16 Jul 30 Aug 13	930.0±79.3^{a*} 792.5±77.5^{a*} 902.5±48.5^{a}	Aug 6 Aug 19 Sep 1	1228.8±131.8^{b*} 821.3±120.9^{a*} 558.8±81.0^{a}
Catechin	$C_{15}H_{14}O_6$ 290.0790 M-H	10.6	Jul 16 Jul 30 Aug 13	1426.3±144.6^{b} 901.3±114.9^{a} 780.0±70.7^{a}	Aug 6 Aug 19 Sep 1	820.0±158.5^{b} 428.8±49.4^{a} 317.5±38.7^{a}
Proantho-cyanidin trimer	$C_{45}H_{38}O_{18}$ 866.2058 M-H, M-2H	11.4	Jul 16 Jul 30 Aug 13	147.5±11.0^{b} 102.5±17.5^{a} 120.0±12.8^{ab}	Aug 6 Aug 19 Sep 1	53.8±9.4^{b} 32.5±4.5^{a} 28.8±3.5^{a}
Proantho-cyanidin dimer	$C_{30}H_{26}O_{12}$ 578.1424 M-H, 2M-H	13.2	Jul 16 Jul 30 Aug 13	111.3±8.1^{a} 113.8±10.8^{a} 136.3±8.4^{a}	Aug 6 Aug 19 Sep 1	65.0±10.9^{a} 50.0±8.5^{a} 53.8±8.9^{a}
Epicatechin	$C_{15}H_{14}O_6$ 290.0790 M-H	14.9	Jul 16 Jul 30 Aug 13	801.3±59.8^{b} 593.8±76.5^{a} 632.5±33.7^{ab}	Aug 6 Aug 19 Sep 1	411.3±67.4^{b} 227.5±26.3^{a} 153.8±21.5^{a}
Proantho-cyanidin dimer monogallate	$C_{37}H_{30}O_{16}$ 730.1534 M-H, 2M-H	18.3	Jul 16 Jul 30 Aug 13	501.3±50.4^{b} 241.3±37.2^{a} 152.5±18.3^{a}	Aug 6 Aug 19 Sep 1	148.8±31.1^{b} 57.5±11.3^{a} 46.3±8.4^{a}
Epicatechin gallate	$C_{22}H_{18}O_{10}$ 442.0900 M-H, 2M-H	22.7	Jul 16 Jul 30 Aug 13	955.0±128.3^{b} 182.5±40.3^{a} 165.0±15.7^{a}	Aug 6 Aug 19 Sep 1	190.0±40.0^{b} 68.8±13.6^{a} 58.8±9.3^{a}

late, epicatechin gallate etc. Although PENA-NEIRA *et al.* (2004) found that with exception of seeds from low vigor vines of *cv* Cabernet Sauvignon, gallic acid concentration decreased during ripening. There is an evidence that extractable flavan-3-ol monomers (Mr: 290-442) and low molecular weight seed tannins (Mr: <900) decrease during grape ripening (KENNEDY *et al.*, 2000; BRAIDOT *et al.*, 2008; OBREQUE-SLIER *et al.*, 2010). The ratio between catechin and epicatechin for Cardinal decreased from 1.8 to 1.2 during last month of ripening, whereas for Alphonse Lavallee was relatively close 1.9-2.1. Similarly, GODJEVAC *et al.* (2010) found in grape seed of some cultivars this ratio between 1 and 2, e.g. 1.25 for Muscat Hamburg the most widespread table grape in Serbia. The relative proportion of flavanol monomers also changed in grape seed of Cardinal, while was almost similar for Alphonse Lavallee (Fig. 2). According to KENNEDY *et al.* (2000), differences in the relative proportions of flavan-3-ol monomers are consistent with a strong relation between the variety and the chemical evolution of monomeric composition in seeds during ripening.

The amount of phenolics was higher in grape seed of Cardinal than of Alphonse Lavallee. Thus, at third sampling date, when total soluble solids was above 17°Brix, proanthocyanidin dimer (t_R=9.8) was even 5-fold, proanthocyanidin trimer and epicatechin were more than 4-fold; proanthocyanidin dimer (t_R=8.9), proanthocyanidin dimer monogallate and epicatechin gallate about 3-fold; gallic acid 2.6-fold; catechin and proanthocyanidin dimer (t_R=13.2) 2.5 and methyl gallate 1.6-fold higher in grape seed of Cardinal. In this regard, literature data also differ. Comparing to our data for both cultivars at third sampling date, in grape seed of Muscat Hamburg (GODJEVAC *et al.*, 2010) much lower concentration of gallic acid, gallic acid glucoside and methyl gallate, but higher of catechin and epicatechin was reported. A concentration of epicatechin gallate was higher than in grape seed of Alphonse Lavallee, but lower than for Cardinal. FULEKI and RICARDO DA SILVA (1997) found concentrations more similar to our results for catechin in grape seeds of Merlot and Riesling as well as for epicatechin in Cabernet Sauvignon and Riesling.

Table 2 - The content (mean ± standard error, n = 8) of nutrients in leaf blade of grapevine. Values with asterisk in the row are not different at $p > 0.05$ and without asterisk are different at $p < 0.05$.

Cultivar	Cardinal	Alphonse Lavallee
Date	16 July	6 August
N (%)	2.52±0.05	1.99±0.04
S (%)	0.36±0.01	0.40±0.01
K (%)	0.73±0.03	0.57±0.04
P (%)	0.18±0.00*	0.19±0.01*
Mg (%)	0.27±0.01	0.46±0.01
Ca (%)	4.70±0.09	4.22±0.14
Fe (mg/kg)	99.95±3.67	114.09±3.27
Mn (mg/kg)	85.90±1.40	63.72±2.30
Zn (mg/kg)	13.87±0.39*	14.09±0.35*
Cu (mg/kg)	4.64±0.22*	4.58±0.21*
B (mg/kg)	36.23±0.57	27.74±0.85

A leaf tissue analysis is commonly used as a diagnostic tool to determine the nutritional program of grapevine. Having in mind that grape quality can be affected by the nutrient composition of leaf (FREGONI, 1998), an objective of this study was to investigate the relationship between macro- and micronutrients in the grapevine leaf and phenolics in the grape seed. The content of nutrients in grapevine leaves just before the last month of grape ripening (Table 2)

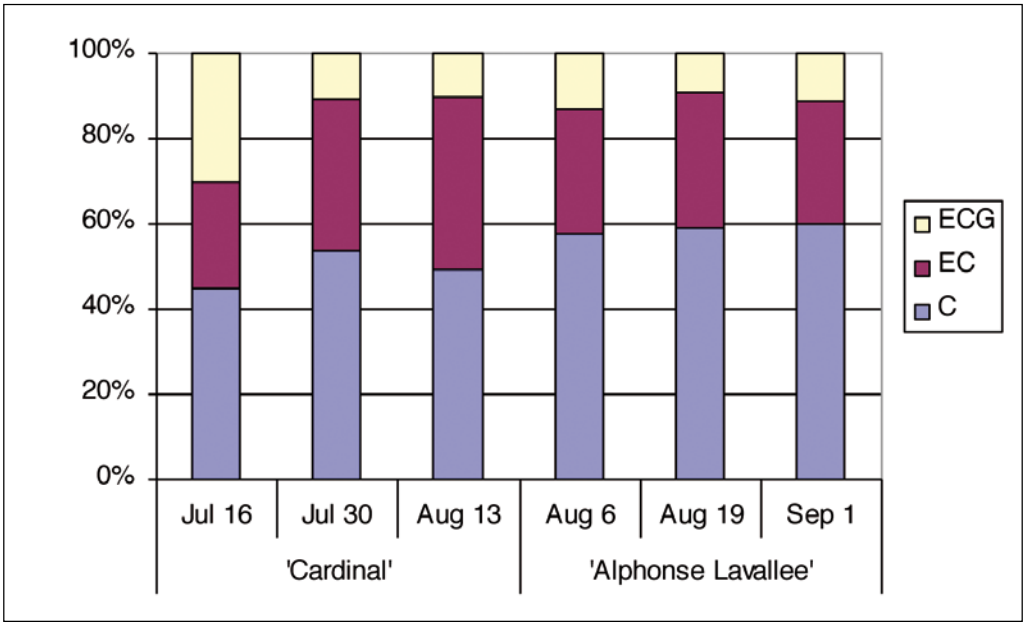

Fig. 2 - Relative proportion of flavanol monomers.

Table 3 - Correlation matrix for macro- and microelements in grapevine leaf blade and phenolics in grape seed of Cardinal (n=8, * significant at < 0.05, ** significant at < 0.01).

	GA	PCD 8.9	PCD 9.8	MG	C	PCT	PCD 13.2	EC	PCDG	ECG
N	0.620	-0.391	-0.678	0.392	0.277	0.157	-0.076	0.475	-0.002	0.248
S	0.204	0.267	0.173	0.128	0.453	0.437	0.375	0.429	0.526	0.500
K	0.913**	0.503	0.124	0.848**	0.771*	0.836**	0.711*	0.936**	0.686	0.647
P	0.681	0.053	-0.235	0.460	0.752*	0.545	0.193	0.598	0.472	0.759*
Mg	-0.378	-0.419	-0.049	-0.227	-0.311	-0.600	-0.215	-0.226	-0.175	-0.450
Ca	-0.782*	-0.312	-0.044	-0.794*	-0.730*	-0.600	-0.706*	-0.909**	-0.677	-0.526
Fe	0.455	-0.216	-0.374	0.208	0.128	0.229	0.053	0.436	0.076	0.156
Mn	-0.366	-0.288	-0.304	-0.484	-0.451	-0.244	-0.221	-0.323	-0.379	-0.279
Zn	-0.660	0.148	0.486	-0.575	-0.077	-0.195	0.019	-0.462	0.127	0.006
Cu	-0.388	0.133	0.457	-0.433	-0.152	-0.078	-0.405	-0.446	-0.049	0.010
B	-0.758*	-0.199	-0.017	-0.736*	-0.616	-0.461	-0.325	-0.674	-0.453	-0.441

GA: gallic acid; PCD 8.9: proanthocyanidin dimer t_R = 8.9; PCD 9.8: proanthocyanidin dimer t_R = 9.8; MG: methyl gallate; C: catechin; PCT: proanthocyanidin trimer; PCD 13.2: proanthocyanidin dimer t_R = 13.2; EC: epicatechin; PCDG: proanthocyanidin dimer monogallate; ECG: epicatechin gallate.

indicated significant differences between studied cultivars for the majority of nutrients, with the exception of P, Zn, and Cu. The leaf blade of Cardinal has higher concentration of N, K, Ca, Mn and B than one of Alphonse Lavallee, which was more abundant in S, Mg and Fe. It has been known that leaf contents of individual elements are not a constant, they keep changing during the growing period. Besides, element contents depend on the variety, soil chemical properties, weather conditions as well as on the anthropogenic impact (fertilization). Taking into account the same soil properties and the same fertilization of vines (similar values of soil agrochemical parameters) as well, these differences might be attributed to sampling date and cultivar characteristics.

For Alphonse Lavallee, there were no significant correlations between above mentioned nutrients in leaves and analyzed seed phenolics (data not shown). For Cardinal (Table 3), the content of gallic acid, methyl gallate, catechin, proanthocyanidin trimer, proanthocyanidin dimer (t_R=13.2) and epicatechin in grape seed increased with increasing of the content of potassium in leaf blade. Moreover, positive correlations were found between catechin, as well as epicatechin gallate and phosphorus. The importance of potassium for photosynthesis, translocation of photosynthates, activation of plant enzymes (among which ones important for pentose phosphate pathway and Krebs cycle when the precursors of secondary metabolites are originated), as well as of phosphorus in energy transfer, photosynthesis, transformation of sugars and starches (SALISBURY and ROSS, 1992) can explain obtained significant correlation of K and P with the content of phenolics.

The negative correlations between some phenolics in grape seed and calcium and boron were likely the consequence of negative correlations between these nutrients and K (data not shown). Namely, the content of gallic acid, methyl gallate, catechin and epicatechin were indirectly proportional with calcium, but gallic acid and methyl gallate showed negative relationships with boron in grapevine leaf.

The investigations indicate that potassium and calcium are antagonistic when the other element is available in higher concentrations (GARCIA *et al.*, 1999). Due to the fact that vineyard soil being highly calcareous, the negative correlation between Ca and K in leaf was expected. Although high potassium concentration can reduce boron, it is difficult to explain the negative correlation between K and B, because their contents in grapevine leaf blade were optimal.

CONCLUSION

The phenolic composition of seed from table grape depends directly on the cultivar and ripening time, as growing conditions being the same for Cardinal and Alphonse Lavallee. In general, the most abundant phenolic in grape seed was gallic acid, followed by methyl gallate and monomeric flavan-3-ols. The amounts of phenolics were higher in grape seed of Cardinal. The majority of phenolics decreased during last month of grape ripening for both cultivars.

Significant correlations between potassium as well as phosphorus in grapevine leaf and some phenolic compounds in grape seed of Cardinal indicate the possible positive effect of these elements on the content of seed phenolics.

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