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Effects of ripening stages and of plant vegetative vigor on the phenolic composition of grapes (*Vitis vinifera* L.) cv. Cabernet Sauvignon in the Maipo Valley (Chile)

A. Peña-Neira[1)], M. Dueñas[2)], A. Duarte[1)], T. Hernandez[2)], I. Estrella[2)] and E. Loyola[1)]

[1)] Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile
[2)] Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Summary

Quantitative changes in the composition of phenolic compounds in skins and seeds were determined during ripening of grape of Cabernet Sauvignon vines growing with low, medium or high vigor. Compounds in the skins were gallic and syringic acid, (+)-catechin, (-)-epicatechin, quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-arabinglucoside, quercetin-3-glucoside, and quercetin-3-rhamnoside, kaempferol-3-rutinoside and kaempferol-3-glucoside. The following compounds were identified in seeds: gallic acid, (+)-catechin, (-)-epicatechin, and procyanidins B1, B2, B3 and B4. The composition of compounds depended on the stage of ripening and vigor. No clear relationship was found between groups of compounds.

K e y w o r d s : phenolic compounds, grape, *Vitis vinifera* L., vigor.

Introduction

The concentration of various compounds in grape berries depends, among other factors, on the stage of development and vegetative vigor which are determined mainly by genetic, environmental and viticultural factors (Ribéreau-Gayon *et al.* 1999; Haselgrove *et al.* 2000; Düring and Davtyan 2002).

There is some evidence that different parts of the berry reach maturity consecutively. The seeds are the first to attain physiological maturity and the ability to germinate in stage II of berry development (Robinson and Davies 2000). After veraison, alterations of the cell walls and an accumulation of secondary metabolites were observed in the pulp and skin. In enological terms, pulp maturity corresponds with an optimal sugar-acid ratio. Skin maturity is reached when some aromatic substances and some phenolic compounds, such as anthocyanins, are highest; moreover the concentration of other tannins will be high and high rates of polymerization will be found (Ribéreau-Gayon *et al.* 1999; Coombe and McCarthy 2000; De Freitas *et al.* 2000; Robinson and Davies 2000).

For cluster development a certain canopy surface area is required which is related to some secondary metabolites in berries, such as anthocyanins, flavanols and flavonols (Ribéreau-Gayon *et al.* 1999; Dry 2000; Poni and Giachino 2000; Petrie *et al.* 2000, Bureau *et al.* 2000).

Comparing the canopy surface area of different trellis systems Smart and Robinson (1991), found that Cabernet Franc and Shiraz wines from vines with a dense canopy had higher pH values, lower color density, lower anthocyanin contents and total phenol concentrations and lower sensory scores than wines from open canopies.

Considering the importance of polyphenols for wine quality, it is worthwhile to study the effect of vigor and harvest time on the composition of phenolic compounds, especially on some flavonols, phenolic acids and flavanols of the berry. In this study, we analyzed the effect of vigor on phenolic compounds of whole berries, skins and seeds of Cabernet Sauvignon during ripening.

Material and Methods

S a m p l e s : Ungrafted Cabernet Sauvignon vines, planted in 1975 and grown in the Maipo Valley in the Central Region of Chile were used.

Vines with high, medium or low vigor were differentiated by their size, diameter of shoots, number of shoots, length of internodes, length of shoots and number of clusters which were estimated visually (Smart and Robinson 1991). Within one parcel (1 ha) three plots of each group of plant vigor (25 plants per plot) were chosen during the growing season in 1999. To study the phenolic compounds during ripening, 200 berries were collected randomly from different positions of the clusters and different vines. Berries were collected from veraison till technological maturity (25-26 °Brix) at about 15-d-intervals, with a total of 5 samples (04/02, 04/03, 18/03, 15/04 and 20/04). Berries of each plot were stored in plastic bags in a portable refrigerator until they were counted and weighed.

S a m p l e e x t r a c t i o n : To study the phenolic composition of berries, skins and seeds were separated, weighed and ground in 30 ml of distilled water after adding 40 ml of an hydro-alcoholic solution ($EtOH/H_2O$; 10:90 v/v). 5 g of tartaric acid were added to the ground material and the final weight of the solution was adjusted to 200 ml by the same hydro-alcoholic solution. After an agitated maceration (2 h at 30 ºC in the dark), the extract was filtered through a glass microfiber (Venencie *et al.* 1997).

An aliquot (100 ml) of macerated seeds and skins was concentrated under vacuum at <35 ºC to 25 % of its initial volume. The concentrate was extracted three times with 25 ml of diethyl ether and three times with 25 ml of ethyl acetate.

Correspondence to: Dr. A. Peña-Neira, Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004 Santiago, Chile. Fax: +56-2- 6785796. E-mail: apena@uchile.cl

The organic fractions were combined and evaporated to dryness under vacuum. The residue was dissolved in 2 ml of methanol/water (1/1, v/v), and analysed by HPLC-PAD and HPLC-PAD-MS.

HPLC-PAD analysis of phenolic compounds: A photodiode-array detector (Model 991, Waters Corp. Milford, M.A. USA) was used. The column was a reversed phase Nova Pack C_{18} (300 mm x 3.9 mm I.D.) with a 4 µ packing. Two mobile phases were used for elution: A [water-acetic acid (98/2), v/v)] and B [water-acetonitrile-acetic acid (78/20/2, v/v/v)]. The gradient profile was 0-55 min, 100-20 % A; 55-70 min, 20-10 % A; 70-90 min, 10-0 % A. Detection was performed by scanning from 210 to 360 nm with an acquisition speed of 1 s. Samples were analyzed in duplicate (PEÑA-NEIRA *et al.* 2000).

Qualitative and quantitative analysis: Specific compounds were identified by comparing their spectra and retention times with those of standards. The standards were purchased from Aldrich (Germany): syringic (4-hydroxy-3,5-dimethoxybenzoic) acid; from Sigma (USA): gallic (3,4,5-trihydroxybenzoic) acid, (+)-catechin, (-)-epicatechin and from Extrasynthese (France): quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-arabinglucoside, quercetin-3-rhamnoside, kaempferol-3-glucoside and kaempferol-3-rutinoside. For procyanidins no standards were available; they were identified by their spectral parameters (BARTOLOMÉ *et al.* 1996, 1997) and confirmed by using HPLC-PAD-MS.

HPLC-PAD-MS conditions: A Hewlet-Packard series 1100 (Palo Alto, CA) chromatograph equipped with DAD and MS detectors and electro spray ionization (ESI) interface was used. A gradient of solvent A (water/acetic acid, 99/1, v/v) and solvent B (water/acetic acid, 90/10, v/v) was applied to a reversed-phase Nova-Pack C_{18} column (300 mm x 3.9 mm ID) as follows: 67 % of B from 0 to 45 min, 67-83 % of B from 45 to 55 min, 83-100 % B from 55 to 75 min. The flow rate was 0.7 ml·min^{-1}. Nitrogen was used as nebulizing and drying gas. ES conditions were as follows: nitrogen pressure 40 psi; drying gas, 10 ml·min^{-1} at 340 ºC; ion spray voltage, 4000 V; and variable fragmentator voltage 100 V (m/z < 200), 200 V (m/z 200-1000); 250 V (m/z 1000-3000). Mass spectra were recorded from m/z 100 to m/z 3000.

Quantitative determinations were derived from calibration curves using the external standard method with commercial standards. Procyanidins were quantified with the (+)-catechin curve.

Data were statistically treated by analysis of variance using STATGRAPHICS. The test for difference between means was based on the least significant method if F value was significant.

Results and Discussion

A statistical analysis of the vegetative parameters indicated they were all different ($p < 0.05$) (Tab. 1).

Phenolic composition in skins: Fig. 1 shows the chromatographic pattern of a skin extract. The following compounds were identified: gallic and syringic acid, (+)-catechin, (-)-epicatechin, quercetin-3-arabinglucoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, and quercetin-3-rhamnoside, kaempferol-3-glucoside and kaempferol-3-rutinoside. The average phenolic compound concentrations, as found in the three samples, are presented in Tabs 2 and 3. In agreement with FLANZY (2000), the concentration of (+)-catechin in skins (Tab. 2) is higher than (-)-epicatechin. The ratio of (+)-catechin/(-)-epicatechin increases considerably at the end of ripening for vines with high and medium vigor. The sum of (+)-catechin plus (-)-epicatechin decreases in general during ripening, which agrees with results of MARQUETTE (1999); it is lower in samples from low vigor plants. At technological maturity (20/04), the skins of berries from medium vigor plants had higher values of (+)-catechin and (-)-epicatechin. This may lead to more astringent or bitter wines from plants with medium vigor (BROSSAUD *et al.* 2001).

During ripening (+)-catechin and (-)-epicatechin have maximum and minimum values in all of the samples from plants with low, medium and high vigor.

Shortly after veraison the concentration of syringic acid increases and remained almost constant thereafter (Tab. 2). At the first dates gallic acid concentration increases slightly in samples from vines with high and medium vigor and decreases during the remaining ripening period. These results coincide with those observed for cv. Airen by FERNANDEZ DE SIMÓN (1992).

Flavonol glycosides (Tab. 3) show also maximum and minimum concentrations during ripening. The concentration of compounds was slightly different between the groups of vigor, with an increase from veraison to technological maturity (20/04) in vines with high and medium vigor; exceptions are quercetin-3-arabinoside and quercetin-3-glucoside,

Table 1

Vegetative parameters used for vigor classification

Vigor	Diameter of shoots (mm)	Number of clusters	Number of shoots	Length of internodes (cm)	Length of shoots (cm)
Low	6.2 ± 0.7 a	41.1 ± 0.4 a	51.7 ± 1.2 a	57.3 ± 2.1 a	93.8 ± 4.1 a
Medium	8.0 ± 0.5 b	61.9 ± 0.2 b	73.3 ± 0.9 b	79.1 ± 2.8 b	160.0 ± 5.6 b
High	8.3 ± 0.2 c	67.8 ± 0.6 c	77.5 ± 1.6 c	95.7 ± 3.1 c	198.0 ± 5.3 c

Data are means from the 25 plants of each group.
Means followed by the same letter in row are not significantly different (LSD, $p < 0.05$).

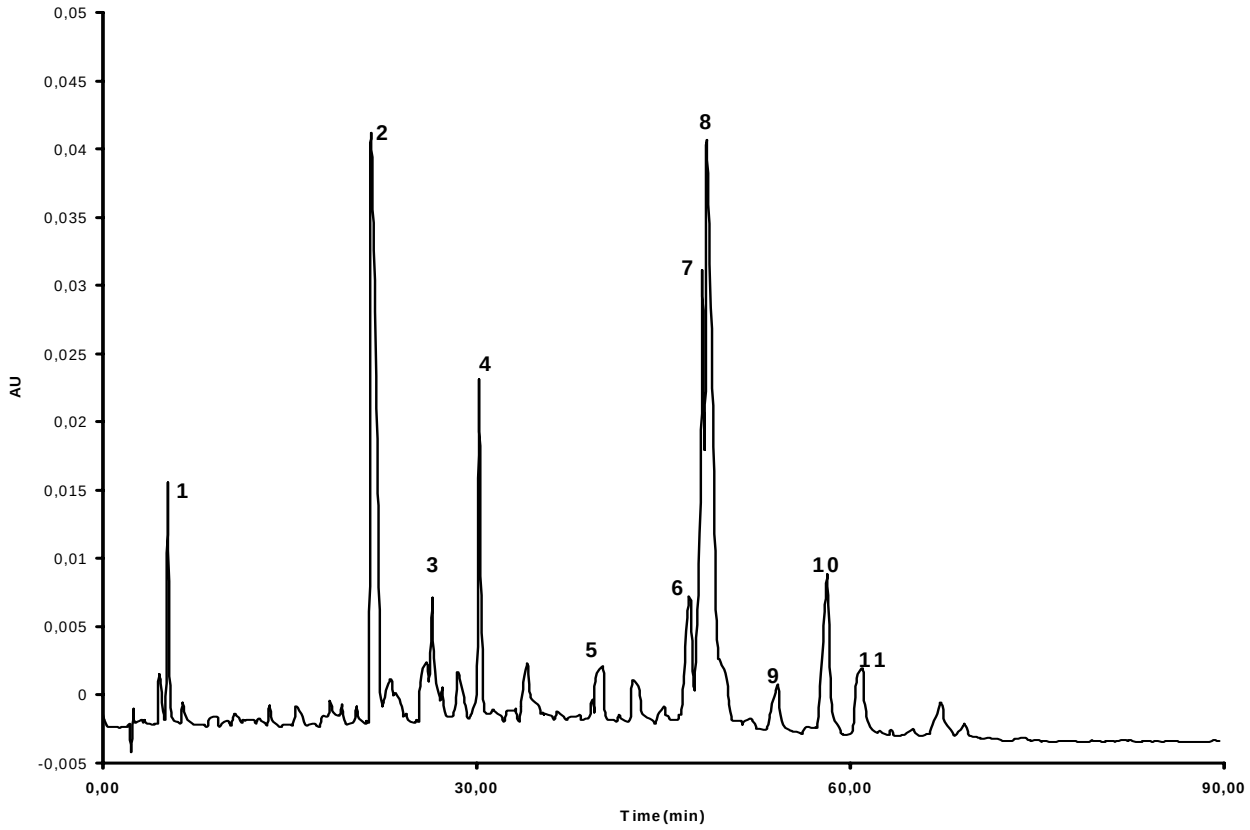

Fig. 1: HPLC chromatogram of berry skins at 280 nm. 1: gallic acid; 2: (+)-catechin; 3: syringic acid; 4:(-)-epicatechin; 5: quercetin-3-arabinglucoside; 6: quercetin-3-rutinoside; 7: quercetin-3-galactoside; 8: quercetin 3-glucoside; 9: kaempferol-3-glucoside; 10: kaempferol-rutinoside; 11:quercetin-3-ramnoside. Abscissa: Time (min).

T a b l e 2

Concentration of some phenolic acids and flavanols (µg 100 berries^{-1}) in the skin of berries, cv. Cabernet Sauvignon

Dates	Vigor	Gallic acid	Syringic acid	(+)Catechin	(-)Epicatechin
04-02-1999	High	28.0 ± 0.6 a[1]	53.1 ± 3.1 a	278.8 ± 5.3 a	133.4 ± 1.8 a
	Medium	33.0 ± 0.7 a	61.6 ± 2.5 b	363.9 ± 4-6 a	181.2 ± 2.7 a
	Low	51.0 ± 1.6 b	65.7 ± 2.6 b	721.5 ± 7.1 b	377.1 ± 5.3 b
04-03-1999	High	49.6 ± 1.1 a	102.5 ± 2.9 a	657.9 ± 4.9 a	310.1 ± 6.4 a
	Medium	37.4 ± 0.9 b	106.6 ± 3.3 a	101.6 ± 4.1 c	36.0 ± 0.7 c
	Low	30.8 ± 1.5 b	87.6 ± 3.1 b	221.6 ± 3.5 b	71.6 ± 1.6 b
18-03-1999	High	38.5 ± 1.4 a	104.2 ± 3.8 a	497.7 ± 4.6 a	256.4 ± 3.2 a
	Medium	28.3 ± 1.7 b	101.0 ± 3.8 a	276.3 ± 3.2 b	124.0 ± 3.5 b
	Low	28.6 ± 2.1 b	90.2 ± 2.6 b	300.6 ± 4.1 b	133.1 ± 2.3 b
15-04-1999	High	18.8 ± 0.8 a	95.5 ± 3.2 a	112.6 ± 2.6 a	31.2 ± 0.8 a
	Medium	20.4 ± 1.1 a	81.4 ± 4.1 b	65.2 ± 3.1 b	52.3 ± 2.9 b
	Low	18.5 ± 1.3 a	82.3 ± 2.9 b	134.2 ± 4.2 a	41.0 ± 1.7 b
20-04-1999	High	16.8 ± 1.4 a	100.1 ± 0.8 a	227.8 ± 2.7 a	14.8 ± 2.5 a
	Medium	25.5 ± 1.1 b	84.9 ± 1.7 b	223.9 ± 3.4 a	85.6 ± 5.2 b
	Low	21.3 ± 1.9 b	97.5 ± 1.3 a	117.4 ± 1.6 b	8.9 ± 1.6 a

[1] Means with different letters in a row differ significantly ($p < 0.05$). Results ± standard deviation are means of three replicates.

Table 3

Concentration of some flavonols (µg 100 berries^{-1}) in the skin of berries, cv. Cabernet Sauvignon

Dates	Vigor	Quercetin-3-arabinoside	Quercetin-glucoside	Quercetin-3-glucoside	Quercetin-3-galactoside	Kaempferol-3-rutinoside	Kaempferol-3-glucoside	Quercetin-3-rutinoside
04-02-1999	High	308.1 ± 4.2 a[1]	78.9 ± 2.6 a	290.0 ± 2.4 a	491.7 ± 6.3 a	49.6 ± 4.4 a	133.1 ± 3.6 a	76.7 ± 2.2 a
	Medium	34.4 ± 2.7 b	71.4 ± 3.7 a	244.0 ± 4.3 a	388.6 ± 5.3 b	44.9 ± 3.6 a	106.0 ± 5.6 a	81.6 ± 2.6 a
	Low	66.0 ± 3.4 b	186.9 ± 4.2 b	522.9 ± 5.7 b	906.4 ± 6.9 c	83.7 ± 3.6 b	232.4 ± 3.2 b	134.9 ± 1.9 b
04-03-1999	High	90.5 ± 6.3 a	114.2 ± 4.3 a	185.5 ± 3.2 a	608.9 ± 3.2 a	85.6 ± 2.7 a	234.8 ± 4.6 a	182.3 ± 3.3 a
	Medium	113.4 ± 4.8 a	169.8 ± 5.7 a	228.8 ± 4.1 b	889.7 ± 4.5 b	127.2 ± 2.3 b	377.5 ± 5.0 b	242.0 ± 3.6 b
	Low	51.9 ± 2.6 b	231.2 ± 7.1 b	152.0 ± 3.6 a	857.5 ± 4.2 b	149.0 ± 4.2 b	240.0 ± 3.5 a	113.8 ± 3.1 c
18-03-1999	High	120.9 ± 3.9 a	142.8 ± 3.9 a	136.3 ± 4.1 a	775.7 ± 6.6a	84.5 ± 3.1 a	331.0 ± 4.3 a	219.9 ± 4.2 a
	Medium	103.3 ± 3.4 a	102.8 ± 2.3 b	141.3 ± 3.9 a	543.1 ± 6.1 b	43.6 ± 2.5 b	209.3 ± 3.8 b	227.4 ± 3.6 a
	Low	151.0 ± 4.1 b	176.2 ± 3.3 a	209.2 ± 2.6 b	952.7 ± 5.2 c	113.9 ± 2.8 c	395.2 ± 3.8 c	265.4 ± 3.5 b
15-04-1999	High	146.8 ± 2.5 a	111.5 ± 2.3 a	146.6 ± 5.1 a	706.5 ± 5.3 a	70.6 ± 2.4 a	250.2 ± 4.1 a	247.8 ± 2.6 a
	Medium	108.9 ± 2.5 b	105.0 ± 2.7 a	96.1 ± 5.6 b	555.0 ± 5.3 b	63.4 ± 2.9 a	222.4 ± 2.9 a	241.2 ± 5.6 a
	Low	87.6 ± 3.2 b	88.1 ± 1.7 b	96.8 ± 3.6 b	528.0 ± 4.6 c	58.4 ± 1.7 a	193.5 ± 3.6 a	196.4 ± 2.8 b
20-04-1999	High	27.0 ± 1.9 a	138.0 ± 5.4 a	197.0 ± 4.1 a	870.6 ± 2.8 a	80.0 ± 1.9 a	276.4 ± 4.0 a	282.4 ± 3.4 a
	Medium	72.9 ± 2.1 b	101.8 ± 3.6 b	136.6 ± 4.8 b	613.0 ± 3.7 b	77.3 ± 3.2 a	227.2 ± 3.2 b	197.4 ± 2.3 b
	Low	29.0 ± 0.4 a	149.5 ± 3.6 a	188.7 ± 5.2 a	829.0 ± 4.2 a	87.7 ± 2.9 a	253.6 ± 3.4 c	264.3 ± 3.9 a

[1]For details see Tab. 2.

which decreased. Vines with low vigor show a more irregular pattern of evolution for flavonol glycosides.

Phenolic composition in seeds: The following compounds were identified in seeds (Fig. 2): gallic acid, (+)-catechin, (-)-epicatechin, and procyanidins B_1, B_2, B_3 and B_4. Tab. 4 shows the average values of the concentration of phenolic compounds.

During ripening, (+)-catechin dominates over all compounds in all samples. This agrees with results of JORDAO *et al.* (2001) for seeds of cv. Castelas Frances. In contrast with our results, JORDAO *et al.* (2001) found higher amounts of (-)-epicatechin than (+)-catechin in grape seeds for cv. Touriga Francesa; this is confirmed for Shiraz by KENNEDY *et al.* (2000) and OSZMIANSKI *et al.* (1986). Comparing the data of the first and the last date of sampling, the ratio (+)-catechin/(-)-epicatechin increases slightly for all groups of vigor.

The concentrations of procyanidins B_2 and B_4 increased slightly at veraison and at the end of harvest in samples from vines with high and medium vigor. In low vigor samples only B_4 increased. Comparing the first and the last harvest date the procyanidins B_1 and B_3 concentration decreased in high and medium vigor samples. The low vigor samples increased conspicuously. With the exception of seeds from low vigor plants, gallic acid concentration decreased during ripening.

As expected, seeds had high concentrations of catechins and procyanidins in comparison with skins. Like the compounds in the skin all the compounds in the seeds showed a similar evolution, with maximum and minimum values during ripening. All compounds in the seeds have a minimum concentration at the third date of sampling, the concentration depending on plant vigor.

At technological maturity the higher concentrations of flavanols in the seeds from plants with low vigor could be explained by over-ripeness of the fruit. This coincides with the results of FERNÁNDEZ DE SIMÓN (1992) for cvs Cencibel and Airen indicating that the date of technological maturity depends on plant vigor.

Conclusions

Vigor alters the concentration of all phenolic compounds in skins and seeds of grape berries during ripening.

At the end of harvest samples from low vigor vines were not distinctly different in their flavonol concentration when compared with the other groups of vigor. However, the concentrations of the most important flavanols, (+)-catechin and (-)-epicatechin, and the majority of the procyanidins, were higher in seeds but lower in skins of low vigor samples as compared with medium and high vigor samples. This may be related to the astringency and bitterness of skins and may have an influence on the time of harvest. Skin tasting is used as a criterion of technological maturity by some winemakers. However, the phenolic composition of wine depends not only on the presence of certain compounds, but also on the circumstances of extraction of these compounds and their chemical interactions.

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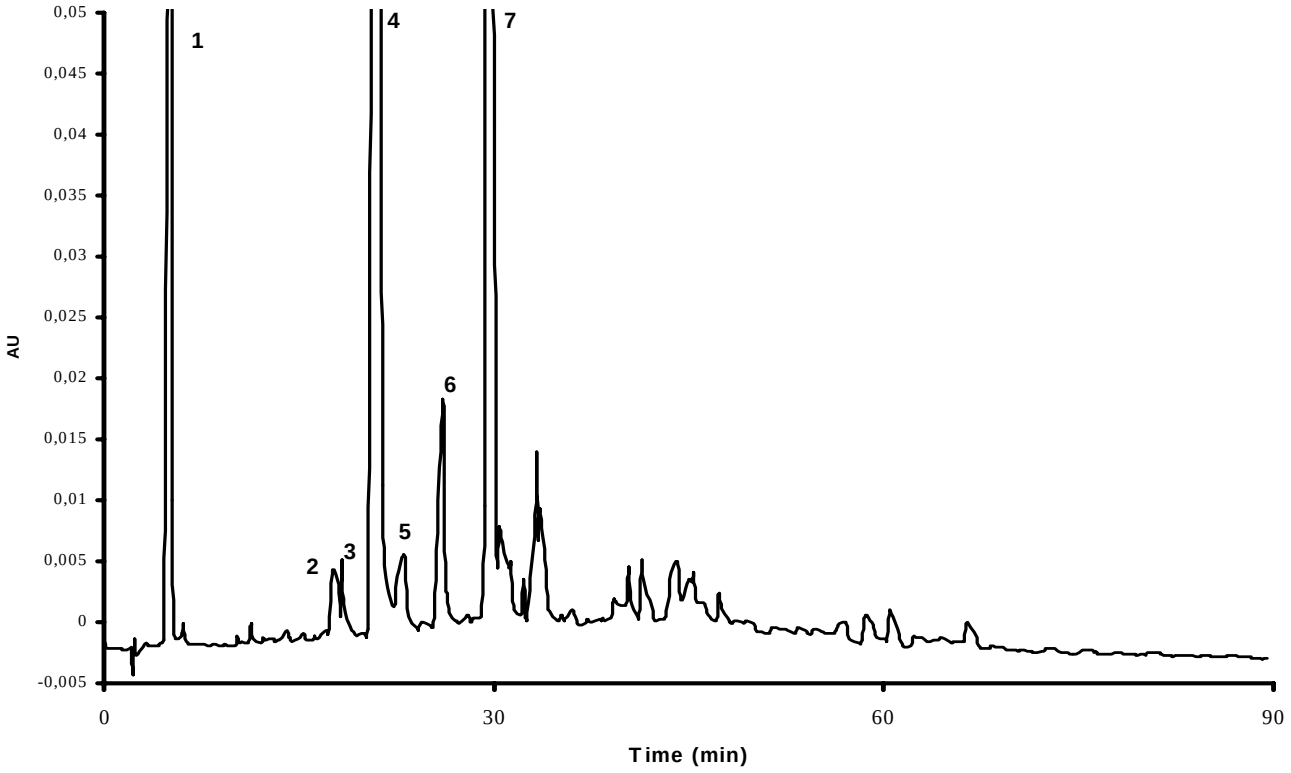

Fig. 2: HPLC chromatogram of seeds at 280 nm. 1: gallic acid; 2:procyanidin B_3; 3: procyanidin B_1; 4: (+)-catechin; 5: procyanidin B_4; 6: procyanidin B_2; 7: (-)-epicatechin.

Table 4

Concentration (mg 100 berries^{-1}) of phenolic compounds in seeds of Cabernet Sauvignon

Dates	Vigor	Gallic acid	Procyanidin B3	Procyanidin B1	(+)Catechin	Procyanidin B4	Procyanidin B2	(-)Epicatechin
04-02-1999	High	0.36 ± 0.04 a[1]	0.19 ± 0.03 a	0.13 ± 0.03 a	9.71 ± 0.3 a	0.09 ± 0.01 a	0.16 ± 0.03 a	5.05 ± 0.6 a
	Medium	0.28 ± 0.06 b	0.34 ± 0.02 b	0.23 ± 0.05 b	5.71 ± 0.9 b	0.19 ± 0.03 b	0.13 ± 0.03 a	2.89 ± 0.5 b
	Low	0.39 ± 0.02 a	0.27 ± 0.03 b	0.21 ± 0.03 b	11.72 ± 0.9 a	0.29 ± 0.03 c	0.26 ± 0.04 b	5.51 ± 0.9 a
04-03-1999	High	0.25 ± 0.05 a	0.20 ± 0.06 a	0.18 ± 0.02 a	7.86 ± 0.6 a	0.23 ± 0.07 a	0.45 ± 0.04 a	4.93 ± 0.3 a
	Medium	0.23 ± 0.01 a	0.15 ± 0.02 a	0.13 ± 0.04 a	5.20 ± 0.8 b	0.15 ± 0.05 b	0.26 ± 0.02 c	2.52 ± 0.3 b
	Low	0.22 ± 0.01 a	0.17 ± 0.03 a	0.16 ± 0.04 a	5.92 ± 0.6 b	0.17 ± 0.07 b	0.33 ± 0.02 b	2.63 ± 0.4 b
18-03-1999	High	0.09 ± 0.01 a	0.08 ± 0.01 a	0.03 ± 0.001 a	2.45 ± 0.4 a	0.09 ± 0.007 a	0.19 ± 0.03 a	0.97 ± 0.1 a
	Medium	0.22 ± 0.02 b	0.07 ± 0.01 a	0.06 ± 0.001 b	5.05 ± 0.7 b	0.06 ± 0.009 a	0.24 ± 0.04 b	2.57 ± 0.4 b
	Low	0.16 ± 0.02 b	0.09 ± 0.01 a	0.06 ± 0.001 b	3.44 ± 0.3 a	0.05 ± 0.007 b	0.17 ± 0.03 a	1.26 ± 0.4 c
15-04-1999	High	0.17 ± 0.03 a	0.12 ± 0.02 a	0.11 ± 0.01 a	5.21 ± 0.7 a	0.20 ± 0.02 a	0.34 ± 0.04 a	2.62 ± 0.6 a
	Medium	0.18 ± 0.01 a	0.12 ± 0.01 a	0.09 ± 0.002 a	5.68 ± 0.5 a	0.16 ± 0.03 b	0.29 ± 0.02 a	2.63 ± 0.6 a
	Low	0.18 ± 0.02 a	0.10 ± 0.01 a	0.08 ± 0.001 a	4.17 ± 0.5 a	0.14 ± 0.04 b	0.23 ± 0.04 b	1.34 ± 0.3 b
20-04-1999	High	0.14 ± 0.04 a	0.12 ± 0.02 a	0.11 ± 0.03 a	3.83 ± 0.2 a	0.13 ± 0.03	0.19 ± 0.04 a	1.38 ± 0.2 a
	Medium	0.26 ± 0.04 b	0.19 ± 0.03 a	0.17 ± 0.03 a	7.19 ± 0.8 b	0.20 ± 0.01	0.35 ± 0.03 b	3.01 ± 0.5 b
	Low	0.58 ± 0.06 c	0.42 ± 0.02 b	0. 52 ± 0.05 b	12.93 ± 0.3 c	0.56 ± 0.04	0.12 ± 0.03 a	5.72 ± 0.3 c

[1]For details see Tab. 2.

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