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Phenolic compounds and antioxidant activity of red and white grapes on different rootstocks

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This study aimed to assess the rootstocks influence over total phenolic compound content, antioxidant activity and its correlation in different red and white grapes cultivars for wine production. This study conducted an experimental three-year-old vineyard, located in Jundiá, in the State of São Paulo, Brazil, from July 2013 to January 2014. Red and white grapes from *Vitis vinifera* L. (Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah and Sauvignon Blanc), *V. labrusca* L. (Isabel and Bordô) and hybrid cultivars (IAC 138-22 Máximo, BRS Violeta, IAC 116-31 Rainha, IAC 21-14 Madalena and BRS Lorena) were grafted on IAC 766 and 106-8 Mgt rootstocks. Samples of grapes were collected and the total carotenoids, chlorophyll, anthocyanin, flavonoid, and phenolic content, and the *in vitro* antioxidant activity determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The rootstocks effects on red grape were mainly observed on the anthocyanins content. The total polyphenols content and the grapes antioxidant activity was virtually not affected by the rootstocks, especially on grapes of BRS Violeta and IAC 138-22 Máximo kinds, which presented the highest content of this compost. Among the white grapes, the 106-8 Mgt rootstock favored the increase of total phenolic compounds content on grapes of Sauvignon Blanc, IAC 116-31 Rainha and IAC 21-14 Madalena kinds. Although the hybrid grapes of IAC 116-31 Rainha e IAC 21-14 Madalena kinds are white grapes, the total phenolic compounds content on them were higher than the ones found on the red grape Isabel.

Key words: *Vitis vinifera*, *Vitis labrusca*, hybrid grapes, total anthocyanin, Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

INTRODUCTION

The composition and phenolic properties of grapes, especially those aimed for wine and juice production, have been constantly studied, and there are more and

more reports of higher amounts of phenolic compounds acting as antioxidants on grapes (Rockenbach et al., 2011). However, most data available in literature about

phenolic compounds in grapes and wines come from traditionally producing countries, mainly from Europe, where the grapes of choice are mostly *Vitis vinifera*. In contrast, in Brazil, more than 85% of the processed grapes volume comes from American cultivars, mainly *Vitis labrusca*, or hybrids, these kinds being considered more fitting to the climate conditions of the country, especially during harvesting season in the South and Southeast Regions of Brazil (Lago-Vanzela et al., 2011).

The *V. labrusca*, Isabel and Bordô grape cultivars are widely farmed in Brazil and, as a way of diversifying the products giving the producers more options, the Campinas Agronomic Institute (Instituto Agronômico de Campinas - IAC) and the Brazilian Company of Farming Research (Empresa Brasileira de Pesquisa Agropecuária - Embrapa), along with their respective genetic improvement programs, have been developing in the past few years new hybrid cultivars for wine production (Biasoto et al., 2014; Burin et al., 2014; Lago-Vanzela et al., 2013), among others there are the IAC 138-22 'Máximo' ('Seibel 11342' x 'Syrah'), IAC 116-31 'Rainha' ('Seibel 7053' x 'Burgunder Kastenholz'), IAC 21-14 'Madalena' ('Seibel 11342' x 'Moscatel de Canelli'), 'BRS Lorena' ('Malvasia Bianca' x 'Seyval') and the 'BRS Violeta' ('BRS Rúbea' x 'IAC 1398-21') grape cultivars. It is known that these varieties present high producing capability and low sensibility to the main fungal diseases that usually attack grapevines. However, there is lack of information about these grapes phenolic compounds and antioxidant activity.

Several studies show that phenolic compounds in grapes may vary due to some specific factors, such as the species, cultivar, climate conditions, geographic region and the grapevines handling practices (Barcia et al., 2014; Burin et al., 2014; Koundouras et al., 2009). On the other hand, studies assessing the rootstocks influence over phenolic compounds content and antioxidant activity, especially on grapes for wine production, are scarce. Some studies have demonstrated that weaker rootstocks enable higher concentrations of anthocyanin and phenolic compounds to accumulate on grapes peel. There were no effects of 1103 Paulsen (*Vitis rupestris* x *Vitis berlandieri*) and SO4 (*Vitis riparia* x *V. berlandieri*) rootstocks over the Cabernet Sauvignon cultivars' anthocyanin content and total phenolic compounds (Koundouras et al., 2009). However, these compounds were strongly influenced on Bordô cultivars, when the cultivar was grafted in different rootstocks (Mota et al., 2009).

Generally, rootstocks are recommended based on, to better fit to, environmental conditions and cultivar compatibility, what directly affects productivity and some of the fruits chemical characteristics, such as the pH,

acidity and soluble solids content. However, the intake of nutrients, phenolic compounds concentration and anthocyanin content are quality parameters that should be taken into account when farming grapes, mainly, to better choose the most efficient combination of cultivar and rootstock (Mota et al., 2009).

Therefore, this study is aimed at assessing the rootstocks influence over phenolic compounds content, antioxidant activity and their correlation in different red and white grapes cultivars for wine production.

MATERIALS AND METHODS

Experimental site and grapevines growing conditions

This study was conducted in an experimental 3-year-old vineyard, located in Jundiaí (23° 06' S, 46° 55' O, altitude 745 m) in the State of São Paulo (SP), Brazil, from July 2013 to January 2014. According to the Köppen classification, the climate is type *Cfb*, that is, subtropical climate with an average temperature of 19.5°C, and the average annual rainfall rate is of 1400 mm, with a tendency for concentrated rainfall during the summer months. The soil of the area was classified as Red Cambisol according to previously published criteria (EMBRAPA, 2006).

The vines were supported by trellising system and spaced 2.5 × 1.0 m apart (4000 vines ha⁻¹). Pruning was performed leaving one bud behind in each of them. Subsequently 5% hydrogen cyanamide was applied on the buds to induce and standardize sprouting. At the changing color stage of berries, plants were protected with anti-hail screens, aiming to protect them against hailstorms, and attack by birds and bees.

Plant material and samples preparation

Red and white grapes from *V. vinifera* L. (Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah and Sauvignon Blanc), *V. labrusca* L. (Isabel and Bordô) and hybrid cultivars (IAC 138-22 Máximo, BRS Violeta, IAC 116-31 Rainha, IAC 21-14 Madalena and BRS Lorena) were grafted in IAC 766 (106-8 Mgt x *V. caribaea*) and 106-8 Mgt [*V. riparia* x (*V. rupestris* x *V. cordifolia*)] rootstocks. All the grapes cultivars were harvested at the stage of their respective technical maturity, according to their soluble solids contents, titratable acidity and pH (Table 1).

10 clusters for each experimental trial were randomly selected. 10 berries from each cluster (from the clusters' top, middle and bottom), were collected adding up to 100 berries per trial, which then were cut in half and had their seeds removed and frozen in liquid nitrogen, pulverized and stored under -20°C up to the moment of analysis.

Chemicals

Methyl alcohol and acetone were obtained from Tedia (Fairfield, Ohio, USA). Tris-(hydroxymethyl)-aminomethane and Folin-Ciocalteu reagents were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,7,8-tetramethylchroman-2-carboxylic acid) and DPPH radical (2,2-Diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

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Table 1. Physicochemical parameters for berries of red and white grape varieties onto different rootstocks.

Cultivars/rootstocks	SS (°Brix)		pH		TA (% tartaric acid)	
	IAC 766	106-8 Mgt	IAC 766	106-8 Mgt	IAC 766	106-8 Mgt
Red cultivars						
Isabel	20.40	18.28	3.30	3.21	1.17	1.14
Bordô	16.76	15.85	3.44	3.49	0.33	0.30
Cabernet Sauvignon	19.68	18.03	3.95	3.82	0.93	1.03
Cabernet Franc	20.68	18.82	4.10	3.97	0.68	0.66
Merlot	20.66	18.13	3.93	3.87	0.67	0.85
Syrah	18.18	17.30	3.85	3.90	0.86	0.88
IAC 138-22 Máximo	17.42	16.00	3.60	3.55	0.90	0.93
BRS Violeta	18.36	18.56	3.55	3.59	0.87	0.79
White cultivars						
Sauvignon Blanc	19.12	19.52	3.18	3.21	0.86	0.88
IAC 116-31 Rainha	20.00	20.82	3.41	3.42	0.90	0.88
IAC 21-14 Madalena	18.82	19.40	3.57	3.50	0.82	0.82
BRS Lorena	22.74	20.50	3.38	3.31	0.77	0.84

SS, Soluble solids content; TA, titratable acidity.

Determination of total phenolic compounds and antioxidant activity

Total anthocyanins, carotenoids and chlorophyll

The pigments based on the methodology proposed by Sims and Gamon (2002) was determined. The extraction of pigments was done in TRIS-HCl-buffered acetone (80:20 of PA acetone and TRIS 0.2 M) in an environment shielded from light. Afterwards the supernatants and the samples' absorbance were retrieved and measured (663 nm for chlorophyll *a*, 647 nm for chlorophyll *b*, 537 nm for anthocyanin and 470 nm for carotenoids). All of the readings were executed with a BEL Photonics®, SP 2000 UV/vis spectrophotometer. Then, the absorbance results were converted to mg 100 g⁻¹ of fresh mass.

Total flavonoids

So the total flavonoids content could be assessed; the extracts were prepared according to the method described by Popova et al. (2004), slightly altered. The extraction was performed through an acidified methanol solution (85:15 of 70% methanol and 10% acetic acid) and later, by adding a 5% aluminum chloride solution. With a spectrophotometer (BEL Photonics®, SP 2000 UV/vis) an absorbance at 425 nm was measured. The total flavonoids content was then calculated by means of quercetin standard curve and the results presented as mg 100 g⁻¹ quercetin equivalent of fresh mass.

Total phenolics content

The grapes' total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi Jr, 1965). The absorbance value at 765 nm was obtained in tests on a UV-Vis SP 2000 BEL Photonics® spectrometer and it was compared with a calibration curve obtained for gallic acid. The results were expressed as mg 100⁻¹ gallic acid equivalents (GAE).

In vitro antioxidant activity

The grapes' *in vitro* antioxidant activity was determined using the DPPH radical scavenging method (Brand-Williams et al., 1995; Rossetto et al., 2009). The analytical standard Trolox was used to build the calibration curves and the results were expressed as mg of Trolox equivalents per g of grape (mg TEAC g⁻¹). Absorbance measurements were performed on a UV-Vis SP 2000 BEL Photonics® spectrometer and the grapes' antioxidant activity was assessed through the decay rate in absorbance at 517 nm. The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) solution was prepared in 80% methanol. For each sample, the absorbance was determined at t = 60 min after adding DPPH radical.

Experimental design and statistical analyzes

The experimental design was a randomized block with 16 treatments for red grapes and 8 treatments for white grapes, both with four replications. All analyses were performed in triplicate. Red and white grapes were assessed separately. The data were subjected to variance analysis, and when this assessment indicated statistically significant treatment effects, the data were subjected to the Scott-Knott means comparison test ($p < 0.05$), aided by the SISVAR statistical program (Ferreira, 2011). The correlation analysis was also conducted ($p < 0.05$ and $p < 0.01$) to investigate the relation between the total phenolic compounds content and antioxidant activity, aided by the ASSISTAT statistical program (Silva and Azevedo, 2002).

RESULTS

The total carotenoid content among red grapes was higher for the BRS Violeta cultivar in 106-8 Mgt rootstocks, followed by the BRS Violeta cultivar in IAC 766 rootstocks, with respective results of 7.59 and 6.48 mg 100 g⁻¹ (Table 2). Regarding the Cabernet Sauvignon

Table 2. Pigments, total phenolic compounds and antioxidant activity of red grapes on different rootstocks.

Rootstock cultivar	Total carotenoids (mg 100 ⁻¹ g)	Total anthocyanin (mg 100 ⁻¹ g)	Total flavonoids ¹ (mg 100 ⁻¹ g)	Total phenolics ² (mg 100 ⁻¹ g)	Antioxidant activity ³ (mg g ⁻¹)
IAC 766 Rootstock					
Isabel	0.62±0.05 ^d	3.63±0.35 ^e	11.15±1.14 ^d	294.3±31 ^d	2.13±0.20 ^d
Bordô	0.95±0.10 ^d	6.00±0.85 ^e	19.51±2.36 ^b	545.2±34 ^b	5.60±0.71 ^b
Cabernet Sauvignon	1.37±0.32 ^c	8.72±2.16 ^d	12.87±1.36 ^d	501.7±38 ^b	4.91±0.88 ^c
Cabernet Franc	1.96±0.26 ^c	12.92±2.00 ^c	17.55±3.60 ^c	481.2±99 ^b	4.77±0.57 ^c
Merlot	1.13±0.03 ^c	7.25±0.27 ^e	15.09±1.69 ^c	386.9±43 ^c	4.03±0.22 ^c
Syrah	0.65±0.09 ^d	3.79±0.68 ^e	12.51±1.59 ^d	414.1±91 ^c	4.28±0.99 ^c
IAC 138-22 Máximo	1.69±0.45 ^c	13.88±2.31 ^c	26.93±6.64 ^a	659.6±99 ^a	6.44±1.50 ^a
BRS Violeta	6.48±0.87 ^b	45.80±5.49 ^b	27.08±2.26 ^a	714.3±183 ^a	7.61±1.12 ^a
106-8 Mgt Rootstock					
Isabel	0.53±0.08 ^d	2.41±0.24 ^e	9.58±2.35 ^d	287.7±41 ^d	2.11±0.50 ^d
Bordô	1.00±0.08 ^d	6.19±0.49 ^e	20.31±2.19 ^b	523.0±64 ^b	5.38±0.74 ^b
Cabernet Sauvignon	0.47±0.03 ^d	2.54±0.17 ^e	10.41±1.51 ^d	474.4±77 ^b	4.76±0.49 ^c
Cabernet Franc	1.28±0.27 ^c	6.44±0.78 ^e	14.51±4.89 ^c	438.4±46 ^b	5.18±0.31 ^b
Merlot	0.74±0.26 ^d	4.28±1.79 ^e	11.17±2.84 ^d	419.0±66 ^c	3.84±0.73 ^c
Syrah	0.69±0.05 ^d	3.84±0.35 ^e	10.50±0.86 ^d	398.0±56 ^c	3.47±0.89 ^c
IAC 138-22 Máximo	1.43±0.06 ^c	8.81±0.38 ^d	21.62±5.35 ^b	669.9±87 ^a	6.63±1.15 ^a
BRS Violeta	7.59±1.34 ^a	54.12±9.96 ^a	28.20±2.55 ^a	722.3±119 ^a	7.41±1.09 ^a
CV (%)	23.69	24.78	18.96	17.20	16.85

Means values ± standard deviation followed by different letters in the same column differ significantly (Scott-Knott test, $p < 0.05$). ¹Total flavonoids expressed as mg 100⁻¹ g equivalent to quercetin. ²Total phenolics measured with Folin-Ciocalteu expressed as mg 100⁻¹ g equivalent to gallic acid. ³Antioxidant activity expressed mg equivalents of Trolox g⁻¹.

and Merlot cultivars, the total carotenoid content of grapes grafted in IAC 766 rootstocks was higher than the ones obtained with the same cultivars in 106-8 Mgt rootstocks.

As well as the results regarding the total carotenoid content, the highest, the highest total anthocyanin content was found in BRS Violeta grapes grafted in 106-8 Mgt rootstocks (54.12 mg 100⁻¹), higher than the one obtained by the same cultivar grafted in IAC 766 rootstocks (45.80 mg 100⁻¹). However, the highest total anthocyanin content in Cabernet Sauvignon, Cabernet Franc and IAC 138-22 Máximo was obtained when these grapevines were grafted in IAC 766 rootstocks.

IAC 138-22 Máximo grafted in IAC 766 rootstock and BRS Violeta in both rootstocks, presented the highest total flavonoid contents (27.40 mg 100 g⁻¹). The total flavonoid content obtained from IAC 138-22 Máximo and Merlot grapes in IAC 766 rootstocks was higher than the ones found in the same grapes grafted in 106-8 Mgt rootstocks. There were no rootstock effects over the total phenolic compound content in red grapes. The highest content was found in BRS Violeta and IAC 138-22 Máximo grapes (718.3 and 664.8 mg 100⁻¹ g, respectively) and these results are 2.38 times higher than the ones obtained from Isabel cultivars (291.0 mg 100⁻¹

g), which presented the lowest total phenolic compound content among the red grapes. Similar to the total phenolic compound content, the highest antioxidant activity among red grapes was found in BRS Violeta and IAC 138-22 Máximo cultivars in both rootstocks studied, 7.51 and 6.54 mg g⁻¹, respectively, and the lowest antioxidant activity was obtained from Isabel cultivars (2.12 mg g⁻¹).

The rootstocks effect over white grapes is shown on Table 3. In both rootstocks studied, grapes from Sauvignon Blanc cultivars presented the highest total chlorophyll content and the lowest total carotenoids content, 424.8 and 0.15 mg 100⁻¹ g, respectively. There were no rootstocks effect over the total flavonoid content in white grapes and the highest contents were found in IAC 116-31 Rainha and IAC 21-14 Madalena cultivars (3.79 and 3.38 mg 100⁻¹ g, respectively).

Grapes from IAC 116-31 Rainha cultivars grafted in 106-8 Mgt rootstocks presented the highest total phenolic compound content (511.8 mg 100⁻¹ g) and the highest antioxidant activity (5.33 mg g⁻¹), superior to those obtained from the fruits of the same cultivar when grafted in IAC 766 rootstocks. On the other hand, Sauvignon Blanc cultivars grafted in IAC 766 rootstocks and BRS Lorena cultivars grafted in both rootstocks had the lowest

Table 3. Pigments, total phenolic compounds and antioxidant activity of white grapes on different rootstocks.

Rootstock cultivar	Total carotenoids (mg 100 ⁻¹ g)	Total chlorophyll (mg 100 ⁻¹ g)	Total flavonoids ¹ (mg 100 ⁻¹ g)	Total phenolics ² (mg 100 ⁻¹ g)	Antioxidant activity ³ (mg g ⁻¹)
IAC 766 Rootstock					
Sauvignon Blanc	0.14±0.01 ^b	407.7±52 ^a	1.53±0.12 ^b	236.1±32 ^d	1.54±0.31 ^c
IAC 116-31 Rainha	0.12±0.01 ^c	191.8±25 ^c	4.08±0.68 ^a	444.9±18 ^b	4.37±0.46 ^b
IAC 21-14 Madalena	0.09±0.01 ^c	241.7±18 ^b	3.13±0.58 ^a	368.3±46 ^c	3.62±0.09 ^b
BRS Lorena	0.10±0.01 ^c	223.8±16 ^c	2.20±0.50 ^b	271.8±27 ^d	2.21±0.78 ^c
106-8 Mgt Rootstock					
Sauvignon Blanc	0.16±0.01 ^a	441.8±72 ^a	2.15±0.47 ^b	320.6±65 ^c	2.12±0.47 ^c
IAC 116-31 Rainha	0.10±0.01 ^c	202.1±30 ^c	3.49±0.65 ^a	511.8±76 ^a	5.33±0.71 ^a
IAC 21-14 Madalena	0.11±0.02 ^c	273.1±58 ^b	3.63±0.89 ^a	426.9±41 ^b	3.68±0.60 ^b
BRS Lorena	0.12±0.00 ^c	266.5±26 ^b	1.79±0.66 ^b	293.5±10 ^d	2.17±0.17 ^c
CV (%)	9.62	14.46	21.08	13.05	16.32

Means values ± standard deviation followed by different letters in the same column differ significantly (Scott-Knott test, $p < 0.05$). ¹Total flavonoids expressed as mg 100⁻¹ g equivalent to quercetin. ²Total phenolics measured with Folin-Ciocalteu expressed as mg 100⁻¹ g equivalent to gallic acid. ³Antioxidant activity expressed equivalents of Trolox g⁻¹.

Table 4. Pearson's correlation analysis between quantification of phenolic compounds and antioxidant activity of red and white grape cultivars measured by DPPH method.

Cultivars	Antioxidant activity (DPPH)	
	Pearson r	p -Values
Red		
Total anthocyanin	0.63	$p < 0.01$
Total carotenoids	0.62	$p < 0.01$
Total flavonoids	0.79	$p < 0.01$
Total phenolics	0.84	$p < 0.01$
White		
Total chlorophyll	-0.63	$p < 0.01$
Total carotenoids	-0.46	$p < 0.01$
Total flavonoids	0.76	$p < 0.01$
Total phenolics	0.91	$p < 0.01$

$p < 0.01$, Significant correlations at 1% probability of error.

total phenolic compound contents and regardless of the studied rootstock, these cultivars also presented the lowest antioxidant activities among white grapes.

There was significant correlation among all of the assessed compounds and the antioxidant activity (Table 4). The total phenolic compound contents had the highest correlation rates regarding the antioxidant activity, both in red grapes ($r = 0.84$, $p < 0.01$) and white grapes alike ($r = 0.91$, $p < 0.01$). Only in white grapes there was a negative correlation between the total chlorophyll content ($r = -0.63$, $p < 0.01$) and total carotenoids ($r = -0.46$, $p < 0.01$) regarding the antioxidant activity.

DISCUSSION

Although the total carotenoids content in grapes for wine production was not varied between rootstocks on most cultivars studies, a big difference was found regarding the pigments content both in red (Table 2) and white grapes (Table 3). Despite the importance these pigments exercise over their own by-products, especially on wines, yet studies regarding carotenoid contents in grapes are rare. However, some specific factors, such as grapes cultivar, farming region, the cluster's exposure to sunlight and its maturation period are known to affect carotenoids concentration in grapes (Mendes-Pinto et al., 2005). The carotenoids, along with pyrazines and terpenes, are responsible for wine's primary scent. These pigments' oxidative decay, which occurs while the berry is growing or even during the must's fermentation process, originates norisoprenoids, thirteen-carbon-hydrocarbons that have no immediate influence over wine's sensory characteristics, however, the products from its decay produce volatile compounds important for its aromatic composition (Jackson, 2000; Mendes-Pinto et al., 2005).

The total chlorophyll content found in hybrid grapes was lower than the one found in *V. Vinifera* and Sauvignon Blanc grapes (Table 3). Several factors may be related to the presence and content of such pigments on grapes. The chlorophyll's synthesis and concentration in grape berries is prompted by exposing the cluster to the sun and in the absence of light, chlorophyll's synthesis is then decreased (Downey et al., 2004). The chlorophyll's content in these fruits may still vary due to the characteristics of each different cultivar, the vineyard's environmental condition and the farming practices applied to the grapevines (Kamffer et al., 2010).

Considering that there was a negative correlation between the total chlorophyll content and the antioxidant activity ($r = -0.63$, $p < 0.01$) in white grapes (Table 4), it's possible to infer that the high content of this pigment in these grapes is unwanted. In this study, the highest total chlorophyll content in grapes from Sauvignon Blanc cultivars was found to present fewer antioxidant activities, along with BRS Lorena.

Total anthocyanin content in red grapes suffered a considerable variation among the cultivars of crops studied (Table 2). Among the cultivars, there was no effect from the rootstocks over the anthocyanin content in Isabel, Bordô, Merlot and Syrah cultivars. A Bordô cultivar grafted in both 'IAC 766' and '106-8 Mgt' rootstocks in Caldas (Minas Gerais) has not presented significant difference regarding the anthocyanin content, however these pigments content (9.86 mg g^{-1} in IAC 766 and 10.47 mg g^{-1} in 106-8 Mgt) were higher comparing to the ones found in our study, it was likely due to the fact that our data reflect the contents in the whole fruit, whilst the aforementioned authors had assessed the presence of anthocyanin only in peels, where this compound's concentration is high.

The difference between rootstocks regarding total anthocyanin content in Cabernet Sauvignon grapes, grafted in 'IAC 766' rootstocks, differs from other studies performed in different regions (Table 2). In these studies, different rootstocks have not been the cause of any alterations on anthocyanin content and other secondary metabolites within the same cultivar. In Davis (USA) there was no effect from the rootstocks 420A (*Vitis berlandieri* × *V. riparia*) and 110R (*V. berlandieri* × *V. rupestris*) over the total anthocyanin content in Cabernet Sauvignon grapes (Lee and Steenwerth, 2013). Likewise, the total anthocyanin content found in the same cultivar grown in Larissa (Greece) did not differ because of the rootstocks 1103 Paulsen (*V. rupestris* × *V. berlandieri*) and SO4 (*V. riparia* × *V. berlandieri*) (Koundouras et al., 2009). In the assessment of the results, it should be noted that different rootstocks was used and the results are likely related to these plants' inner characteristics, to its vigor, in addition to environmental conditions in the harvest place.

The high anthocyanin content found in BRS Violeta grapes grafted in both rootstocks, IAC 766 (45.80 mg g^{-1}) and 106-8 Mgt (54.12 mg g^{-1}), is related to an innate characteristic from the cultivar, which is also described by other authors both in fruits (Barcia et al., 2014), and in wines and juices made from these fruits (Lago-Vanzela et al., 2014; Lima et al., 2014). BRS Violeta grapes are covered by a very thick skin, which contains the highest concentration of phenolic compounds, mainly anthocyanin, predominantly (major compound) anthocyanidin 3,5-diglucoside (Rebello et al., 2013).

Total flavonoid contents have varied among the cultivars, with a higher content among red grapes. This result had already been described in other studies stating

that total flavonoid content in Cabernet Sauvignon and Merlot grapes are higher than the ones found in Sauvignon Blanc grapes (Burin et al., 2014). These authors observed, however, that although it's a white grape cultivar of crop, total flavonoid content found in these grapes was higher than the ones from red grapes cultivars, Isabel and Bordô, differing from our results. It is possible that the anthocyanin contents taken from grapes in the present study have affected positively the high total flavonoid rates found through spectrophotometry, making it necessary for the analyses in successive cycles or even so the use of more sophisticated techniques in order to confirm the results obtained. Significant variations on total flavonoid levels might be attributed to several factors, such as genetic, climate, vineyard handling, grape maturation level, berry size (Rockenbach et al., 2011), extraction method and these compounds assessment alike. The species, cultivar and part of the grape (peel, pulp or seed) assessed yet affects the presence and quantity of grapes main flavonoids (Burin et al., 2014; Rockenbach et al., 2011). It is important to notice that flavonols are the best cofactors for a wine's anthocyanin copigmentation and that the higher the grape's flavonoids content is, the higher is also the amount of anthocyanin transferred into the wine during the wine production process (Schwarz et al., 2005).

There was no effect from rootstocks over total phenolic compounds of red and white grapes. Usually, different rootstocks effects on grapes' phenolic compounds content are likely related to several factors, such as vigor, water and nutrients absorption capability, resistance to diseases and their interaction with the grapevine, possibly affecting directly these plants' primary and secondary metabolites and, consequently, the grapevines' productive characteristics and quality of the grapes (Lee and Steenwerth, 2013; Tecchio et al., 2014). The fact that rootstocks have no effect on the total phenols content in Bordô grapes has already been described in previous studies. When grown in Caldas (Minas Gerais, Brazil), this cultivar's total phenolic compounds content has not suffered any effects from 'IAC 766' and '106-8 Mgt' rootstocks, presenting a total phenols average rate of 11.65 and 12.58 mg g^{-1} in their peels, respectively (Mota et al., 2009). These authors observed that the contents were higher than the ones found on other studied rootstocks, such as 'IAC 572' and 'RR 101-14' and they came to the conclusion that rootstocks of less vigor prompt a higher concentration of phenolic compounds in grapes' skins.

As the rootstocks 'IAC 766' and '106-8 Mgt' have not affected Cabernet Sauvignon cultivar's total phenols content, other researches demonstrate that some rootstocks have no effects over these grapes' total phenols content. When the 'SO4' and '1103 Paulsen' rootstocks were used in Greece (Koundouras et al., 2009) it was observed that the total phenols content did not differ significantly due to the studied rootstocks

contents of 26.9 mg g^{-1} were found in the grape's skin, in both rootstocks. These results are higher than the ones found in the present study along the same cultivars, however, it must be noted that analysis were performed from the grape's skin, where these compounds highest concentrations are found, whilst in the current study both skin and pulp were assessed.

Among all the cultivars studied, the highest total phenol content and antioxidant activity were found in BRS Violeta and IAC 138-22 Máximo grapes. Although determining phenol compounds and antioxidant activity in grapes is important for producers in order to decide which cultivar to choose or even a basis for genetic improvement projects; most studies in literature is aimed at the analysing these compounds and their by-products, especially juices and wines. BRS Violeta cultivars have been widely researched in several Brazilian grape producing regions for industrial purposes due to, among other reasons, the high phenolic compounds content and antioxidant activity presented by this cultivar (Lago-Vanzela et al., 2014; Lima et al., 2014; Rebello et al., 2013), however, few studies are available regarding these compounds content in IAC 138-22 Máximo grapes, which presented results close to those found for BRS Violeta.

High total phenolic compounds content was also found in IAC 116-31 Rainha ($511.8 \text{ mg } 100^{-1}$) and IAC 21-14 Madalena ($426.9 \text{ mg } 100^{-1}$) cultivars, when grafted in 106-8 Mgt rootstocks. Consequently, these grapes antioxidant activity was high (5.33 and 3.68 mg g^{-1} , respectively). Even though these grapes are white, the phenolic compounds content, as well as the antioxidant activity found in these cultivars were higher than those from Isabel red grapes, which contain $291 \text{ mg } 100^{-1}$ of total phenols and 2.12 mg g^{-1} of antioxidant activity taken via DPPH. Isabel grapes are one of the main Brazilian grapes for juice and wine production, and many other studies present these compounds have low content in the fruits, as well as in juices (Burin et al., 2014; Nixford and Hermosín-Gutiérrez, 2010). In spite of the results found and the possibility of producing white wines with high total phenol content and antioxidant activity equivalent or superior to those of red wines, studies involving IAC 116-31 Rainha and IAC 21-14 Madalena grapes are limited in literature.

Regarding antioxidant activities, many studies have reported difficulties in obtaining similar data among grapes, making it difficult to compare results, due to factors such as the use of different analytic methods (DPPH, ABTS, FRAP, ORAC, among others), norms and unities of measurement or even due to the differences in reference materials taken from grapes (the whole grape; skin, pulp or seeds individually, bagasse) (González-Centeno et al., 2013; Lago-Vanzela et al., 2011).

Besides total phenolic compounds ($r = 0.84$, $p < 0.01$), other compounds found in red grapes such as total anthocyanin ($r = 0.63$, $p < 0.01$), total carotenoids ($r =$

0.62 , $p < 0.01$) and total flavonoids ($r = 0.79$, $p < 0.01$) present positive correlation to antioxidant activity. This is in accordance with many authors who have also observed positive correlation between total phenols and antioxidant activity in red grapes (Burin et al., 2014; Rockenbach et al., 2011; Xu et al., 2010). On the other hand, a few other studies demonstrated no correlations while others presented a negative correlation between these variables (Nixford and Hermosín-Gutiérrez, 2010), which means variation is due to different cultivars, rootstocks and farming sites. Among white grapes, the antioxidant activity is correlated positively to flavonoids ($r = 0.76$, $p < 0.01$) and, mainly to total phenols ($r = 0.91$, $p < 0.01$). A positive correlation between phenol contents and antioxidant activity has also been demonstrated by González-Centeno et al. (2013) in white grapes Chardonnay, Macabeu, Parellada and Premsal Blanc; all the *V. vinifera* were grown along the Balearic Islands, Spain.

Conclusions

In conclusion, the farming of red grapes Cabernet Sauvignon, Cabernet Franc and IAC 138-22 Máximo while using IAC 766 rootstocks has higher anthocyanin content. BRS Violeta grapes show a higher concentration of these pigments when 106-8 Mgt rootstocks are used. Neither the total phenols, nor the antioxidant activity, were influenced by the rootstocks, especially in BRS Violeta and IAC 138-22 Máximo grapes, which presented the highest contents of these compounds using both studied rootstocks. Among white grapes, however, Sauvignon Blanc, IAC 116-31 Rainha and IAC 21-14 Madalena grapes farmed over 106-8 Mgt rootstocks presented higher total phenol levels. Despite being white, hybrid grapes in fruits from IAC 116-31 Rainha, Madalena and IAC 21-14 cultivars, these compounds content, as well as the antioxidant activity, were superior to those found in red grapes from Isabel cultivars.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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