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Estudo do perfil de compostos bioativos da cultivar de uva Isabel precoce (*Vitis labrusca* l) cultivado no cerrado brasileira

Study of the profile of bioactive compounds of the early Isabel grape cultivar (*Vitis labrusca* l) cultiated in the brazilian savanna

Estudio del perfil de compuestos bioactivos de cultivar uva Isabel prematura (*Vitis labrusca* l) cultivado en el cerrado brasileño

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Resumo

O objetivo do presente estudo foi avaliar o comportamento dos compostos bioativos durante o desenvolvimento fisiológico da cultivar Isabel precoce (*Vitis labrusca L*), cultivada no cerrado brasileiro, com vistas a aplicações futuras. Trata-se de um estudo quantitativo realizado em campo e em laboratório. A caracterização físico-química foi realizada com compostos fenólicos totais, atividade antioxidante e perfil dos compostos fenólicos por HPLC. As transformações físicas e químicas dos frutos foram muito mais intensas a partir dos 60 DAA. As sementes apresentaram alto potencial antioxidante nos dois métodos analisados. Três foram identificados compostos fenólicos, estando presente o ácido clorogênico nas bagas, peles e polpas, sendo a catequina presente nas maiores quantidades, seguida pela epicatequina, presente nas sementes. Esse conhecimento é importante para futuras extrações e aplicações em alimentos e cosméticos.

Palavras chave: Antese; Uva; Caracterização; HPLC; Isabel precoce.

Abstract

The objective of the present study was to evaluate the behavior of the bioactive compounds during the physiological development of the early Isabel grape cultivar (*Vitis labrusca* L), cultivated in the Brazilian savanna, with a view to future applications. This is a quantitative study carried out in the field and in the laboratory.The physical and chemical characterization was performed total phenolic compounds, antioxidant activity and the profile of the phenolic compounds by HPLC. The physical and chemical transformations of the berries were much more intense as from 60 DAA. The seeds presented high antioxidant potential in two analyzed methods. Three were phenolic compounds identified, chlorogenic acid being present in the berries, skins and pulps, catechin being present in the largest amounts, followed by

epicatechin, present in the seeds. Such knowledge is important for future extractions and applications in foods and cosmetics.

Keywords: Anthesis grape, Characterization; HPLC; Early isabel.

Resumen

El objetivo del presente estudio fue evaluar el comportamiento de los compuestos bioactivos durante el desarrollo fisiológico del cultivar Isabel prematura (*Vitis labrusca L*), cultivado en el cerrado brasileño, con vistas a futuras aplicaciones. Este es un estudio cuantitativo realizado en el campo y en el laboratorio. La caracterización fisicoquímica se realizó con compuestos fenólicos totales, actividad antioxidante y perfil de los compuestos fenólicos por HPLC. Las transformaciones físicas y químicas de los frutos fueron mucho más intensas después de 60 DAA. Las semillas mostraron un alto potencial antioxidante en los dos métodos analizados. Se identificaron tres compuestos fenólicos, con ácido clorogénico presente en las bayas, pieles y pulpas, con catequina presente en las mayores cantidades, seguido de epicatequina, presente en las semillas. Este conocimiento es importante para futuras extracciones y aplicaciones en alimentos y cosméticos.

Palabras clave: Antesis; Uva; Caracterización; HPLC; Isabel prematura.

1. Introduction

In Brazil grape production is growing, not only in the southern and northeastern regions, but also in other regions such as the center-west. The center-west region is part of the Brazilian Savanna biome having a tropical seasonal climate, and with the use of irrigation two harvests can be obtained per year. Seasonal climatic variations can define phenological changes in the plants (Aubert; Chalot, 2018; Almeida, Ferri, Seraphin & Moraes, 2017; Coelho et al., 2017).

The main varieties cultivated in the state of Goiás are: white and pink Niagara (*Vitis labrusca* L.), early Isabel (*Vitis labrusca* L.), Italian (*Vitis vinifera* L.), Ruby, Thompson (*Vitis vinifera* L.), Bordeaux (*Vitis labrusca* L.) and Muscat (*Vitis vinifera* L.) (Monteiro, 2010). The early Isabel cultivar (*Vitis Labrusca* L.) originated from a spontaneous somatic mutation of the variety Isabel (*Vitis labrusca* L.), showing similar characteristics to those of the original cultivar but anticipating its ripening by up to 35 days (Camargo, 2004).

The grape and its byproducts are sources of polyphenol compounds, which exert antioxidant functions. An increasing number of studies have been reported in recent decades

related to the consumption of bioactive compounds and their possible beneficial effects on health, such as the prevention of various chronic pathologies including cardiovascular diseases, diabetes, neurodegenerative diseases and some types of cancer, and also cardioprotective and vasodilatory effects, amongst others (Acuña-Avila, Vasquez-Murrieta, Franco & López-Cortez, 2016; Ribeiro, Ribani, Francisco, Soares, Pontarolo, & Haminiuk, 2015).

The phenolic compounds are the most important secondary metabolites in grapes and are present in the berries and in varying amounts in the skin, pulp and seeds. The concentration increases during ripening, the influence depending on the cultivar, geographic location, climate, soil and principally the viticulture practices (Carrera, Rodriguez, Palma & Barroso, 2012; Lima et al., 2014).

Thus, the objective of this study was to evaluate the behavior of the phenolic compounds and their antioxidant activity during the physiological development of the early Isabel cultivar (*Vitis labrusca* L.) cultivated in the Brazilian Savanna.

2. Material and methods

2.1 Obtaining the grape samples

The experiment was carried out during the 2017 harvest at the Goiás winery in the municipality of Itaberai (GO).Plants of the early Isabel cultivar (*Vitis labrusca*) were used. At anthesis 200 plants were selected at random and marked. The first fruit collection was 10 days after anthesis (DAA) and then at 10-day intervals up to 90 DAA, when the fruits reached the optimum harvesting point.

Each collection was divided into two batches, the berries in the first batch being kept whole and *in natura*, the analyses being carried out after each collection. In the case of the second batch, the unripe berries were maintained whole (10-50 DAA) and during the ripening phase (60-90 DAA) they were manually fractioned into skin, pulp and seed.

The fruits in the first batch were evaluated whole (berries) and *in natura* up to harvest (90 DAA), carrying out the analyses of mass, transversal and longitudinal diameters, pH, titratable acidity and soluble solids.

The fruits from the second batches were quick-frozen in liquid nitrogen after each collection, stored at -18ºC, freeze-dried (Liotop, L101, Brazil) and ground (IKA, A11 Basic, China) in order to prepare the extracts used to determine the total phenolic compound

contents, antioxidant potential (reduction of the DPPH* (2,2-diphenol-1-picryl-hydrazil) radical, capture of the free $ABTS^{+}$ radical, inhibition of the formation of the phosphomolybdenum complex) and the phenolic compound profile.

2.2 Methods

It is a quantitative study, in which part was carried out in field(collection of fruits and preparation) and part in the laboratory(preparation of the samples the grape, determination of compounds and data analysis) (Pereira et al., 2018).

2.2.1 Obtaining the extracts

The total phenolic compound contents and antioxidant activities were determined as from four extracts: ether extract (EE), alcoholic extract (ALE), aqueous extract (AQE) and acetone + methanol extract (AME) and analyzed according to the methodology used.

The ether (EE), alcoholic (AE) and aqueous (AQE) extracts were prepared sequentially according to the methodology of Borguini (2006).To obtain the acetone + methanol extract (AME) (Rufino et al., 2007), a 2.5 g sample (berry, skin, pulp and seed).

2.2.2 Physical and chemical characterizations

2.2.2.1 Physical & chemical characterization

The mass of the berries was determined using an analytical balance (Marte, Shimadzu, AY-220, Japan) and the results expressed in grams (g). The fruits were measured using a digital pachymeter (0-150 mm Lotus Plus, China) the results expressed in millimeters (mm). Evaluated with fifteen repetitions. The absolute and relative growth rates of the mass (g/day;g/g.day) and the longitudinal and transversal diameters (mm/day; nn/day) were calculated using formulas 1 and 2:

$$AGR = \frac{(V1-V0)}{(T1-T0)} \quad (1)$$

where AGR = the Absolute growth rate; V = values of the parameters evaluated; V_0 = initial value; V_1 = final value; T = time of evaluation (days after anthesis); T_0 = initial time; T_1= final time

$$RGR = \frac{lnV1 - lnV0}{T1 - T0} \quad (2)$$

where RGR = the Relative growth rate; V = values of the parameters evaluated; V_0 = initial value; V_1 = final value; T = time of evaluation (days after anthesis); T_0 = initial time; T_1 = final time; *Ln* = Negative logarithm

The pH was determined using a pH meter (model HI-9224, Steinham, Germany). The soluble solids content (SS) was determined using a digital refractometer (Reichert, AR200, USA), with the results expressed in ºBrix. All analyses were carried out according to the Association of Official Agricultural Chemists - AOAC (2010) and evaluated with 15 repetitions. The total titratable acidity (TTA) was determined by titration with 0.1N NaOH and expressed as g tartaric acid per 100 mL.

The color was determined at three distinct points on the grape skins (15 repetitions) using a colorimeter (Hunterlab, ColorQuest II) in the CIE L*a* b* mode. The values obtained for a* and b* were used to calculate the color index Chroma (C*) and the total color difference delta E (ΔE) with the aid of equations 3 and 4.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

$$\Delta E = \frac{[\Delta L^2 + \Delta a^2 + \Delta b^2\ ]}{1/2} \quad (4)$$

2.2.2.2. Total phenolic compounds

The total phenolic compound content was determined for the EE, ALE, AQE and AME using the Folin Ciocalteau reagent, taking the readings at 700 nm in a spectrophotometer (Biospectro SP-220). A standard curve was prepared in the range from 5 to 50 mg L^{-1} and the results expressed in milligrams of gallic acid equivalents (GAE) per 100 grams (Zielinski & Kozlowska, 2000).

2.2.2.3. Determination of the antioxidant activity

Three assays were used to evaluate the antioxidant activity: DPPH (2,2 diphenyl -1-picrylhydrazil), ABTS radical (2,2' azinobis- (3- ethylbenzthiazoline-6-sulfonic acid) and phosphomolybdenum (Borguini, 2006; Rufino et al.,2007; Prieto, Pineda & Aguilar, 1999). All the assays were carried out in triplicate.

DPPH: The DPPH method was evaluated with respect to the antioxidant activity of the sample by way of the de-staining rate at 517 nm measured spectrophotometrically for the EE, ALE and AQE with a concentration of 0.2 $mg.mL^{-1}$, and the results expressed in MIC_{50} (mean inhibitory concentration of 50% of the total activity). The spectrophotometric readings were taken (Biospectro SP-220) after 30 min of reaction time, and the calculations done with the aid of equation 5:

$$\%\ destaining\ of\ DPPH = (1 - \left(\frac{Abs\ extra - Abs\ blank}{Abs\ control}\right) * 100 \quad (5)$$

ABTS: The antioxidant potential of the ABTS radical was determined from the rate of fall of the absorbance at 734 nm (Biospectro SP-220) in the EE, ALE, AQU and AME. The standard curve was linear between 100 and 1500 μM Trolox and the results were expressed in μM Trolox/g.

Phosphomolybdenum: The antioxidant activity was measured spectrophotometrically at 695 nm in a plate reader (Perkin Elmer, Enspire, Brazil) for the ALE and AQE. The standard curve was linear between 0.025 and 0.2 mg/mL of ascorbic acid and the results were expressed in milligrams ascorbic acid equivalents per gram (mg AAE/g).

2.2.2.4. Obtaining the extracts for the analysis of the phenolic compound profiles by HPLC

In order to analyze the phenolic compound profiles, the extracts were prepared according to the methodology proposed by Gómez-Alonso, Garcia-Romero and Hermosín-Gutiérrez (2007). Aliquots of 20 g were used for the berries, skin and pulp and 2 g for the seeds, being dissolved in 150 mL of a 50:48.5:1.5 (v/v) solution of methanol/water/formic acid by homogenizing in a shaker (Eppendorf, Innova 44, Brazil) at 120 rpm and 27.1ºC for 15 hours and then centrifuging (HERMLE, Z326K, Germany) at 2500 g and 5ºC for 15 minutes. The supernatant was filtered through a 0.45 μm nylon membrane and injected into vials (2 mL) for reading using the liquid chromatograph.

2.2.2.5. Determination of the phenolic compounds

The chromatographic profile was obtained by HPLC (Leite et al., 2014). The injection volume for the samples and standards was 10.0 µL and an Elite® LaChrom system was used for the analysis, equipped with a L2200 automatic injector, L2130 pump, L2300 column at 25ºC, and a L2455 diode array detector (DAD) (Hitachi®, Tokyo, Japan). The sample components were separated on a C-18 reverse phase column (5 µm particles, 150 mm x 4.6 mm) coupled to an appropriate pre-column (4.0 mm x 4.00 mm; 5 µm particles) (Merck®, Germany), and the analysis carried out at fixed wavelengths of 280, 354 and 510 nm.

The mobile phase consisted of a gradient system composed of 1% phosphoric acid (v/v) (channel A) and acetonitrile (channel B) with a flow rate of 0.6 mL/min., and the data were retained by the Elite® ExChrom software (SPI version 3.3.2) (Scientific Software Inc.). The compounds present in the samples were compared with commercial phenolic compound samples according to their UV/visible spectra (230 to 400 nm) and retention times, The following gradient was used for the separation: 0 min: 90% A & 10% B; 40min: 70% A & 30% B; 50min: 50% A & 50% B; 51 min: 90% A & 10% B; 55 min: 90% A & 10% B, where solvent A was a 1% phosphoric acid solution and solution B was acetonitrile.

Each component was quantified by way of a calibration curve prepared with the corresponding standard, using the following compounds for comparison: chlorogenic acid, catechin and epicatechin. The results were expressed in µg/ml and analyzed with 3 repetitions.

2.3. Statistical analyses

A completely random design was used for the experiment and the data of the physicochemical variables (pH, titratable acidity, soluble solids and color) were submitted to a linear regression analysis and to the Scott-Knott means test for significant sources of variation (Scott & Knott, 1974).

A 4x4x9 factorial design was used for the analyses of the total phenolic compounds and antioxidant potential, analyzing four types of extract (EE, ALE, AQE and AME), four types of sample (berry, skin, pulp and seeds) and 9 times (10 to 90 days after anthesis with ten-day intervals).

The data showing the greatest phenolic compound and antioxidant extraction yields were selected for the DPPH, ABTS and Phosphomolybdenum assays. For the extracts used

conforming the methodology in question, that is ethereal (EE), alcoholic (ALE), aqueous (AQE) and methanol with acetone (AME), the greatest compound yield was chosen for each repetition and time (DAA). This resulted in four-column matrixes with variable responses (phenolic compounds, DPPH, ABTS and Phosphomolybdenum) and three repetitions in the lines, with five times for the berries (10, 20, 30, 40 and 50 DAA) and four evaluation times for the skin, pulp and seeds (60, 70, 80 and 90 DAA).

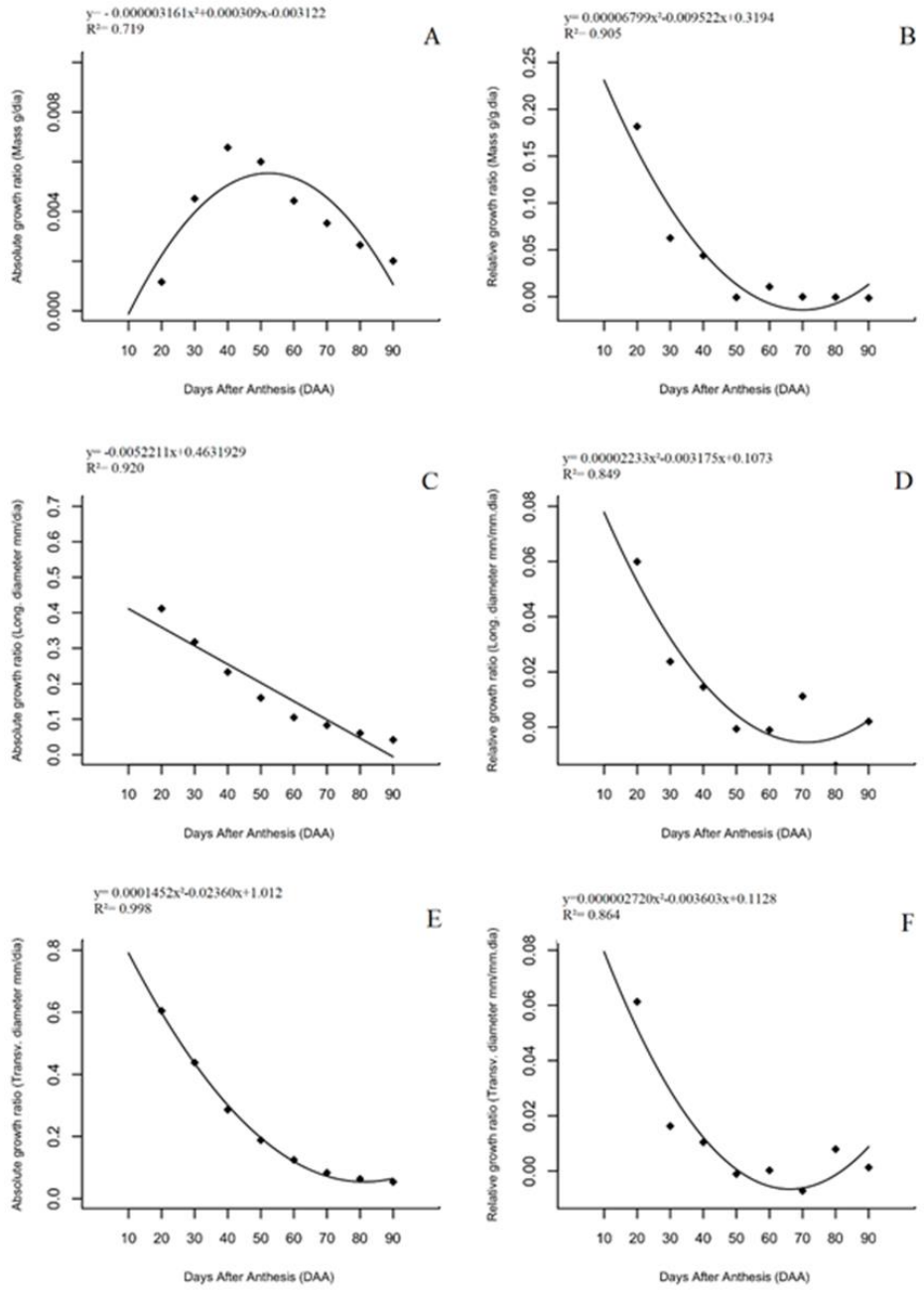
The data for total phenolic compounds and antioxidant potential were submitted to a 4x4x9 factorial variance analysis, using the Scott-Knott test to establish the differences between the means for the total phenolic compounds, antioxidant potential and chromatographic profiles. Tukey's test was used to evaluate the greatest contents with time for the analyses of total phenolic compounds and antioxidant activity during ripening (60 to 90 days after anthesis – DAA). All the statistical analyses were implemented using the R software (R Development Core Team, 2018).

3. Results and discussion

3.1. Physical and chemical characterization

The analyses of variance of the physicochemical analyses showed significant variances in relation to the ripening time.

Figure 1: Absolute growth rate (AGR) and Relative growth rate (RGR) for the mass and longitudinal and transversal diameters.

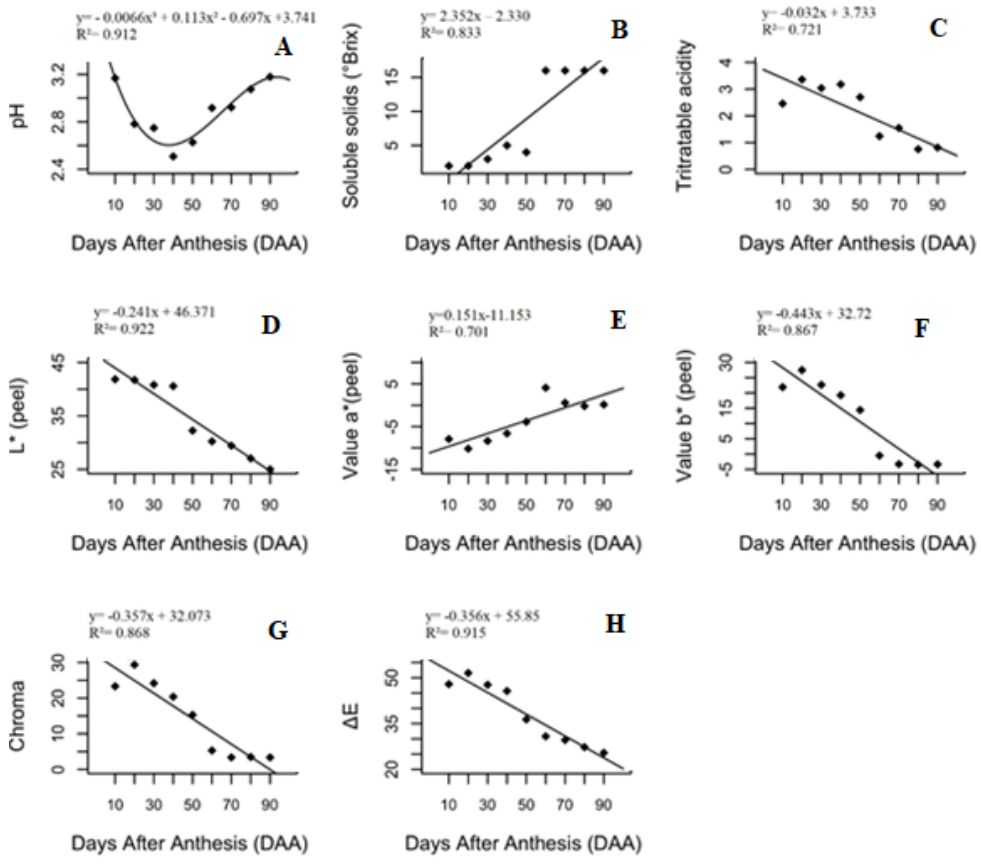


A: Absolute growth rate of the mass; B: Relative growth rate of the mass; C: Absolute growth rate of the longitudinal diameter; D: Relative growth rate of the longitudinal diameter; E: Absolute growth rate of the transversal diameter; F: Relative growth rate of the transversal diameter.Source: Author (2018).

According to Figure 1, the highest values for the AGR and RGR were presented during phase I (10-40 DAA). The AGR and RGR variables of longitudinal and transversal diameters and the RGR variable of mass showed their maximum values 20 DAA

and the RGR variable of mass 40 DAA. During this phase there was an accelerated increase in berry mass and volume, corresponding to cell divisions and expansions, the greater part of seed growth occurring during this phase, which also contributes to growth of the berry. Structural changes were less expressive in phase II, mostly occurring chemical changes which introduce the ripening phase (III), such as, for example, color variations (Figure 2H). Structural changes occurred to a smaller degree in phase III, possibly due to the accumulation of solutes only via the xylem route. In this phase the chemical changes were more intense, such as the degradation of organic acids (Figure 2C), the accumulation of sugars (Figure 2B) and the metabolism of the phenolic compounds. Variations in the RGR were observed during phase III and could be attributed to a lack of uniformity between the fruits (Borghezan, 2017).

Figure 2: Physical and chemical characterization of the grape berries of the early Isabel cultivar during their physiological development.



A: pH; B: Soluble solids; C: Titratable acidity (g tartaric acid/ 100 mL); D: L*; E: value of a*; F: value of b*; G: Chroma; H: Total color difference (ΔE). Source: Author (2018).

The values for pH decreased up to 40 DAA, englobing phase I (Figure 2A), with a significant increase in phase III (ripening), showing the highest value in 90 DAA. The increase in pH during the ripening phase (stage III) could be related to salification of the organic acids in regions with higher temperatures. The increase in pH occurred due to the elevated alkalinity of the salts (ash) and the decline in malic and tartaric acids, since the increase in pH was related to the reduction in acidity (Rizzon, Mielle & Meneguzzo 2000).

The soluble solids content showed a gradual increase during phases I and II up to the start of phase III (60 DAA), as can be seen in Figure 2B, and there was no significant difference throughout the ripening phase (III) up to harvest. The increase in the soluble solids content during ripening could be attributed to displacement of the xylem to the phloem of the berries, which occurred during the veraison phase, thus indicating an increase in non-volatile water-soluble compounds such as sugars and organic acids (Griesser et al., 2018).

The titratable acidity (TA) increased after 20 DAA, with a significant decrease throughout development of the fruit up to harvest (Figure 2C). During the first rapid growth period of the berry (phase I), the acid concentration was higher, since the synthesis of acids resulted in a secondary photosynthesis reaction, being synthesized both in the leaves and berries up to the veraison phase (stage II). As the ripening process started so the concentration of malic acid decreased with a consequent progressive decrease in acidity. Due to the organic acids being among the main substrates of the grape (Rizzon & Saganzerla, 2006).

Kurt, Torun, Colak, Seiler, Hayirloglu-Ayaz and Ayaz, (2017) evaluated the ripening of the cultivar Isabel with a pH value of 3.5 and TA of 0.0098 g of tartaric acid per g. Samoticha et al. (2017) investigated grape varieties, both white and red, and observed mean values for soluble solids of 17.4ºB, titratable acidity of 0.93 g tartaric acid per g and a pH value of 3.94 for the red varieties. Figures 2 D, E and F present the results obtained for the color parameters, with L* and b* showing reductions during development and a* an increase. This behavior became more evident from the end of phase II (50 DAA) and during phase III (60 to 90 DAA), that is, a decline in green color and increase in red or blue, giving the brownish and red/blue coloration to the berries indicating degradation of the chlorophyll and biosynthesis of anthocyanins. The reduction in Luminosity (L*) on ripening could also be attributed to the larger amount of epicuticular wax covering the berries (Olivares et al., 2017).

Such color change behavior could be confirmed by evaluating the saturation (Chroma) and from the color difference (ΔE). The values for chroma and ΔE showed a decrease during phase I (20 DAA) up to the start of phase III, then remaining statistically stable up to harvest (Figure 2H).

The data analyzed conformed with those obtained by Ferreira et al. (2017b), who analyzed four varieties of white and red grapes and found similar behavior for the parameters evaluated (L*, a*, b*, Chroma and ΔE) for the red varieties. Pinillos, Chiamolera,; Ortiz, Hueso & Cuevas (2016) evaluated the Crimson seedless cultivar during two harvests (2012 and 2013) and obtained values for L* of 35.00 and 38.3 and for Chroma of 12.4 and 13.1, respectively.

3.2. Total phenolic compounds and antioxidant activity

The 4x4x9 factorial analysis of variance for the phenolic compounds and antioxidants showed significance for the interactions between the factors: extraction method, grape part (berry, skin, pulp and seed) and the time of evaluation at all levels. This signifies that the best extraction yield was not associated with a specific extract.

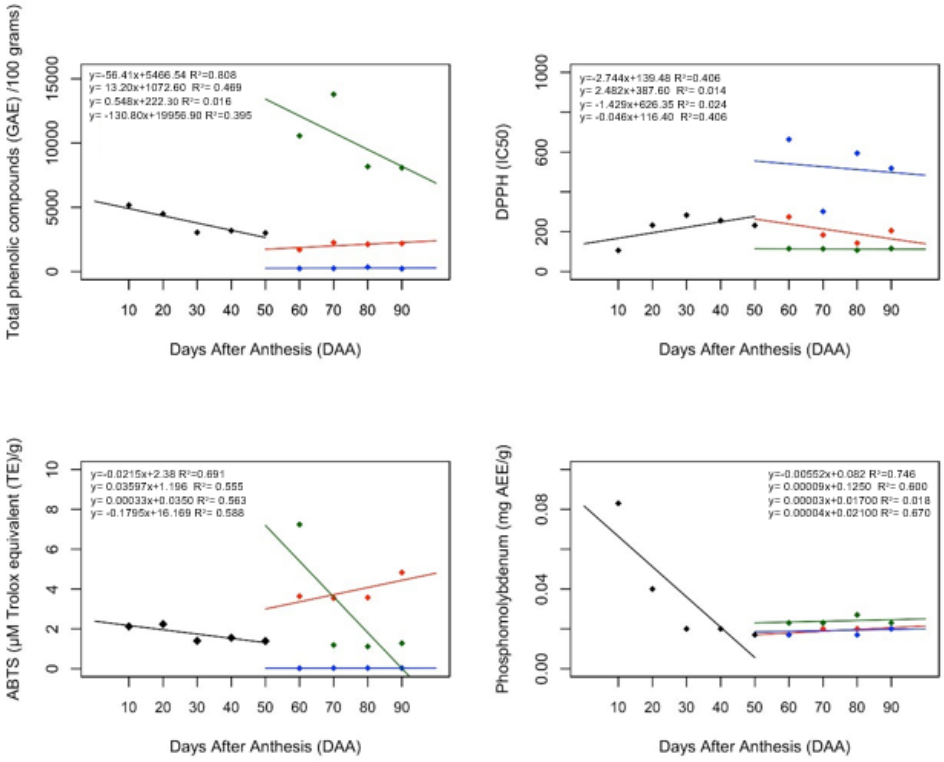
The effect of the extraction method on the phenolic compounds and antioxidants depended on the part of the grape evaluated and the time of development of the fruit. That is, for each part of the grape associated with a determined developmental time, there was a specific more efficient extract for the extraction of each compound.

The means of the various responses obtained from the ethereal (EE), alcoholic (ALE), aqueous (AQE) and methanol + acetone (AME) extractions made during the physiological development of the berries, were evaluated according to the total phenolic compound and antioxidant potential assays, and the extract presenting the highest content at each collection time evaluated, selected.

With respect to the total phenolic compounds, it was shown that during the physiological development of the berries the AME was the extract with the greatest yield with the exception of 80 DAA, when the AQE showed the greatest extraction yield. This variability with time in the extracts with greater extraction yield could be attributed to the fact that they were a group of compounds with great structural diversification since they possess different polarities and solubilities, and could interact with different molecules (Jakobek, 2015).

As shown in Figure 3A, the total phenolic compound content was highest in the seed, followed by the skin and pulp during the whole of phase III (60 to 90 DAA).

Figure 3: Means for the total phenolic compound contents and antioxidant activity (DPPH, ABTS and Phosphomolybdenum) in the berries during physiological development. Different letters signify statistical difference ($p \leq 0.05$) according to Tukey's test. A: total phenolic compounds; B: DPPH (IC_{50} -µg/ml); C: ABTS; D: phosphomolybdenum (mg AEE/g).
Black: berry; red: skin; blue: pulp; green: seed.



Source: Author (2018).

The antioxidant activity evaluated during the physiological development (Figure 3) of the grapes was associated with different contents of bioactive compounds. The data obtained from the different extracts: ethereal (EE), alcoholic (ALE), aqueous (AQE) and methanol + acetone (AME), analyzed according to specific methodology, were submitted to a means test, and selected according to the greatest extraction yield in relation to time.

The extract showing the highest yields in the DPPH assays throughout physiological development was the EE, with some exceptions. For the berries at 10 DAA and the skin at 90 DAA, the highest yield was from the ALE, and for the seeds at 80 DAA, it was from the AQE. The greatest antioxidant activities presented in the DPPH assay during phase III were obtained from the seed, skin and pulp, respectively (Figure 3B).

In the ABTS assays the extracts showing the greatest yields were the AME and ALE, the AME for the berries, skins and seeds at 60 DAA, and the ALE for the pulps and seeds at

70 to 90 DAA. The seed presented the highest antioxidant activity at 60 DAA, and after the period from 70-90 DAA, the skin presented the highest antioxidant activity (Figure 3C).

With respect to the evaluation of the activity inhibiting formation of the phosphomolybdenum complex, the extract showing the greatest yield was the ALE obtained from all samples at all times. During phase III, the seeds presented the greatest antioxidant activity (Figure 3D).

Xi et al. (2013) determined the antioxidant capacity in grapes,by ABTS and DPPH assay, where they obtained results with greater antioxidant activity by the ABTS than DPPH assay, similar to the data obtained for the skin (phase III) in the present experiment.

Similar behavior was also observed by Baydar; Ozkan & Yasar (2007) when analyzing the antioxidant capacity of the seed and bagasse of the Narince grape cultivar by the phosphomolybdenum method, where the seeds presented greater antioxidant activity.

3.3 Phenolic compound profile

The time significantly influenced the phenolic compounds evaluated, that is chlorogenic acid, catechin and epicatechin. Table 1 shows the amounts of the different phenolic compounds obtained.

Table 1: Means obtained for the phenolic compound contents in the early Isabel cultivar grapes during 50 days for the berries and during 90 days for the skin, pulp and seed (µg/mL).
DAA: Days after anthesis. Different letters in the same column are significantly different (p≤0.05) by the Scott-

Sample	DAA	Chlorogenic acid	Catechin	Epicatechin
Berry	10	3033.00a	-	-
	20	1789.60b	-	-
	30	1591.90b	-	-
	40	533.80c	-	-
	50	500.30c	-	-
Skin	60	396.17[a]	-	-
	70	333.48b	-	-
	80	236.57c	-	-
	90	224.45c	-	-
Pulp	60	40.64[a]	-	-
	70	45.77[a]	-	-
	80	50.67[a]	-	-
	90	48.87[a]	-	-
Seed	60	-	12848.09[a]	7732.16[a]
	70	-	10965.22b	6348.04[a]
	80	-	7324.47c	2483.03b
	90	-	6390.30c	2039.74b

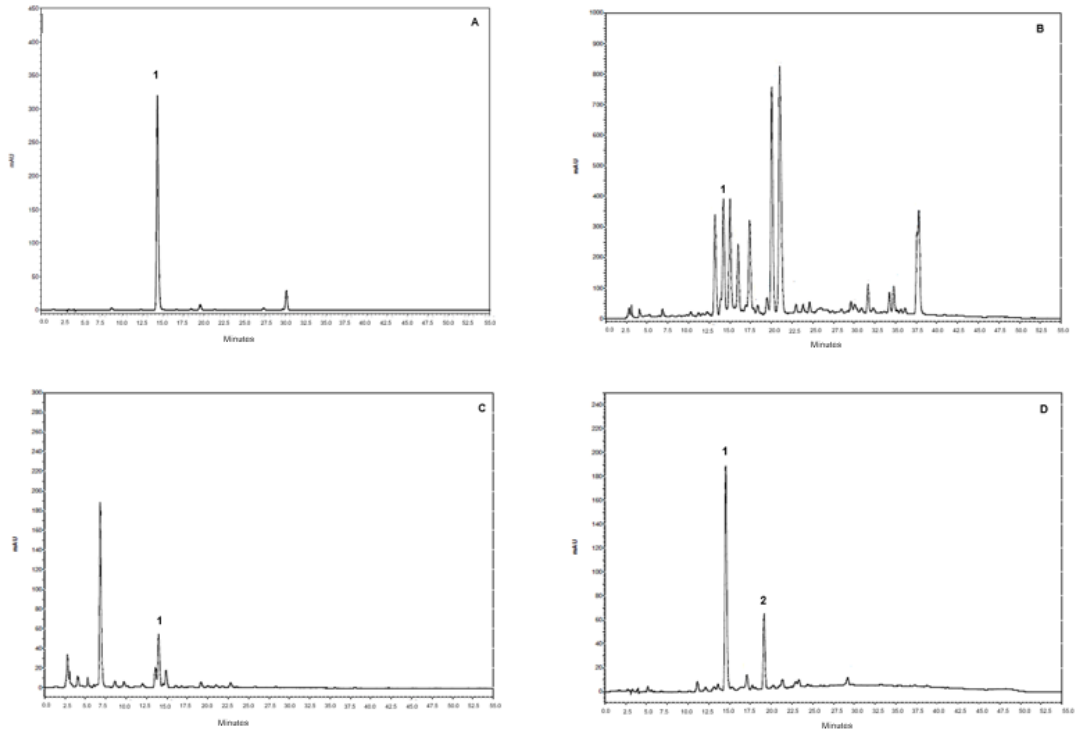
Knott test for each part of the grape. Source: Author (2018).

Chlorogenic acid was synthesized in phase I of berry development, as can be seen in Table 1. The amount subsequently decreased during berry development, since it was oxidized in the Kreb's cycle (cell respiration). This decrease was also visible in the phenolic compound content during phase I (Figure 3A) and reflected in the antioxidant activity (Figures 3B, C and D) (Ferreira et al., 2016a).

In the seeds catechin showed the highest content, followed by epicatechin (Table 1). According to Borghezan (2017), during ripening the tegument changes in color due to tissue lignification and oxidation of the phenolic compounds. This can be observed from the decrease in catechin and epicatechin during phase III, behavior reflecting directly on the total phenolic compound content (Figure 3A) but not totally affecting the antioxidant activity, whose values, as from 70 DAA and at 90 DAA, showed no significant difference. Montealegre et al. (2006) evaluated the seeds of four different grape varieties and found more catechin than epicatechin in all of them.

Figure 4 presents examples of the phenolic compound profiles, where chlorogenic acid is represented in figures 4A (berry), 4B (skin), and 4C (pulp) as peak 1. Catechin and epicatechin are represented in figure 4D (seed) by peaks 1 and 2, respectively.

Figure 4: Examples of HPLC chromatograms recorded at 280 nm, (A) berries – 10 DAA, (B) skin – 90 DAA, (C) pulp – 90 DAA, (D) seed – 90 DAA.



Source: Author (2018).

4. Final Considerations

The predominant phenolic compound found during phase 1 was identified as chlorogenic acid, showing a high antioxidant potential. The chlorogenic acid content decreased with time, being identified in the skin and pulp together with other non-identified compounds. The skin showed similar behavior to that of the berry, decreasing with time probably because it was a precursor of lignin. In phase III, the phenolic compound content and antioxidant activity in the skin increased with time, possibly due to the synthesis of compounds related to the pigments responsible for the color of the skin.

The bio-compound content of the pulp varied little with time, being the tissue containing the smallest amount as compared to the others (berry, skin and seed). Catechin and epicatechin were identified in the seed.

In grape industrialization, the skin and seed are considered sub-products rich in bio-active compounds. An understanding of the synthesis of the bio-active compound profile during the physiological development of the different tissues would allow extractions and future applications, for the development of functional foods, food supplements, food additives and cosmetics.

Declaration of conflict of interest

The authors have no conflicts of interest to declare.

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