Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration

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ABSTRACT

The effect of maceration process on the profile of phenolic compounds, organic acids composition and antioxidant activity of grape juices from new varieties of Vitis labrusca L. obtained in industrial scale was investigated. The extraction process presented a high yield without pressing the grapes. The use of a commercial pectinase resulted in an increase on extraction yield and procyanidins B1 and B2 concentrations and a decrease on turbidity and concentration of catechins. The combination of 60 °C and 3.0 mL 100 kg^{-1} of enzyme resulted in the highest extraction of phenolic compounds, reducing the content of acetic acid. The juices presented high antioxidant activity, related to the great concentration of malvidin, cyanidin, catechin and caffeic, cinnamic and gallic acids. Among the bioactive compounds, the juices presented high concentration of procyanidin B1, caffeic acid and trans-resveratrol, with higher levels compared to those reported in the literature.

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1. Introduction

The world consumption of grape juice has grown each year, being the Brazil and United States the largest producers and consumers (OIV, 2011). In Brazil, the production of juices has been spread in the region of Vale do Submédio São Francisco (VSF), located at the Northeast region. The main grape varieties for the juice production are Isabel Precoce (Vitis labrusca) and the hybrids BRS Cora and BRS Violeta, new Brazilian varieties for the production of juices of high quality (Lima et al., 2014).

The grape juice is a representative source of phenolic compounds and studies have demonstrated that the consumption of this beverage is associated to several health benefits to consumers (Krikorian et al., 2012; Vauzour, Rodriguez-Mateos, Corona, Oruna-Concha, & Spencer, 2010). The phenolic compounds associated with these benefits are, mainly, flavonols, flavanols, anthocyanins, phenolic acids and stilbenes (Krikorian et al., 2012; Sautter et al., 2005; Xia, Deng, Guo, & Li, 2010). Among the

biological activities related to the phenolic compounds, the antioxidant activity is one of the main characteristics of grape juices (Burin et al., 2010; Dani et al., 2007; Dávalos, Bartolome, & Gómez-Cordovés, 2005; Xia et al., 2010). Several factors exert influence on the juice composition, as the processing techniques (Morris & Striegler, 2005). Among these, variables as temperature, maceration time and use of enzymes have influence on the analytical characteristics, phenolic compounds, antioxidant activity and yield of juices (Fuleki & Ricardo-da-Silva, 2003; Leblanc, Johnson, & Wilson, 2008; Mojsov, Ziberoski, & Bozinovic, 2011; Talcott & Lee, 2002).

In an industrial scale process, the techniques of juices production are divided basically in "Hot press" (HP) and "Cold press" (CP), corresponding to the pressing the heated grape (HP) or pressing at ambient/chilling temperature (CP) (Morris, 1998; Morris & Striegler, 2005). In the HP process the crushed grape is heated at 60–62 °C, pectinase enzyme is added and samples are placed in stainless steel tanks with shaking to promote the extraction of compounds present in grape films, step known as maceration, for 30–60 min. Then, the juice is drained and the bagasse is pressed, obtaining a cloudy juice, which will be submitted to clarification

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treatments to remove suspended solids, using vacuum rotary filters or industrial centrifuges. After, the juice is stabilized, pasteurized and bottled hot (Iyer, Sacks, & Padilla-Zakour, 2010; Morris, 1998).

In the CP process, the maceration of crushed grape is carried out at ambient/chilled temperature, with addition of sulfur dioxide to inhibit the action of oxidative enzymes, and addition of pectinases to degrade the structures of grape films, facilitating the liberation of phenolic compounds to the juice (Leblanc et al., 2008; Morris & Striegler, 2005).

Another technique used is the "Hot Break" (HB), where the grapes are crushed and heated at temperatures higher than 75 °C, for a short time (5–10 min), to deactivate the polyphenoloxidases (PPO), and cooled at 60 °C to add pectinase, following the procedures used in HP process (Iyer et al., 2010; Morris & Striegler, 2005).

In the preparation of grape juices, the maceration process has relevant importance, since is in this phase where the incorporation of the compounds present on the grape film to the juice occurs, as phenolic compounds and aroma components (Fuleki & Ricardo-da-Silva, 2003; Iyer et al., 2010). The heating of crushed grape has as objective to promote the plasmolysis of the membrane and ruptures on the fruit cell wall, facilitating the liberation of liquid and anthocyanins responsible for the color. The enzymes (pectinases) on the grape juices are used to hydrolyze the pectin present on the film, facilitating the liberation of shell compound; to reduce the viscosity of the juice and to increase the juice yield and also to reduce the turbidity (Cascales, Garcia, Roca, & Plaza, 2012; Gomes, Guez, Martin, & Silva, 2007). The addition of pectinases during the grape maceration can be considered a complex process, resulting in important alterations on the chemical composition of grape juice, mainly related to phenolic compounds. There are several commercial enzyme formulations available for application in fruit maceration, consisting of pectinases, cellulases and galactosidades (Arnous & Meyer, 2010; Landbo & Meyer, 2004).

The maceration process comprehends a critical step on grape juice production, directly related to the phenolic quality and bioactive properties of the product. Chemical modification as monomeric anthocyanins degradation and increase of phenolic acids concentration has been reported in grape derivatives treated with enzymatic pool based on pectinases and cellulase (Arnous & Meyer, 2010; Toaldo et al., 2014). However, the chemical alterations from the grape maceration and their influence on grape juices processed in industrial scale are not yet known. In this context, the objective of this work was to evaluate the influence of maceration process at industrial conditions using a commercial enzymatic pool, based on pectinases, on the main group of phenolic compounds, organic acids and antioxidant activity of grape juices from new varieties of *V. labrusca* L.

2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol, potassium persulfate and Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Methanol, acetonitrile and 85% phosphoric acid were obtained from Vetec Ltda (Rio de Janeiro, Brazil), J.T. Baker (Phillipsburg, NJ, USA) and Fluka (Switzerland), respectively. Analytical standards of tartaric, malic, citric, succinic, lactic, acetic and ascorbic acids were purchased from Vetec Ltda (Rio de Janeiro, Brazil). Malvidin 3,5-diglucoside, cyanidin 3,5-diglucoside,

malvidin 3-glucoside, cyanidin 3-glucoside, peonidin 3-glucoside, delphinidin 3-glucoside and pelargonidin 3-glucoside, kaempferol 3-glucoside, myricetin, quercetin, rutin (quercetin 3-rutinoside), isorhamnetin 3-glucoside, (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, procyanidins A2, B1 and B2, and *trans*-resveratrol were purchased from Extrasynthese (Genay, France). Gallic, cinnamic and caffeic acids were purchased from Chem Service (West Chester, USA). *p*-Coumaric and chlorogenic acids were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained by purification using a Purelab Option Q Elga System (USA).

2.2. Grape samples

The grapes of Isabel Precoce and BRS Cora varieties were collected from a specific area destined to the production of commercial juice at Empresa Brasileira de Frutas Tropicais (EBFT/ASA), located at Projeto de Irrigação Senador Nilo Coelho – lote 07, PA III, Petrolina, Pernambuco State, Brazil, situated at 09° 27′S latitude and 40° 38′W longitude, at an altitude of approximately 350 m. The grape juices were produced in an industrial facility belonging to EBFT/ASA.

The plants were grown in vineyards with an average age of tree years, grafted on *IAC 572* rootstock, planted in a field with 3.0 and 2.0 meters of spacing between lines and plants, respectively, and following a trellis system. The irrigation was by micro-aspersion and the vineyards were pruned (production pruning) on October and the grapes were harvested on February, when they reached the required standard maturation: soluble solids between 18 and 20 °Brix, titratable acidity (TA) from 0.7 to 0.9 g 100 mL^{-1} of must, expressed as tartaric acid, and °Brix/TA ratio between 20 and 25.

The climatic data (average/month) for the region in the months of October and February, that is, from pruning to harvest time, were: temperature 28.4 °C, rainfall 16.6 mm, relative humidity 51.4%, evaporation 9.1 mm, luminance 546.3 lx/day and insolation 7.6 h, measured at the weather station of Bebedouro (Petrolina, Pernambuco State, Brazil, 09°09′S 40°22′W).

2.3. Grape juice processing

The juice was elaborated following the formulation adopted by industries of Northeast of Brazil, a mixture of Isabel Precoce 80% and BRS Cora 20%, the cut (blend) was made by mixing the grapes at weighing. All juices were obtained by hot extraction without bagasse pressing, in an in-line system manufactured by JAPA® (Garibaldi, Rio Grande do Sul State, Brazil). The grapes were destemmed and crushed in an automatic equipment model DZ-35, with the addition of an enzymatic liquid mixture based on pectinase, called Endozym® Pectofruit PR, produced by Spindal – Pascal Biotech (Gretz-Armainvilliers, France) at doses of 0, 1.5 and 3.0 mL 100 kg^{-1} of fresh grapes. The grapes were then pumped into a maceration tank with controlled temperature and the mixture was heated at 50 and 60 °C and held at this temperature for 1 h with constant pumping of the liquid. After maceration the juice was separated by draining, aided by a suction pump. This procedure did not require pressing of the bagasse. The juice was homogenized and then submitted to pasteurization at 85 °C for 60 s in a tubular pasteurizer. The samples were then packaged, through hot filling of non-colored glass bottles of 300 mL capacity manufactured by Saint-Gobain® (São Paulo-SP, Brazil), using a gravimetric automatic filling machine (EVR12 model). The filled bottles were capped, closed and tumbled. The closed bottles were cooled in a cooling tunnel by water spraying until reaching an average temperature of 45 °C.

The juices were obtained by the following different maceration conditions: 50 °C without the addition of pectinase (T50E0), 60 °C

without the addition of pectinase (T60E0), 50 °C with the addition of pectinase (1.5 mL 100 kg^{-1} of fresh grape) (T50E1.5), 60 °C with the addition of pectinase (1.5 mL 100 kg^{-1} of fresh grape (T60E1.5), 50 °C with addition of pectinase (3.0 mL 100 kg^{-1} of fresh grape) (T50E3) and 60 °C with the addition of pectinase (3.0 mL 100 kg^{-1} of fresh grape) (T60E3).

2.4. Determination of quality parameters of grape juice

The analysis of juice quality was carried out by determining the pH (potentiometer pH Analyser – Tecnal (Brazil)); soluble solids (°Brix) (digital refractometer HI 96801 Hanna®, USA) and titratable acidity (TA), following the methodologies described in OIV, 2011. The color intensity was determined by obtaining the indices at 420, 520 and 620 nm with a UV–Vis UV 2000A spectrophotometer, Instrutherm® (Brazil), using glass cuvettes with a path length of 0.5 cm (OIV, 2011). The turbidity was measured by nephelometry using a turbidimeter Model HI 98703 Hanna® (USA). The yield of juice was calculated by the volume (liters) obtained using 100 kg of fresh grape.

2.5. Determination of total phenolics content, total monomeric anthocyanins and in vitro antioxidant activity of grape juice

The total phenolics content of grape juices was determined using the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). Briefly, grape juice samples were treated with saturated sodium carbonate solution, followed by the addition of the Folin–Ciocalteu reagent. The mixture was kept in dark at room temperature (24 °C) for 120 min. Absorbance was determined at 765 nm on an UV–Vis L-2010 Hitachi® spectrometer (Tokyo, Japan). Gallic acid solutions were used for construction of calibration curve and results were expressed as mg of gallic acid equivalents (GAE)/L of grape juice.

The total monomeric anthocyanins content was determined using the pH-differential method, as described by Giusti and Wrolstad (2001). Grape juice samples were diluted with buffer solutions of KCl (0.025 M) pH 1.0 and CH$_3$COONa (0.4 M) pH 4.5 and absorbance measurements were performed at 520 and 700 nm, respectively. The total monomeric anthocyanins content was expressed as cyanidin-3-glucoside equivalents in mg L^{-1} of grape juice.

The in vitro antioxidant activity was determined using the DPPH and ABTS radical scavenging methods, as described by Kim, Guo, and Packer (2002) and Re et al. (1999), respectively. Trolox was used as standard for the calibration curves and results were expressed as equivalents of Trolox per liter of grape juice (mM TEAC L^{-1}). Absorbance measurements were performed on a UV–Vis L-2010 Hitachi® spectrometer (Tokyo, Japan). For the analysis, solutions of DPPH and ABTS radicals were prepared in ethanol and diluted to an absorbance of 0.900 ± 0.050 and 0.700 ± 0.030, respectively. The antioxidant activity of grape juices was assessed through the rate of decay in absorbance at 517 nm for DPPH and at 754 nm for ABTS. In both procedures, absorbance of the radical solutions was determined before and after the addition of grape juice samples. In the DPPH method, absorbance was measured at time $t = 0$ min and at time $t = 30$ min after the addition of grape juice. In the ABTS method, absorbance was determined at time $t = 0$ min and at time $t = 6$ min after the addition of samples. All the analyses were performed in triplicates.

2.6. Determination of organic acids of grape fruits

The quantification of tartaric, malic, citric, lactic, succinic, acetic and ascorbic acids was carried out by High-Performance Liquid Chromatography (HPLC) using a WATERS (model Aliance e2695)

chromatograph coupled with diode-array detection (DAD), following the methodology described by Rybka et al. (2012). The juice samples were filtered through a 0.45 μm membrane and injected in triplicate. For the acid determination the DAD wavelength was maintained at 250 nm for ascorbic acid and 210 nm for tartaric, malic, citric, lactic, succinic and acetic acids, with a run time of 15 min, flow rate of 0.6 mL min^{-1}, 26 °C and volume injection of 10 μL. The column used was a Gemini-NX C18 (150 × 4.60 mm, with 3 μm internal particles) and the pre-column was a Gemini-NX C18 (4.0 × 3.0 mm), both manufactured by Phenomenex®. The liquid phase was comprised of a 0.025 M solution of KH$_2$PO$_4$ acidified with H$_3$PO$_4$ until pH 2.6. The R^2 values obtained ranged from 0.9984 to 0.9998. The lower and upper values for the limits of detection (LOD) were 0.03 and 3.59 mg L^{-1}, respectively, for ascorbic and citric acids.

2.7. Determination of individual phenolic compounds of grape juices

The phenolic compounds were determined by HPLC on a WATERS chromatograph (model Aliance e2695), equipped with a quaternary solvent pump and automatic injector, coupled with DAD and fluorescence detection (FD), according to the methodology described by Natividade, Corrêa, Souza, Pereira, and Lima (2013). The data collection and analysis were carried out using the software Empower™ 2 (Milford, EUA). In the DAD, the detection of compounds was performed at 220 nm for gallic acid, (−)-epicatechin gallate, (−)-epigallocatechin and procyanidin B1; 320 nm for t-resveratrol, caffeic acid, cinnamic acid, p-coumaric acid and chlorogenic acid; 360 nm for the flavonols kaempferol, myricetin, quercetin, rutin and isorhamnetin; and 520 nm for the anthocyanins malvidin 3,5-diglucoside, cyanidin 3,5-diglucoside, malvidin 3-glucoside, cyanidin 3-glucoside, peonidin 3-glucoside, delphinidin 3-glucoside and pelargonidin 3-glucoside. In the FD, the photon excitation was carried out at 280 nm and the emission at 320 nm for (+)-catechins, procyanidin B2, procyanidin A2 and (−)-epicatechin. The limit of detection ranged from 0.001 to 0.19 mg L^{-1} and the R^2 was always higher than 0.983 for all the assayed compounds.

The column used was a Gemini-NX C18, 150 × 4.60 mm, with 3 μm internal particles, and the pre-column was a Gemini-NX C18, 4.0 × 3.0 mm, both manufactured by Phenomenex. The oven temperature was maintained at 40 °C, the injection volume was 10 μL (juice previously filtered through a 0.45 μm membrane; Allcrom-Phenomenex, USA) and the flow rate was 0.5 mL min^{-1}. The gradient used in the separation was 0 min: 100% A; 10 min: 93% A and 7% B; 20 min: 90% A and 10% B; 30 min: 88% A and 12% B; 40 min: 77% A and 33% B; 45 min: 65% A and 35% B and 55 min: 100% B, where solvent A is 0.85% phosphoric acid solution and solvent B is acetonitrile.

2.8. Statistical analysis

Six samples of juice produced by three repetitions were evaluated, each repetition composed by a process tank containing 3000 kg of grape fruit, totaling 18 tanks and 54,000 kg of grapes. The results obtained for the variables studied were subjected to analysis of variance (ANOVA) and followed by comparisons performed using the Tukey test with a probability of error of 5%. The principal component analysis (PCA) was carried out to evaluate the relation among the different conditions of maceration employed and the main phenolic classes and antioxidant activity of the grape juice produced. The statistical analysis was carried out using the SPSS version 17.0 statistical package for Windows (SPSS, Chicago, USA).

Table 1
Quality parameters of grape juices obtained in different conditions of maceration.

Temperature	50 °C			60 °C		
Enzyme dose	0	1.5	3.0	0	1.5	3.0
pH	3.39 ± 0.2 a	3.38 ± 0.01 a	3.37 ± 0.04 a	3.39 ± 0.07 a	3.39 ± 0.08 a	3.35 ± 0.02 a
Soluble solids (°Brix)	19.2 ± 0.2 a	19.0 ± 0.4 a	19.2 ± 0.1 a	19.2 ± 0.3 a	18.7 ± 0.6 a	19.2 ± 0.0 a
Titratable acidity (TA)	0.83 ± 0.02 a	0.85 ± 0.04 a	0.84 ± 0.02 a	0.85 ± 0.04 a	0.85 ± 0.04 a	0.85 ± 0.03 a
°Brix/TA	23.2 ± 0.9 a	22.5 ± 0.8 a	22.9 ± 0.7 a	22.6 ± 0.9 a	22.2 ± 1.9 a	22.8 ± 0.9 a
Turbidity (NTU)	147 ± 6 a	113 ± 5 bc	100 ± 5 cd	135 ± 15 ab	100 ± 9 cd	84 ± 15 e
Color intensity	6.56 ± 1.04 a	6.00 ± 0.51 a	5.78 ± 0.39 a	5.38 ± 0.43 a	5.94 ± 0.29 a	5.77 ± 0.44 a
% juice yield	59 ± 0.6 c	64.1 ± 0.1 b	64.7 ± 0.3 ab	59 ± 0.4 c	64.1 ± 0.3 b	65 ± 0.2 a

Means followed by the same letters in the same lines do not differ by Tukey test at 5% probability.
Dose of enzyme as mL 100 kg^{-1} of grape fruit; TA – as g 100 mL^{-1} tartaric acid; SS – % (°Brix); NTU – nephelometric turbidity units.

Table 2
Mean values of organic acids of grape juices obtained in different conditions of maceration.

Temperature	50 °C			60 °C		
Enzyme dose	0	1.5	3.0	0	1.5	3.0
Organic acids						
Tartaric (g L^{-1})	5.64 ± 0.22 a	5.32 ± 0.47 ab	4.75 ± 0.56 ab	5.33 ± 0.29 ab	5.26 ± 0.41 ab	4.30 ± 0.19 b
Malic (g L^{-1})	3.46 ± 0.18 b	3.65 ± 0.04 ab	3.77 ± 0.02 ab	3.50 ± 0.06 ab	3.80 ± 0.03 a	3.70 ± 0.21 ab
Citric (mg L^{-1})	313 ± 6 b	290 ± 35 b	470 ± 26 a	317 ± 6 b	345 ± 40 b	503 ± 30 a
Ascorbic (mg L^{-1})	8.3 ± 3.2 a	11 ± 0.3 a	9.6 ± 0.6 a	9.2 ± 1.0 a	9.4 ± 0.4 a	9.6 ± 0.2 a
Lactic (mg L^{-1})	227 ± 46 bc	373 ± 97 abc	603 ± 227 ab	200 ± 10 c	693 ± 237 a	278 ± 6 bc
Succinic (mg L^{-1})	87 ± 25 a	73 ± 15 a	70 ± 20 a	43 ± 15 a	87 ± 6 a	80 ± 72 a
Acetic (mg L^{-1})	537 ± 58 ab	510 ± 70 ab	420 ± 10 b	637 ± 55 a	450 ± 17 b	460 ± 121 ab
Total organic acids (g L^{-1})	10.27 ± 0.54	10.23 ± 0.73	10.09 ± 0.86	10.07 ± 0.44	10.64 ± 0.74	9.33 ± 0.63

Means followed by the same letters in the same lines do not differ by Tukey test at 5% probability.
Dose of enzyme as mL 100 kg^{-1} of grape fruit.

3. Results and discussion

3.1. Grape juice quality

The results for quality physico-chemical analyses of grape juices are presented in Table 1. The maceration conditions did not influence the soluble solid (SS) content, pH, titratable acidity (TA) and SS/TA ratio. All the values are in accordance with the Brazilian regulation for integral juice: 14 °Brix (minimum for SS), minimum of 0.41 g 100 mL^{-1} TA in the must, and SS/TA ratio of between 14 and 45 (Brazil, 2000). The mean values for the SS content (19.1 ± 0.4) in the juices were higher than that obtained by Rizzon and Miele (2012) for commercial Brazilian juices (16.2 ± 1.1).

The color intensity also presented no significant difference among the maceration conditions tested, with mean values from 5.38 to 6.56, according to Brazilian grape juices (Burin et al., 2010; Rizzon & Miele, 2012). The values of turbidity varied from 84 to 147 NTU, decreasing with the increase of enzyme dose and maceration temperature. Temperature did not influence the juice yield, but the use of pectinases enhanced the yield from 59% to 65% (liters of juice per ton of grape fruit), using 3.0 mL 100 kg^{-1} of grape. According to Morris (1998) the yield of grape juice can reach 73.8% (HP process) and 64.3% (CP process), depending on the cultivar of fruit and efficiency of pressing. Leblanc et al. (2008) obtained an increase on juice yield from 41.5% to 61.1% (grape "Carlos") and from 40.8% to 56.2% (grape "Noble") by the addition of 10 g pectinase per 100 kg of fruit, after 14 h of maceration at 7 °C. The same authors reached a yield from 49% to 52% for the same cultivars in HP process at 60 °C without the addition of enzyme. Mojsov et al. (2011) obtained yields from 44.8% (without enzyme) to 50.3% (Vonozyn Vintage FCE®; 4.0 g 100 kg^{-1} of grape) for grapes cultivar "Vranec" by cold pressing, in industrial scale process.

The decrease of turbidity with increase of enzyme content can be associated with the degradation of soluble pectin and other polysaccharides presents in the juice by pectinases. These compounds can act as colloids, increasing the turbidity of the juice (Arnous & Meyer, 2010; Landbo & Meyer, 2004).

The results obtained in the present work demonstrate that the methodology for production of grape juice presented here led to good yields because even without the addition of enzyme a yield of 59% was obtained, which can be considered high compared to the works form the literature. The yields with addition of enzyme increased about 5%, and are in accordance with other works (Morris, 1998; Mojsov et al., 2011). An enzyme dose of 1.5 mL 100 kg^{-1} of grape showed a yield of 67%, considered good since the manufacturer recommendation is the use of a dose from 2.0 to 4.0 mL 100 kg^{-1} for grapes maceration.

3.2. Content of organic acids of grape juices

The content of organic acids for the different formulations of grape juice is presented in Table 2. The tested maceration treatments resulted in significant differences on the composition of organic acids. For tartaric acid, the main organic acid of grapes and derivatives, it was observed a decrease with the treatment of 60 °C with addition of 3.0 mL of enzyme, compared to that obtained at 50 °C without addition of enzyme. The mean values varied from 5.64 to 4.30 g L^{-1}. Citric acid content did not varied significantly in treatments without and with the addition of 1.5 mL of enzyme, but compared to the formulation obtained with the addition of 3.0 mL of enzyme a considerable increase on the concentration of this acid was observed, from 290 to 503 mg L^{-1}. The contents of succinic and ascorbic acids on grape juices did not were influenced by the maceration treatments employed and presented mean values from 43 to 87 mg L^{-1}, and 8.3 to 11.0 mg L^{-1}, respectively.

In a general way, the content of lactic acid in the juice increased with the addition of enzyme. Acetic acid, the most undesirable acid in juices and wines, presented a significant reduction using

Table 3
Phenolics compounds of grape juices obtained in different conditions of maceration.

Temperature	50 °C			60 °C		
Enzyme dose	0	1.5	3.0	0	1.5	3.0
Flavanols						
(+)-Catechin	48.1 ± 13.4 a	9.8 ± 0.9 b	9.9 ± 0.5 b	50.8 ± 4.7 a	14.9 ± 0.6 b	11.2 ± 3.4 b
(−)-Epicatechin	1.0 ± 1.0 a	0.6 ± 0.1 a	0.8 ± 0.7 a	1.4 ± 0.5 a	1.3 ± 0.1 a	1.7 ± 0.3 a
(−)-Epicatechin gallate	1.6 ± 0.1 a	1.4 ± 0.1 a	2.2 ± 1.2 a	2.4 ± 3.0 a	0.8 ± 0.0 a	0.8 ± 0.1 a
(−)-Epigallocatechin gallate	1.4 ± 0.6 a	3.1 ± 0.4 a	2.1 ± 0.5 a	5.0 ± 1.1 a	3.9 ± 0.6 a	1.8 ± 0.3 a
Procyanidin A2	2.8 ± 0.1 a	2.8 ± 0.2 a	2.6 ± 0.2 a	2.6 ± 0.3 a	3.2 ± 0.7 a	3.0 ± 0.2 a
Procyanidin B1	5.9 ± 5.5 b	7.3 ± 0.5 b	34.4 ± 2.4 a	11.6 ± 2.8 b	9.9 ± 1.0 b	36.8 ± 0.8 a
Procyanidin B2	9.3 ± 0.4 b	9.0 ± 1.5 b	13.1 ± 3.1 ab	11.3 ± 0.9 ab	10.4 ± 0.6 b	16.1 ± 2.5 a
Total Flavanols quantification	70.1 ± 21.1	34 ± 3.7	65.1 ± 8.6	109.7 ± 13.3	44.4 ± 3.6	71.4 ± 7.6
Flavonols						
Kaempferol	0.7 ± 0.5 b	1.4 ± 0.4 ab	0.9 ± 0.2 ab	1.5 ± 0.3 a	1.0 ± 0.1 ab	1.2 ± 0.2 ab
Myricetin	0.1 ± 0.1 a	0.2 ± 0.0 a	0.2 ± 0.1 a	0.2 ± 0.1 a	0.2 ± 0.1 a	0.3 ± 0.1 a
Isorhamnetin	0.8 ± 0.6 a	1.3 ± 0.2 a	1.2 ± 0.6 a	1.5 ± 0.2 a	1.4 ± 0.3 a	1.6 ± 0.2 a
Rutin	0.9 ± 0.6 b	1.4 ± 0.2 ab	1.5 ± 0.1 ab	1.5 ± 0.1 ab	1.4 ± 0.3 ab	1.8 ± 0.3 a
Quercetin	0.01 ± 0.01 c	0.03 ± 0.06 b	0.10 ± 0.00 ab	0.10 ± 0.00 ab	0.07 ± 0.06 ab	0.17 ± 0.06 a
Total flavonols quantification	2.7 ± 1.8	4.3 ± 1.5	3.9 ± 1.0	4.8 ± 0.7	4.1 ± 0.9	5.1 ± 0.9
Stilbene						
trans-Resveratrol	0.67 ± 0.1 a	0.67 ± 0.1 a	0.90 ± 0.3 a	0.70 ± 0.1 a	0.73 ± 0.1 a	0.90 ± 0.4 a
Anthocyanins						
Malvidin 3,5-diglucoside	6.6 ± 2.0 a	4.6 ± 0.6 a	5.2 ± 1.0 a	5.6 ± 0.6 a	6.1 ± 0.3 a	6.4 ± 0.1 a
Malvidin 3-glucoside	11.0 ± 4.9 a	7.1 ± 0.8 a	8.1 ± 3.6 a	8.8 ± 1.5 a	10.5 ± 0.5 a	11.2 ± 0.5 a
Cyanidin-3,5-diglucoside	5.6 ± 0.9 a	3.9 ± 1.0 a	4.8 ± 1.0 a	4.7 ± 0.5 a	5.7 ± 0.3 a	5.6 ± 1.0 a
Cyanidin 3-glucoside	7.9 ± 1.9 a	5.8 ± 1.2 a	5.2 ± 3.2 a	6.6 ± 0.4 a	7.3 ± 0.3 a	8.0 ± 0.3 a
Delphinidin 3-glucoside	6.0 ± 3.5 a	1.2 ± 0.3 a	3.3 ± 2.8 a	4.6 ± 1.7 a	5.7 ± 1.5 a	6.0 ± 1.2 a
Peonidin 3-glucoside	3.1 ± 1.5 a	2.0 ± 0.1 a	2.2 ± 1.0 a	2.4 ± 0.4 a	2.4 ± 0.2 a	3.0 ± 0.1 a
Pelargonidin 3-glucoside	3.9 ± 1.9 a	0.4 ± 0.2 a	2.4 ± 1.4 a	2.5 ± 1.3 a	3.5 ± 0.4 a	3.6 ± 0.4 a
Total anthocyanins quantification	44.1 ± 16.6	25 ± 4.2	31.2 ± 14	35.2 ± 6.4	41.2 ± 3.5	43.8 ± 3.6
Phenolics acids						
Gallic acid	2.7 ± 0.7 a	2.2 ± 0.1 a	2.3 ± 0.4 a	3.4 ± 1.1 a	2.8 ± 0.3 a	2.8 ± 0.5 a
Caffeic acid	17.9 ± 1.6 a	15.3 ± 0.2 a	15.7 ± 1.0 a	16.5 ± 1.1 a	17.2 ± 0.9 a	17.2 ± 1.6 a
Cinnamic acid	1.2 ± 1.0 a	0.5 ± 0.1 a	1.2 ± 0.7 a	1.4 ± 0.8 a	1.7 ± 0.3 a	2.0 ± 0.2 a
Chlorogenic acid	1.8 ± 0.4 a	3.2 ± 2.0 a	2.3 ± 0.1 a	2.4 ± 1.2 a	2.7 ± 0.1 a	2.5 ± 0.3 a
p-Coumaric acid	1.1 ± 0.4 b	1.4 ± 0.1 ab	1.4 ± 0.0 ab	1.5 ± 0.5 ab	1.6 ± 0.2 ab	1.7 ± 0.1 a
Total phenolics acids quantification	24.7 ± 4.1	22.4 ± 2.5	22.9 ± 2.2	29.2 ± 4.7	26 ± 1.8	26.2 ± 2.7
Total monomeric anthocyanins[†]	155 ± 46 a	107 ± 22 a	117 ± 39 a	123 ± 2 a	103 ± 6 a	129 ± 21 a
Total phenolics[§]	1343 ± 218 ab	959 ± 33 b	1088 ± 183 ab	1296 ± 39 ab	1384 ± 207 a	1203 ± 101ab

Means followed by the same letters in the same lines do not differ by Tukey test at 5% probability.
Dose of enzyme as mL 100 kg^{-1} of grape fruit.
[†] Total monomeric anthocyanins quantified by the technic of difference of pH and expressed as equivalent to cyanidin 3-glucoside.
[§] Total phenolics measured with Folin–Ciocalteu expressed as mg L^{-1} equivalent to gallic acid.

pectinase, from 637 to 420 mg L^{-1}. The content of tartaric and malic acids obtained here are in accordance with the literature for Brazilian grape juices (Lima et al., 2014; Rizzon & Miele, 2012) and also juices of different cultivars of *Vitis vinífera* obtained by CP process (Soyer, Koca, & Karadeniz, 2003). The citric acid content obtained presented values higher than the specific literature, where the contents vary from 31 to 181 mg L^{-1} (Soyer et al., 2003).

3.3. Content of total phenolics and flavanol compounds of grape juices

The results for phenolics compounds quantified in grape juices are presented in Table 3. The mean values of total phenolics varied from 959 to 1384 mg L^{-1} in the different samples of juice obtained in the present work. The concentration of total phenolics was similar among the treatments, with a low value for the juice obtained in experiment T50E1.5. The values of total phenolics obtained here are in agreement with results from the literature for commercial Brazilian grape fruits (270–3433 mg L^{-1}, with mean values among 1430 and 1915 mg L^{-1}) (Burin et al., 2010; Malacrida & Motta, 2005; Sautter et al., 2005). The results were also similar to commercial Spanish juices (mean values from 705 to 1177 mg L^{-1}) (Dávalos et al., 2005; Moreno-Montoro, Olalla-Herrera, Gimenez-Martinez, Navarro-Alarcon, & Rufián-Henares, 2015).

For the flavanols epicatechin, epicatechin gallate, epigallocatechin and procyanidin A2, no significant differences were observed for different maceration treatments. Temperatures of 50 and 60 °C did not show influence on catechin concentration, but the treatments without the addition of enzyme (T50E0 and T60E0) presented higher contents of catechin, with mean values from 48.1 to 50.8 mg L^{-1}, related to the juices with doses of 1.5 and 3.0 mL of enzyme (T50E1.5, T50E3, T60E1.5 and T60E3), with mean values from 9.8 to 14.9 mg L^{-1}. Procyanidins B1 and B2 contents did not differ among the treatments T50E0, T60E0, T50E1.5 and T60E1.5; but using 3.0 mL of enzyme (T50E3 and T60E3) the concentration of these compounds presented a significant increase, mainly for procyanidin B1 (from 5.9 and 11.6 to 34.4 and 36.8 mg L^{-1}), respectively. The main flavanols found in the juices were catechin and procyanidins B1 and B2, compounds directly associated with high antioxidant activities (Muselík, García-Alonso, Martín-López, Žemlička, & Rivas-Gonzalo, 2007). The work by Fuleki and Ricardo-da-Silva (2003) with Concord grape juice obtained by HP process and addition of pectinase 50 mg L^{-1} and maceration at 60 °C presented flavanols contents similar to those obtained in our work. These authors also observed that the pasteurization step enhanced the concentration of procyanidins B1 and B2. Mean values of flavanols quantified by HPLC on juices produced here varied
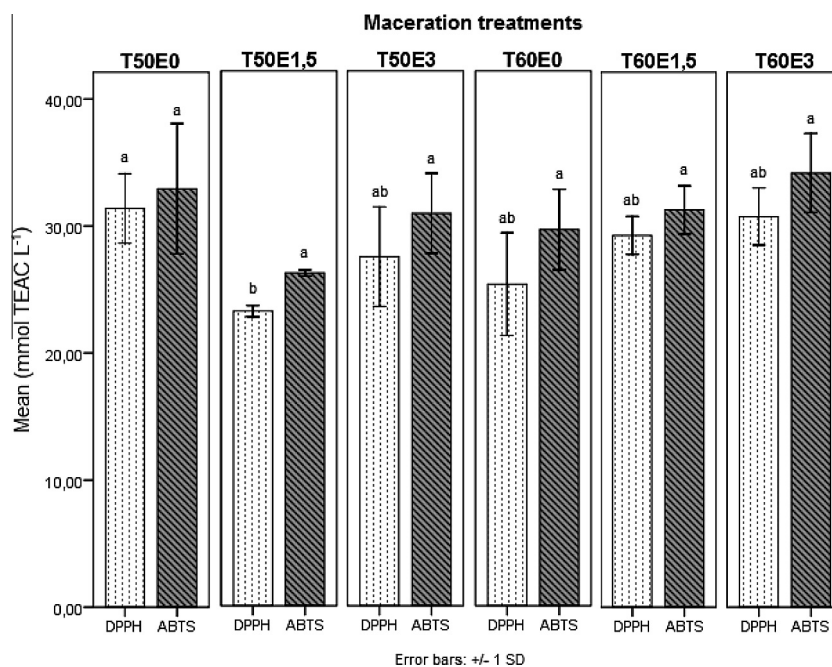
Fig. 1. Mean values of antioxidant activity of grape juices from different maceration conditions. Mean bars followed by the same letters, between juices, do not differ according to the Tukey test at 5% probability. T50E0 – 50 °C without pectinase; T60E0 – 60 °C without pectinase; T50E1.5 – 50 °C + 1.5 mL 100 kg^{-1} pectinase; T60E1.5 – 60 °C + 1.5 mL 100 kg^{-1} pectinase; T50E3 – 50 °C + 3.0 mL 100 kg^{-1} pectinase and T60E3 – 60 °C + 3.0 mL 100 kg^{-1} pectinase.

from 34 to 109.7 mg L^{-1}, higher than those mentioned by Moreno-Montoro et al. (2015) for Spanish grape juices, with mean reported values of 0.53 and 3.07 mg L^{-1}, for white and red juices, respectively.

The total quantified flavanols decreased in the treatments with 1.5 mL kg^{-1} of enzyme (T50E1.5 and T60E1.5) compared to the treatments without enzyme (T50E0 and T60E0) and those using 3.0 mL kg^{-1} (T50E3 and T60E3). In the first case (without enzyme) due to the presence of higher concentrations of catechins, and, in the second case (3.0 mL kg^{-1} of enzyme dose) by the higher extraction of compounds as procyanidins B1 and B2.

3.4. Content of flavonols and trans-resveratrol of grape juices

The flavonols content presented significant difference only for the juice obtained in the experimental condition T50E0, with lower values of kaempferol, quercetin and rutin. The other conditions of maceration did not influence significantly the flavanols quantified. The sum of flavanols (kaempferol, isorhamnetin, quercetin, rutin and myricetin) varied from 2.7 to 5.1 mg L^{-1}, values lower than those mentioned in the literature for grape juices (Talcott & Lee, 2002). Lima et al. (2014) also mentioned low values of flavanols in grape juice produced by the varieties Isabel Precoce and BRS Cora planted in VSF.

For the stilbene *trans*-resveratrol no significant difference was observed among the juices from different maceration conditions, the values varied from 0.67 to 0.90 mg L^{-1}. The values of *trans*-resveratrol obtained here can be considered high, since several authors mentioned concentrations of this compound in grape juices from different cultivars, region of origin and process of elaboration varying from 0.00 to 0.44 mg L^{-1} (Dani et al., 2007; Leblanc et al., 2008; Natividade et al., 2013; Sautter et al., 2005).

The values of *trans*-resveratrol for the juices elaborated in this work can be associated to combined factors as management of vines, climatic conditions of the region and process used, which may have favored the extraction of stilbene (Lima et al., 2014).

3.5. Profile of anthocyanins of grape juices

The concentration of total monomeric anthocyanins in the juices did not present significant differences ($p < 0.05$) among the maceration treatments used in this work, the mean values varied from 103 to 155 mg L^{-1}. The total sum of anthocyanins quantified by HPLC varied from 25.2 to 44.1 mg L^{-1} for the juices obtained from different conditions of maceration, mainly malvidin 3-glucoside with mean values among 7.1 and 11.2 mg L^{-1}, followed in descending order by cyanidin 3-glucoside (5.2–8.0 mg L^{-1}), malvidin 3,5 diglucoside (5.2–6.6 mg L^{-1}), cyanidin 3,5 diglucoside (3.9–5.7 mg L^{-1}), delphinidin 3-glucoside (1.2–6.0 mg L^{-1}), pelargonidin 3-glucoside (0.4–3.9 mg L^{-1}) and peonidin 3-glucoside (2.0–3.1 mg L^{-1}), where a homogeneous distribution of anthocyanins was observed in the grape juices.

The variation of total anthocyanins quantified by HPLC at 50 and 60 °C is associated with the high variance in the treatments, observed by high standard deviation in treatments T50E0 and T60E3, explained by the heterogeneity of the material (grape fruit) as a result of ambient/production factors, since each repetition was original of a tank containing 3000 kg of grape from different lotes.

The variety Isabel Precoce is the main cultivar used for the formulation of the juices, since the variety BRS Cora was used to improve the color of commercial grape juice. The profile of anthocyanins obtained for the grape juices produced with these two cultivars in different conditions of maceration are in accordance with previous works related to the profile of this compound in *V. labrusca* grape juices (Lima et al., 2014; Natividade et al., 2013). Also the total monomeric anthocyanins content are in accordance with results from the literature for artisanal and commercial Brazilian grape juices, which varied from 25.6 to 450.4 mg L^{-1}, and commercial Spanish grape juices with values from 129 to 535 mg L^{-1} (Burin et al., 2010; Lima et al., 2014; Malacrida & Motta, 2005; Moreno-Montoro et al., 2015; Toaldo et al., 2014), but lower than the values mentioned by Iyer et al. (2010) for grape juices from "Concord" cultivar (850 mg L^{-1}) elaborated in industrial condition by HP process in the United States of America, maceration at 60 °C
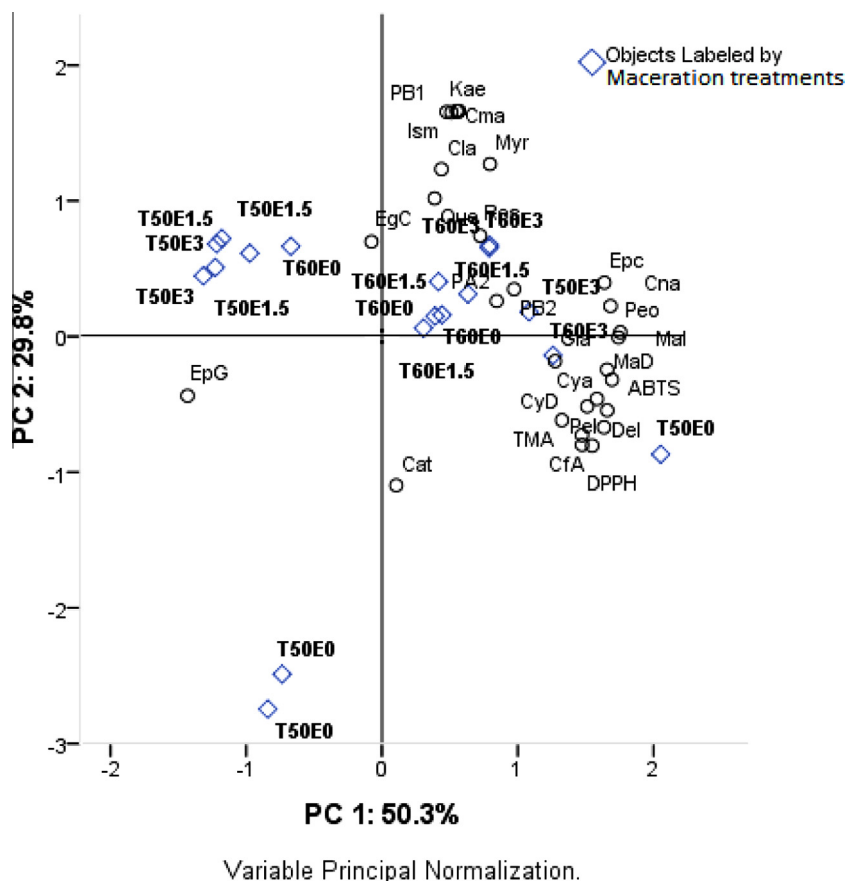
Fig. 2. Principal component analysis of the maceration treatments as a function of phenolics compounds and antioxidant activity of the grape juices produced industrially. EpG – epicatechin gallate; Cat – catechin; Epc – epicatechin; EgC – epigallocatechin; PB1 – procyanidin B1; PB2 – procyanidin B2; PA2 – procyanidin A2; Kae – kaempferol; Que – quercetin; Ism –isorhamnetin; Rut – rutin; Myr – myricetin; Res – *trans*-resveratrol; Cma – coumaric acid; Cla – chlorogenic acid; Cna – cinnamic acid; Gla – gallic acid; Cfa – caffeic acid; Cya – cyanidin 3-glucoside; CyD – cyanidin 3,5-diglucoside; Mal – malvidin 3-glucoside; MaD – malvidin 3,5-diglucoside; Pel – pelargonidin 3-glucoside; Del – delphinidin 3-glucoside; Peo – peonidin 3-glucoside; TMA – total monomeric anthocyanins; TP – total phenolics; DPPH – antioxidant activity; ABTS – antioxidant activity.

during 30 min and addition of 3.5 mL of enzymatic pool per 100 kg of grape fruit.

The sum of anthocyanins quantified by HPLC in the juices produced here under different conditions of maceration are also in accordance with the data obtained by Capanoglu, Vos, Hall, Boyacioglu, and Beekwilder (2012) for grape juices pasteurized and clarified at industrial scale in Turkey (maceration at 40 and 50 °C, during 30–45 min with the addition of pectinase).

Several studies have also shown that the concentration and profile of anthocyanins vary as a function of processing techniques and cultivars used (Dani et al., 2007; Lima et al., 2014; Talcott & Lee, 2002; Toaldo et al., 2014) and steps as pasteurization and treatments of clarification, centrifugation and filtration result in decrease in the concentration of anthocyanins of juices produced in industrial scale (Capanoglu et al., 2012).

3.6. Content of phenolic acids of grape juices

The sum of total phenolic acids quantified in this work varied from 22.4 to 29.2 mg L^{-1} for the different formulations. No significant difference ($p < 0.05$) was observed for the phenolic acids identified. The major compound was caffeic acid (values from 15.3 to 17.9 mg L^{-1}), followed by gallic (2.2 to 3.4 mg L^{-1}), cinnamic (0.5 to 2.0 mg L^{-1}), chlorogenic (1.8 to 3.2 mg L^{-1}) and *p*-coumaric (1.1 to 1.7 mg L^{-1}) acids. The values obtained for these last acids are in accordance with those mentioned by other authors (Moreno-Montoro et al., 2015; Natividade et al., 2013; Stalmach, Edwards, Wightman, & Crozier, 2011) however for caffeic acid

the values obtained here were higher than those on the literature. Mudnic et al. (2010) observed that the phenolic acids present a high antioxidant activity, suggesting an effective contribution of caffeic acid to this bioactive property observed on the *V. labrusca* grape juice produced in this work.

3.7. In vitro antioxidant activity of grape juices

The antioxidant activity of grape juices produced at different conditions of maceration is presented in Fig. 1. The antioxidant activity of juices (AOX) was expressed as equivalents (mM) of Trolox per liter of juice (mM TEAC L^{-1}). The values of AOX varied from 23.20 to 31.38 mM by the DPPH and from 26.20 to 34.17 mM by the ABTS methods. These results are in agreement with the AOX values of Brazilian grape juices, which present mean values from 2.12 to 54.6 mM TEAC L^{-1} (Burin et al., 2010; Lima et al., 2014) and Spanish grape juices, with mean values from 15.1 to 27.1 mM TEAC L^{-1} (Moreno-Montoro et al., 2015).

In a general way, the AOX values increased with the increase of enzyme dose and temperature, possibly due to the higher extraction of antioxidant phenolic compounds, except for treatment T50E0, which presented high activity using lower temperature and without addition of enzyme. This fact can be associated to higher concentration of phenolics in grapes of lotes destined to the elaboration of juices.

Finally, the antioxidant activity determined for the juices produced here is considered high and can be related to the characteristics of the viticulture in the region of origin of grapes (Lima et al.,

2014). In spite of the high values of AOX found here, no significant difference ($p < 0.05$) was observed for the different juices obtained for each different process.

3.8. Principal Components Analysis (PCA)

The Principal Components Analysis (PCA) was applied in the different maceration treatments as a function of phenolic compounds and antioxidant activity, resulting in 28 principal components. The components 1 and 2 (PC1 and PC2, respectively) explained 80.1% of the variance of the experiment, PC1 explained 50.3% and PC2 29.8%. The factor af analysis showed that the variables with higher contribution for the separation of the maceration treatments on PC1 with loading > 0.70 were total phenolics (TP), total monomeric anthocyanins (TMA), antioxidant activity (DPPH and ABTS), epicatechin (Epc), epicatechin gallate (loading negative), gallic acid (Gla), caffeic acid (Cfa), cinnamic acid (Cna), pelargonidin 3-glucoside (Pel), cyanidin 3-glucoside (Cya), cyanidin 3,5-diglucoside (CyD), malvidin 3-glucoside (Mal), malvidin 3,5-diglucoside (MaD), delphinidin 3-glucoside (Del) and peonidin 3-glucoside (Peo). For PC2 the variables that more contributed on the separation (loading > 0.70) were: procyanidin B1 (PB1), p-coumaric acid (Cma), kaempferol (Kae), isorhamnetin (Ism) and rutin (Rut).

The components PC1 and PC2 separated the treatments of maceration in distinct groups. The component 1 (PC1) with a greater importance on explained variance (50.3%) separated the maceration treatments in two groups, the processes at 50 °C (T50E0, T50E1.5 and T50E3) and at 60 °C (T60E0; T60E1.5 and T60E3), influenced mainly by the positive correlations among the treatments at 60 °C with TP, TMA, DPPH, ABTS, Epc, Gla, Cfa, Cna, Pel, Cya, CyD, Mal, MaD, Del, Pel and Peo. The component 2 (PC2) separated the treatments in one group, (T60E0, T50E1.5; T50E3, T60E1.5 and T60E3), influenced by the positive correlations among the treatments T60E0, T50E1.5, T50E3, T60E1.5 and T60E3 with PB1, Cma, Kae, Ism and Rut. Based on the separations obtained in PC1 and PC2 it was evidenced that the increase on the temperature was the factor that more contributed to a higher extraction of phenolic compounds, mainly anthocyanins. The use of a enzymatic pool for the maceration was the factor that most influenced the increase of concentrations of procyanidin B1, p-coumaric acid, kaempferol, quercetin, isorhamnetin and rutin. The combination of temperature of 60 °C with the use of enzymatic pool evidenced a higher extraction of almost all quantified phenolic compounds, except for epicatechin gallate, and on the increase of antioxidant activity of the juice. Fig. 2 presents the principal component analysis of the maceration treatments as a function of phenolic compounds and antioxidant activity of the grape juices produced industrially.

Cabrera et al. (2009) showed that an increase on temperature of the maceration process from 60 to 80 °C contributed to an increase of total phenolics, total monomeric anthocyanins and antioxidant activity of grape juices, corroborating the results obtained here. Leblanc et al. (2008) observed that the HP process combined with the use of pectinase on maceration step also enhanced the stilbenes concentrations, concluding that the use of heat was the main factor related to the increase of concentration of this class of compounds.

4. Conclusions

The process used for production of grape juice presented high yield without the pressing the fruit. The temperature of 60 °C with addition of pectinase resulted in an increase on the yield and decrease of turbidity. The use of enzyme decreased the concentration of catechins and increased the content of procyanidin B1 and

B2. The temperature of maceration of 60 °C contributed to the higher extraction of phenolic compounds, mainly anthocyanins. The combination of temperature 60 °C with the use of enzymatic poll resulted in higher extraction of all analyzed phenolic compounds, increase of almost all organic acids and decrease of acetic acid content. The antioxidant activity was high and can be related to the contents of anthocyanins, total phenolics, catechins, caffeic acid, cinnamic acid and gallic acid. Among the quantified bioactive compounds, the caffeic acid, procyanidin B1 and trans-resveratrol presented higher contents compared to juices of other regions. The analysis of principal components was efficient to show the effect of maceration treatments, giving evidence of positive effect of temperature and pectinases addition on the extraction of desirable grape compounds. A high quality juice was obtained in industrial scale, contributing to the process development taking into account the bioactive properties of the product.

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