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Physicochemical stability of grape juice produced on industrial scale by different commercial enzyme preparations

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Abstract

The present work evaluated the analytical characteristics, total phenolic contents (TPC), antioxidant activities (AOX), sugars and organic acids by RP-HPLC/DAD/RID of grape juices produced on an industrial scale, with three different commercial pectinases. They were evaluated by colour, total monomeric anthocyanins (TMA) and AOX of the juices stored for six months. Newly produced juices did not present differences in the classical parameters such as pH, °Brix, titratable acidity, colour and yield. There was no difference in the organic acids profile of the obtained juices. The juice obtained with predominant presence of hemicellulases presented rhamnose in its composition, indicating degradation of polysaccharides of the grape peel. The juices showed no difference in TPC and AOX between the studied pectinases. In storage studies, there were fast colour evolution, TMA and AOX in all samples. The juice obtained with the predominance of hemicellulases presented more accelerated loss of TMA and AOX. The results obtained in the present work demonstrated the importance of knowing the influence of the use of commercial pectinases in the process of producing grape juice, not only at the time of production, but during its shelf life. The comparison of the different pectinases used under real processing conditions showed that choice of enzymes used in the preparation could be decisive for the shelf life after bottling the product.

Introduction

Grape juice is one of the most consumed juices, due to its pleasant sensory acceptance related to flavour, aroma, colour and freshness (Granato *et al*., 2016). Another factor that popularised the consumption of grape juices is its functional properties *in vitro* and *in vivo*, and potential health benefits (Mcgill *et al*., 2013; Lima *et al*., 2014; Toaldo *et al*., 2015; Toscano *et al*., 2015; Toaldo *et al*., 2016).

Several factors influence the physicochemical composition and bioactive potential of grape juice such as geographical origin, variety, the grape cultivation system and methods of production (Margraf *et al*., 2016; Granato *et al*., 2016; Padilha *et al*., 2017). Recently, several works reported on the influence of different maceration treatments, quantities and types of enzyme preparations in the physicochemical characteristics and bioactive potentials of grape juices (Lima *et al*., 2015; Lambri *et al*., 2015; Aguilar *et al*., 2016). It is known that commercial enzyme preparations based on pectinases (CEPP), used in the maceration of grapes, has improved the process yield, decreased the viscosity, improved the colour extraction and increased the bioactive content and antioxidant activity of grape juice (Aguilar *et al*., 2016; Magro *et al*., 2016). To date however, no studies have been done to evaluate the influence of CEPP in the physicochemical stability of grape juice over shelf life.

In a work carried out by Talcott and Lee (2002), a considerable loss of anthocyanin monomers and antioxidant activity was observed in grape juices produced by different methods and stockpiled for 60 days. This reinforces the importance of studying the physicochemical stability of this beverage over shelf life.

The results obtained for the quantification of antioxidant activity can vary according to the methods used. For grape juices, several techniques have been tested (Lima *et al*., 2014, Padilha *et al*., 2017). The International Organization of Vine and Wine (OIV) recommends the use of a sensitive colorimetric free radical scavenger method using 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH) (Organisation Internationale de la Vigne et du Vin, 2011).

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The addition of pectinases during the grape maceration can be considered a complex process, resulting in important alterations in the chemical composition of grape juice, mainly related to the phenolic compounds involved (Lima *et al*., 2015). There are several commercial enzyme formulations available for application in fruit maceration, consisting of pectinases, cellulases and galactosidases (Arnous and Meyer, 2010).

Given this, we chose to evaluate the influence of three commercial pectic enzyme preparations on the physicochemical characteristics of grape juice produced on an industrial scale, and to assess the colour and antioxidant activity of the juices over six months storage at room temperature.

Materials and methods

Chemical products

Folin-Ciocalteu, ethylic alcohol and sulphuric acid were purchased from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained by purification in a PureLab Option Q Elga System (USA). Tartaric, malic, lactic, citric and acetic acids were purchased from Química Vetec (Rio de Janeiro, Brazil) with a purity level of ≥ 98%. Glucose, fructose and rhamnose were purchased from Chem Service (West Chester, USA).

Grape samples

Grapes of "Isabel Precoce" and "BRS Cora" varieties were collected from a specific area destined for the production of commercial juice at the 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 40º 38'W longitude. The grape juices were produced in an industrial facility belonging to EBFT/ASA. The plants were grown in vineyards with an average age of five 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) in July, and the grapes were harvested in November 2014, 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 of must, expressed as tartaric acid, and °Brix/TA ratio between 20 and 28.

Grape juice processing

Grape juice was produced following the formulation adopted by industries of the Northeast of Brazil, a mixture of Isabel Precoce 80% and BRS Cora 20%; and the cut (blend) was made by mixing the grapes at weighing (Lima *et al*., 2015). 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 machine, model DZ-35, with the addition of an enzymatic liquid mixture based on pectinases ED, EV and RO (Table 1) at a dose of 3.0 mL/100 kg of fresh grapes. The grapes were then pumped into a maceration tank with controlled temperature and the mixture was heated to 60°C and held at this temperature for 1 h with constant pumping of the liquid. Following 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 subjected to pasteurisation at 85°C for 60 s in a tubular pasteuriser. The samples were then packaged, through hot filling of non-coloured glass bottles of 500 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

Table 1. Liquid preparations based on pectinases used in the production of grape juices on an industrial process scale.

Enzyme name	Activitya	Main activity	Optimum temp.	Production strain
Endozym Pecto Fruit PR (ED)[b]	Not given	Pectinliase, polygalacturonase, pectinmethylesterase and cellulase	45-55°C	*Aspergillus niger*
Everzym Thermo (EV)[c]	Not given	Hemicellulase	20-55°C	Not given
Rohapect 10 L (RO)[d]	>70,000 ADJU/ mL	Polygalacturonase	50°C	*Aspergillus niger*

[a] Activity given by the suppliers' data sheets

[b] Obtained from AEB Biochemistry Latin America (São José do Pinhais, PR, Brazil)

[c] Obtained from Ever Intec (Garibaldi, RS, Brazil)

[d] Obtained from Amazon Group (Monte Belo do Sul, RS, Brazil).

bottles were stored at room temperature and analysed 30 d after processing.

Classical analyses and colour measurements

The classical analysis of juice was carried out by determining the pH (potentiometer pH Analyser - Tecnal (Brazil)); Total soluble solids (°Brix) (digital refractometer HI 96801 Hanna®, USA) and titratable acidity (TA), following the methodologies described in OIV (2011). The juice yield was calculated by the volume (L) obtained using 100 kg of fresh grape. The colour intensity was determined by sum of absorbance at 420, 520 and 620 nm, and tint was calculated as the relation between absorbance at 420 nm and at 520 nm with a UV-Vis UV 2000A spectrophotometer, Instrutherm® (Brazil), using glass cuvettes with a path length of 0.5 cm (Organisation Internationale de la Vigne et du Vin, 2011). Other variables calculated were red, yellow, and blue percentages, according to Glories (1984).

HPLC determination of organic acids and sugars

The analyses were performed using an HPLC system model Agilent 1260 Infinity LC (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary solvent pump (G1311C model), degasser, thermostatic column compartment (G1316A model) and an autosampler (model G1329B) coupled to a Diode Arrangement Detector (DAD) (G1315D model) and Refractive Index Detector (RID) (G1362A model). Data were processed using OpenLAB CDS ChemStation EditionTM software (Agilent Technologies, USA).

Analytical procedure was performed following chromatographic conditions previously described by Ball (2011). A 500 µL aliquot of grape juice was diluted in 1.5 mL of ultrapure water, filtered through a 0.45 µm nylon membrane (Chromafil® Xtra, Macherey-Nagel - Germany), and a volume of 10 µL was injected into a column. The column used was that of Agilent Hi-Plex H (300 × 7.7 mm) ion exchange with internal particles of 8.0 µm protected by a PL Hi-Plex H (5 × 3mm) guard column (Agilent Technologies, Santa Clara, CA, USA). The temperature of the column compartment was maintained at 70°C, and RID flow cell at 50°C. The flow used was 0.5 mL/min with a run time of 20 min. The phase was H2SO4 0.004 mol/L in ultrapure water. For the determination of tartaric, malic, lactic, citric and acetic acids, detection was made in the DAD at 210 nm. For glucose, fructose and rhamnose sugar, detection was made in the RID.

Bioactive total content – total phenolic contents and monomeric anthocyanins

Total phenolic contents were determined by the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965). The absorbance of the samples, read at 765 nm was compared to a calibration curve of the gallic acid. The results were expressed as mg/kg gallic acid equivalents (mg/kg GAE). The analysis of total monomeric anthocyanins in juices was performed using the pH differential method, according to Giusti and Wolstrad (2001). Two buffer solutions were taken. The former was 0.025 M of KCl at pH 1.0, the latter was 0.4 M CH_3COONa at pH 4.5. Samples were diluted with buffer solutions and read at 520 nm and 700 nm with a spectrophotometer. Pigments concentration in the juices was expressed as mg/L equivalents of cyanidin 3-glucoside.

Antioxidant activity

In vitro antioxidant activities of the juices were determined by the method of free radicals scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Kim *et al*. (2002). An analytical standard Trolox was used to construct the calibration curves. Results were expressed as Trolox equivalents per litre of grape juice (mM TE/L). Absorbance readings were made using a UV-Vis 2000A spectrophotometer (Instrutherm, Brazil). A solution of DPPH 1.0 mM were prepared in ethanol and diluted to an absorbance of 0.900 ± 0.050 (100 µmol/L). The antioxidant activity of the samples was assessed through the rate of decay in absorbance at 517 nm. The absorbance of free radical solution was determined before and after the addition of grape juice samples, and measured at time t = 0 min and at time t = 30 min after the addition of grape juice. All analyses were performed in triplicate.

Statistical analysis

Treatments consisted of three tanks containing 3,000 kg grape, harvested on the same day and coming from the same area. These were processed to obtain juices using three different commercial liquid preparations based on pectinases: Endozyn Pecto Fruit PR (ED), Rhoapect 10 L (RO) and Everzyn Thermo (EV). Statistical analysis was performed using SPSS version 17.0 for Windows (SPSS, Chicago, USA). Results of the analyses of the juices, carried out 30 d after preparation, were subjected to Analysis of Variance (one-way ANOVA), and followed by comparisons performed using the Tukey's test with a probability of error of 5%. For assessing physicochemical stability, the juices were analysed during storage at 30, 60, 90, 120, 150 and

180 d after filling. Results obtained were subjected to linear regression analyses. For each storage time, three bottles of each treatment (process tank) were evaluated and all analyses carried out in triplicate.

Results and discussion

Quality classical analyses and colour measurements

Results obtained for the classical analyses of grape juices are presented in Table 2. There were no significant differences among the juices produced, with respect to pH, °Brix and titratable acidity. Average values of °Brix and titratable acidity in the samples studied were 18.7 and 0.80%, respectively. The values obtained for juices varied between 62.1 and 62.8% for the ED and EV enzymes respectively. For colour analyses, the juice obtained with the ED enzyme was more intense in colour (12.82), greater %red (57.1) and had a lower tint (0.56). Juices obtained with the RO and EV enzymes obtained on average equal values for %red and tint (55.5 and 0.60, respectively). Juice obtained with the EV enzyme yielded a higher value of %yellow (33.1) compared to those produced with ED and RO enzymes (32.2 and 32.6, respectively). Generally, the produced juices presented similar values for yield and colour, the juice obtained with the ED enzyme, however, stood out by having greater intensity of colour, higher %red and lower tint (yellow/red ratio) than that obtained with the RO and EV enzymes.

According to Magro *et al*. (2016) the yield of juices obtained with different enzymes varied from 69 to 75%, where the CEPP that obtained the highest yield presented balance between the enzymes polygalacturonase, pectinmethylesterase and pectinliase. This indicates that the CEPP exerted little influence on the basic analytical characteristics of the juice as pH, °Brix and acidity titratable, but the different types of pectinases that comprise the CEPP might affect the juice yield.

Organic acids and sugars quantified by RP-HPLC/DAD/RID

A typical chromatogram of grape juices was obtained from Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) coupled with diode-array detection (DAD), and refractive index Detector (RID). The juices produced with different CEPP differed significantly in relation to organic acids and sugars as quantified by the HPLC. The main organic acid present in the samples studied was tartaric acid (5.10-5.33 g/L), followed by malic acid (1.78-1.85 g/L) and citric acid (0.28-0.30 g/L). In relation to acetic acid, the values obtained varied from 0.02 to 0.03 g/L (Table 2). The profile and values of organic acids found in the samples of the present work are in agreement with those mentioned in the literature for grape juices from the São Francisco Valley, in the northeast of Brazil, the same region producing the grapes used in the present work (Lima *et al*., 2014). The values obtained for acetic acid (0.02-0.03 g/L) were below the maximum permitted value by Brazilian legislation for grape juice (0.50 g/L) (Brasil, 2004), giving evidence of the health of the grapes used and that the enzymes studied had not exerted influence on the formation of this undesirable organic acid. The profile and concentration of organic acids are important parameters for the processing and chemical composition of grape juices because their presence causes a reduction of pH of the resulting products and consequently increase the colour stability, since anthocyanins retain the red colour at low pH as well as ensure a taste balance between sweet and sour (Silva *et al*., 2015).

In relation to grape juice sugar content, the evaluated CEPP did not exert a significant influence on their values. Glucose and fructose were the main sugars of the grape and their values varied from 87.9 to 88.1 and 77.2 to 78.2 g/L for glucose and fructose, respectively. Rhamnose was detected only in the juice produced with the EV enzyme, with a value obtained of 0.16 g/L. Although the CEPP studied did not exert a significant influence on the concentration of grape juices sugar, the presence of rhamnose in the juice produced with the enzyme EV indicated a greater degradation of the polysaccharides of the film by this enzyme. According to Apolinar-Valiente *et al*. (2015) grape derivatives can also have other sugars from hydrolysis of polysaccharides by action of pectinase enzymes, such as rhamnose.

Total bioactive content and in vitro antioxidant activity

The total bioactive content and the antioxidant activity of the juices produced with different CEPP are presented in Table 2. The total phenolic content was influenced by the CEPP; the juices produced with the ED and RO enzymes obtained higher values (1661 and 1512 mg/L, respectively); and the juice produced with the EV enzyme obtained an average value of 1425 mg/L. For total monomeric anthocyanins, the juice produced with the EV enzyme obtained the highest average value (257.7 mg/L), followed by the juices obtained with the ED and RO enzymes, with approximate values of 239 mg/L. There was no significant difference in the *in vitro* antioxidant activity of the juices obtained with the different CEPP, where the values obtained

Table 2. Analytical characteristics, colour and total bioactive content of grape juices produced on an industrial scale by different commercial enzyme preparation.

Classical parameters	Enzymes		
	ED	EV	RO
pH	3.27 ± 0.02[a]	3.30 ± 0.03[a]	3.33 ± 0.02[a]
ºBrix	18.70 ± 0.10[a]	18.60 ± 0.10[a]	18.90 ± 0.20[a]
Titratable acidity %	0.80 ± 0.01[a]	0.82 ± 0.02[a]	0.80 ± 0.01[a]
Ratio (ºBrix/acidity%)	23.40 ± 0.30[a]	22.70 ± 0.50[a]	23.60 ± 0.30[a]
Juice yield %	62.10	62.80	62.20
Colour analysis			
%Red	57.10 ± 0.50[a]	55.40 ± 0.10[b]	55.60 ± 0.10[b]
%Yellow	32.20 ± 0.30[b]	33.10 ± 0.10[a]	32.60 ± 0.10[b]
%Blue	10.70 ± 0.83[a]	11.50 ± 0.00[a]	11.80 ± 0.00[a]
Colour Intensity	12.82 ± 0.11[a]	10.89 ± 0.01[c]	11.84 ± 0.01[b]
Tint	0.56 ± 0.00[b]	0.60 ± 0.01[a]	0.59 ± 0.00[a]
Organic acids g/L			
Tartaric	5.14 ± 0.23[a]	5.33 ± 0.13[a]	5.10 ± 0.14[a]
Malic	1.85 ± 0.08[a]	1.78 ± 0.08[a]	1.85 ± 0.05[a]
Lactic	ND	ND	ND
Citric	0.28 ± 0.03[a]	0.29 ± 0.05[a]	0.30 ± 0.02[a]
Acetic	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]
Sugars g/L			
Glucose	87.90 ± 0.30[a]	87.90 ± 0.40[a]	88.10 ± 0.30[a]
Fructose	77.20 ± 0.80[a]	78.20 ± 1.40[a]	77.70 ± 1.50[a]
Rhamnose	ND	0.16 ± 0.02	ND
Total bioactive content			
Total phenolics mg/L	1661 ± 79[a]	1425 ± 39[b]	1512 ± 73[a]
Total monomeric anthocyanins mg/L	239.40 ± 3.80[b]	257.70 ± 5.20[a]	238.00 ± 1.70[b]
Antioxidant activity (DPPH) mM TE/L	11.21 ± 0.25[a]	10.82 ± 0.26[a]	11.24 ± 0.13[a]

Legend: ED – Endozym Pectofruit PR; EV – Everzym Thermo; RO – Rohapect 10 L. Means followed by the same letters in the same lines do not differ by Tukey's test at 5% probability.

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 equivalent to gallic acid.

were 11.21, 10.82, and 11.24 mM TE/L for ED, EV and RO, respectively. The total phenolic values, monomeric anthocyanins and antioxidant activity by DPPH of the juices obtained were similar to that reported in the literature for commercial juices of northeast of Brazil, the same region of origin of the grapes used in the present work (Padilha *et al*., 2017). The presence of rhamnose in grape juice might indicate a high degradation of the grape film structure since this monosaccharide is one of the constituents of structural polysaccharides such as hemicellulose (Fasoli *et al*., 2016). Based on this, the highest values of monomeric anthocyanins in the juice obtained with the EV enzyme can be associated with a higher efficiency of grape film polysaccharide degradation by hemicellulases action, the predominant enzyme in the EV formulation (Table 1).

The total phenolic content and antioxidant activity of the juices obtained with different CEPP under industrial conditions did not display very much difference among the enzymes evaluated. In a study conducted by Magro *et al*. (2016) eight CEPP were tested in grape juices, and significant differences were obtained in the phenolic compositions. The highest values of antioxidant activity, total monomeric anthocyanins and total phenolic contents were obtained with the enzyme Lallzyme Beta[@] whose principal activities are pectinase and polygalacturonase. It should be noted that the experimental condition cited was on a laboratory scale using only 100 g of grape to obtain each juice.

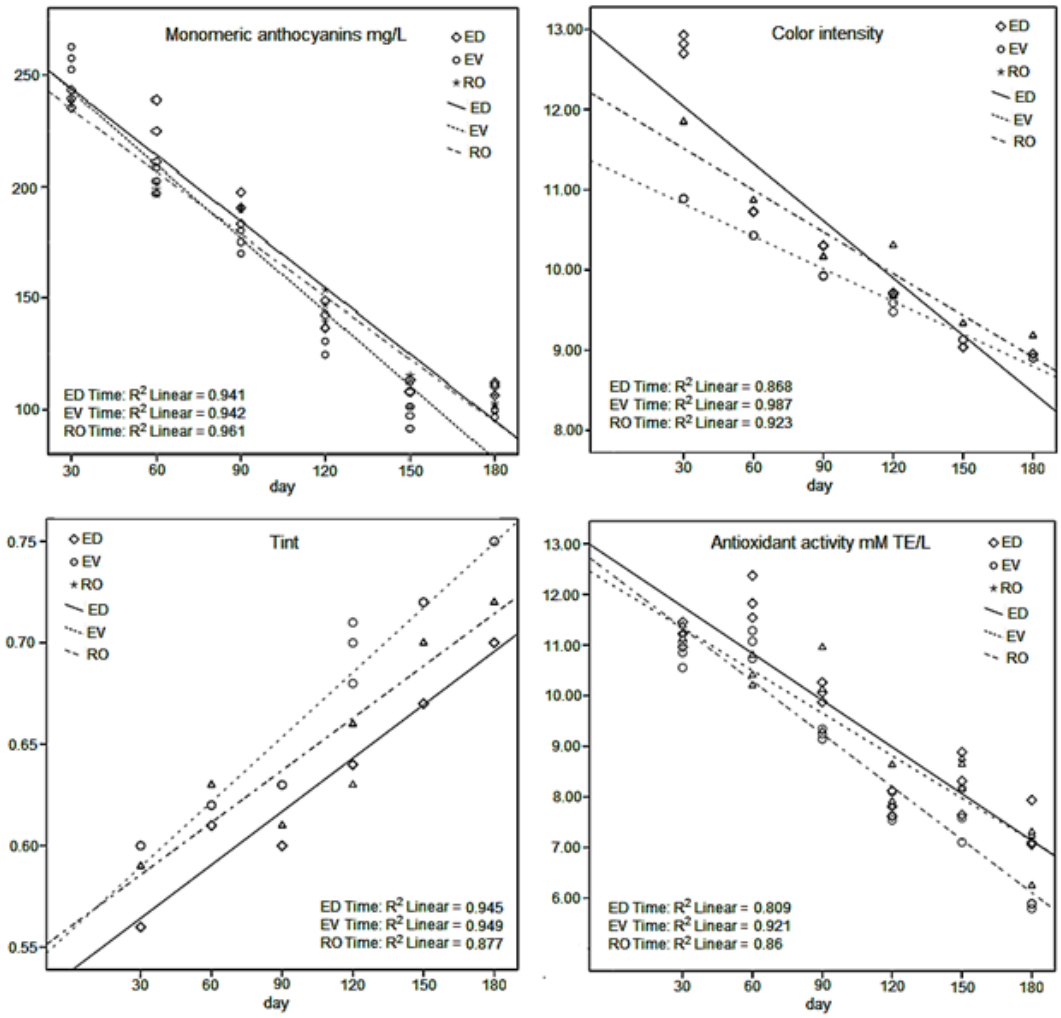

Figure 1. Evolution of colour, total monomeric anthocyanins and antioxidant activity of grape juices obtained through the industrial condition of process with different preparations based on pectinases. Legend: ED –Endozym Pectofruit PR; EV – Everzym Thermo; RO – Rohapect 10 L.

Evolution, monomeric anthocyanins and antioxidant activity of grape juices during storage

To verify the influence of CEPP in the evolution of the colour and antioxidant activity of the juices, both important quality parameters, samples were evaluated during six months storage. The results obtained for total monomeric anthocyanins (TMA), colour intensity (CI), tint (TN), and antioxidant activity (AOX) are shown in Figure 1. A considerable decrease can be seen in TMA, CI and AOX of the juices obtained with the CEPP, over the six months storage, indicating the need to identify the physicochemical properties of grape juice, not just immediately subsequent to their production. The decrease in TMA, CI and AOX, and the increase in TN presented linear behaviour, confirmed by the values of R^2 obtained in the adjustment of the data to the model "$y=bx+a$" (R^2 varying from 0.809 to 0.987), where "y" corresponded to the values obtained in the parameters studied and "x" corresponded to the time of storage. The grape juice produced with the EV enzyme had highest decrease in TMA, starting at an average of 257.7 mg/L and after six months storage showing 98.7 mg/L. The juices produced with the ED and RO enzymes began the storage period with an approximate TMA of 239 mg/L, and at the end of the storage period presented average values of 109.7 and 103.7 mg/L, respectively. These results highlight a more accelerated loss of red colour in the juice produced with the EV enzyme during shelf life, which could be confirmed by the TN in this juice which was the component that developed the fastest.

The tint is the red/yellow colour ratio (absorbance at 420 nm/520 nm), which during the natural evolution of grape juice tends to increase due to several chemical reactions resulting in the decrease in TMA (substances responsible for the colour red) and the increase of compounds responsible for the yellow colour. The main chemical pathways involved in the reduction of monomeric anthocyanins in grape

derivatives, such as wines and juices, are by oxidation or by co-pigmentation anthocyanin/anthocyanin and anthocyanin/tannins (Talcott and Lee, 2002; Ribereau-Gayon *et al*., 2003).

In relation to CI, the juice that presented the most accelerated loss of the total colour was the one produced with the ED enzyme, which started with an average of 12.82, and after six months storage decreased to 8.95. The antioxidant activity of the juice produced decreased significantly during storage, compared to the juice produced with the EV enzyme which presented a more accelerated loss, starting at 10.82 mM TE/L and after six months decreased to 5.85. The juices produced with the ED and RO enzymes presented an initial value of approximately 11.21 mM TE/L, and after storage 7.20 mM TE/L. Talcott and Lee (2002) mentioned losses of over 50% of the antioxidant activity (ORAC method) and TMA in grape juices stored at 25°C and 37°C, but over a period of only 60 days.

The results obtained in the present work demonstrated the importance of knowing the characteristics of CEPP used in the process of producing grape juice, not only after production, but also during its shelf life. In six months storage, expressive changes physicochemical parameters of quality were observed, such as colour, monomeric anthocyanins and antioxidant activity. Considering that the period of validity of grape juices in Brazil is established at a maximum of 24 months, the results obtained in the present work showed the importance of the choice of CEPP that produces greater stability of the product during storage.

Conclusions

Grape juices produced under industrial condition, with three different commercial pectinases showed no considerable difference in classical process parameters such as pH, °Brix, titratable acidity, colour and juice yield. There was also no significant difference for organic acids: tartaric, malic, citric, lactic and acetic quantified by RP-HPLC/DAD/RID. The juice obtained with a predominant preparation of hemicellulases presented rhamnose in its composition, which can indicate a greater degradation of complex polysaccharides of the grape film. For total bioactive content, total phenolic compounds and monomeric anthocyanins, and antioxidant activity, there was no considerable difference between the juices produced. In the study of physicochemical stability over the six months storage, there were considerable losses in colour, total monomeric anthocyanins and antioxidant activity in all the samples studied. The juice obtained with the highest amount of hemicellulases had a more accelerated loss of monomeric anthocyanins, antioxidant activity and increase in tonality, indicative of the rapid evolution of the colour in the bottle. The results obtained in the present work demonstrated the importance of knowing the influence of pectinases in the production of grape juice, not only at the time of production, but also during its shelf life.

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