Abstract
The extraction, and stability of polyphenols from the grape residue were studied. The extractions were performed following the Box-Behnken design, and the surface response methodology was used to model the extraction of total anthocyanins (TA), flavonols (TF), and phenolics (TP). The extraction was optimized simultaneously by the desirability function. The degradation kinetics of monomeric anthocyanins, and the increase in polymeric color were modeled under refrigeration conditions. The extraction with a temperature of 60 °C, solid:liquid ratio of 1/25 g/mL, and time of 80 min, maximized the recovery of TA (30.96 mg/100 g), TF (73.34 mg/100 g), and TP (856.78 mg EAG/100 g). The degradation of monomeric anthocyanins, and the increase in polymeric color followed a kinetic first-order reaction, with reaction rates (k) of $4.10 \times 10^{-1}$ days$^{-1}$, and $3.46 \times 10^{-3}$ days$^{-1}$, respectively. The half-life ($t_{1/2}$) of anthocyanins was 169 days. The ethanol-citric acid solution allowed polyphenols to be efficiently extracted from the grape residue, and had a positive effect on the stability of anthocyanins.

Keywords: Box-Behnken design; phytochemicals; grape residue; solid-liquid extraction.

Practical Application: Extraction of antioxidant compounds from agroindustrial residues of grapes for food use.

1 Introduction
One of the industries historically, and economically important in many countries of the world is the processing of grapes, as they are obtained from various products such as wine, raisins, juices, jellies, among others. The grape fruit is recognized for its nutritional properties and beneficial to health due to its bioactive compounds (Dhekney, 2016).

The wine, and grape juice industries generate abundant waste, which causes additional costs for its elimination (Devesa-Rey et al., 2011; Mammadova et al., 2020). These residues are approximately 30% by weight of the fruit used in processing, consisting of seeds, husks, and stalks (Teixeira et al., 2014). Many investigations point out that residues derived from fruit processing present phytochemicals (Morais et al., 2015), with antioxidant properties (O’Shea et al., 2012; Ribeiro et al., 2018), of which phenolic compounds have been reported frequently (Bataglion et al., 2015).

The grape residue is a potential source of phenolic compounds (Goula et al., 2016), of this group, stands out the anthocyanins for their ability to confer color, and functional properties, which can be used in the elaboration of functional foods (Aguilera et al., 2016). Recently, powdered grape residues were used in the ice cream formulation (Vital et al., 2018), and yogurt (Mammadova et al., 2020), improving their functional properties. Grape seed extracts were used to enrich milk for yogurt processing, observing an alteration of the fermentation time, and the yogurt quality attributes (Alwazeer et al., 2020). Although the results are promising, the use of HCl in the obtaining bioactive extracts represents a potential risk to human health.

The conventional solid-liquid extraction method is alternatively used to obtain phytochemicals. Extraction occurs as result of diffusion of the compound of interest to the solvent, this phenomenon is produced by the affinity and selectivity of the solvent used (Takeuchi et al., 2009). Solvents such as ethanol, methanol, and acetone are often used in the extraction of phytochemicals, these solvents are acidified with hydrochloric acid in order to improve the stability of anthocyanins (Lees & Francis, 1972; Rodriguez-Saona & Wrolstad, 2001). The concern about the use of toxic solvents in the extraction of phytochemicals was approached by Pedro et al., (2016), observed good results in the extraction of phytochemicals with ethanol acidified with citric acid. Some factors that significantly influenced the extraction were temperature, solvent concentration, solid-liquid ratio, and time (Li et al., 2012; Pedro et al., 2016). It was observed that in the extraction of anthocyanins other compounds are also extracted (Pedro et al., 2016).

Due to the current interest in the consumption of foods with functional properties, it is necessary to develop efficient processes, using non-toxic chemicals reagents for the safe extraction of phytochemicals. For this reason, the present research aimed to optimize the extraction of polyphenols from grape residue from...
the juice industry, aiming their use in food processing, and to evaluate their stability during storage.

2 Materials and methods

2.1 Sample

A total of 25 kg of grape residue from cv. Isabel (Vitis labrusca) was supplied by a pulp processing industry in the municipality of Goiana, Pernambuco, Brazil. The residue was homogenized and divided into sample units of 800 g each and stored at -18 ± 1 ºC.

2.2 Chemical reagents

The reagents used in the extraction were: absolute ethanol 99.9% (Merck KGaA, Emsure, Germany) and citric acid 99.5% (Quimica Moderna, Brazil). The reagents used in the quantitative analyzes were: hydrochloric acid, potassium chloride, sodium acetate, chloroform and acetone (Fmaia, Brazil); Folin-Ciocalteu’s reagent (Merck, Germany); Gallic acid 98% (Vetec Química fina Ltda, Brazil); Sodium carbonate (Sigma-Aldrich, Brazil); Gallic acid 98% (Vetec Química fina Ltda, Brazil); Sodium carbonate (Sigma-Aldrich, Brazil) and potassium metabisulphite 96% (K₂S₂O₅) (Dinâmica, Química Contemporânea Ltda, Brazil).

2.3 Preparation of the residue for extraction

The residue was oven dried (MARCONI, MA035, Brazil) with air circulation at 40 h. The dried residue was ground in a knife mill with cooling (TECNAL, TE-631/2, Brazil) for 1 min at 7,000 rpm. The flour was sieved sequentially with two stainless steel sieves (Bertel, Caieiras, Brazil) of 42 and 80 mesh. The residue with a particle size between 355-180 μm was selected for the experiments. This residue was vacuum packed at 98.66 kPa in a vacuum sealer (SELOVAC, 200B, Brazil) and stored protected from light at -18±1 ºC until the experiments were run.

2.4 Characterization of the wet and dry residue

Moisture content

The moisture of the residue was determined by infrared radiation before and after drying, an infrared apparatus (Marte, ID50, Brazil) was used with a constant temperature of 105 ºC.

Quantification of total anthocyanins, total flavonols and total phenolics

Total anthocyanins (TA) and total flavonols (TF) were quantified following the methodology of Lees & Francis (1972). Using 3 g of the wet residue and 2 g of the dried residue for extraction. Absorbance readings were performed on a UV-Visible spectrophotometer (SHIMADZU, UV-1650PC, Japan) at 535 and 374 nm, for TA and TF, respectively. Calculations were performed with Equations 1 and 2, TA were expressed in mg of cyanidin-3-glycoside per 100 g of sample and TF in mg equivalent in quercetin per 100 g of sample.

\[ TA = \frac{Abs_{535 \text{ nm}} \times DF}{98.2} \]  

\[ TF = \frac{Abs_{374 \text{ nm}} \times DF}{76.5} \]

Where: DF – Dilution factor; Abs – Absorbance; TA - Total anthocyanins (mg/100g); TF - Total flavonols (mg/100g)

Total phenolics (TP) were quantified from the extracts obtained to quantify TA and TF, following the methodology of Wettasinghe & Shahidi (1999). The absorbance was read at 725 nm on the UV-Visible Spectrophotometer. TP was calculated with a standard curve constructed with gallic acid and the results were expressed in mg equivalent in gallic acid (EAG) per 100 g of sample.

2.5 Experimental design for extractions

The effect of temperature (X₁), solid:liquid ratio (X₂) and time (X₃) on TA, TF and TP extraction were investigated. The tests were performed according to the Box-Behnken design adjusted to the three independent variables, with total of 15 assays including three replicates at the center point (Table 1). The levels of the variables were adjusted according to the experiences of Pedro et al. (2016), as well as the extraction assays. The extractions were performed in a rotary evaporator (Heidolph, Laborota 4000), with agitation of 90±2 rpm at atmospheric pressure according to the experimental design. 2 g of the dry residue was mixed with the extraction solvent (ethanol acidified with 1.5 mol/L citric acid solution in a ratio of 80/20 vol/vol). After completion of the extraction, the sample-solvent mixture was filtered and the volume of the filtrate was checked to 100 mL with the extraction solvent. The extracts were stored at -18 ± 1 ºC in amber glass vials. The TA, TF and TP were quantified by reading the absorbance of the extracts after 24 h, as described by Lees & Francis (1972).

2.6 Elaboration and characterization of the optimized extract concentrate

The optimized concentrate extract (OCE) was prepared following the flowchart as shown in Figure 1. For each extraction was used 20 g of the dried residue. The parameters of each operation were established in the extraction assays and after the optimization of the extraction. The concentration was added to the process for the purpose of removing the ethanol.

The OCE was analyzed to quantify the monomeric anthocyanins in mg/L of Malvidine-3,5-diglucoside (Toaldo et al., 2013) by the pH-differential method (Lee et al., 2005). TP and TF were quantified by reading the absorbance according to the methodology described by Lees & Francis (1972). The pH was measured with the aid of a pH meter, TECNAL, Tec-3MP, Brazil (Association of Official Analytical Chemistry, 2002). Solids soluble in °Brix were determined by reading on an automatic refractometer at 25 ºC (REICHERT, r2i300, USA). The water activity was determined by the direct method at 25 ºC in an Aqualab (4T analyzer, DECAGON DEVICES, Brazil). The color was characterized using the CIELAB parameters (L* a* b*) as described by McGuire (1992).
The percentage of the polymeric color was determined according to the Giusti, & Wrolstad (2001), with Equation 3.

\[
\text{Polymeric color} = \frac{\text{Polymeric color}}{\text{Color density}} 
\times 100
\]

(3)

The polymeric color and color density were calculated with Equation 4, using absorbance readings of the OCE diluted with bleaching treatment and without bleaching, respectively.

\[
\left( A_{420\text{nm}} - A_{700\text{nm}} \right) + \left( A_{520\text{nm}} - A_{700\text{nm}} \right) \times DF
\]

(4)

Where: \(A\) – Absorbance; \(DF\) – Dilution factor

### Table 1. Box-Behnken design with experimental and fitted data for the extraction of polyphenols from grape residue cv. Isabel.

| Run | Real value and coded independent variables | Response variables |
|-----|-------------------------------------------|--------------------|
|     | \(X_1\) | \(X_2\) | \(X_3\) | \(TA\) | \(TF\) | \(TP\) |
| 1   | 20 (-1) | 1/15 (-1) | 50 (0) | 16.91 ± 1.22 | 18.19 | 56.12 ± 2.00 |
| 2   | 20 (-1) | 1/25 (1) | 50 (0) | 16.49 ± 1.55 | 16.85 | 56.06 ± 2.56 |
| 3   | 60 (1)  | 1/15 (-1) | 50 (0) | 21.19 ± 1.71(e) | 20.83 | 68.01 ± 3.02(b) |
| 4   | 60 (1)  | 1/25 (1) | 50 (0) | 31.25 ± 1.86(c) | 29.97 | 75.88 ± 3.99(a) |
| 5   | 20 (-1) | 1/20 (0) | 20 (-1) | 18.26 ± 1.03(d) | 18.01 | 55.32 ± 1.67(c) |
| 6   | 20 (-1) | 1/20 (0) | 80 (1)  | 18.46 ± 1.03(d) | 17.07 | 58.89 ± 3.43(abc) |
| 7   | 60 (1)  | 1/20 (0) | 20 (-1) | 19.97 ± 0.73(bcd) | 21.36 | 65.84 ± 1.07(bcd) |
| 8   | 60 (1)  | 1/20 (0) | 80 (1)  | 29.24 ± 1.71(a) | 29.49 | 77.92 ± 2.42(b) |
| 9   | 40 (0)  | 1/15 (-1) | 20 (-1) | 17.29 ± 1.10(c) | 16.26 | 59.57 ± 1.95(abc) |
| 10  | 40 (0)  | 1/15 (-1) | 80 (1)  | 19.42 ± 1.07(bcd) | 19.53 | 59.98 ± 1.04(abc) |
| 11  | 40 (0)  | 1/25 (1) | 20 (-1) | 19.94 ± 1.63(bcd) | 19.83 | 59.94 ± 2.86(abc) |
| 12  | 40 (0)  | 1/25 (1) | 80 (1)  | 22.72 ± 1.93(a) | 23.75 | 65.35 ± 2.76(a) |
| 13(C)| 40 (0)  | 1/20 (0) | 50 (0)  | 18.85 ± 0.31(bcd) | 19.60 | 60.72 ± 0.83(b) |
| 14(C)| 40 (0)  | 1/20 (0) | 50 (0)  | 18.06 ± 0.56(bcd) | 19.60 | 59.58 ± 0.93(bcd) |
| 15(C)| 40 (0)  | 1/20 (0) | 50 (0)  | 21.89 ± 2.16(bcd) | 19.60 | 65.89 ± 3.62(bcd) |

\(X_1\): Temperature (°C); \(X_2\): solid/liquid rate (g/mL); \(X_3\): Time (min); TA: Total anthocyanins (mg/100g); TF: Total flavonols (mg/100 g); TP: Total phenolics (mg EAG/100 g); (C): central point; *Mean values ± standard deviation (n = 3). The means in the column follow equal letters in the experimental data indicate statistically significant differences, according to Tukey test (p ≤ 0.05).

### Figure 1. Flowchart of the process of elaboration of optimization extract concentrate of grape residue.
the increase of the polymeric color were determined using the first order reaction model as proposed by Sharma et al., (2016), using Equation 5.

\[ C = C_0 e^{2k_1t} \]  

Where: \( t \) – Time; \( k \) – The first-order kinetic rate constant; \( C_0 \) – Concentrations of monomeric anthocyanins (mg.L\(^{-1}\)) and polymeric color (%) at time zero; \( C \) – Concentrations of monomeric anthocyanins (mg.L\(^{-1}\)) and polymeric color (%) at time \( t \).

The half-life time of the monomeric anthocyanins was calculated with Equation 6.

\[ t_{1/2} = \frac{\ln(2)}{k} \]

Where: \( k \) – The first-order kinetic rate constant; \( t_{1/2} \) – Half-life time

2.8. Statistical analysis

The data of the assays for optimization of the extraction were submitted to analysis of variances (ANOVA) and the differences among the means by Tukey test (\( p < 0.05 \)). The normality and homoscedasticity of the ANOVA residues were obtained by the Anderson-Darling and Breush-Pagan tests, respectively. The analysis of multiple linear regression by response surface methodology (RSM) was performed using the Equation 7 model:

\[ Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j=1}^{i} \beta_{ij} X_i X_j \]  

Where the response function (\( Y \)) is composed of linear, quadratic and interactive components. The constant \( \beta_0 \) denotes the intercept of the model; \( \beta \), \( \beta_i \) and \( \beta_{ij} \) represent the coefficients of the linear, quadratic and iterative components of the model, respectively. \( X \) and \( X_i \) are the independent variables, and \( k \) represents the number of factors that were investigated. The quality of the fit of the experimental data to the model was evaluated by the test of lack of fit, coefficient of ordinary and adjusted regression. The assumption of normality of residuals was verified by Anderson-Darling test. Simultaneous optimization of TA, TF and TP extraction was performed using the desirability function (Derringer & Suich, 1980).

The degradation kinetics of monomeric anthocyanins and the increase in polymeric color of OCE were adjusted by non-linear regression. The normality of the residues and the ordinary determination coefficient were used to evaluate the quality of the adjustment. Statistical analyzes were performed with the aid of MATLAB* R2010a 7.10.0.499 software (MathWorks, USA).

3 Results and discussion

3.1 Characterization of the wet and dry residue

The moisture content of the wet residue was 51.39±1.53 g/100g wet basis (w.b.), a near content was observed in the grape residue of vinification by Minjares-Fuentes et al. (2014). The dried residue had a moisture content of 5.33±0.44 g/100g w.b., a similar value was reported by Ribeiro et al. (2015). The TA content of the residue before and after drying was 11.51±0.95 mg/100g and 28.32±0.34 mg/100g, respectively. The Higher value was observed by Liazid et al. (2011) in the grape skin of cv. Tintilla Rota (V. vinifera), reporting a content of 154.59 mg/100g. The TA content observed in this work is probably due to the low anthocyanin content of the cv. Isabel (Yamamoto et al., 2015), and/or the culture conditions (Zhou et al., 2020; Sun et al., 2019).

The TP content of the wet and dry residue was 273.20 ± 18.27 mg EAG/100 g and 804.26 ± 44.53 mg EAG/100 g, respectively. The phenolic content of the grape residue is influenced by the grape cultivar and the processing conditions (Abe et al., 2007). This fact was observed in the skin of grapes of several cultivars derived from ten different vinification processes (Harsha et al., 2013).

The TF content in the wet and dry residue was 21.65 ± 0.64 mg/100 g and 58.95 ± 0.48 mg/100 g, respectively. Harsha et al. (2013) reported similar contents in grape skins derived from various vinification processes. In a similar study, it was reported close levels in Cabernet Franc and Sauvignon grapes skins derived from vinification (Barcia et al., 2014).

3.2 Surface responses

The experimental data from the extraction assays are presented in Table 1. The normality and homoscedasticity of ANOVA residues and multiple linear regression analysis were verified by the Anderson-Darling and Breush-Pagan test (\( p > 0.05 \)), respectively. Norman (2010), say that parametric statistics are robust when data are not normal. However, obtaining efficient estimates is linked to adherence to the ANOVA assumptions for the residues.

Significant statistical differences (\( p < 0.05 \)) were found between the essays and the means of the essays, by ANOVA and the Tukey test, respectively. The regression results are shown in Table 2, the quadratic model was significant (\( p < 0.05 \)) for TA, TP and TF. Likewise, there was no lack of fit significant (\( p > 0.05 \)) for the three data groups. The adjusted models for TA, TP and TF explain 93%, 94% and 94%, respectively, the variation of the response variables.

Response surface for extraction of total anthocyanins

In Table 2, it is observed that the linear components of temperature (\( X_1 \)), solid/liquid ratio (\( X_2 \)), time (\( X_3 \)), the interaction between the two (\( X_1 X_3 \)) and the intercept were significant and positive. This indicates that these components increase the value of the TA content. The temperature influenced significantly the extraction of anthocyanins from residues of Tulipa gesneriana.
The microwave-assisted extraction of phenolic compounds in grape skins (Cvjetko Bubalo et al., 2016). Extraction of phenolic compounds from grape residue was significantly influenced by temperature and solid:liquid ratio (Pinelo et al., 2005).

Response surface for extraction of total flavonols

The coefficients of the linear components of temperature ($X_1$), time ($X_3$), of the interaction between the temperature with time ($X_1X_3$) and the intercept were significant and positive (Table 2). The temperature had a significant effect on the microwave-assisted extraction of phenolic compounds in grape skins (Cvjetko Bubalo et al., 2016). Extraction of phenolic compounds from grape residue was significantly influenced by temperature and solid:liquid ratio (Pinelo et al., 2005).

Response surface for extraction of total phenolics

The coefficients of the linear components of temperature ($X_1$), time ($X_3$) and intercept were significant (p<0.05) and positive in grape skins and Nitraria tangutorum seeds (Sang et al., 2017).
positive indicating that these components tend to increase TF extraction. The time had a significant influence on the extraction of flavonols from citrus flowers (González-Centeno et al., 2014).

### 3.3 Extraction optimization

Equations 8, 9 and 10, with significant components determined by RSM, were used for optimization. Ex extractions of TA, TF and TP were optimized simultaneously by the desirability function. The individual desirability (d₁, d₂ and d₃) was calculated for TA, TP and TF by unilateral transformation. The global desirability (D) was obtained by the geometric mean of the individual desirability's.

\[
TA = 20.66 - 3.94X_1 - 1.94X_2 - 1.79X_3 + 2.61X_1X_2 \quad (8)
\]

\[
TF = 63.00 + 7.65X_1 + 2.68X_3 \quad (9)
\]

\[
TP = 682.49 - 75.14X_1 - 32.55X_3 + 66.59X_1X_3 \quad (10)
\]

Where: TA – Total anthocyanins; TF – Total flavonols; TP – Total phenolics; X₁ – Temperature (°C); X₂ – Solid/liquid rate (g/mL); X₃ – Time (min)

The conditions that maximize the three response variables obtained for a D = 0.77, was the temperature of 60 °C, solid/liquid ratio of 1/25 g/mL and 80 min. Under these conditions it is possible to extract 30.96 mg/100g of TA; 73.34 mg/100g TF and 856.78 mg EAG/100g TP. Similar optimized extraction temperature was observed in the extraction of anthocyanins from grape skins by Li et al. (2012). Different optimized conditions were observed in the extraction of anthocyanins from black rice (Pedro et al., 2016).

The predicted value for TP extraction was higher than that reported by González-Centeno et al. (2014), on ultrasound extraction of grape residue. In another study, higher near total phenolics content of 957 mg EAG/100g was reported in the extraction of grape residue. In another study, higher near total phenolics content of 957 mg EAG/100g was reported in the extraction of grape residue (Goula et al., 2016). The value predicted for the extraction of total flavonols was higher than reported by González-Centeno et al. (2014), on ultrasonic extraction from grape residue.

### 3.4 Elaboration and characterization of the optimized extract concentrate

Approximately 200 mL of OCE was obtained for each liter of alcoholic extract. Table 3 shows the results of the characterization. Most soluble solids in the extract are assumed to be the citric acid used in the acidification of the extraction solvent. Water activity indicates that there is water available for chemical and enzymatic reactions, which could accelerate the degradation of phytochemicals (Schwartz et al., 2010). The pH of the extract was similar to that reported as convenient to maintain the stability of anthocyanins (Sui et al., 2014).

The positive and superior value of parameter a* compared to b* indicates that OCE has a predominantly red color, with luminosity (L *) of 24.32. The similar color was observed in wines without aging by Avizcuri et al. (2016). The percentage of OCE polymeric color indicates the presence of degraded and/or polymerized anthocyanins, probably generated in the grape processing in the industry (Kirca, & Cemeroglu, 2003). Extracts with composition and similar characteristics obtained in this work are being applied in yogurts (Chouchouli et al., 2013).

### 3.5 Stability of anthocyanins

Table 4 shows the mean values of the monomeric anthocyanin content and the percentage of polymeric color determined periodically during 83 days of storage at 4 °C. In Table 5, the kinetic analysis of anthocyanin degradation is reported, to determine the stability of anthocyanins. The study of anthocyanin degradation in OCE is important, given the stability of anthocyanins in this extract and their potential use in the production of new processed foods, particularly in yogurts and cheeses.

| Days | Monomeric anthocyanins (mg/L) | Polymeric color (%) |
|------|-------------------------------|---------------------|
| 0    | 40.73 ± 0.94                  | 45.40 ± 1.00        |
| 3    | 37.95 ± 3.50                  | 46.17 ± 0.17        |
| 6    | 37.27 ± 2.08                  | 48.63 ± 0.47        |
| 12   | 37.73 ± 1.81                  | 47.96 ± 0.49        |
| 15   | 37.99 ± 1.93                  | 48.58 ± 0.42        |
| 18   | 35.99 ± 1.48                  | 49.97 ± 0.42        |
| 21   | 36.49 ± 0.91                  | 49.96 ± 0.40        |
| 31   | 35.51 ± 1.71                  | 51.38 ± 0.25        |
| 36   | 35.18 ± 1.25                  | 51.40 ± 0.51        |
| 41   | 34.46 ± 0.88                  | 52.86 ± 0.46        |
| 46   | 32.50 ± 0.64                  | 53.39 ± 0.51        |
| 51   | 31.85 ± 1.21                  | 53.35 ± 0.59        |
| 58   | 32.77 ± 0.79                  | 55.83 ± 0.52        |
| 63   | 30.51 ± 0.63                  | 55.36 ± 0.42        |
| 68   | 29.54 ± 1.24                  | 57.03 ± 1.06        |
| 73   | 31.79 ± 1.96                  | 58.63 ± 1.69        |
| 78   | 30.22 ± 0.59                  | 55.77 ± 2.95        |
| 83   | 31.56 ± 1.17                  | 57.75 ± 0.99        |

*Mean (n = 3) ± standard deviation. Means in the columns, followed by equal letters, do not differ statistically among themselves at a 5% probability level by the Tukey Test.
parameters for the degradation of monomeric anthocyanins and the increase of the polymeric color are observed. A 50% lower reaction rate for anthocyanin degradation was observed in extracts of Hibiscus sabdariffa, during storage at 4 °C (Sinela et al., 2017). Cissé et al. (2012) reported a degradation rate of anthocyanins in Hibiscus sabdariffa extract, similar to that found in this present study. They also reported that the increase in polymer color is directly related to the degradation of anthocyanins.

### 4 Conclusions

The optimization of the polyphenol extraction of the grape residue with ethanol acidified with citric acid showed good results. It was observed that temperature and time significantly influenced the extraction of the three phytochemicals. While the solid:liquid ratio was only significant for the extraction of anthocyanins. The quadratic model used to adjust the experimental data was significant, although only linear and interaction components were significant. The obtained extract is rich in polyphenols and from the toxicological point of view is safe, due to the use of only ethanol and citric acid in the extraction, both considered nontoxic. Therefore, the extract can be used in the preparation of food safely. The degradation of monomeric anthocyanins and the increase of polymeric color followed a first order kinetics. The degradation rate of anthocyanins was similar to that reported in other studies, therefore, it can be inferred that the ethanol acidified with the citric acid used in the extraction of polyphenols has a positive effect on the stability of anthocyanins under refrigeration.

### Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Authors are also acknowledged with the National Institute of Science and Technology of Tropical Fruits and CNPq for the financial support; to the Organization of American States (OAS) and to the Grupo Coimbra das Universidades Brasileiras (GCUB). The authors are also grateful to the Company “Agroindustria Frutana” for providing the agro industrial waste of grape cv. Isabel used is this research.

### References

Abe, L. T., Mota, R. V., Lajolo, F. M., & Genovese, M. I. (2007). Compostos fenólicos e capacidade antioxidante de cultivares de uvas Vitis labrusca L. e Vitis vinífera L. *Food Science and Technology (Campinas)*, 27(2), 394-400. http://dx.doi.org/10.1590/S0101-20612007000200032.

Aguilera, Y., Mojica, L., Rebollo-Hernanz, M., Berhow, M., De Mejia, E. G., & Martín-Cabrejas, M. A. (2016). Black bean coats: new source of anthocyanins stabilized by B-cyclodextrin copigmentation in a sport beverage. *Food Chemistry*, 212, 561-570. http://dx.doi.org/10.1016/j.foodchem.2016.06.022. PMid:27374568.

Alwazeer, D., Bulut, M., & Tunçtürk, Y. (2020). Fortification of milk with plant extracts modifies the acidification and reducing capacities of yoghurt bacteria. *International Journal of Dairy Technology*, 73(1), 117-125. http://dx.doi.org/10.1111/1471-0307.12643.

Arici, M., Karasu, S., Baslar, M., Toker, O. S., Sagdic, O., & Kayaagacli, M. (2016). Tulip petal as a novel natural food colorant source: extraction optimization and stability studies. *Industrial Crops and Products*, 91, 215-222. http://dx.doi.org/10.1016/j.indcrop.2016.07.003.

Association of Official Analytical Chemistry – AOAC. (2002). *Official methods of analysis of the Association of Official Analytical Chemists* (16th ed.). Gaithersburg: AOAC.

Avizcuri, J.-M., Sáenz-Navajas, M.-P., Echávarri, J.-F., Ferreira, V., & Fernández-Zurbano, P. (2016). Evaluation of the impact of initial red wine composition on changes in color and anthocyanin content during bottle storage. *Food Chemistry*, 213, 123-134. http://dx.doi.org/10.1016/j.foodchem.2016.06.050. PMid:27451163.

Barcia, M. T., Pertuzatti, P. B., Rodrigues, D., Gómez-Alonso, S., Hermosín-Gutiérrez, L., & Godoy, H. T. (2014). Occurrence of low molecular weight phenolics in Vitis vinifera red grape cultivars and their winemaking by-products from São Paulo (Brazil). *Food Research International*, 62, 500-513. http://dx.doi.org/10.1016/j.foodres.2014.03.051.

Bataglion, G. A., Da Silva, F. M. A., Eberlin, M. N., & Koolen, H. H. F. (2015). Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. *Food Chemistry*, 180, 280-287. http://dx.doi.org/10.1016/j.foodchem.2015.02.059. PMid:25766829.

Chouchouli, V., Kalogeropoulos, N., Korteles, S. J., Karvela, E., Makris, D. P., & Karathanos, V. T. (2013). Fortification of yoghurts with grape (Vitis vinifera) seed extracts. *Lebensmittel-Wissenschaft + Technologie*, 53(2), 522-529. http://dx.doi.org/10.1016/j.lwt.2013.03.008.

Cissé, M., Bohuon, P., Sambe, F., Kane, C., Sakh, M., & Dornier, M. (2012). Aquous extraction of anthocyanins from Hibiscus sabdariffa: Experimental kinetics and modeling. *Journal of Food Engineering*, 109(1), 16-21. http://dx.doi.org/10.1016/j.jfoodeng.2011.10.012.

Cvjetko Bubalo, M., Ćurko, N., Tomašević, M., Kovačević Ganić, K., & Radojičić Redovniković, I. (2016). Black bean coats: new source of molecular weight phenolics in Vitis vinifera seed extracts. *Lebensmittel-Wissenschaft + Technologie*, 53(2), 2327-2335. http://dx.doi.org/10.1016/B978-0-12-384947-2.00360-3.

Dhekney, S. A. (2016). Grape. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 261-265). London: Elsevier. http://dx.doi.org/10.1016/B978-0-12-384947-2.00360-3.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy.
Polyphenols extraction from grape residues

O’Shea, N., Arendt, E. K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science & Emerging Technologies*, 16, 1-10. http://dx.doi.org/10.1016/j.ifset.2012.06.002.

Pedro, A. C., Granado, D., & Rosso, N. D. (2016). Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa L.*) by modeling and assessing their reversibility and stability. *Food Chemistry*, 191, 12-20. http://dx.doi.org/10.1016/j.foodchem.2015.02.045. PMid:26258066.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry*, 53(6), 2111-2117. http://dx.doi.org/10.1021/jf0488110. PMid:15769143.

Ribeiro, L. F., Ribani, R. H., Francisco, T. M. G., Soares, A. A., Pontarolo, R., & Haminiuk, C. W. I. (2015). Profile of bioactive compounds from grape pomace (*Vitis vinifera* and *Vitis labrusca*) by spectrophotometric, chromatographic and spectral analyses. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 1007, 72-80. http://dx.doi.org/10.1016/j.jchromb.2015.11.005. PMid:26590878.

Ribeiro, T. P., Lima, M. A. C., Alves, R. E., Gonçalves, A. L. S., & Souza, A. P. C. (2018). Chemical characterization of winemaking by-products from grape varieties cultivated in Vale do São Francisco, Brazil. *Food Science and Technology (Campinas)*, 38(4), 577-583. http://dx.doi.org/10.1590/fst.01116.

Rodríguez-Saona, L. E., & Wrolstad, R. E. (2001). Extraction, isolation, and purification of anthocyanins. *Current Protocols in Food Analytical Chemistry*, 1, F1.1.1-F1.1.11. https://doi.org/10.1002/0471142913.faf0101s00.

Sang, J., Sang, J., Ma, Q., Hou, X., & Li, C. Q. (2017). Extraction optimization and identification of anthocyanins from Nitraria tangutorum Bohr. seed meal and establishment of a green analytical method of anthocyanins. *Food Chemistry*, 218, 386-395. http://dx.doi.org/10.1016/j.foodchem.2016.09.093. PMid:27719225.

Schwartz, S. J., von Elbe, J. H., & Giusti, M. M. (2010). Colorants. In S. M. Parkin, & O. R. Fennema (Eds.), *Colorants* (pp. 140-142). London: CRC Press, Taylor & Francis Group.

Sinha, R., Gupta, R. C., Singh, S., Bansal, A. K., & Singh, I. P. (2016). Stability of anthocyanins- and anthocyanidins-enriched extracts, and formulations of fruit pulp of *Eugenia jambolana* (‘jamur’). *Food Chemistry*, 190, 808-817. http://dx.doi.org/10.1016/j.foodchem.2015.06.029. PMid:26313042.

Sinela, A., Rawat, N., Mertz, C., Aehrich, N., Fulcrand, H., & Dornier, M. (2017). Anthocyanins degradation during storage of *Hibiscus sabdariffa* extract and evolution of its degradation products. *Food Chemistry*, 214, 234-241. http://dx.doi.org/10.1016/j.foodchem.2016.07.071. PMid:27507471.

Sui, X., Dong, X., & Zhou, W. (2014). Combined effect of pH and high temperature on the stability and antioxidant capacity of two anthocyanins in aqueous solution. *Food Chemistry*, 163, 163-170. http://dx.doi.org/10.1016/j.foodchem.2014.04.075. PMid:24912712.

Sun, X., Liu, L., Ma, T., Yu, J., Huang, W., Fang, Y., & Zhan, J. (2019). Effect of high Cu²⁺ stress on fermentation performance and copper biosorption of *Saccharomyces cerevisiae* during wine fermentation. *Food Science and Technology (Campinas)*, 39(1), 19-26. http://dx.doi.org/10.1590/1678-457x.24217.

Takeuchi, T. M., Pereira, C. G., Graga, M. E. M., Marostica, M. R., Leal, P. F., & Meireles, M. A. A. (2009). Low-pressure solvent extraction (solid–liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants. In M. A. A. Meireles (Ed.), *Extracting bioactive compounds for food products: theory and applications* (pp. 140-142). London: CRC Press, Taylor & Francis Group.
Chañi-Paucar et al.

Teixeira, A., Baenas, N., Domínguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., & García-Viguera, C. (2014). Natural bioactive compounds from winery by-products as health promoters: a review. *International Journal of Molecular Sciences*, 15(9), 15638-15678. http://dx.doi.org/10.3390/ijms150915638. PMid:25192288.

Toaldo, I. M., Fogolari, O., Pimentel, G. C., de Gois, J. S., Borges, D. L. G., Caliari, V., & Bordignon-Luiz, M. (2013). Effect of grape seeds on the polyphenol bioactive content and elemental composition by ICP-MS of grape juices from Vitis labrusca L. *Lebensmittel-Wissenschaft + Technologie*, 53(1), 1-8. http://dx.doi.org/10.1016/j.lwt.2013.02.028.

Vital, A. C. P., Santos, N. W., Matumoto-Pintro, P. T., da Silva Scapim, M. R., & Madrona, G. S. (2018). Ice cream supplemented with grape juice residue as a source of antioxidants. *International Journal of Dairy Technology*, 71(1), 183-189. http://dx.doi.org/10.1111/1471-0307.12412.

Wettasinghe, M., & Shahidi, F. (1999). Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *Journal of Agricultural and Food Chemistry*, 47(5), 1801-1812. http://dx.doi.org/10.1021/jf9810416. PMid:10552455.

Yamamoto, L. Y., de Assis, A. M., Roberto, S. R., Bovolenta, Y. R., Nixdorf, S. L., García-Romero, E., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2015). Application of abscisic acid (S-ABA) to cv. Isabel grapes (*V. vinifera × V. labrusca*) for color improvement: effects on color, phenolic composition and antioxidant capacity of their grape juice. *Food Research International*, 77, 572-583. http://dx.doi.org/10.1016/j.foodres.2015.10.019.

Zhou, S. H., Guo, R. R., Wei, R. F., Liu, J. B., Yu, H., Shi, X. F., Zhang, Y., Xie, T. L., & Cheng, G. (2020). Effects of bagging or the combination of umbrella and bag treatments on anthocyanin accumulation in the berry skin of ‘Kyoho’ (*Vitis labruscana*) grape. *Food Science and Technology (Campinas)*, 40(2), 394-400. http://dx.doi.org/10.1590/fst.41218.