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Simplified process of extraction of polyphenols from agroindustrial grape waste

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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 x 10^{-3} $days^{-1}$, and 3.46 x 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.

Pratical 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

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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 °C.

2.2 Chemical reagents

The reagents used in the extraction were: absolute ethanol 99.9% (Merck KGaA, Emsure, Germany) and citric acid 99.5% (Química 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) and potassium metabisulphite 96% ($K_2S_2O_5$) (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 °C for 18 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°C 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 °C.

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.

$$TA = \frac{Abs_{535\,nm}\, x\, DF}{98.2} \quad (1)$$

$$TF = \frac{Abs_{374\,nm}\, x\, DF}{76.5} \quad (2)$$

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 (X_1), solid:liquid ratio (X_2) and time (X_3) 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 °C 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 °C (REICHERT, r2i300, USA). The water activity was determined by the direct method at 25 °C in an Aqualab (4T analyzer, DECAGON DEVICES, Brazil). The color was characterized using the CIELAB parameters (L* a* b*) as described by McGuire (1992).

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					
				TA		TF		TP	
	X_1	X_2	X_3	Experimental*	Fitted	Experimental*	Fitted	Experimental*	Fitted
1	20 (-1)	1/15 (-1)	50 (0)	16.91 ± 1.22^{e}	18.19	56.12 ± 2.00^{f}	56.65	625.00 ± 13.94^{jk}	606.77
2	20 (-1)	1/25 (1)	50 (0)	16.49 ± 1.55^{e}	16.85	56.06 ± 2.56^{f}	56.07	625.00 ± 11.81^{jk}	632.07
3	60 (1)	1/15 (-1)	50 (0)	21.19 ± 1.71^{bcd}	20.83	68.01 ± 3.02^{b}	67.99	785.71 ± 5.91^{b}	778.64
4	60 (1)	1/25 (1)	50 (0)	31.25 ± 1.86^{a}	29.97	75.88 ± 3.99^{a}	75.35	742.56 ± 4.65^{c}	760.79
5	20 (-1)	1/20 (0)	20 (-1)	18.26 ± 1.03^{cde}	18.01	55.32 ± 1.67^{f}	56.28	623.51 ± 16.75^{jk}	644.53
6	20 (-1)	1/20 (0)	80 (1)	18.46 ± 1.03^{cde}	17.07	58.89 ± 3.43^{cdef}	57.39	586.31 ± 7.84^{l}	576.45
7	60 (1)	1/20 (0)	20 (-1)	19.97 ± 0.73^{bcde}	21.36	65.84 ± 1.07^{bd}	67.34	651.79 ± 19.07^{fghij}	661.64
8	60 (1)	1/20 (0)	80 (1)	29.24 ± 1.71^{a}	29.49	77.92 ± 2.42^{a}	76.96	880.95 ± 15.19^{a}	859.93
9	40 (0)	1/15 (-1)	20 (-1)	17.29 ± 1.10^{de}	16.26	59.57 ± 1.95^{cdef}	58.08	636.16 ± 8.05^{fghik}	633.37
10	40 (0)	1/15 (-1)	80 (1)	19.42 ± 1.07^{bcde}	19.53	59.98 ± 1.04^{cdef}	60.95	671.13 ± 16.15^{eg}	699.22
11	40 (0)	1/25 (1)	20 (-1)	19.94 ± 1.63^{bcde}	19.83	59.94 ± 2.86^{cdef}	58.97	665.92 ± 7.18^{eh}	637.83
12	40 (0)	1/25 (1)	80 (1)	22.72 ± 1.93^{b}	23.75	65.35 ± 2.76^{be}	66.84	699.40 ± 17.34^{de}	702.19
13(C)	40 (0)	1/20 (0)	50 (0)	18.85 ± 0.31^{bcde}	19.60	60.72 ± 0.83^{bf}	62.06	663.69 ± 6.82^{ei}	681.30
14(C)	40 (0)	1/20 (0)	50 (0)	18.06 ± 0.56^{cde}	19.60	59.58 ± 0.93^{cdef}	62.06	671.88 ± 8.05^{ef}	681.30
15(C)	40 (0)	1/20 (0)	50 (0)	21.89 ± 2.16^{bc}	19.60	65.89 ± 3.62^{bc}	62.06	708.33 ± 7.84^{cd}	681.30

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 ($p \leq 0.05$).

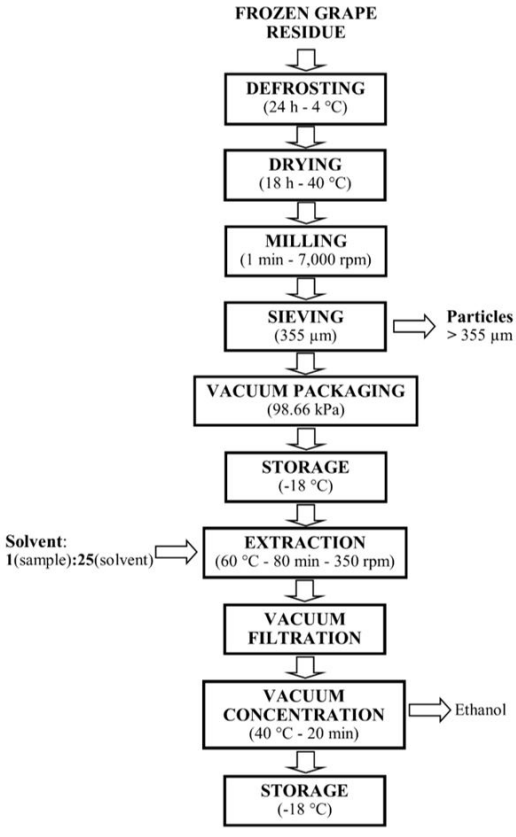

Figure 1. Flowchart of the process of elaboration of optimization extract concentrate of grape residue.

Percentage of polymeric color of optimized extract concentrate

The percentage of the polymeric color was determined according to the Giusti, & Wrolstad (2001), with Equation 3. The diluted OCE (1 part OCE and 4 parts distilled water) was used for the determinations.

$$Polymeric\ color\ (\%) = \left(\frac{Polymeric\ color}{Color\ density} \right) \text{x}100 \quad (3)$$

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.

$$\left[\left(A_{420nm} - A_{700nm} \right) + \left(A_{520\,nm} - A_{700nm} \right) \right] x\, DF \quad (4)$$

Where: *A* – Absorbance; *DF* – Dilution factor

2.7. Stability study of anthocyanins

The OCE was packed in 20 mL amber vials and stored at 4 °C in a refrigerated incubator (BOD, TECNAL, TE-371, Brazil). The degradation of the monomeric anthocyanins was monitored by the pH-differential method (Lee et al., 2005) and the polymeric color by the method described in item 2.6.1. The determinations were performed initially every 3 days and after 21 days every 5 days for a period of 83 days. The degradation kinetics of the monomeric anthocyanins and

the increase of the polymeric color were determined using the first order reaction model as proposed by Sharma et al., (2016), using Equation 5.

$$C = C_0 e^{\pm k*t} \quad (5)$$

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.

$$t_{1/2} = \frac{\ln(2)}{k} \quad (6)$$

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:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i>j}^{k} \beta_{ij} X_i X_j \quad (7)$$

Where the response function (Y_i) is composed of linear, quadratic and interactive components. The constant β_0 denotes the intercept of the model; B_i, β_{ii} and β_{ij} represent the coefficients of the linear, quadratic and iterative components of the model, respectively. X_i and X_j 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_1X_2) 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*

Table 2. Estimated regression coefficients for the model quadratic polynomial and the ANOVA of the fitted model.

Source	Sum of Squares	DF	Mean square	Coefficients of the model	*F-value*	*p-value*
Total anthocyanins						
Constant				19.60		0.0000*
X_1	124.29	1	124.29	3.94	34.85	0.0019*
X_2	30.41	1	30.41	1.95	8.53	0.0330*
X_3	25.86	1	25.86	1.79	7.25	0.0431*
X_1X_2	27.41	1	27.41	2.62	7.68	0.0392*
Model	239.91	9	26.66		7.47	0.0280*
Lack of fit	9.64	3	3.21		0.79	0.6022
Pure error	8.19	2	4.09			
Total	257.74	14	18.41			
Ordinary R^2	0.931					
Adjusted R^2	0.806					
Total phenolics						
Constant				681.30		0.0000*
X_1	45178.18	1	45178.18	75.15	49.47	0.00089*
X_3	8477.11	1	8477.11	32.55	9.28	0.02852*
X_1X_3	17738.23	1	17738.23	66.59	19.43	0.00697*
Model	73336.79	9	8148.53		8.92	0.01334*
Lack of fit	3436.09	3	1145.37		2.03	0.3471
Pure error	1129.71	2	564.85			
Total	77902.60	14	5564.47			
Ordinary R^2	0.941					
Adjusted R^2	0.835					
Total flavonols						
Constant				62.06		0.00000*
X_1	469.09	1	469.09	7.66	65.59	0.00046*
X_3	57.60	1	57.60	2.68	8.06	0.03632*
Model	618.32	9	68.70		9.61	0.01132*
Lack of fit	13.19	3	4.39		0.39	0.77592
Pure error	22.56	2	11.28			
Total	654.07	14	46.72			
Ordinary R^2	0.945					
Adjusted R^2	0.847					

DF: Degrees of freedom; *Means significance ($p < 0.05$);X_1: Temperature (°C). X_2: solid/liquid rate (g/mL) and X_3: Time (min).

L. petals (Arici et al., 2016) and in *Nitraria tangutorun* seeds (Sang et al., 2017).

Response surface for extraction of total phenolics

The coefficients of the linear components of the 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 flavonols

The coefficients of the linear components of temperature (X_1), time (X_3) and intercept were significant ($p<0.05$) and

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. Extractions of TA, TF and TP were optimized simultaneously by the desirability function. The individual desirability (d_1, d_2 and d_3) 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; X_1 – Temperature (°C); X_2 – Solid/liquid rate (g/mL); X_3 – 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/100 g was reported in the 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

Table 3. Result of the characterization of the optimized extract concentrate.

Analysis	Value*
Soluble solid (°Brix)	34.07 ± 2.18
Water activity	0.94 ± 0.01
Color:	
L*	24.32 ± 1.88
a*	26.32 ± 4.12
b*	5.14 ± 3.27
pH	2.14 ± 0.15
Monomeric anthocyanins (mg/L)	39.95 ± 1.39
Total flavonols (mg/L)	118.49 ± 1.21
Total phenolics (mg EAG/L)	2257.44 ± 78.14
Polymeric color (%)	46.63 ± 1.46

*Mean values ± standard deviation (n = 3).

Table 4. Variation of monomeric anthocyanins content and percentage of polymeric color in the concentrated extract stored at 4 °C

Days	Monomeric anthocyanins ($mg.L^{-1}$)*	Polymeric color* (%)
0	40.73 ± 0.94^{a}	45.40 ± 1.00^{l}
3	37.95 ± 3.50^{ab}	46.17 ± 0.17^{kl}
6	37.27 ± 2.08^{ae}	48.63 ± 0.47^{ghijk}
12	37.73 ± 1.81^{ad}	49.76 ± 0.49^{ghij}
15	37.99 ± 1.93^{ac}	48.58 ± 0.42^{ghijk}
18	35.99 ± 1.48^{bcdeg}	49.97 ± 0.42^{fj}
21	36.49 ± 0.91^{acdf}	49.96 ± 0.40^{fi}
31	35.51 ± 1.71^{bcdeh}	51.38 ± 0.25^{defh}
36	35.18 ± 1.25^{bcdei}	51.40 ± 0.51^{dfg}
41	34.46 ± 0.88^{bcdej}	52.86 ± 0.46^{cf}
46	32.50 ± 0.64^{fghijl}	53.39 ± 0.51^{cd}
51	31.85 ± 1.21^{fghijm}	53.35 ± 0.59^{ceg}
58	32.77 ± 0.79^{efk}	55.83 ± 0.52^{ac}
63	30.51 ± 0.63^{ijk}	55.36 ± 0.42^{bc}
68	29.54 ± 1.24^{klmn}	57.03 ± 1.06^{a}
73	31.79 ± 1.96^{fhijn}	58.63 ± 1.69^{a}
78	30.22 ± 0.59^{jk}	55.77 ± 2.95^{adef}
83	31.56 ± 1.17^{ghijk}	57.75 ± 0.99^{ab}

*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.

Table 5. Kinetic parameters of the degradation of monomeric anthocyanins and generation of polymeric color of the concentrated extract in storage at 4 °C.

Variables	C_0	k_1 (days^{-1})	$t_{1/2}$ (days)	SE	R^2
Monomeric anthocyanins (mg.L^{-1})	40.73	4.096 x 10^{-3}	169.22	0.0002192	0.81
Polymeric color (%)	45.39	3.465 x 10^{-3}	-	0.0001509	0.83

k_1: Constant of the reaction of the first order model; C_0: Initial concentration; $t_{1/2}$: Half-life time; SE: standard error; R^2: Coefficient of ordinary determination.

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.

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