



Purification of phenolic compounds from genipap (*Genipa americana* L.) extract by the ultrasound assisted ultrafiltration process

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ABSTRACT. The aim of this study was to evaluate the potential of an integrated membrane process for purification of polyphenol and genipin components from genipap fruit extract. Optimal conditions for aqueous extraction of polyphenols from genipap fruit pulp were at 71°C for 49 min using a fruit pulp:water ratio of 1:3 (w w⁻¹). Microfiltration with membranes of different pore sizes (0.22, 0.3 and 0.8 µm) resulted in a clarified permeate with similar physical-chemical properties, but the permeate flux through the 0.8 µm membrane was higher than through the other membranes. The sequential ultrafiltration process reduced even more total and soluble solids of the microfiltered extract. Purities of polyphenols and genipin were, respectively, 2.5 and 1.2 times greater in the permeate than in the feed stream. Application of ultrasound during the ultrafiltration increased the steady state permeate flux and the purities of polyphenols and genipin in the permeate stream. Results suggest application of microfiltration as a clarification step followed by the ultrasound assisted ultrafiltration to produce a purified genipap extract with high contents of polyphenols and genipin.

Keywords: membrane; extraction; genipap extract; clarification; purification; phenolic compounds.

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Introduction

Extraction, purification and concentration of high added-value compounds from natural sources are of great interest to pharmaceutical and food industries. Genipap (*Genipa Americana* L.) is a soft brown berry fruit widely distributed in tropical Central and South America (Porto et al., 2014). Genipap fruit has considerable concentrations of phytosterols, such as campesterol, stigmasterol, and β -sitosterol, which presents anti-obesity, antioxidant and antiproliferative functional properties (Bailao, Devilla, Conceicao, & Borges, 2015). Moreover, genipap contains an iridoid cross-linking compound named genipin, which forms a blue pigment by a spontaneous reaction with amine groups and has a great potential to be used by the food industry (Bentes, Souza, Amaya-Farfan, Lopes, & Faria, 2015). Proposition of processes for genipap pulp fruit processing is rarely reported in the literature, although the importance of this fruit had already been highlighted (Porto et al., 2014). Pena, Renard, Montanez, Reyes-Vega, and Contreras-Esquivel (2016) recently presented a review on extraction and purification methods of genipin from *Genipa americana* L. (Rubiaceae) and *Gardenia jasminoides* Ellis (Rubiaceae). Purification methods include ionic exchange resins, chromatographic columns and ultrafiltration process (Pena, Renard, Montanez, Reyes-Vega, & Contreras-Esquivel, 2015; Pena et al., 2016). However, a deep study on extraction and purification conditions of bioactive compounds from genipap pulp fruit should be still carried out.

Membrane filtration is a suitable alternative for fruit extract treatment, since the process is carried out at low temperatures and preserves the food functional nutrients with a minimum energy demand. The membrane filtration process has been successfully applied for the treatment of several fruit extracts, including açai (*Euterpe oleracea* Mart.) (Silva, Rossi, Cardoso, & Reis, 2016), passion fruit (Oliveira, Docê, & Barros, 2012; Domingues, Ramos, Cardoso, & Reis, 2014), pineapple (Barros, Andrade, Mendes, & Peres, 2003), cashew apple (Das & Arora, 2017), pequi (Magalhães, Cardoso, & Reis, 2018), and others. However, one of the main drawbacks in applying the membrane filtration process is fouling, which causes a drastic

decline in the permeate flux. Application of ultrasound during membrane filtration may result in the decrease of cake formation with consequent increase in permeate flux, as observed by Aghdam, Mirsaeedghazi, Aboonajmi, and Kianmehr (2015) for the ultrasound assisted membrane filtration of pomegranate juice.

Here, we propose a suitable process for extraction and purification of polyphenol and genipin compounds from genipap fruit pulp. The main objectives of this study are twofold: to find the best conditions of temperature and time for aqueous extraction of polyphenol content from genipap pulp by using the response surface methodology, and to evaluate a sequential process of micro- and ultrafiltration for clarification of genipap extract and for purification of polyphenol compounds, respectively. The effect of using ultrasound during ultrafiltration was also evaluated. Additionally, fouling occurrences were analyzed by using a mathematical flux decay model.

Material and methods

Material

Analytical grade reagents were purchased from Vetec (Brazil) for physical-chemical analyses. The standard genipin ($\geq 98\%$, HPLC, powder, Sigma Aldrich) was used for chromatographic analyses. Ripe genipap fruit were purchased from a local market (Uberlândia, state Minas Gerais, Brazil).

Extraction process

Genipap fruits were manually washed and sanitized (10 ppm sodium hypochlorite). The mesocarp was crushed in a domestic food processor to obtain the pulp, which was stored in plastic packages at -20°C . The aqueous extract of genipap was prepared by using a mass ratio of 1:3 (pulp:distilled water), as suggested by Machado, Mello, and Hubinger (2015) for extraction of phenolic compounds from fruit pulp. Extractions were performed using a magnetic stirrer (400 rpm) and, after the required extraction time (obtained by experimental design), the extract was filtered through a strainer ($3\ \mu\text{m}$) to remove rough particles.

The response surface methodology (RSM) was applied to verify the effects of the independent variables (temperature, X_1 and extraction time, X_2) on the efficiency of extracting phenolic compounds from genipap pulp. Experiments were performed according to a central composite design consisted of 11 experimental runs with three replicates at the central point and using an alpha for orthogonality of 1.1474. Table 1 lists the independent variables and their coded and real values. The applied levels were chosen based on preliminary assays and on values recommended in the literature (Yang, Liu, & Gao, 2009).

Data were analyzed by multiple regressions through the least-square method. A second-order polynomial equation was used to express the response (phenolic content, Y) as a function of the coded independent variables, as presented in Equation 1.

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k b_{ij} X_i X_j \quad (1)$$

where:

X_i and X_j are the independent variables for the response Y , k is the number of variables and b_0 , b_i , b_{ii} and b_{ij} represents the regression coefficients of the variables for intercept, linear, quadratic and interaction terms, respectively.

The analysis of variance (ANOVA) was applied to check the significance of the terms in the obtained model. The RSM was applied to the experimental data using the Statistica 8.0 software. Optimization of the extraction conditions was carried out as suggested by Myers, Montgomery, and Anderson-Cook (2009) by calculating the critical point of the obtained model. Coefficients of Equation 1 were written as vectors and matrices as presented in Equation 2.

$$Y = a + bX^T + X^T C X \quad (2)$$

where:

a is a scalar for the intercept term in the response surface equation, b is a vector for the linear terms, C is a matrix for the quadratic and interaction terms and X is a matrix for the independent variables.

The critical point (X_c) is obtained by solving the linear systems which is resulted from the equality of the partial derivatives of the independent variables to zero ($X_c = -0.5C^{-1}b$). The critical point is characterized (maximum, minimum or saddle point) from the signal of the autovalues associated to the C matrix of the response surface model.

Table 1. Independent variables and their coded and real values employed in a central composite design for aqueous extraction of phenolic compounds from genipap fruit pulp.

Independent variable	Coded levels				
	-1.1474 (- α)	-1	0	+1	1.1474 (+ α)
Temperature (°C), X ₂	37	40	60	80	83
Time (min), X ₂	28	30	45	60	62

Membrane processes

Flat microfiltration membranes of cellulose ester with different pore sizes (Millipore®, 0.22, 0.3 and 0.8 μm) were evaluated for the pre-treatment of genipap extract. Microfiltration processes were carried out at room temperature (approximately 25°C) and at transmembrane pressure of 0.5 bar in a dead-end module with a permeation area of 6.36x10⁻³ m². Ultrafiltration processes were carried out sequentially to the microfiltration processes using a flat membrane of polyethersulfone (NADIR®) with molecular weight cut-off (MWCO) of 50 kDa in a filtration module with permeation area of 3.84x10⁻³ m². Operation conditions were transmembrane pressures of 3 and 5 bar and temperature of 25°C. Ultrafiltrations were performed with and without ultrasound. In the ultrasound assisted ultrafiltration, the filtration module was inserted in an ultrasound bath of 40 Hz (Ultracleaner, model 1650A). The temperature was controlled at 25°C during the process by water recirculation.

The Hermia model (Hermia, 1982) (Equation 3) was applied to adjust the flux decay data.

$$-\frac{dJ}{dt} = kJ^{3-n} \quad (3)$$

where:

J is the mass flux (kg h⁻¹ m⁻²), t is the filtration time (min), k is the equation constant and n is the number indicating fouling mechanism.

The experimental flux data were compared to the flux curves considering the ‘n’ values that represent each fouling mechanism (n = 2 for complete pore blocking, n = 1.5 for internal pore blocking, n = 1 for partial pore blocking and n = 0 for cake formation). Differential equations were numerically solved by the Levenberg–Marquardt method, combining the Gauss and the Steepest Descent methods. An integration step of 10⁻³ and a precision of 10⁻⁸ were applied.

Analytical determinations

The obtained pulp was analyzed for pH, acidity, moisture, ash, protein and lipid content according to the AOAC methods (AOAC). Total carbohydrate content was determined by the difference from the sum of moisture, protein, lipids and ash content. Analyses were done in triplicate.

The genipap extract and the micro- and ultrafiltered permeates were analyzed for pH, total solids, acidity, brix, turbidity, color, total polyphenol content and genipin concentration. Values of pH were measured using a Digital pHmeter model PG2000. Total solids were measured by weighting 5.0 mL of the sample before and after drying at 105°C for 24 hours. Titratable acidity measurements were carried out by titrating 10 mL of the sample with a solution of NaOH at 0.1 mol L⁻¹ and using phenolphthalein as indicator, as recommended by AOAC (AOAC). Total soluble solids were measured with a Hanna Instruments HI 96801 refractometer (USA) and were expressed as °Brix. Turbidity was measured with a Nova Organica HD 114 turbidimeter. The color parameters L, a* and b* were determined using a CR-400 Minolta colorimeter, and readings were obtained in the CIEL*a*b* scale.

Total polyphenols were determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). The absorbance was read in an UVmini-1240 Shimadzu spectrophotometer at 760 nm. Gallic acid was used as the standard for calibration curve, and results of total phenolic content were expressed in mg of gallic acid equivalents (mg GAE g⁻¹ of sample). Genipin concentrations were determined by liquid chromatography in a Shimadzu HPLC (model LC-20A Prominence) chromatograph equipped with a Discovery HS C18 column. Chromatographic analyses were carried out at 280 nm and 40°C. A sample of 10 μL was automatically injected at a flow rate of 0.7 mL min⁻¹. The mobile phase consisted of 2% (v v⁻¹) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (50:50 v v⁻¹, eluent B) using a gradient program, as suggested by Ribeiro et al. (2015).

All analyses were done in triplicate. Results were expressed as mean ± standard deviation and analysis of variance (ANOVA) and correlations were performed with the software Statistic version 8.0 (StatSoft, Inc.,

Tulsa, OK, USA). A significance level of 5% was adopted for rejection of the null hypothesis ($p < 0.05$) for the Tukey's test.

Results and discussion

Effect of temperature and time on the efficiency of extracting phenolic compounds from genipap pulp

Prior to the aqueous extraction process, the pulp obtained from the genipap fruit was characterized and presented moisture, protein, lipid, ash and carbohydrate contents of 74.53, 0.59, 0.36, 1.02, 23.5 and 0.6 g 100 g⁻¹, respectively, and a pH value of 3.8. The characteristics of the genipap pulp are similar to those reported in the literature (Souza, Pereira, Queiroz, Borges, & Carneiro, 2012). According to its high moisture content, genipap can be classified as a fleshy and succulent fruit, which is a common characteristic of tropical fruits. Moreover, the genipap fruit is mild in flavor due to its intermediate acidity value, besides being a low fat fruit (Souza et al., 2012).

Aqueous extractions of polyphenolic compounds from genipap fruit pulp were performed at different conditions of temperature and time, according to a central composite design. Experimental results of total polyphenol content (TPC) at all operational conditions are listed in Table 2. Table 3 summarizes the results of analysis of variance (ANOVA) and the regression coefficients obtained by multiple regressions through the least-square method.

ANOVA results in Table 3 show that the obtained model is suitable to represent the experimental data. According to the value of the coefficient of determination (R^2), which was calculated from the quadratic regression model, 98% of the variability in the response can be explained by the fitted model. The value of the adjusted coefficient of determination (R^2 Adjusted) was close to the R^2 value, indicating a high degree of correlation between experimental and predicted values. Furthermore, the mathematical model is statistically acceptable due to non-significant lack of fit (lack of fit p -value > 0.05). All these results illustrated that the model was satisfactory for predicting the total polyphenol content in genipap extracts under any combination of the variables within the applied range.

Considering the terms significant at $p < 0.05$, Equation 4 represents the predicted response for the total phenolic content in terms of coded factors. The values of the coefficient of determination and the adjusted coefficient of determination for the reduced model (Equation 4) were $R^2 = 0.9815$ and $\text{Adj. } R^2 = 0.9692$, respectively. The coefficient of variation (CV) of the reduced model was 1.82%, which represents the low dispersion of the data ($\text{CV} < 10\%$).

$$Y = 3.2437 + 0.1808 X_1 - 0.1778 X_1^2 + 0.2325 X_2 - 0.3932 X_2^2 \quad (4)$$

where:

X_1 and X_2 are the coded values of the test variables extraction temperature and time (min), respectively.

$$Y = 3.238 + 0.237 X_1 - 0.415 X_1^2 + 0.181 X_2 - 0.161 X_2^2 \quad (R^2 = 0.986).$$

Figure 1 presents the response surface and contour plots for total polyphenol content as a function of time and temperature of extraction according to the proposed model (Equation 4).

Interactions between time and temperature did not significantly influence the total polyphenolic compounds in the extract (X_{12} p -value > 0.05 , Table 3). The convex curvature of the presented response surface plot (Figure 1a) is associated with the negative quadratic terms related to both temperature and time, as presented in Equation 4. Individually and linearly, both variables presented a positive influence on the extraction of polyphenolic compounds from genipap pulp, by means that, within the considered range for the independent variables, the increase in extraction time and in temperature increased the efficiency of extracting polyphenolic compounds from genipap pulp using water as solvent. Moreover, the influence of the applied extraction time was slightly greater than the influence of the applied temperature. The same behavior was observed by Yang et al. (2009) and by Andrade, Maciel, Santos, and Melo (2015) for the extraction of bioactive compounds from gardenia fruits and from cashew apple agro-industrial residues, respectively.

The maximum point of Equation 4 was calculated as 71°C and 49 min, which represents the optimal condition for extraction of phenolic compounds from genipap pulp fruit. The experimental validation of the optimal point was carried out and the experimental value (3.18 ± 0.12 mg GAE g⁻¹) was in agreement with the predicted value (3.33 mg GAE g⁻¹) for total polyphenolic content. The optimal

conditions found to extract polyphenol compounds from genipap pulp is close to the values suggested by Vuong, Golding, Stathopoulos, Nguyen, and Roach (2011) and Sousa, Cabral, Madrona, Cardoso, and Reis (2016) for the optimal aqueous extraction of polyphenol compounds from green tea leaves. Yang et al. (2009) also applied RSM to optimize the extraction of bioactive compounds from gardenia fruits using ethanol as solvent and reported that the maximum polyphenol extraction was at 72.9°C and 34.6 min. These conditions (71°C and 49 min) were applied to obtain the genipap extract for the further microfiltration experiments.

Results of total polyphenolic content show that the genipap extract has greater concentration of polyphenolic compounds than other exotic fruit, such as *Platonia insignis* (bacuri) (0.238 mg GAE g⁻¹), *Spondias mombin* (cajá) (0.720 mg GAE g⁻¹) and *Byrsonima crassifolia* (L.) Kunth (murici) (1.599 mg GAE g⁻¹) (Rufino et al., 2010; Almeida et al., 2011), or even compared to some conventional fruit, such as avocado (1.06 mg GAE g⁻¹) and mango (1.21 mg GAE g⁻¹) (Gregoris et al., 2013).

Table 2. Results of total phenolic content at different extraction conditions according to a central composite design.

Run	Extraction conditions		Response
	X ₁ , Temperature (°C)	X ₂ , Time (min)	Y, TPC (mg GAE g ⁻¹)
1	40	30	2.289
2	40	60	2.705
3	80	30	2.519
4	80	60	3.096
5	37	45	2.787
6	83	45	3.291
7	60	27	2.452
8	60	63	2.958
9	60	45	3.186
10	60	45	3.256
11	60	45	3.251

Table 3. Regression coefficients of the predictive second-order polynomial model and results of analysis of variance (ANOVA) for effect of extraction temperature and time on total phenolic content.

Source	Coefficient estimated	SS	DF	MS	F-ratio	p-value
Model	3.2437					
X ₁	0.1808	0.2173	1	0.2173	143.33	0.0069*
X ₁ ²	-0.1775	0.1096	1	0.1096	72.31	0.0135*
X ₂	0.2325	0.3718	1	0.3718	245.32	0.0041*
X ₂ ²	-0.3932	0.5933	1	0.5933	391.43	0.0025*
X ₁₂	0.0402	0.0065	1	0.0065	4.2731	0.1747
Lack of fit		0.0145	3	0.0048	3.1821	0.2482
Pure error		0.0030	2	0.0015		
Total SS		1.2954	10			
R ² = 0.9865	Adjusted R ² = 0.9730				CV = 1.53%	

SS: Sum of square; DF: degrees of freedom; MS: Mean Square; CV: coefficient of variation; X₁ and X₂ are the coded symbols for extraction temperature and time variables, respectively; *significant at p < 0.05.

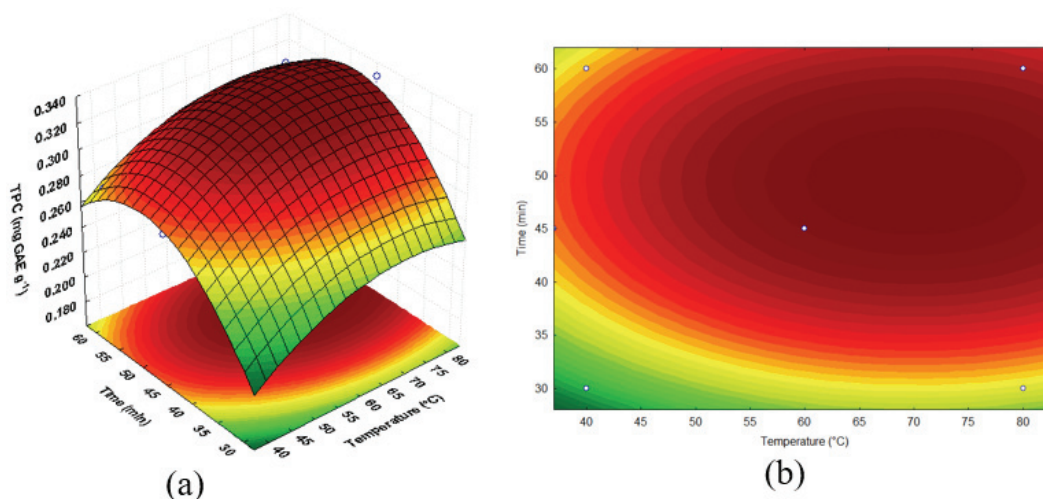


Figure 1. Response surface (a) and contour (b) plots for total polyphenol content as a function of time and temperature of extraction.

Membrane filtrations of genipap extract

The aqueous genipap extract was microfiltered through membranes of different pore sizes (0.8, 0.3 and 0.22 μm , named in Table 2 as M08, M03 and M022, respectively) in order to propose a proper pre-treatment for the further ultrafiltrations. Ultrafiltrations with a membrane of 50 kDa MWCO and under different conditions of transmembrane pressures (3 and 5 bar, named in Table 4 as U50-3 and U50-5, respectively) were carried out with genipap extract that was previously microfiltered through the membrane of 0.8 μm . Ultrasound assisted ultrafiltrations were carried out only at 5 bar transmembrane pressure (named in Table 4 as UU50-5), since the statistical analysis showed no significant difference ($p < 0.05$) for most results at different transmembrane pressures (3 and 5 bar) and the flux was considerable higher at 5 bar than at 3 bar. Physical-chemical characteristics of feed and permeate streams are listed in Table 4.

The microfiltration process did not alter the acidity and the pH value of the genipap extract. All evaluated microfiltration membranes were equally efficient (no statistical differences at $p \leq 0.05$) to reduce turbidity (about 50%), soluble solids ($^{\circ}\text{Brix}$, about 21%) and total solids (about 35%), acting as an efficient clarification treatment for subsequent ultrafiltration. The color analyses showed that the extract (feed) was darker than the permeate, with lower tendency to green and yellow. Bentes et al. (2015) evaluated the color of genipap fruit and observed similar color values for genipap mesocarp. The microfiltration membranes did not retain polyphenol and genipin compounds (no significant difference was detected in this case). Thus, the use of microfiltration as a pre-treatment of the genipap extract for further purification processes is a suitable alternative since the microfiltration process produced a clarified extract without any loss of polyphenol compounds. The obtained genipin concentration is similar to the concentration reported by Pena et al. (2015) in the permeate with a membrane of 50 kDa, but is lower than the concentration in the retentate, probably because Pena et al. (2015) applied a sonic extraction followed by an enzymatic treatment for genipin extraction from genipap pulp.

Figure 2a presents experimental flux data of genipap extract through all evaluated microfiltration membranes. Calculated data obtained by the adjustment of the experimental data to the model proposed by Hermia (1982) for the membrane of 0.8 μm are illustrated in Figure 2b. Similar behavior was observed for the other membranes.

Table 4. Physical-chemical characteristics of feed and permeate genipap extract.

	Feed	M022	M03	M08	U50-3	U50-5	UU50-5
$^{\circ}\text{Brix}$	6.6 \pm 0.005	5.2 \pm 0.002	5.2 \pm 0.001	5.3 \pm 0.002	4.8 \pm 0.01	4.6 \pm 0.03	4.6 \pm 0.05
Total solids (g 100 g $^{-1}$)	46105 \pm 1.5	29000 \pm 5.9	32240 \pm 0.9	30520 \pm 6.8	10220 \pm 9.5	10340 \pm 4.8	10130 \pm 7.2
Turbidity (NTU)	13.3 \pm 0.5	7.1 \pm 0.3	7.0 \pm 0.4	7.9 \pm 0.6	7.1 \pm 0.5	6.2 \pm 0.4	6.2 \pm 0.1
Acidity (g 100 g $^{-1}$)	0.50 \pm 0.03	0.51 \pm 0.02	0.51 \pm 0.03	0.52 \pm 0.03	0.49 \pm 0.09	0.50 \pm 0.08	0.51 \pm 0.09
pH	3.75 \pm 0.01	3.76 \pm 0.02	3.76 \pm 0.00	3.77 \pm 0.02	3.81 \pm 0.2	3.80 \pm 0.8	3.78 \pm 0.2
L	68.75 \pm 3.43	79.35 \pm 2.64	80.36 \pm 0.40	80.52 \pm 3.58	81.04 \pm 1.0	82.00 \pm 0.2	84.5 \pm 0.5
-a*	3.68 \pm 0.14	4.71 \pm 0.02	4.72 \pm 0.00	4.55 \pm 0.11	4.22 \pm 1.0	4.31 \pm 0.30	4.41 \pm 0.14
b*	4.48 \pm 0.14	8.85 \pm 0.01	8.87 \pm 0.29	8.55 \pm 0.03	7.02 \pm 0.17	7.13 \pm 0.09	7.12 \pm 0.27
TPC(mg GAEg $^{-1}$)	3.18 \pm 0.04	2.53 \pm 0.11	2.77 \pm 0.05	3.08 \pm 0.09	2.55 \pm 0.25	2.75 \pm 0.10	3.08 \pm 0.95
Genipin (mg mL $^{-1}$)	1.57 \pm 0.20	1.02 \pm 0.02	1.23 \pm 0.40	1.23 \pm 0.03	1.26 \pm 0.02	1.34 \pm 0.07	1.79 \pm 0.09

Values are expressed as mean \pm standard deviation of triplicate analyses. Mean values followed by a different letter in the same row are significantly different at $p \leq 0.05$.

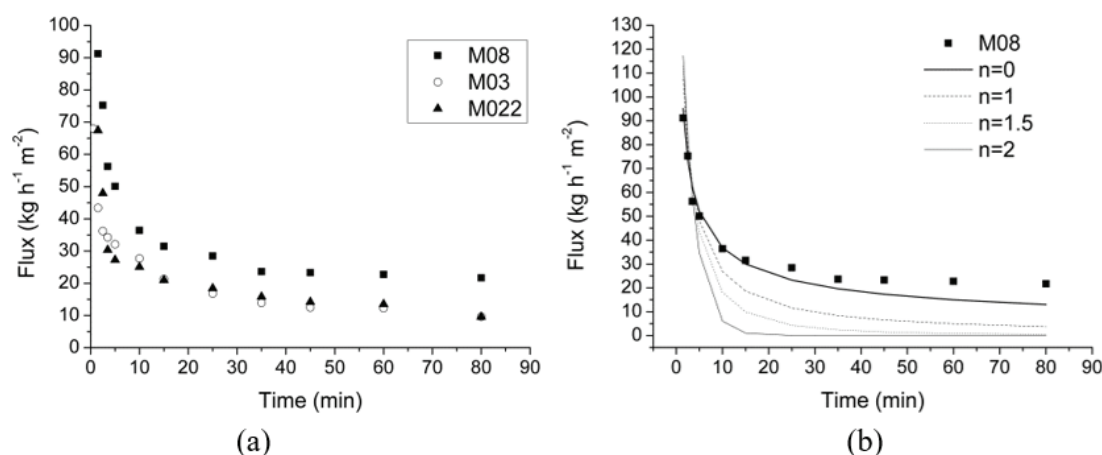


Figure 2. (a) Experimental flux data for genipap extract microfiltration with membranes of 0.22, 0.3 and 0.8 μm (M022, M03 and M08, respectively). (b) Fouling analysis of flux decay for the membrane of 0.8 μm .

Figure 2 shows the typical behavior of a microfiltration flux curve in dead-end operation. The permeate flux decreases abruptly during the first minutes of operation and then the decrease rate diminishes with time, becoming almost constant for times greater than 40 min. The steady-state fluxes through the membranes of 0.8, 0.3 and 0.22 μm were of 21.68, 9.65 and 9.50 $\text{kg hour}^{-1}\text{m}^2$. Considering that the flux through the membrane of 0.8 μm was higher than through the others, and considering the fact that no significant differences ($p < 0.05$) were observed in the physical-chemical characteristics of permeates (Table 2), microfiltration through the membrane of 0.8 μm was chosen as a pre-treatment before ultrafiltration process. The flux modeling (Figure 2b) showed that all the fouling mechanisms can be responsible for the flux decay in the first 5 min of filtration. This behavior should be expected due to the high solid concentration in the feed stream and was also reported by Sousa et al. (2016). The stabilized flux is better described by the cake formation model ($n = 0$). The cake formation is the most reported fouling mechanism during microfiltration of extracts with high solid contents. Zhu et al. (2015) also concluded that the cake formation was the predominant fouling mechanism for dead-end microfiltrations (0.1 μm membrane) of purple sweet potato extract. Nandi, Das, and Uppaluri (2012) applied the Hermia model to describe the fouling during the filtration of orange juice and verified the best adjust to the cake formation. Domingues et al. (2014) also reported that cake formation ($n = 0$) was the major fouling factor during the microfiltration of passion fruit juice.

The sequential ultrafiltration process did not alter most of the analyzed physical-chemical parameters, except for soluble and total solids and color. All proposed ultrafiltration process (at both transmembrane pressures and with and without ultrasound) reduced by $12 \pm 1.8\%$ soluble solids, while reduction in total solids was $66 \pm 0.3\%$, on average. Similar reductions were reported by Cassano, Jiao, and Drioli (2004) after ultrafiltration of kiwifruit juice. This great reduction in total solids confirms the importance of applying the sequential ultrafiltration process for the purification of genipap extract. All the ultrafiltration permeates presented higher values of L than the feed stream in addition to the lower tendency to yellow (b^*), which shows the efficiency of the sequential ultrafiltration membranes for the clarification of genipap extract. The pressure elevation from 3 to 5 bar did not affect any of the evaluated physical-chemical parameters. Application of ultrasound increased the permeation of polyphenol and genipin compounds. Some factor may have contributed to the higher concentration of polyphenol and genipin compounds in the permeate of the ultrasound assisted process, such as the minimization of cake formation in the ultrasound assisted process that contributed to the permeation of these molecules. Although the applied ultrafiltration membrane had not retained polyphenol or genipin compounds, the purity of these compounds in the ultrafiltration permeate was greater than in the feed stream. Considering the purity factor as a ratio between the concentration of polyphenol or genipin to the concentration of total solids, as also analyzed by Balyan and Sarkar (2016), purities of polyphenol and genipin were, respectively, at least 2.5 and 1.2 times greater in the permeate than in the feed stream. Balyan and Sarkar (2016) also observed an increase in polyphenol purity in the permeate using a membrane of 100 kDa for the ultrafiltration of jamun (*Syzygium cumini* L.) seed extract. Purities of polyphenol and genipin were, respectively, 14 and 19% greater when using the ultrasound assisted process than using the conventional one. These results suggest that the application of ultrasound assisted ultrafiltration is a suitable alternative for the purification of polyphenol and genipin compounds from genipap extract.

Figure 3 shows experimental and calculated flux profiles during genipap extract ultrafiltration. Calculated data were obtained by the adjustment of the experimental data to the model proposed by Hermia (1982).

As presented in Figure 3, all fouling mechanisms were responsible for the flux decay in the first 5 min of filtration for all operation conditions. For the ultrafiltration process without ultrasound at 3 bar (Figure 3a), the transition from the accentuated flux decline to the steady state flux was better described by the internal pore blocking ($n = 1.5$) followed by the partial pore blocking ($n = 1$). When the pressure was increased from 3 to 5 bar in the process without ultrasound (Figure 3b), the internal pore blocking ($n = 1.5$) was the main fouling mechanism to represent the flux profile from the accentuated flux decline to the steady state flux. The increase in pressure caused the internal and complete pore blockages more than the partial pore blockage. A similar behavior was observed in the ultrasound assisted process (Figure 3c). In all evaluated conditions, the steady state flux profiles were worse described by the cake formation model ($n = 0$), probably because the macromolecules were eliminated in the previous microfiltration process. The steady state-fluxes for ultrafiltration without ultrasound at 3 and 5 bar and for the ultrasound assisted ultrafiltration at 5

bar were of 4.52, 5.30 and 9.52 kg h⁻¹m⁻², respectively. The increase in the transmembrane pressure from 3 to 5 bar increased the steady state flux by 17%. The application of ultrasound increased the steady state flux in approximately 80%, which is a positive improvement to apply the ultrafiltration process for juice treatments. Liu, Vorobiev, Savoie, & Lanoiselle (2013) applied an ultrasound assisted microfiltration process for the treatment of grape pomace extract and also verified an increase of approximately 50% in the permeate flux. Aghdam et al. (2015) studied the effect of ultrasound on the clarification of pomegranate juice by membrane and observed in scanning electron microscopy images that the cake formed during the filtration without ultrasound was 4 times greater than that using ultrasound. Aghdam et al. (2015) concluded that the membrane processing with ultrasonic treatment decreases the limitation against industrialization of the membrane clarification of fruit juices.

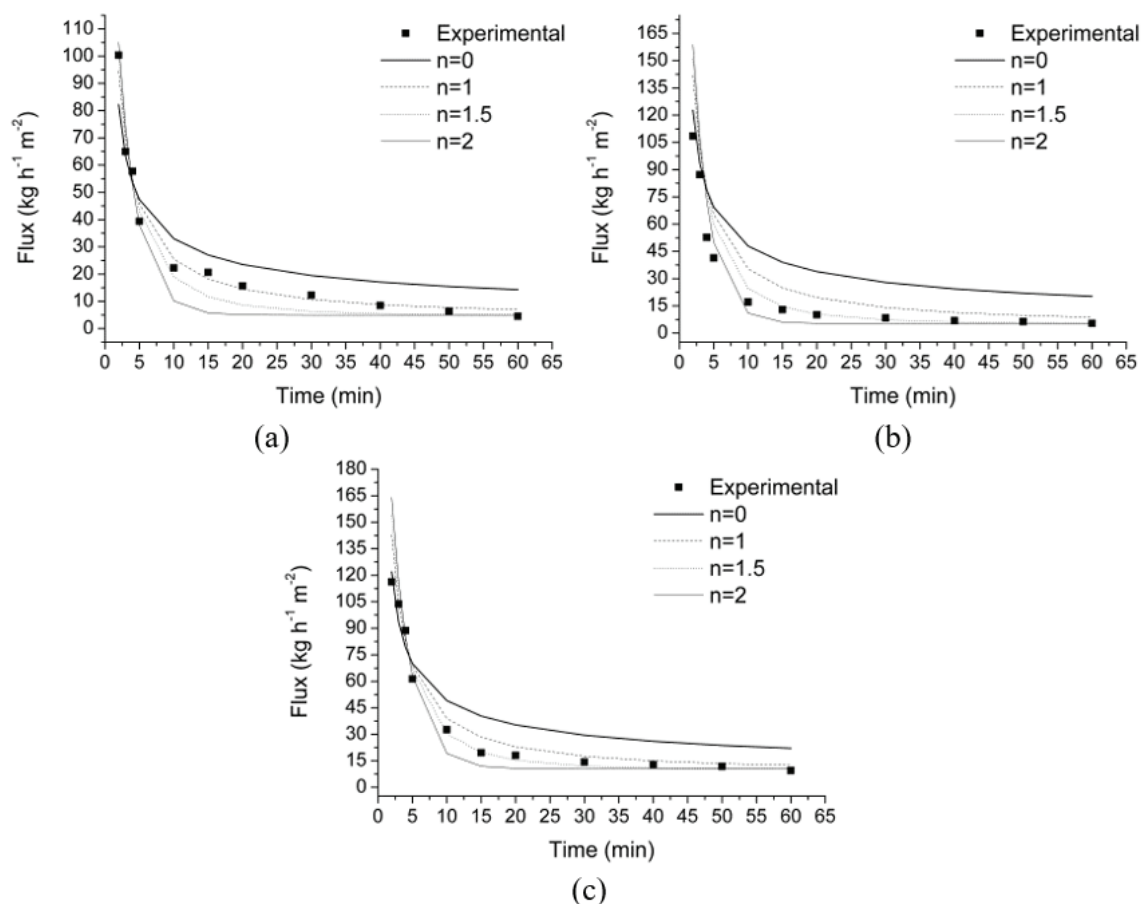


Figure 3. Calculated and experimental flux data for genipap extract ultrafiltrations with the membrane of 50 kDa and conditions of (a) 3 bar without ultrasound, (b) 5 bar without ultrasound, and (c) 5 bar with ultrasound.

Conclusion

Optimized conditions for polyphenol extraction from genipap fruit were temperature at 71°C and time of 49 min resulting in an extract with 3.18 mg GAE g⁻¹. All evaluated microfiltration membranes (0.22, 0.3 and 0.8 µm) were equally efficient for genipap extract clarification with no retention of polyphenol and genipin compounds. Ultrafiltration process with the membrane of 50 kDa was efficient in reducing color and soluble and total solids of the microfiltered genipap extract. Application of ultrasound during ultrafiltration resulted in a permeate stream with a higher purity than when using the process without ultrasound, besides the increase in the permeate flux.

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