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Optimization of blueberry flour processing and anthocyanin extraction

Abstract – The objective of this work was to determine the ideal conditions for the processing of flour and extraction of anthocyanins from blueberries (*Vaccinium corymbosum*). A central composite design (CCD) with response surfaces was used. For the processing of blueberry flour, different dehydration temperatures (53ºC–67ºC) and times (43.18–48.82 hours) were used. The extraction of anthocyanins was performed with ethanol solutions (15–85% v/v) at different pH values (1.0–4.0).). The total monomeric anthocyanin content was 1,538.39±25.63 mg 100 g^{-1} (0.89 desirability value) for fresh blueberries in the optimal extraction condition (53.5% ethanol, 2.5 pH) and 3,101.04±11.57 mg 100 g^{-1} (0.96 desirability value) for blueberry flour in the optimum processing condition (53ºC, 46 hours). The optimized extraction and dehydration of blueberries provide high levels of anthocyanins in the samples.

Index terms: *Vaccinium corymbosum*, dehydration, natural antioxidant, pigments.

Otimização do processamento da farinha e da extração de antocianinas do mirtilo

Resumo – O objetivo deste trabalho foi determinar as condições ideais para o processamento da farinha e a extração de antocianinas de mirtilos (*Vaccinium corymbosum*). Utilizou-se um delineamento composto central (DCC) com superfícies de resposta. Para o processamento da farinha de mirtilo, utilizaram-se diferentes temperaturas (53ºC–67ºC) e tempos (43,18–48,82 horas) de desidratação. A extração de antocianinas foi feita com soluções de etanol (15–85% v/v) com diferentes valores de pH (1,0–4,0). O teor de antocianinas monoméricas totais foi de 1.538,39±25,63 mg 100 g^{-1} (valor de desejabilidade de 0,89) para mirtilos frescos, na condição ótima de extração (53,5% etanol, pH 2,5), e de 3.101,04±11,57 mg 100 g^{-1} (valor de desejabilidade de 0,96) para farinha de mirtilo, na condição ótima de processamento (53ºC, 46 horas). A extração e a desidratação otimizadas dos mirtilos proporcionam a obtenção de altos teores de antocianinas nas amostras.

Termos para indexação: *Vaccinium corymbosum*, desidratação, antioxidante natural, pigmentos.

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Introduction

Blueberry (*Vaccinium* spp.) is a bluish-colored fruit with a bittersweet flavor, originating in Europe and North America, belonging to the Ericaceae family and *Vaccinium* genus (Retamales & Hancock, 2018). Its world production grew from 143.7 to 850.8 thousand tonnes between 1998 and 2020, and the United States is its largest world producer (FAO, 2022). Because it is a seasonal fruit, it is widely marketed in frozen form to be used in culinary preparations. New forms of conservation and consumption are being sought by local producers to add value to the product (Antunes & Raseira, 2006).

Blueberry health benefits are due to the numerous bioactive compounds present in this plant, mainly anthocyanins, whose most important property is its antioxidant activity (Zang et al., 2022). Because of this property, these pigments have been proposed for use as nutraceuticals in food formulations (López et al., 2019). Food industries are increasingly interested in the use of bioactive compounds present in fruits, as they are a good source of nutrients and functional components (Kowalska et al., 2017).

Dehydrated and powdered fruits and vegetables are known to be good sources of nutrients (Almeida et al., 2020). Different physical and chemical methods are used in the food industries to reduce their moisture content and transform them into powders (Salehi & Aghajanzadeh, 2020). Despite the great number of studies on the innovation and improvement of unconventional technologies for blueberry dehydration, including spray drying (Correia et al., 2017), refractance window (Rurush et al., 2022), and far-infrared radiation heating-assisted pulsed vacuum drying (Liu et al., 2022), the conventional dehydration (convection drying) is actually still dominant in the processing of berries (Li et al., 2017). In processes involving food dehydration, time and temperature are indispensable factors for the quality control and maintenance of essential nutrients (Sharif et al., 2018).

The convection drying, as employed in the present work, is relatively inexpensive and it is widely used for the dehydration of fruits and vegetables (Roratto et al., 2021). The proper selection of technique and processing conditions should be evaluated along with technological investment, processing cost, and product quality.

Nevertheless, this kind of general consideration of the process (and optimization) is rarely reported in the literature (Ngamwonglumlert et al., 2020). The use of a central composite design (CCD) as a statistical tool to obtain response surfaces makes it possible to determine the optimized conditions and the building of a mathematical model that accurately describes the overall process (Kidane, 2021).

If fruits and vegetables are dried in the place where they are produced, food losses are reduced, and this helps small farmers to earn a higher income (Roratto et al., 2021).

The production of blueberry flour can be a viable alternative to extend the useful life of fruit properties, adding value to the product for the elaboration of ingredients with a high anthocyanin content. The optimization of dehydration process and construction of mathematical models can turn the flour production viable and easily reproducible. Likewise, this study can be able to provide the optimum extracting parameters to achieve the highest anthocyanin extraction yield from blueberries, eliminating laboratory testing and replication.

The objective of this work was to determine the ideal conditions for the processing of flour and for the anthocyanins extraction of blueberries.

Materials and Methods

Ripe blueberry fruit of Misty and Emerald (*Vaccinium corymbosum* L.) cultivars were used in the experiment. These cultivars were grown in an organic production system, in the municipality of Itá (27º17'26"S, 52º19'23"W, at 385 m altitude), in the state of Santa Catarina, Brazil, and they were harvested in 2019/2020. The selected fruit did not conform to the size and/or appearance standards required for market in natura. Thus, after being collected, fruit were rinsed in water to remove dirt and sanitized by immersion in sodium hypochlorite at 200 mg kg^{-1}, for 15 min. Then, fruit received another rinse with water and were stored in polyethylene packages containing 1000 g of blueberry in each bag. The blueberries were frozen at temperatures between -10 ºC and -15 ºC.

In order to optimize the anthocyanin extraction process, a central composite design (CCD) (Kidane, 2021) with two independent variables was used. The solvent chosen was an ethanol / water solution, due to its good affinity with anthocyanin pigments and low toxicity (Ardestani et al., 2016). The ethanolic solutions were acidified with 1.5 mol L^{-1} HCl. The ethanol content and pH were evaluated, using a factorial design strategy 2^2, with three replicates of the central point and four axial points, to calculate the effects and analyze the surface. The dependent variable (response) was the content of monomeric anthocyanins in blueberry fruit. Through the response surface methodology, eleven combinations (Table 1) were evaluated to investigate the effect of the variables (% ethanol and pH) on the anthocyanins content obtained from each extraction.

Likewise, for the production of blueberry flour, a CCD with two independent variables was also used (Kidane, 2021). Time and temperature were evaluated, using a factorial 2^2 strategy with a central point and four axial points, to calculate the effects and analyze the response surface. The dependent variables (responses) were the content of monomeric anthocyanins and the moisture content of blueberry flour. A total of eleven different combinations (Table 1) were studied, using the response surface methodology to investigate the effect of process variables on dependent variables.

In the experimental tests, 10 g blueberry fruit and 100 mL solvent were used in accordance with the concentration of ethanol and pH values (Table 1). The pH values were measured before the beginning of each extraction. The mixture (fruit and solvent) was ground for 3 min in a blender. It was kept under magnetic stirring for 2 hours in the dark, the vacuum filtered and made up to 100 mL in a volumetric flask with ethanol (Table 1). All analyses were performed in triplicate. Likewise, in order to determine the content of total monomeric anthocyanins in the blueberry flour, an extract was prepared with each sample at 1:10 ratio, in triplicate. The quantification of monomeric anthocyanins was performed according to Lee et al. (2005). The analyses were performed in triplicate. The results were multiplied by 100 and reported as monomeric anthocyanins (mg 100 g^{-1}), expressed as cyanidin-3-glycoside equivalent.

For the development of the flour, the blueberries were thawed under refrigeration for a period of 24 hours. Portions of approximately 500 g were weighed and dehydrated in an oven with air circulation, in accordance with the time and temperature previously established. The objective of these procedures was to optimize the best combination of time and temperature, in order to guarantee the highest anthocyanin content and 15% as maximum moisture content, according to the Brazilian regulation established for fruit flours (Anvisa, 2005). After the drying process, the fruit were crushed in a Willey knife mill with a number 10 mesh sieve. The produced flour was packed in polyethylene packages with hermetic closure, protected from light, and kept under refrigeration (2 – 8ºC). The moisture content of each flour type was evaluated immediately after preparation, using method 44-15.02 of the American Association of Cereal Chemistry International (AACC, 1999).

The performance of the processes, both for the extraction of anthocyanins and for the production of blueberry flour, was evaluated by analyzing the responses (Y), which depends on the input factors x_1, x_2, x_k, and the relationship between the response and the parameters of the process, as described by following equation: $Y = f(x_1, x_2, ..., x_k) + e$, where: f is the actual response function, whose format is unknown; and e is the error that describes the differentiation.

Table 1. Values used in the central composite design (CCD) for anthocyanin extraction and blueberry (*Vaccinium corymbosum*) flour development.

Test	Coded levels		Uncoded levels (anthocyanin extraction)		Uncoded levels (flour development)	
	x_1	x_2	Ethanol (% v/v)	pH	Temperature (ºC)	Time (h)
1	-1	-1	25	1.5	55	44
2	1	-1	75	1.5	65	44
3	-1	1	25	3.5	55	48
4	1	1	75	3.5	65	48
5	-1.41	0	15	2.5	53	46
6	1.41	0	85	2.5	67	46
7	0	-1.41	50	1	60	43.18
8	0	1.41	50	4	60	48.82
9 to 11	0	0	50	2.5	60	46

The response surface behavior was investigated for the response function (Y_i), using a second order polynomial equation whose generalized response surface model is described in the equation below:

$$Y=\beta_0+\sum_{j=1}^{k}\beta_j x_j+\sum_{j=1}^{k}\beta_{jj} x_j^2+\sum_{i}\ \sum_{<j=2}^{k}\beta_{ij} x_i x_j+e_i$$

where: Y is the answer; x_i and x_j are variables (i and j vary from 1 to k); β_0 is the model's intercept coefficient; β_j , β_{jj} and β_{ij} are linear, quadratic, and second order interaction coefficients, respectively; k is the number of independent parameters (k=2, in this study); and e_i is the error (Kidane, 2021). The calculations to determine the models for comparisons of the experimental results with the predicted ones, and to generate the response surfaces were performed using the software Statistica version 12 (Statsoft Inc., USA). The analysis of variance was performed at 5% probability. The content of monomeric anthocyanins was determined after both the extraction and preparation under optimized conditions. The experimental and predicted values were compared to determine the validity of the models.

Results and Discussion

The relationship between the experimental results based on the CCD and the input variables was expressed by a second order polynomial equation with terms of interaction. The final equations obtained concerning the uncoded factors are the following:

Total monomeric anthocyanin (mg 100 g^{-1} fresh blueberry) =
$$1{,}567.798 + 44.116x^{1} -181.735x_1^2 -5.824x_2 -57.228x_2^2 +12.25x_1x_2$$

Total monomeric anthocyanin (mg 100 g^{-1} blueberry flour) =
$$2{,}552.794 - 219.407x_1 +128.060x_1^2 -56.386x_2 -156.103x_2^2 -54.608x_1x_2$$

Moisture (% blueberry flour) =
$$12.98 - 2.854x_1 -0.49611x_1^2 -0.40099x_2 +0.05037x_2^2 -0.20235x_1x_2$$

The results of the analysis of variance indicated that the equations adequately represented the real value of the relationship between the independent variables and responses (Table 2). The p values were less than 0.05, showing that the terms are statistically significant. Thus, for the CCD of the anthocyanin content of fresh blueberries, the variable pH (x_2) in the linear model and the effect of the interaction were not considered statistically significant. These factors do not have a significant influence on the responses; however, they are part of the model. The same way, the moisture content showed neither a significant influence of time (x_2) on the quadratic model nor any effect of the interaction on the experimental planning for the production of blueberry flour.

The high F values of the models show that most of the response variations can be explained by the regression equation. It can be inferred from the model that these p values were statistically significant.

The regression model adjustment was verified by the coefficient of determination (R^2) (Table 3). The R^2 values for anthocyanin content in fresh blueberries (R^2=0.9164), anthocyanin content in blueberry flours (R^2=0.9007), and moisture in blueberry flours (R^2=0.9008) indicate a high degree of correlation between the response and the independent variables (experimental and predicted values). Therefore, the result suggests that the models used in the present study were able to identify operational conditions for anthocyanin extraction in fresh blueberries and development of blueberry flour, with the optimization of the anthocyanin content and adequate moisture content.

Blueberries showed high values of total monomeric anthocyanins from 1,117.0 to 1,576.5 mg 100 g^{-1} (Table 4), which are superior to others reported in the literature. Total anthocyanins were found in blueberries grown in Brazil as 1,182.0 mg 100 g^{-1} from the Rabbiteye group (*Vaccinium ashei*), according to Concenço et al. (2014). In a study with blueberries (*Vaccinium corymbosum*) grown in Australia, the authors evaluated different extraction methods and solvents and found 792.0 mg 100 g^{-1} as the maximum value of anthocyanins (Singh et al., 2022). As well as the genotype, the antioxidant capacity and anthocyanin content can be affected by location, growing season, cultural management, maturity, handling, and post-harvest storage (Retamales & Hancock, 2018), in addition to the organic and conventional production system (Çelik et al., 2013).

Blueberry flours also showed high anthocyanin values from 2,040.74±20.42 to 3,138.11±56.80 mg 100 g^{-1}, which shows the potential of this fruit to become an ingredient with a high antioxidant effect. In addition, the moisture content was maintained in accordance with the Brazilian regulation (Anvisa, 2005), being characterized as fruit flour. It was possible to prove that high temperatures for prolonged periods are shown to cause the degradation of these pigments. According to Brownmiller et al. (2008), processing blueberries into various forms resulted in significant losses of monomeric anthocyanins (28% to 59%), which can be attributed to the enzymatic polymerization and/or degradation of anthocyanins prior to pasteurization or polymerization reactions

Table 2. Analysis of variance and statistical parameters of the models for the extraction of fresh blueberry (*Vaccinium corymbosum*) anthocyanins and development of blueberry flour.

Factor	Coefficient	Sum of squares	DF	Mean square	F test	p-value
Fresh blueberry anthocyanins						
(1) Ethanol (L)	44.116	30,828.4	1	30,828.4	16.0719	0.001487
Ethanol (Q)	-181.735	355,778.5	1	355,778.5	185.4796	<0.00001
(2) pH (L)	-5.824	576.5	1	576.5	0.3006	0.592819
pH (Q)	-57.228	44,575.5	1	44,575.5	23.2387	0.000334
1L by 2L interaction	12.250	1,200.5	1	1,200.5	0.6259	0.443072
Lack of fit		10,571.3	3	3,523.8		
Pure error		24,936.0	13	1,918.2		
Total sum of squares		424,480.4	21			
Model	1567.798				74.90	< 0.001
Blueberry flour anthocyanins						
(1) Temperature (L)	-219.407	979,087	1	979,087.5	1277.891	<0.00001
Temperature (Q)	128.060	259,372	1	259,371.8	338.528	<0.00001
(2) Time (L)	-56.386	78,760	1	78,760.1	102.797	<0.00001
Time (Q)	-156.103	377,656	1	377,655.6	492.911	<0.00001
1L by 2L interaction	-54.608	42,528	1	42,528.4	55.507	<0.00001
Lack of fit		255,107	3	85,035.6		
Pure error		18,388	24	766.2		
Total sum of squares		2,754,343	32			
Model	2,552.794				453.50	< 0.0001
Blueberry flour moisture						
(1) Temperature (L)	-1.42700	41.41606	1	41.41606	221.6080	<0.00001
Temperature (Q)	-0.49611	3.89270	1	3.89270	20.8290	0.000126
(2) Time (L)	-0.40099	3.98326	1	3.98326	21.3135	0.000110
Time (Q)	0.05037	0.03933	1	0.03933	0.2104	0.650558
1L by 2L interaction	-0.20235	0.58396	1	0.58396	3.1246	0.089830
Lack of fit		3.06883	3	1.02294		
Pure error		4.48533	24	0.18689		
Total sum of squares		76.13845	32			
Model	12.98				87.90	< 0.0001

DF, degree of freedom; L, linear; Q, quadratic; (1), first-order equation; (2), second-order equation.

with anthocyanins and other phenolic compounds. Thermal treatment leads to brown products, which is a consequence of anthocyanins degradation, especially in the presence of oxygen (Herrera-Balandrano et al., 2021).

In a study with dried strawberries, previously cut into cubes, 26% of the anthocyanin content had been lost at 50°C, while at 60°C, the loss reached 45%, showing that the thermal exposure had a strong impact on the retention of the anthocyanins (Méndez-Lagunas et al., 2017).

Based on the regression coefficients (Table 3), a response surface was generated as a function of the variables (Figure 1). The p values show that there was no significant interaction between the variables (ethanol concentration and pH) in the anthocyanin extraction process from fresh blueberries, but both were significant separately in both the linear and quadratic models, influencing the extraction yield.

In the planning for the production of blueberry flour, both independent variables (in the linear, quadratic, and interaction models) were statistically

Table 3. Regression coefficients (RC) and analysis of variance of the regression models for the content of anthocyanins in fresh blueberries (*Vaccinium corymbosum*) and the content of anthocyanins and moisture in blueberry flour.

Factor	Fresh blueberry anthocyanins (R^2=0.9164)		Factor	Blueberry flour anthocyanins (R^2=0.9007)		Blueberry flour moisture (R^2=0.9008)	
	RC	p-value		RC	p-value	RC	p-value
Mean/Intercept (β_0)	470.7588	0.002137	Mean/Intercept (β_0)	-72727.1	<0.00001	-61.3127	0.369699
(1) Ethanol (L)	29.6173	<0.00001	(1)Temperature (L)	-407.4	<0.00001	3.0267	0.000065
Ethanol (Q)	-0.2908	<0.00001	Temperature (Q)	5.1	<0.00001	-0.0198	0.000126
(2) pH (L)	255.8160	0.002320	(2)Time (L)	3889.8	<0.00001	-0.1450	0.956198
pH (Q)	-57.2279	0.000334	Time (Q)	-39.0	<0.00001	0.0126	0.650558
1L by 2L interaction	0.4900	0.443072	1L by 2L interaction	-5.5	<0.00001	-0.0202	0.089830

Table 4. Matrix of the experimental design with the respective experimental values and the predicted values of total monomeric anthocyanins of the extraction from fresh blueberries (*Vaccinium corymbosum*), and the experimental and predicted values of total monomeric anthocyanins and moisture in blueberry flours.

Test	Ethanol (% v/v)	pH	Fresh blueberry anthocyanins (mg 100 g⁻¹)			Temperature (ºC)	Time (h)	Blueberry flour anthocyanins (mg 100 g⁻¹)			Blueberry flour moisture (%)		
			Experimental	Predicted	% Error			Experimental	Predicted	Error (%)	Experimental	Predicted	Error (%)
1	25	1.5	1,325.0±79.2	1,302.79	1.70	55	44	2,615.05±11.56	2,745.93	-4.77	14.43±0.78	14.16	1.93
2	15	2.5	1,117.0±9.9	1,149.83	-2.86	65	44	2,304.45±3.34	2,416.34	-4.63	11.60±0.13	11.71	-0.97
3	75	3.5	1,350.5±12.0	1,379.38	-2.09	55	48	2,821.14±31.60	2,742.38	2.87	14.38±0.71	13.76	4.53
4	25	3.5	1,288.5±27.6	1,266.65	1.73	65	48	2,259.51±14.00	2,194.35	2.97	10.55±0.12	10.5	0.44
5	85	2.5	1,313.0±72.1	1,273.36	3.11	53	46	3,138.11±56.80	3,110.96	0.87	13.45±0.25	14.01	-3.97
6	75	1.5	1,338.0±14.1	1,366.52	-2.09	67	48	2,230.97±12.04	2,207.68	1.05	9.51±0.21	9.38	1.42
7	50	1.0	1,450.5±3.5	1,447.77	0.19	60	43.2	2,491.47±51.41	2,325.77	7.12	13.63±0.46	13.64	-0.10
8	50	4.0	1,433.5±0.7	1,430.30	0.22	60	48.8	2,040.74±20.42	2,167.89	-5.86	12.05±0.59	12.52	-3.78
9	50	2.5	1,576.5±47.4	1,567.80	0.56	60	46	2,563.50±27.81	2,552.79	0.42	13.04±0.23	12.98	0.46
10	50	2.5	1,569.5±82.7	1,567.80	0.11	60	46	2,543.11±14.00	2,552.79	-0.38	13.07±0.59	12.98	0.72
11	50	2.5	1,558.0±17.0	1,567.80	-0.62	60	46	2,561.64±8.50	2,552.79	0.35	12.92±0.15	12.98	-0.49

significant for the anthocyanin content, but only the temperature showed statistical significance for the moisture content. Time had no influence on the moisture of the flour nor on the interactive effect between the two factors.

From the visualization of the level curve for the values of anthocyanins in fresh blueberries, in surface response 3D plots, it is observed that both the pH and the ethanol concentration are influential in the extraction of the pigment, in an optimum region of approximately

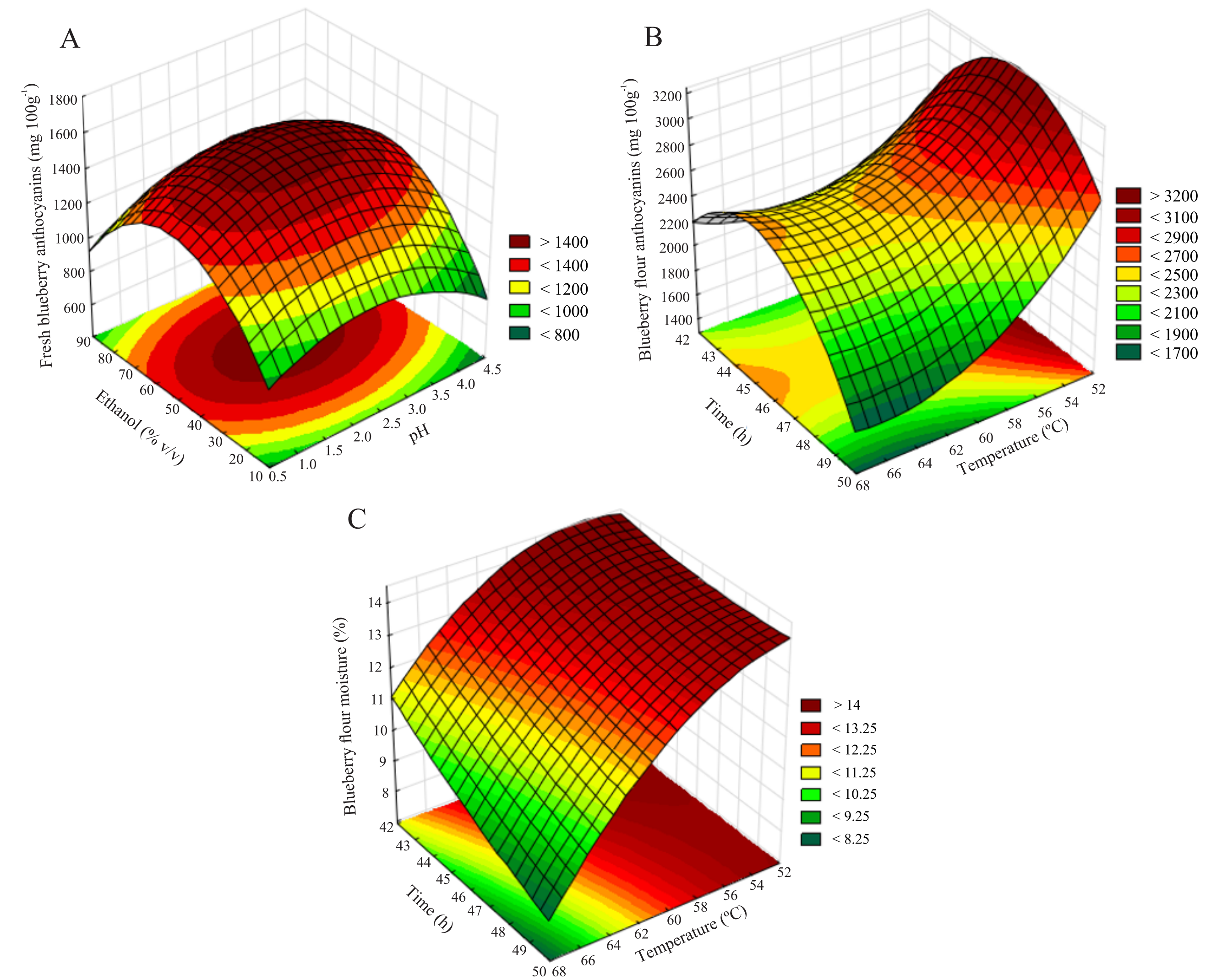

Figure 1. A) Response surface graphs and level curves for the content of total monomeric anthocyanins (mg 100 g^{-1}) of fresh blueberries, according to the different ethanol concentrations (% v/v) and pH values of the solvent. Total monomeric anthocyanins = 470.7588 + 29.6173*x_1 - 0.2908*x_1^2 + 255.8160*x^2 - 57.2279*x_2^2 + 0.4900*x_1 x_2, R^2 = 0.91635; x_1, variable coded for ethanol concentration and x_2, variable coded for pH. B) Response surface graphs and contour lines for the total monomeric anthocyanins content (mg 100 g^{-1}) of blueberry flour, according to the different temperature (ºC) and time (h) conditions. Total monomeric anthocyanins = -72,727.1 - 407.4*x_1 + 5.1*x_1^2 + 3,889.8*x^2 - 39.0*x_2^2 - 5.5*x_1 x_2, R^2 = 0.9007; x_1, variable coded for temperature and x_2, time; C) Graphs of the response surface and level curves for blueberry flour, according to the different conditions of temperature (ºC) and time (h). Moisture content (%) = -61.3127 + 3.0267*x_1 - 0.0198*x_1^2 - 0.1450*x^2 + 0.0126*x_2^2 - 0.0202*x_1 x_2, R^2 = 0.90078; x_1, variable coded for temperature and x_2, time.

45 to 60% ethanol and values pH between 2.0 to 3.0 (Figure 1). Values below or above this region decrease the extraction yield. The anthocyanins are stable between pH 2–3 and unstable between pH 6–8, causing a reduction of their bioavailability (Herrera-Balandrano et al., 2021). The stability of the two anthocyanins (cyanidin and peonidin) were observed to decrease gradually with increasing pH, which shows that anthocyanins are sensitive to pH due to the pyrylium ring of anthocyanins skeleton which is easily opened and formed a chalcone structure, subsequently resulting in anthocyanins degradation, according to Sui et al. (2014).

Anthocyanin stability is affected by temperature and exposure time, degrading with the increase of these two factors, as it is possible to see in the response surface graph of the anthocyanin content of blueberry flours (Figure 1). At temperatures above 55ºC, there was a gradual decrease in the anthocyanin content, even with small variations of the time duration of the drying process. As anthocyanins are thermosensitive, their degradation is observed during the thermal processing, especially at temperatures above 70ºC (Routray & Orsat, 2011) that favors the opening of the heterocyclic ring and greater cleavage (Santos-Buelga & González-Paramás, 2019). The optimum condition was found between 45 to 48 hours, with temperatures between 52 to 55ºC, in which the moisture content-limiting compliance with the Brazilian regulation (Anvisa, 2005) (< 15%) was also reached.

Therefore, to optimize the extraction of anthocyanins from fresh blueberries, the following restrictions were taken for the concentration of ethanol (15−85%), and the pH (1.00− 4.00), for maximum convenience. For the development of blueberry flour in optimized conditions, the parameters temperature (53−67ºC) and time (43.2−48.8 hours) were used for to attain a greater efficiency of the anthocyanin contents with limited moisture content (maximum 14%).

Applying the desirability function methodology, the optimal level of the parameters was obtained and indicated that the ethanol concentration of 53.5% (v/v) and pH of 2.5 can provide 1,570.41 mg 100 g^{-1} anthocyanin content of fresh blueberries, with desirability value of 0.89. Regarding blueberry flour, the optimum level was established at 53ºC for a period of 46 hours, to obtain an anthocyanin content of 3,111.6 mg 100 g^{-1} and 13.99% moisture. For this optimization, the desirability value was 0.96.

The adequacy of the model equations to predict the best response values was tested under the following conditions: ethanol concentration at 53.5% (v/v) and pH 2.5, for the extraction of anthocyanins from fresh blueberries; temperature at 53ºC and time of 46 hours, for the development of blueberry flour. These conditions were determined as optimal by the response surface analysis and were used to confirm the validity of the optimized process.

All experimental values obtained are in accordance with the 95% confidence interval range of the predicted values (Table 5). These values indicate the suitability of the system developed by the models and show that such ideal conditions are valid within the range specified by the process parameters.

Table 5. Predicted and experimental values of responses under ideal conditions.

Optimum levels of process parameters	Total monomeric anthocyanins (mg 100 g^{-1})	
	Optimized value(1)(predicted value)	Experimental value(2)
Anthocyanins extraction		
Ethanol (% v/v) = 53,5 and pH = 2,5	1,570.41 (1,532.06 – 1,608.76)(3)	1,538.39±25.63
Blueberry flour		
Temperature (ºC) = 53 and Time (h) = 46	3,111.6 (3,085.0 – 3,138.2)(3)	3,101.04±11.57
Moisture (%)		
Temperature (ºC) = 53 and Time (h) = 46	13.99 (13.58 – 14.41)(3)	13.98±1.23

(1)Value predicted by the quadratic response surface model. (2)Mean ± standard deviation of triplicate determinations of experiments. (3)Confidence interval.

Conclusions

1. Ethanol concentration and pH have a significant influence on the extraction of total monomeric anthocyanins in fresh blueberries, reaching the highest yield between 45 to 60% ethanol and pH values between 2.0 to 3.0.

2. Time and temperature have a significant influence on the degradation of total monomeric anthocyanins, during dehydration of blueberries by convective drying, increasing the loss of these pigments with the increase of these parameters.

3. The drying condition of blueberry fruit that shows the highest retention of total monomeric anthocyanins content is between 45 and 48 hours, at temperatures between 52 and 55ºC.

4. The developed models of the second order polynomial equation are able to predict the content of total monomeric anthocyanins in fresh blueberries and the content of total monomeric anthocyanins and moisture in blueberry flour.

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