Chapter

Elaboration of a Purple Corn Drink with Maximum Retention of Anthocyanins

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Abstract

In the present work, the anthocyanin extraction process was characterized during the elaboration of a purple corn drink, using the response surface analysis method with temperature intervals between 47.57 and 132.43°C, and times from 11, 36 at 138.64 minutes. In addition, with the stationary point technique, the maximum retention of anthocyanin (33.99 mg/g) was determined at a temperature of 98.39°C at a time of 105.89 minutes of extraction. Since this time is too long and to reduce production costs, without resorting to considerable losses of anthocyanins, canonical analysis was used, redefining the optimal extraction parameters at a temperature of 100°C for 60 minutes with a reduction of the anthocyanin content of 2.49% (33.14 mg/g) concerning the maximum, a value that is within the optimum area of performance of the process. With the extract obtained under optimal conditions, a drink was prepared and, using the differential pH method and Student's t-test ($p = 0.05$), its anthocyanin content was quantified and compared with that of a commercial drink with typical characteristics. Similar, observing that the elaborated drink presents higher contents, whose difference varies within the range of 2.79 and 4.72 mg/mL. Finally, using a satisfaction test with a nine-point hedonic scale, it was determined that the beverage was "very well liked" by a semi-trained sensory panel.

Keywords: purple corn, anthocyanin, optimization, response surface, canonical analysis

1. Introduction

The cultivation of purple corn is of growing economic importance in Peru, mainly for producers in the mountains who have few possibilities of generating economic income from the sale of agricultural products that they produce on their plots. In recent years, the consumption of purple corn has intensified, in the country and abroad, because the purple pigment that this type of corn has (anthocyanins) prevents diseases such as colon cancer, and reduces obesity and diabetes, among other

diseases; likewise, it is a natural colorant for the industry. Among the anthocyanins of purple corn, cyanidin-3-glucoside is found in greater quantity, constituting a powerful natural antioxidant [1].

Purple corn is a vegetable resource native to Andean Peru and to which an interesting biological activity as an antioxidant is attributed, due to the type of bioactive compounds it contains. Due to the increased demand for this plant resource and its derivatives in the national and international market, efforts have been made in the country to expand the cultivation areas and introduce improved varieties of purple corn that can be adapted to these new cultivation areas. and that they improve the production and commercialization of this resource [2].

Antioxidants are responsible for stabilizing free radicals by transferring electrons and hydrogen atoms, and they also have the ability to inhibit oxidative degradation such as lipoperoxidation. For this reason, they play an important role in the prevention of various degenerative diseases such as cancer, diabetes, obesity, high blood pressure [3–5].

Within this group of antioxidants are anthocyanins, natural dyes that belong to the group of flavonoids, because they have a characteristic structure of C6-C3-C6 (**Figure 1**). Its basic structure is the flavylium group (2-phenylbenzopyrylium). These pigments are responsible for giving the pink, red, blue, mauve and violet color of flowers, fruits and vegetables, they are polar compounds, which allows them to be soluble in ethanol and water. Anthocyanins are glycosides that have a sugar in position 3 linked by the ß-glycosidic bond that, when broken, forms the aglycone, known as anthocyanidin, the most common being: pelargonidine, cyanidin, delphinidin, peonidin, malvidin and petunidin [6–10]. On the other hand, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside and the acylated forms of each of them were found in purple corn from the Andean region of Peru [11].

In the Peruvian market, two commercial beverages based on purple corn are offered; however, the industry has prioritized the sanitary quality of the product before the beneficial effect on health provided by anthocyanins due to their antioxidant capacity and the phenolic compounds present in their chemical structure [12].

The extraction of anthocyanins depends on the temperature and extraction time, being favored by the 20% ethanolic medium and pH between 1 and 4 [13]. While, used solutions as solvents hydroalcoholic (ethanol or methanol) acidified with acetic acid, concluding that the acid methanol is more effective for extraction, although its toxicity prevents it from being used when the extracted substances will be used for human consumption [8]. On the other hand, recommend using water, methanol, or

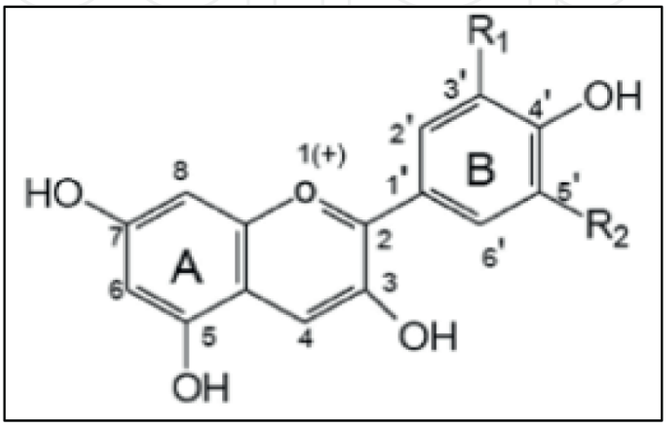

Figure 1.
Basic structure of anthocyanins.

ethanol acidified with hydrochloric acid at a pH between 4 and 5 at a temperature between 70 and 100°C as solvents, to avoid pigment degradation [14]. The extraction using an ethanolic solution as solvent acidified with hydrochloric acid by immersion for 15 minutes in an ultrasound bath; finally, the appropriate extraction conditions take place in an aqueous medium with a contact time of 120 minutes and a limit temperature of 50°C [15, 16]. All the aforementioned extraction techniques were carried out under laboratory conditions using inorganic acids as acidifying agents and alcoholic solutions as solvents; conditions that cannot be reproduced on an industrial scale due to the high toxicity of said compounds.

In this sense, the present work aims to determine the optimal physical parameters of time and temperature for the extraction of anthocyanins from corn earns to fortify a commercial purple corn drink.

2. Materials and method

A Central Composite Rotational Design (DCCR) was carried out with 4 factorial points, 4 axial points, and 5 repetitions in the central points, having a total of 13 treatments. The independent variables or "Factors" of the design were factor A (X1): Temperature, with a low level at 60°C, a high level at 120°C, the central point at 90°C and axial points at 47.57°C and 132.43°C); and factor B (X2): Time, with a low level at 30 min, high level at 120 min, a central point at 75 min and its axial points at 11.36 min and 138.64 min as shown in **Table 1**.

The purple corn cobs used came from the Majes district of the department of Arequipa, Peru, located between 200 and 800 meters above sea level, with an average annual temperature between 14 and 32°C.

Natural Variables		**Coded Variables**	
Factor A: Temperature	**Factor B: Time**	**X1**	**X2**
60	30	−1	−1
120	30	+1	−1
60	120	−1	+1
120	120	+1	+1
90	75	0	0
90	75	0	0
90	75	0	0
90	75	0	0
90	75	0	0
132.43	75	√2	0
47.57	75	−√2	0
90	138.64	0	√2
90	11.36	0	−√2

Source: self made.

Table 1.
Design of Experiments Matrix.

The present research work was carried out in the Food Technology laboratories of the Chucuito Pilot Plant, of the Faculty of Fisheries and Food Engineering of the National University of Callao, and Bromatology of the Faculty of Pharmacy and Biochemistry of the University Inca Garcilaso de la Vega.

2.1 Obtaining the anthocyanin extract

The ears were selected to discard those that have symptoms of deterioration or perceptible damage, then they were shelled to remain only with the shelled ears in the next stage of the process, later they were rolled in a circular shape with an approximate thickness of 3 mm and dried. in an oven at a temperature of 65°C for 2 hours, until reaching an approximate humidity of 8%.

The shelled and dehydrated cobs were ground with a manual mortar and diluted in a ratio of 2.5 g. in 100 mL of extraction solution (treated water adjusted to pH 2 with citric acid), applying the times and temperatures as indicated in **Table 1**, to develop the experimental model.

Subsequently, the temperature of the extract was brought below 30°C to filter it through a 1 mm diameter mesh.

2.2 Anthocyanin quantification

The quantification of the content of anthocyanins was expressed as mg of cyanidin 3-glucoside/g of shelled cob, was used, where an aliquot of 0.3 ml of extract was diluted in 2.7 mL of buffer solution of potassium chloride (pH 1) and sodium acetate (pH 4.5), separately, leaving it to stand for 20 minutes [17]. Finally proceeding to read their respective absorbances as indicated in the following expression:

$$\text{Total anthocyanins}\left(\text{mg / L}\right) = \text{A x PM x FD x 1000} / \left(\text{n x l}\right)$$

Where:

TA = cyanidin 3-glucoside content; A = (A510 – A700) pH 1 - (A510 – A700) Ph 4.5; MW = molecular weight; DF = dilution factor; 1000 = conversion factor from grams to milligrams; ϵ = molar extinction factor (26900) for cyanidin 3-glucoside; l = cell length.

Subsequently, the data were analyzed to obtain the response surface, identifying the point of maximum performance through the stationary point methodology,

2.3 Response surface methodology

The response surface methodology was applied to the response variable using the commercial statistical software Design Expert Version 5.0 (Stat-Ease, Minneapolis, USA). Second-order polynomials were fitted to the data to obtain regression equations for the response variables analyzed. The graph of the response surfaces, the variance analysis, and the determination coefficients (R^2) was generated with the same software. Then, the canonical analysis of the data was performed to adjust the optimal point of the process.

2.4 Preparation of the drink and comparison of the anthocyanin content with that of a commercial drink

For this, the purple corn drink was prepared, for which the purple corn extract obtained using the optimal parameters determined in the previous point was diluted

with treated water at a temperature of 78°C in a volumetric ratio of 2 of treated water and 1 of extract. Subsequently, the drink was standardized until it reached a pH of 3, an acidity content of 0.2% citric acid, and 13°Brix. The mixture was pasteurized at 72°C for 10 minutes and then bottled in glass bottles with a capacity of 250 mL, amber color, and screw cap.

Finally, the anthocyanin content was determined in triplicate, in the drink made under the aforementioned conditions, and in a commercial purple corn drink. The mean values were compared using the t-Student test ($p = 0.05$).

2.5 Satisfaction degree test

It was carried out with 50 students of the eighth cycle of the Professional School of Food Engineering of the National University of Callao, who had completed the Sensory Analysis of Food subject, following the methodology [18].

3. Results and discussion

3.1 Anthocyanin quantification and application of the response surface methodology

The yield of anthocyanins in the extracts obtained with the different treatments tested is shown in **Table 2**.

To determine if the anthocyanin extraction levels were within the region of maximum yield, the fit was made to a second-order polynomial model [19] (**Table 3**).

Based on the fact that the Fc of the lack of fit is much lower than the Ft, for a significance level of 0.5%, it can be concluded with a statistical significance level

Natural Variables		Anthocyanin Content (mg/g)
Factor A: Temperature	**Factor B: Time**	
60	30	25.856
120	30	31.056
60	120	28.918
120	120	32.802
90	75	32.761
90	75	33.012
90	75	33.405
90	75	33.212
90	75	34.219
132.43	75	29.642
47.57	75	25.971
90	138.64	33.489
90	11.36	29.969

Source: self made.

Table 2.
Design of Experiments Matrix.

Source of Variation	Degrees of freedom	Sum of squares	Middle Square	F_c	F_t
Model	5	93.34	18.67	60.49	22.46
Factor A	1	25.47	25.47	82.55	31.33
Factor B	1	11.97	11.97	38.79	31.33
Factor A^2	1	54.30	54.30	175.94	31.33
Factor B^2	1	4.75	4.75	15.39	31.33
Interaction AB	1	0.43	0.43	1.40	31.33
Residual	7	3.26	0.45		
Lack of Adjustment	3	1.92	0.64	2.08	31.33
Mistake	4	1.23	0.31		
Total	12	96.49			

Table 3.
Analysis of variance table for a second order model.

of 99.5% that the second-order model is an adequate approximation to the actual behavior of the experiment. Therefore, the second-order equation was established to predict anthocyanin yields when the food matrix is subjected to the extraction factors that are the object of this study. The analysis of the yields of anthocyanins obtained with the Desing Expert Version 5.0 software, allowed us to calculate the values of the regression coefficients that are presented in **Table 4**.

The equation obtained to predict the content of anthocyanins was:

$$Y = -3.07 + 0.64X1 + 0.11X2 - 0.00024X1X2 - 0.003X12 - 0.82X22$$

Where: Y = yield of anthocyanins in mg of cyanidin 3-glucoside/g of shelled cob; X1 = temperature in °C; X2 = time in minutes. This corroborates that the established mathematical model describes the anthocyanin extraction process very closely to reality under the pre-established experimental conditions and within the study region.

Coefficients	**Anthocyanin content (mg/g)**
Constant β_o	−3.07
Linear	
β_1 (Temperature)	0.64
β_2 (Time)	0.11
Interaction	
β_3 (Time*Temperature)	−0.00024
Quadratic	
β_4 (Time*Time)	−0.003
β_5 (Temperature*Temperature)	−0.82
R^2	0.96

Source: self made.

Table 4.
Values of the regression coefficients obtained in the anthocyanin extraction process.

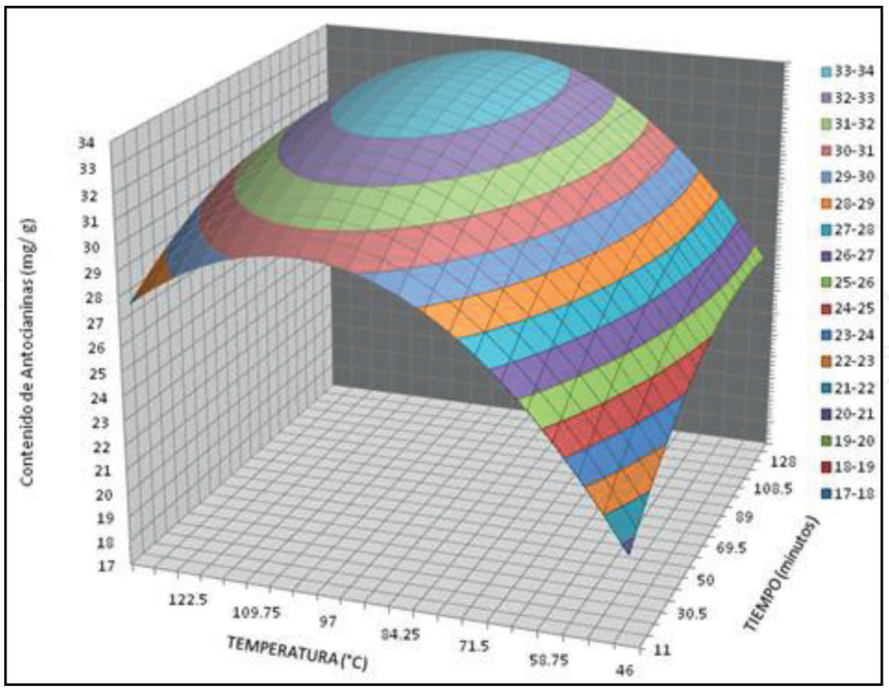

Figure 2.
Response surface of the experiment.

This is important to highlight, since the equation obtained should not be used for extrapolate data outside the study range or for conditions other than those pre-established in the design. With this equation, we proceeded to characterize the response surface shown in **Figure 2**, for this the adjusted data of the experiment were used.

Figure 2 shows the behavior of the process against the factors of temperature and time, where overexposure to high temperatures and times causes a decrease in | performance by degradation of anthocyanins. The auxiliary graph or contour graph was also made to facilitate the interpretation of the response surface and the region of the maximum performance of the process, which can be seen in green in **Figure 3**.

With the use of the stationary point methodology, it was obtained that the highest yield of anthocyanins (33.99 mg/g) is obtained at a temperature of 98.39°C and 105.89 minutes of extraction, maintaining preset pH values and the shelled cob/solvent ratio. However, a time of approximately 106 minutes is excessive and represents high power consumption. From the analysis of **Figure 3**, it was deduced that the extraction process has a greater dependence on temperature than on extraction time, so it was decided to reduce the time to the minimum possible without leaving the zone of maximum yield and using parameters of the time and temperature variables that present operational ease in a process at an industrial level. For this reason, the canonical analysis of the results was performed, obtaining an extraction yield of 33.08 mg/g for a temperature of 96.29°C and a time of 59.50 min. These new extraction parameters are very advantageous compared to those with higher yields, however, in an industrial process, they are difficult to maintain constant and even to achieve exactly, so it was decided to explore the neighborhood of the new parameters obtained, in the order to establish as optimal points of the process, those that are also easy to operationalize.

Based on the results in **Table 5**, it was decided to operate the extraction process at a temperature of 100°C for 60 minutes, reaching an anthocyanin yield of 33,144 mg/g. Comparing these parameters with the highest yield initially obtained, it can be seen

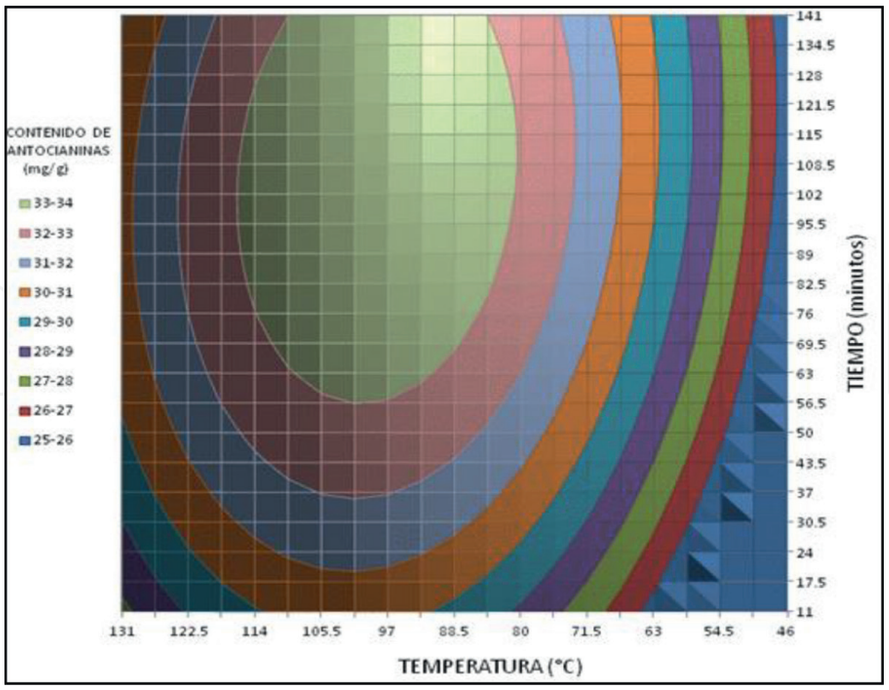

Figure 3.
Contour plot of the response surface of the experiment.

Natural variables		Performance (mg/g)
Temperature (X_1)	Time (X_2)	
95	55	32,860
100	55	32.950
95	60	33.061
100	60	33.144

Source: self made.

Table 5.
Exploration of the optimal point of the extraction process.

that with a temperature increase of 2°C the extraction time was reduced by 45% with only a 2.49% decrease in the yield of anthocyanins.

3.2 Comparison of the anthocyanin content of the beverage made with a commercial beverage

Table 6 shows the average values and standard deviation of the anthocyanin content in the samples evaluated. The difference test showed that the elaborated beverage had a significantly higher average anthocyanin content ($p = 0.05$) than that of the commercial beverage, this difference is found in a range between 2.79 and 4.72 mg/mL.

3.3 Satisfaction degree test

According to the hedonic scale of acceptability, the attribute color and flavor obtained average ratings of 8.28 and 7.64 (**Figure 4**), which is equivalent to saying

Nomenclature	Variable	Value
Average content of anthocyanins of the beverage made with the optimal parameters (mg/mL)	Y_1	33.138
Average anthocyanin content of the commercial beverage (mg/mL)	Y_2	29.380
Estimation of the sample variance	S_p^2	1.4250
Sample size of the brewed beverage	n_1	13
Commercial drink sample size	n_2	13
Significance level	A	0.05
Calculated statistical variable	T_c	8.0246
Tabulated statistical variable	T_t	2.064

Source: self made.

Table 6.
Summary of the comparison of means test.

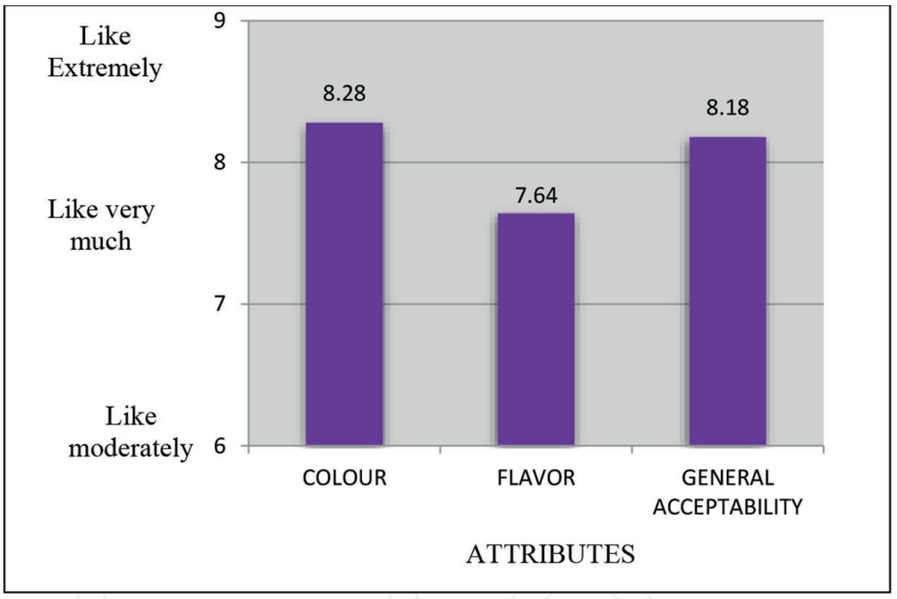

Figure 4.
Degree of satisfaction with the drink.

that the color was "liked a lot" and the flavor "liked a lot", respectively. The average of the general acceptability was 8.18, that is, "liked a lot", which indicated that the flavor attribute does not significantly affect (p = 0.05) the general acceptability of the beverage made with the optimal parameters.

4. Discussions

Carried out the extraction of anthocyanins from shelled ears of purple corn at different pH, solvents, temperatures, and times, observing that for a process at pH 2, and using water as the solvent, a maximum yield was obtained (33.509 mg/g) at a temperature of 90°C and a time of 240 minutes, yield very similar to that obtained in the present work (33.14 mg/g), using the same pH and solvent, but at a temperature

of 100°C and a time of 60 minutes [13]. That is, by increasing the extraction temperature by 10°C, it is possible to reduce the process by 3 hours, which allows for increasing the production volumes of a purple corn drink and a significant reduction in the cost of producing the product.

On the other hand, it has been shown that the anthocyanin extraction process depends mainly on the temperature, rather than on the extraction time; because in recent works, an anthocyanin content of 22.68 mg/g in an extract obtained from the same raw material (shelled cobs), using water as a solvent, but at a temperature of 50°C with contact times of 120 minutes [16].

An important factor to consider is the type of statistical treatment used in the study of the extraction process only carried out the variance analysis of the different factors considered, which allowed to select the best combination of the different levels of the tested factors; while in the present study the response surface analysis was carried out, the same one that allows characterizing the entire process within the range under study and optimizing it [13, 16].

5. Conclusion

In this study, the response surface analysis was a useful technique to forecast a hight anthocyanin yield in an extraction process of this molecule from earns corn. We obtained the highest anthocyanin yield (33.99 mg/g) at 98.39°C after 105.89 minutes of extraction, maintaining preset pH values and the hulled ears/solvent ratio. In addition, the results showed that the extraction process has a higher dependence on temperature than the extraction time.

The canonical analysis of the anthocyanin retention results in the vicinity of the maximum retention temperature made it possible to select an extraction temperature of 100°C for a period of 60 minutes, with which an anthocyanin yield of 33.144 mg/g (2.49% below optimal yield), but with a 45% reduction in extraction time.

Author details

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