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ORIGINAL ARTICLE

Optimization of hydroalcoholic extraction of antioxidant compounds from *Syzygium cumini* L. pulp: effect of ethanol concentration and extraction time

Optimización de la extracción hidroalcohólica de compuestos antioxidantes desde la pulpa de *Syzygium cumini* L.: efecto del etanol y tiempo

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Abstract The hydroalcoholic extraction process of black cherry (Syzygium cumini) pulp was optimized, considering the yield of total polyphenols, anthocyanins, and antioxidant capacity as response variables. The highest yields of phenolic and anthocyanin compounds were achieved when prolonged extraction times and an ethanol concentration close to 80% (v/v) were used, with statistically significant differences $(p \le 0.05)$. These conditions did not show a relevant effect on antioxidant capacity from a practical point of view. The extract obtained under optimal conditions, with 83.23% ethanol and 12 hours of extraction, presented yield values for polyphenols (25.19%), anthocyanins (23.24%), and antioxidant activity (11886 mg/100 mL) that were lower than the values predicted by the optimization model. Visually, the extract exhibited an intense purple hue, indicative of the presence of anthocyanins, such as pelargonidin and delphinidin, as well as their glycosylated derivatives.

Keywords *Syzygium cumini*, hydroalcoholic extraction, antioxidant compounds, total polyphenols, anthocyanins, extraction optimization.

Resumen Se llevó a cabo la optimización del proceso de extracción hidroalcohólica de la pulpa de cerezo negro (Syzygium cumini), considerando como variables de respuesta el rendimiento de polifenoles totales, antocianinas y la capacidad antioxidante. Los mayores rendimientos de compuestos fenólicos y antociánicos se alcanzaron al emplear tiempos prolongados de extracción y una concentración de etanol cercana al 80 % (v/v), con diferencias estadísticamente significativas (p \leq 0,05). Estas condiciones no mostraron un efecto relevante en la capacidad antioxidante desde el punto de vista práctico. El extracto obtenido bajo condiciones óptimas, 83,23 % de etanol y 12 horas de extracción, presentó valores de rendimiento en polifenoles (25,19 %), antocianinas (23,24 %) y actividad antioxidante (11886 mg/100 mL) por debajo de los valores predichos por el modelo de optimización. Visualmente, el extracto mostró una tonalidad morada intensa, indicativo de la presencia de antocianinas como pelargonidina, delfinidina y sus derivados glicosilados.

Palabras clave *Syzygium cumini*, hydroalcoholic extraction, antioxidant compounds, total polyphenols, anthocyanins, extraction optimization.

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Introduction

In recent years, there has been growing interest in replacing artificial colorants with natural alternatives that, in addition to providing an attractive appearance, offer biological benefits and maintain stability during storage (Srinivasan & Rana, 2025). However, many natural pigments, especially anthocyanins, have low stability against factors such as pH, temperature, light, and oxygen, which limits their application in food products (Rodríguez, 2023; Molina et al., 2023; Xue et al., 2024).

Anthocyanins are phenolic compounds of the flavonoid subgroup, widely present in fruits, flowers, and berries, and responsible for colors ranging from red to blue. These pigments have gained relevance not only for their sensory value but also for their antioxidant properties and potential protective effects against cardiovascular disease, cancer, diabetes, and cognitive impairments (Kaur et al., 2024; Srinivasan & Rana, 2025). Therefore, in addition to their role as natural colorants, anthocyanins are of interest for developing value-added functional ingredients.

Recent studies have shown that the use of modern techniques, such as ultrasound-assisted extraction or the use of green solvents (aqueous glycerol), significantly improves the yield and stability of anthocyanins (De Sousa et al., 2021; Kaur & Qadri, 2024). However, there is still a need to optimize hydroalcoholic extraction processes from black cherry (*Syzygium cumini* L.) pulp, a fruit rich in anthocyanins that still requires validation of methods to maximize its yield and functionality under industrial conditions (Kaur et al., 2024). The objective of this work was to optimize the hydroalcoholic extraction of anthocyanins and total polyphenols from black cherry pulp, thereby maximizing their content and antioxidant capacity, using experimental design techniques.

Methodology

The fruits were harvested manually, selecting those with uniform characteristics of size, color, vegetative state, and no visible defects. From each fruit, the pulp and skin were separated using a No. 3 scalpel, and both parts were crushed and homogenized with an Ultra-Turrax IKA T25 (model T25 D S25).

Design-Expert 8.0.6 software (Stat-Ease Inc., Minneapolis, USA) was used to design a factorial experiment with two factors: percentage of ethanol (A) and extraction time (B), evaluating the yield of total polyphenols, anthocyanins, and antioxidant activity as responses. Numerical optimization was applied using a response surface (optimal type IV), fitting second- and third-degree polynomial models for the response variables. Sixteen runs were defined, including four replicates, with factorial sets covering the levels of A and B according to the specified ranges.

The experimental conditions evaluated were ethanol percentages ranging from 30 to 70% and extraction times ranging from 30 to 60 minutes. Each combination was performed in triplicate across 16 runs, resulting in a total of 64 determinations. Data were analyzed using the response surface model using Design-Expert software, with significance considered at $p \le 0.05$.

For each run, dry matter was mixed with solvent in a 1:5 (m/v) ratio, using the established ethanol and time conditions. Extraction was performed on a sieve at 260 m⁻¹, at room temperature (25 °C). After extraction, the mixture was filtered to separate the extracts from the solid residues.

Total polyphenols were determined by reaction with the Folin–Ciocalteu reagent (Slinkard & Singleton, 1977), absorbance measurement at 765 nm, and expression of the content as mg gallic acid/100 mL of extract. The yield was calculated as a proportion of the total content in the original pulp. Total anthocyanins were determined using the differential pH method (Lee et al., 2005), which involved measuring absorbance at 510 and 700 nm with buffers at pH 1.0 and 4.5, and calculating the content as cyanidin-3-glucoside equivalents based on the extinction coefficient. The yield was expressed in the same way as for polyphenols. The antioxidant capacity was determined using the ABTS* radical assay (Re et al., 1999), measured at 734 nm after a 10-min reaction with the extract at 25 °C; results are expressed as mg Trolox equivalent/100 mL of extract.

Results and discussion

Considering the presence of phenolic compounds and anthocyanins in black cherry, it was decided to evaluate the effectiveness of the extraction process based on the extraction yield of total polyphenols and anthocyanins, as well as the antioxidant capacity of the hydroalcoholic extracts for each of the conditions tested (Table 1).

It is observed that runs 2 and 5, replicates of the extraction test, showed higher (p \leq 0.05) yields of total polyphenols and anthocyanins, which may be because these runs corresponded to the longest extraction time and the use of ethanol at 80.1% (v/v), a concentration proposed by other works as the most appropriate for the extraction of phytochemical constituents, while runs 3 and 15 (replicates), with the shortest extraction time and a similar ethanol concentration, showed lower values for the response variables mentioned above.

Table 2 shows the three statistical models used to analyze the effects of ethanol percentage (A) and extraction time (B) on three response variables: total polyphenols yield (TPY), antioxidant capacity (AC), and anthocyanin yield (AY).



Table 1. Yield of total polyphenols, anthocyanins, and antioxidant capacity of hydroalcoholic extracts of black cherry

Run	Ethanol (%)	Extraction time (h)	Total polyphenol yield (%)	Anthocyanin yield (%)	Antioxidant capacity (mg/100 mL) ¹
1	70.8	9.8	23.52	20.10	12000
2	80.1	12	29.60	24.83	19302.8
3	84.9	6	22.33	24.26	13708.3
4	78.9	9.46	28.95	24.34	11613.89
5	80.1	12	29.38	24.29	18488.9
6	70	6	27.57	24.50	14077.78
7	70	12	28.76	25.46	11705.56
8	90	12	25.38	21.33	15244.44
9	90	8.1	22.07	23.94	12658.33
10	77.6	6	28.03	24.43	14422.22
11	74	7.86	29.45	24.66	13977.78
12	78.9	9.46	27.32	24.66	15005.56
13	90	8.1	23.06	23.18	13186.1
14	86	10.11	29.38	23.86	13877.78
15	84.9	6	22.82	22.53	14786.11
16	78.9	9,462	25.81	23.18	16341.67

^{1:} Expressed as Trolox.

Table 2. Analysis of variance for total polyphenols yield

Fountain -	p-value				
rountain –	TPY	AC	AY		
Model	0.0076	0.0371	0.0372		
A	0.0562	0.0436	0.3296		
В	0.0559	0.0295	0.1000		
AB	0.0037	0.1419	0.1629		
A^2	0.0013	0.0036	0.0142		
\mathbf{B}^2	0.1182	0.3816	0.0722		
A^2B	0.0298	0.1094	-		
AB^2	0.0077	0.0042	-		
A^3	0.0648	0.1119	-		
\mathbf{B}^3	0.0368	0.0300	-		
\mathbb{R}^2	0.9302	0.8752	0.6492		
Lack of fit	0.1578	0.4131	0.5193		

A: percentage of ethanol; B: extraction time; TPY: total polyphenols yield; AC: antioxidant capacity; AY: anthocyanin yield.

The cubic model applied to evaluate total polyphenol yield was statistically significant at the 95% confidence level, with a coefficient of determination (R^2) of 93.02%, indicating an adequate fit of the model to the experimental data. Although the main factors, percentage of ethanol and extraction time, did not show significant individual effects on the response, their interaction was significant at various levels ($p \le 0.05$).

The analysis of anthocyanin yield revealed that the cubic model was adequate, accounting for 87.52% of the variability (R^2). In this case, the individual factors, the quadratic term

 (A^2) , their interaction (AB^2) , and the cubic term (B^3) were statistically significant $(p \le 0.05)$. The quadratic model was significant $(p \le 0.05)$ for antioxidant capacity, with an R^2 of 64.92%, reflecting a strong relationship between the study factors and the dependent variable. In this model, only the quadratic term for the percentage of ethanol (A^2) was statistically relevant.

Together, these models demonstrate the complexity of bioactive compound behavior under different extraction conditions. The significance of interactions and nonlinear terms suggests the need for careful optimization to maximize both polyphenol and anthocyanin content and yield, similar to that reported by studies such as Gaibor et al. (2016) on black cherry and phenolic compound extraction, and Araújo et al. (2023) on anthocyanin optimization studies using response surface designs.

The influence of these factors on the extraction yield of total polyphenols and anthocyanins, as well as the antioxidant capacity, can be best observed in Figure 1. It was evident that, in none of the evaluated models, maximum antioxidant capacity values similar to those obtained experimentally in the runs with the best results were achieved. This discrepancy can be attributed to the absence of a direct linear relationship between antioxidant capacity and total polyphenol and anthocyanin concentrations. Antioxidant activity is influenced by multiple factors, including the compounds present and their structural characteristics, so a higher concentration of polyphenols or anthocyanins does not guarantee a higher antioxidant capacity, as also pointed out by Prior et al.



(2005) and de la Rosa et al. (2014).

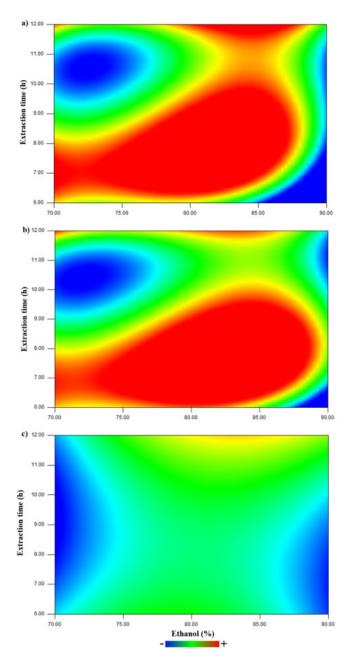


Figure 1. Influence of ethanol percentage and extraction time on a) total polyphenol extraction yield; b) anthocyanin extraction yield; and c) antioxidant capacity.

On the other hand, it was observed that the highest yield values for total polyphenols were achieved with ethanol concentrations of approximately 85% (v/v) and extraction times ranging from 6 to 10 hours. These results are consistent with those reported by Vergara-Salinas et al. (2012), who evaluated the extraction of polyphenols in maqui (Aristotelia chilensis) fruits and highlighted that high ethanol concentrations favor the recovery of phenolic compounds. The significant differences ($p \le 0.05$) in yield between studies can be at-

tributed to the physicochemical characteristics of the plant material used, including the plant matrix, the type and location of phenolic compounds, and genetic variability within the species.

Table 3 presents the established ranges for the parameters evaluated during the optimization process of the hydroalcoholic extraction of bioactive compounds from black cherry pulp. The independent variables defined were the percentage of ethanol (between 70 and 90%) and the extraction time (from 6 to 12 hours), both within a range considered adequate to guarantee process efficiency. The dependent variables included the total polyphenol yield, the anthocyanin yield, and the antioxidant capacity of the extract, which were defined using the maximization criterion.

Table 3. Restrictions for the optimization of the extraction process

Parameter	Lower limit	Upper limit	Criterion
Ethanol (%)	70	90	In the interval
Extraction time (h)	6	12	In the interval
Total polyphenol yield (%)	22,0677	29,6007	Maximize
Anthocyanin yield (%)	20,0965	25,4567	Maximize
Antioxidant capacity (mg/100 mL)	11613.9	19302.8	Maximize

Table 4 presents the three best solutions obtained during the optimization process of hydroalcoholic extraction of bioactive compounds from black cherry pulp, based on different combinations of ethanol and extraction time. Three responses were evaluated: total polyphenol yield, anthocyanin yield, and antioxidant capacity, in addition to the statistical convenience value, which indicates the suitability of each solution according to the established optimization criteria.

Solution 1, with 83% ethanol and a 12-hour time, achieved the highest yields of total polyphenols (30.77%) and exhibited good antioxidant capacity. This finding aligns with results from fruit extractions, such as S. cumini, where ethanol concentrations of around 70–80% were found to favor the optimal recovery of anthocyanins and polyphenols (De Sousa et al., 2021). Likewise, the maximum yield values reported for jaboticaba (Myrciaria cauliflora) with 74% ethanol support the positive effect of high ethanol concentrations on extraction efficiency (Nunes et al., 2021).



Table 4. Optimized solutions that meet the constraints

Parameter	Solution			
rarameter	1	2	3	
Ethanol (%)	83.23	79.23	82.70	
Extraction time (h)	12	6.34	9.45	
Total polyphenol yield (%)	30,7684	29,6007	30,3069	
Anthocyanin yield (%)	25,0582	26,1989	25,4567	
Antioxidant capacity (mg/100 mL)	17715	15206.6	14885.8	
Statistical convenience	0.902	0.776	0.752	

Solution 2, with a shorter extraction time (6.34 h) and 79.23% ethanol, yielded the highest anthocyanin content (26.20%), although with a lower antioxidant capacity. Studies using ultrasound-assisted extraction in jaboticaba have also shown that moderately short times can better preserve anthocyanins, reducing their thermal degradation and generating greater selectivity (Sharma & Dash, 2022).

In all solutions, the antioxidant capacity was lower than the theoretical maximum values, which coincides with observations in jaboticaba, where higher anthocyanin contents did not always correspond to higher antioxidant capacities, according to the ABTS assay, due to antagonistic effects or lower solubility of antioxidant compounds in high-concentration ethanol.

Solution 1 presented the highest statistical convenience (0.902), suggesting an adequate combination of factors to maximize multiple responses. This is consistent with the principles of response surface design, where the balance between variables allows for robust and replicable results, as demonstrated by numerous optimization studies on anthocyanin extractions (Miranda-Medina et al., 2018; Khan et al., 2020).

The results obtained in this study reflect trends observed in the scientific literature: ethanol concentrations around 80% combined with moderate to long times favor high yields of polyphenols and anthocyanins. However, higher concentrations or excessive times may not significantly improve antioxidant capacity, due to possible limitations in the solubilization or stability of specific compounds. In this context, solution 1 represents the best alternative analyzed, reconciling high total yield with considerable functional activity and statistical robustness.

Several biological and environmental factors contribute to the variability observed among different studies on phenolic compounds, including cultivar, ripening stage, and climatic conditions, among others (Tomás-Barberán & Espín, 2001; Bouaziz et al., 2004). Furthermore, the absence of a standardized method for measuring phenolics complicates the comparison between results, given that the Folin-Ciocalteu assay, although widely used, is influenced by parameters such as temperature, sodium carbonate concentration, and the type of standard used (Singleton et al., 1999).

Slinkard and Singleton (1977) proposed modifications to the classical method, improving its specificity by standardizing the ratio of reagent to alkaline substance, reaction time and temperature, absorbance wavelength (765 nm), and the use of gallic acid as a reference (Slinkard & Singleton, 1977). However, numerous published works fail to strictly follow these recommendations strictly, adopting instead alternative standards such as catechin, chlorogenic acid, caffeic acid, or tannic acid, which introduces comparative biases between studies.

The lack of standardization in analytical methods can lead to significant differences in the results obtained. For example, in the determination of total phenols in blueberries, values ranging from 22 to 4180 mg/100 g of fresh weight are reported, mainly due to variations in test conditions (Lee, 2005). All of this limits the comparability of results with other sources.

When analyzing the visible spectrum (Figure 2), a well-defined peak was observed between 520 and 560 nm, consistent with the UV-Vis spectrum of anthocyanins in general, as reported by Giusti and Wrolstad (2001).

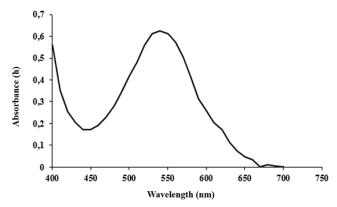


Figure 2. Spectrum of absorption of the extract hydroal-coholic optimized of cherry tree black.

The shape of the UV-Vis spectrum of anthocyanins (490-550 nm) can provide information about their concentration and the type of anthocyanin present. The anthocyanins pelargonidin (520 nm) and delphinidin (546 nm), as well as their glycosidic variants (10 to 15 nm lower), could be found in the optimized extract.

Conclusions

The highest yields of total polyphenols and anthocyanins were obtained using 80% (v/v) ethanol and a prolonged



extraction time (up to 12 h), although these conditions did not significantly influence the antioxidant capacity. Process optimization defined optimal extraction conditions (83.23% ethanol and 12 h), obtaining values lower than those estimated by the model. The optimized extract presented an intense purple color, possibly associated with the presence of anthocyanins such as pelargonidin and delphinidin, suggesting its potential as a source of natural colorants with functional properties.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization: Claudia E. Restrepo-Flórez, Yenis E. Castillo. Data curation: Yenis E. Castillo. Formal analysis: Claudia E. Restrepo-Flórez, Yenis E. Castillo. Research: Claudia E. Restrepo-Flórez, Yenis E. Castillo. Methodology: Claudia E. Restrepo-Flórez, Yenis E. Castillo. Supervision: Claudia E. Restrepo-Flórez. Validation: Claudia E. Restrepo-Flórez. Visualization: Yenis E. Castillo. Writing-original draft: Claudia E. Restrepo-Flórez, Yenis E. Castillo. Writing-review & editing: Claudia E. Restrepo-Flórez, Yenis E. Castillo.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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