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ORIGINAL ARTICLE

Obtaining freeze-dried powdered dye from *Hibiscus rosa-sinensis* flowers

Obtención de colorante en polvo liofilizado a partir de la flor de *Hibiscus rosa-sinensis*

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Abstract Currently, there is growing interest in bioactive plant compounds, such as anthocyanins, pigments widely distributed in fruits and flowers, which not only provide color but also antioxidant properties with preventive effects against coronary heart disease and certain types of cancer. The objective of this study was to develop a natural powdered dye by freeze-drying Hibiscus rosa-sinensis flowers, contributing to the agri-food heritage through the agro-industrial use of plant species. The collected flowers were subjected to hydroalcoholic extraction, concentration, and freeze-drying according to the proposed experimental design. The optimal formulation was obtained with 8.2% maltodextrin, 5% starch, and 1.863% xanthan gum. The resulting dye achieved an encapsulation efficiency of 83.67%, moisture content of 9.48%, hygroscopicity of 17.98%, and solubility of 90.23%, in accordance with the theoretical values determined by numerical optimization. These findings demonstrate the potential for innovation in the natural colorant industry, aligning the valorization of plant resources with the principles of sustainable agri-food heritage.

Keywords *Hibiscus rosa-sienensis* L., natural coloring, freeze-drying, anthocyanins, optimization.

Resumen Actualmente, existe un creciente interés por los compuestos bioactivos vegetales, como las antocianinas, pigmentos ampliamente distribuidos en frutas y flores, que no solo aportan color sino también propiedades antioxidantes con efectos preventivos frente a enfermedades coronarias y ciertos tipos de cáncer. El objetivo de este estudio fue desarrollar un colorante natural en polvo mediante liofilización de flores de *Hibiscus rosa-sinensis*, contribuyendo al patrimonio agroalimentario a través del aprovechamiento agroindustrial de especies vegetales. Las flores recolectadas fueron sometidas a extracción hidroalcohólica, concentración y liofilización conforme al diseño experimental propuesto. La formulación óptima se obtuvo con 8,2 % de maltodextrina, 5 % de almidón y 1,863 % de goma xantana. El colorante resultante alcanzó una eficiencia de encapsulación del 83,67 %, humedad del 9,48 %, higroscopicidad del 17,98 % y solubilidad del 90,23 %, en concordancia con los valores teóricos determinados mediante optimización numérica. Estos hallazgos evidencian el potencial de innovar en la industria de colorantes naturales, alineando la valorización de recursos vegetales con los principios del patrimonio agroalimentario sostenible.

Palabras clave *Hibiscus rosa-sienensis* L., colorante natural, liofilización, antocianinas, optimización.

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Introduction

The demand for natural food colorants has increased significantly, driven by consumer preference for clean-label products and growing toxicological concerns associated with synthetic colorants (Gaibor et al., 2022). The ban and regulatory reevaluation of several of these additives by agencies such as the FDA have led to a boom in the search for safe natural alternatives. In this context, anthocyanins, water-soluble pigments responsible for red, blue, and purple hues, are emerging as highly promising candidates (Mejía et al., 2020).

The flowers of *Hibiscus rosa-sinensis* L. are a significant source of anthocyanins, predominantly cyanidin-3-sophoroside. Previous studies have demonstrated the feasibility of their extraction and application as colorants and in smart packaging systems, including solid-liquid kinetics analysis with ethanol-water solvents and evaluation of their antioxidant capacity (Pérez-Orozco et al., 2020; Mejía et al., 2023).

However, the application of anthocyanins is limited by their marked instability in the face of environmental factors such as light, pH, oxygen, and temperature (Mohammadalinejhad & Kurek, 2021). In this context, microencapsulation has established itself as an effective strategy for protecting the bioactive compound by incorporating it into wall matrices, increasing its stability and shelf life during storage (de Moura et al., 2018). Although this technique has been applied to anthocyanins from various sources, its optimization for *H. rosa-sinensis* remains poorly documented, constituting a knowledge gap that the present study addresses.

In this context, the present study aims to optimize a microencapsulation process to obtain powdered dye from *H. rosa-sinensis* flowers. The research is distinguished by using food-grade solvents and the selection of wall materials aimed at maximizing encapsulation efficiency. The resulting product combines color stability and antioxidant functionality, providing a relevant techno-functional benefit for food preservation and positioning itself as a differentiated alternative to synthetic dyes.

Methodology

HRS flowers were collected and selected for uniformity in size, color, and absence of visible morphological alterations. Petals and pistils were separated and dried in an oven (YLD-6000, AISET, China) at 40 °C for 72 hours. They were then ground with a handmade grinder, sieved (0.5 mm), and stored in amber bottles with ground glass stoppers in a desiccator until use (Arencibia et al., 2023).

The extract was obtained by maceration for 24 hours at 60 °C, with 50% ethanol (v/v) acidified with 0.5% citric acid, at a solid/liquid ratio of 1 g/20 ml (Arencibia et al., 2023). The mixture was then filtered using a vacuum pump (UL-VAC DTC-21), and the filtrate was concentrated to 7% total solids by rotary evaporation (RV 10 Basic, IKA, Argentina) at 40 °C and 85 s^{-1}.

To 35 ml of the concentrated extract (7% total solids), different combinations of between 5 and 9% maltodextrin (MX) DE 20 and modified starch (MS) were added, together with xanthan gum (XG) at concentrations between 0 and 2%, according to the experimental design in Table 1. The formulations were homogenized in a magnetic stirrer (ASIN MS-500) at 750 rpm, adjusting the total solids between 20 and 25% according to the corresponding run. Finally, the samples were subjected to freeze-drying (Telstar Lioalfa-6 Lyophyliser), operating at -50 °C and 10^{-2} Pa for 36 hours.

The encapsulation efficiency (EE) was determined by quantifying total and surface anthocyanins according to Lee et al. (2005). Absorbances at 510 and 700 nm were measured in buffer solutions at different pH values using spectrophotometry (Rayleigh UV-1601, Beijing), applying the equations proposed by Robert et al. (2010).

Moisture content (Hu) was determined by drying in an oven at 105 °C (YLD-6000 AISET, China), calculated by the difference in weight before and after drying (da Rosa et al., 2019). Hygroscopicity (Hi) was determined by exposing 1 g of dye to a controlled atmosphere with saturated sodium chloride solution (75.29% RH at 25 °C) and was expressed as g of water/100 g of dry solids (Tonon et al., 2008). Solubility (Sol) was evaluated by quantifying the percentage of powder dissolved in distilled water through stirring, centrifugation, and drying of the recovered solids. It was calculated by comparing the initial weight with that of the dry solids (Cano-Chauca et al., 2005).

The characterization of the optimal extract replicated the methodologies described above. The procedures were replicated three times to ensure accuracy. Statistical analysis was performed using simple ANOVA in Statistics (version 7, 2004, StatSoft Inc., Tulsa, USA), applying Duncan's test ($p \leq 0.05$) for comparison of means.

Results and discussion

The results shown in Table 1 indicate that, among the formulations evaluated, runs 3, 4, 6, and 8 presented high EE (≥ 84%) and Sol above 86%, accompanied by low levels of Hy (16.0–17.3%), suggesting the formation of denser and more cohesive matrices. Run 3 achieved the highest EE (85.7%) and one of the lowest Hy (16%), supporting the hypothesis that a balanced proportion of the three components promotes the formation of a continuous and highly soluble encapsulating matrix. Likewise, run 4 recorded the highest Sol value (88.6%). In contrast, the formulations corresponding to runs 5 and 7 showed lower

EE values of 61.0 and 66.4%, respectively, accompanied by high Hy (up to 21.9%) and lower Sol levels, which can be attributed to the low concentration of encapsulating agents and the absence of XG in the mixture.

Table 1. Experimental design and response variables

Run	A (%)	B (%)	C (%)	EE (%) Mean (Standard deviation)	Hu (%) Mean (Standard deviation)	Hy (%) Mean (Standard deviation)	Sol (%) Mean (Standard deviation)
1	7.2	6.8	0.043	71.5 (0.6) c	8.76 (0.02) bc	19.4 (0.2) ef	84.7 (0.8) ab
2	9	9	0	70.8 (0.7) c	9.8 (0.6) de	16.0 (1) a	83.9 (2.7) a
3	9	7.5	2	85.7 (0.5) e	10.1 (0.8) e	16.0 (0.9) a	87.0 (1.3) bc
4	7.2	8.94	1.1	84.2 (1) e	9.5 (0.2) de	16.8 (0.2) ab	88.6 (1.9) d
5	5	5	0	61.0 (3) a	7.91 (0.11) a	21.9 (0.5) g	85.4 (0.2) abc
6	9	5	0.74	84.7 (0.2) e	9.31 (0.41) cd	17.7 (0.4) c	85.2 (0.5) abc
7	5	9	0	66.4 (0.6) b	8.42 (0.15) ab	18.2 (0.4) cd	85.3 (0.8) abc
8	7.2	8.94	1.1	84.3 (0.8) e	9.5 (0.6) de	17.3 (0.3) bc	86.5 (0.4) abc
9	5.08	6.8	1.1	77.1 (0.6) d	8.39 (0.06) ab	19.85 (0.08) f	86.8 (1.7) abc
10	6.46	5	2	76.5 (0.4) d	9.2 (0.2) cd	18.8 (0.3) de	87.9 (1.8) cd
11	5.08	6.8	1.1	76.3 (0.2) d	8.39 (0.01) ab	19.0 (0.2) def	86.4 (1.7) abc
12	5	9	2	78.0 (3) d	9.22 (0.08) cd	18 (1) bc	87.9 (1.7) c
13	7.2	6.8	0.043	71.3 (0.3) c	8.7 (0.1) bc	19.3 (0.3) ef	84.7 (0.4) ab

A: maltodextrin, B: modified starch, C: xanthan gum, EE: encapsulation efficiency, Hu: humidity, Hy: hygroscopicity, Sol: solubility. Different letters indicate significant differences ($p \leq 0.05$).

Table 2 presents the results of the analysis of variance (ANOVA) of the coded models used to describe the effect of the independent variables on EE, Hu, Hi, and Sol of the encapsulated dye. Terms with ≤ 0.05 were considered significant.

Table 2. Analysis of variance of coded models for encapsulation efficiency, moisture content, hygroscopicity, and dye solubility

Parameter	p value			
	EE	Hu	Hy	Sol
Model	< 0.0001	< 0.0001	< 0.0001	0.0023
A	< 0.0001	< 0.0001	< 0.0001	0.1238
B	< 0.0001	< 0.0001	< 0.0001	0.4711
C	< 0.0001	< 0.0001	0.0021	0.0004
AB	< 0.0001	0.0187	-	-
AC	0.0002	-	-	-
BC	-	-	-	-
A^2	< 0.0001	-	-	-
B^2	< 0.0001	0.0004	-	-
C^2	< 0.0001	0.0076	-	-
Lack of fit	0.7671	0.2733	0.3081	0.6690

A: maltodextrin, B: modified starch, C: xanthan gum, EE: encapsulation efficiency, Hu: humidity, Hy: hygroscopicity, Sol: solubility.

The models adjusted for each response variable were statistically significant, indicating that at least one term contributes to explaining the observed variability. The lack of adjustment was not significant, supporting the validity and representativeness of the models.

In the EE model, all main terms (A, B, C), interactions (AB, AC), and quadratic terms (A^2, B^2, C^2) were significant, showing linear, nonlinear, and interactive effects. For Hu, A, B, C, the AB interaction, and the quadratic terms B^2 and C^2 were significant, reflecting combined and curvilinear effects. In Hy, A and B were significant, while C showed a relevant effect without interaction or quadratic terms. In Sol, only C was significant, indicating XG as a determining component. Although non-significant terms were eliminated, Gutiérrez & De la Vara (2012) recommend preserving the hierarchy for a robust statistical interpretation. The R^2 values were high (78.5–99.9%).

The influence of A, B, and C on the response variables (EE, Hu, Hy, and Sol) was evaluated using response surface graphs, which show the average behavior according to the adjusted model (Gutiérrez & De la Vara, 2012). The joint analysis of Figures 1-4 reveals the differential role of XG (C), MX (A), and MS (B) on the response variables evaluated.

In Figure 1, XG has a positive and significant effect on EE, especially from 0.74% onwards, reaching values close to 90% in the optimal zone. In the absence of XG, EE depended more on A and B, but its combination with C generated synergistic interactions that stabilize the response (EE).

In Figure 2, XG does not have a significant linear effect on Hu at levels below 2%, maintaining values between 7.5 and 10.5%. However, at 2%, a moderate increase is observed associated with its water retention capacity. MX has a reducing effect (Nik Abd Rahman et al., 2024), while MS slightly increases it at high concentrations due to its hydrophilic nature.

In Figure 3, Hy is dominated by A and B, with a significant positive effect of MX and a negative effect of MS, like the

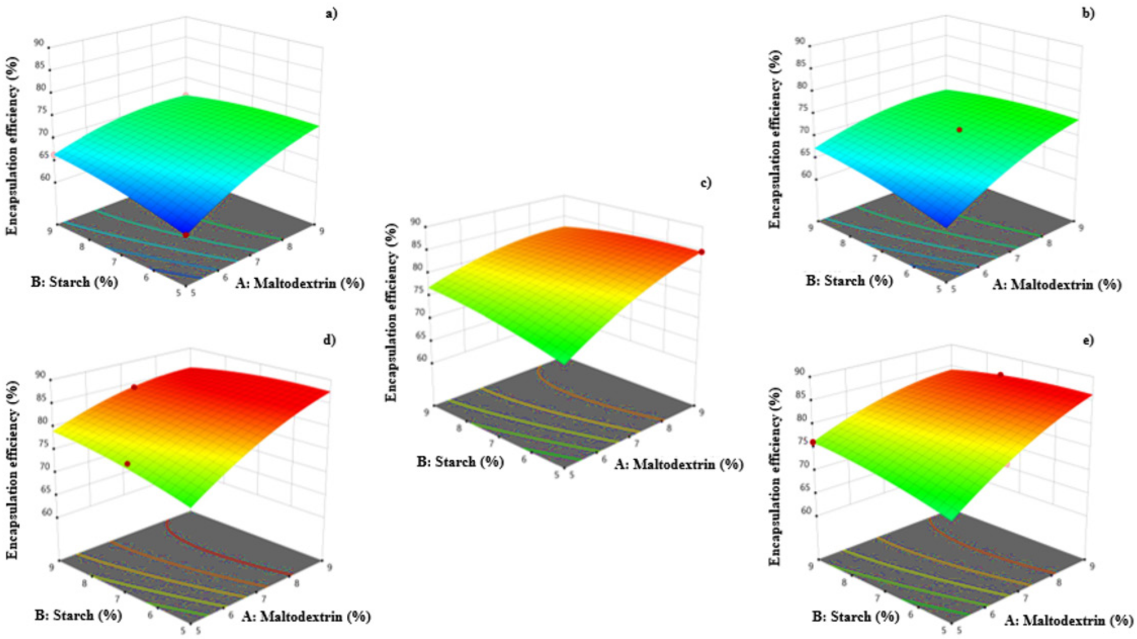

Figure 1. Influence of xanthan gum on EE. a) 0%; b) 0.0434446%; c) 0.74%; d) 1.1% y e) 2%.

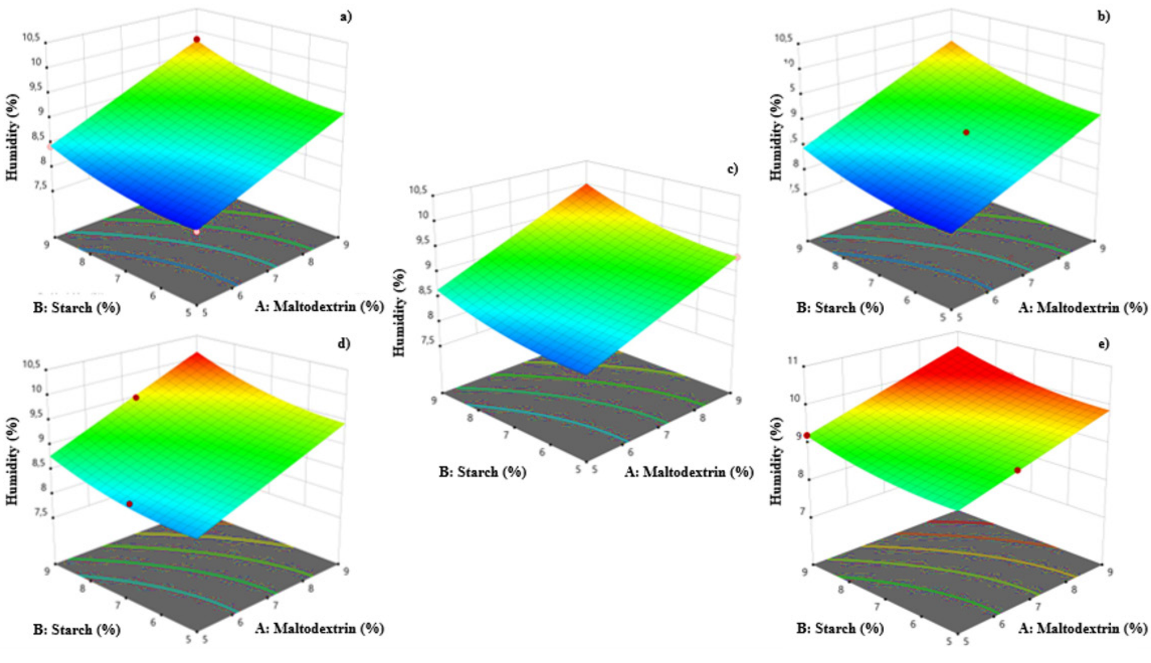

Figure 2. Influence of xanthan gum on humidity. a) 0%; b) 0.0434446%; c) 0.74%; d) 1.1% y e) 2%.

study by Tonon et al. (2008). XG acts as a modulator (C and C², $p < 0.01$), smoothing the gradients and stabilizing Hyat intermediate and high levels, which could be related to a denser matrix that limits Hu absorption.

In Figure 4, XG shows a positive and significant effect on Sol (C and C², $p < 0.01$), with the maximum at 2%. Its high solubility and rapid hydration facilitate dye dispersion. Meanwhile, A and B show no significant effects or interactions with C, indicating that Sol is mainly determined by XG content.

Overall, XG appears to be the main control factor in EE and Sol, a modulator of Hy, and to have a limited effect on Hu, while MX and MS play opposite and complementary roles in Hy and Hu, helping to optimize the encapsulating matrix.

Optimization of response variables

After adjusting and validating the model, the response

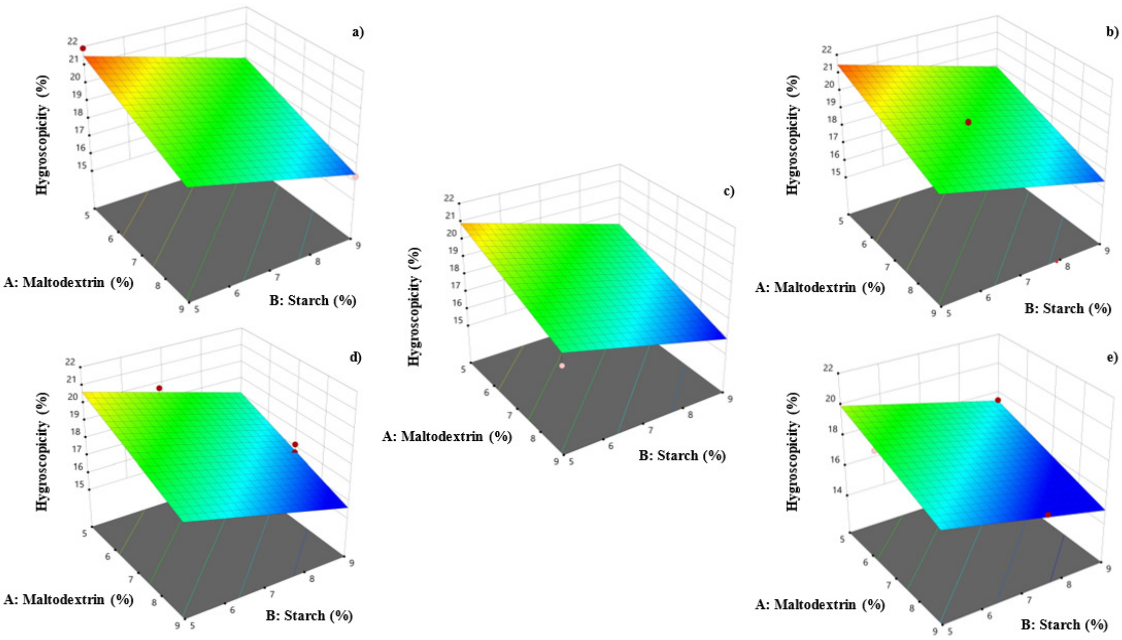

Figure 3. Influence of xanthan gum on hygroscopicity. a) 0%; b) 0.0434446%; c) 0.74%; d) 1.1% y e) 2.2%.

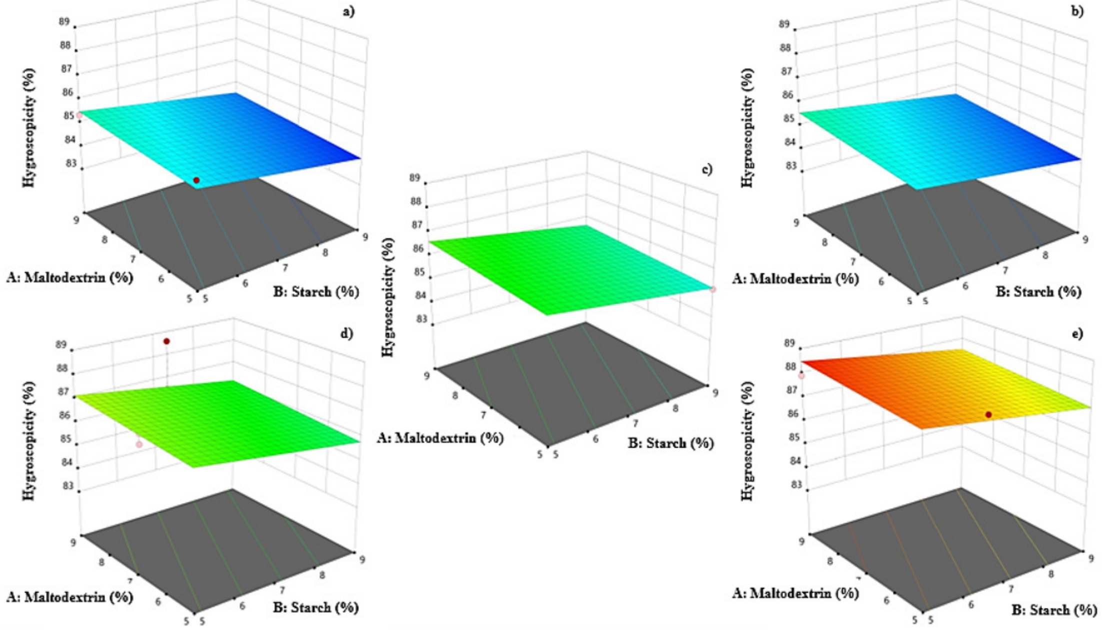

Figure 4. Influence of xanthan gum on solubility. a) 0%; b) 0.0434446%; c) 0.74%; d) 1.1% y e) 2%.

surface was explored to define optimal conditions or directions for future experimentation (Gutiérrez & De la Vara, 2012). In this study, the validated model made it possible to establish the ideal conditions for obtaining a natural powdered dye from HRS flowers, simultaneously considering all process variables.

Table 3 shows the constraints applied to optimize EE, Hu, Hy, and Sol. Fourteen feasible solutions were obtained, with solution 1 being selected for its greater statistical convenience: 8.2% MX, 5% MS, and 1.863% XG. Under these conditions, 85.28% EE, 9.57% Hu, 18.00% Hy, and 87.11% Sol were predicted, with a convenience of 0.701.

The dye obtained under optimal conditions showed an EE of 83.67% (Table 4), which is lower than that estimated by numerical optimization. However, the relative percentage error was 1.89%, indicating high precision and experimental reliability. A lower percentage of Hu was obtained than estimated by the model. XG, with limited environmental absorption but high internal water retention, probably modulated the free water in the formulation, reducing the final Hu of the product to 9.48% (Patel et al., 2020; Berninger et al., 2021).

Table 3. Constraints for optimizing the dye production process

Parameter(%)	Minimum limit	Maximum limit	Criterion
Maltodextrin	5	9	Maximize
Modified starch	5	9	Minimize
Xanthan gum	0	2	Maximize
Encapsulation efficiency	61.0	85.7	Maximize
Humidity	7.9	10.1	Minimize
Hygroscopicity	16.0	22.0	Minimize
Solubility	83.9	88.6	Maximize

Table 4. Physical and chemical indicators of the optimal dye obtained

Parameter	Mean	Standar deviation
Encapsulation efficiency (%)	83.67	0.09
Humidity (%)	9.48	0.02
Hygroscopicity (%)	17.98	0.14
Solubility (%)	90.23	0.36

The optimal formulation yielded a Hy of 17.98%, a moderate value suggesting that XG stabilized the product against RH variations without increasing its absorption. Unlike other hygroscopic polysaccharides, XG is not particularly hygroscopic (Berninger et al., 2021), regulating internal Hu and water-powder balance.

Due to its high solubility and ability to form viscous solutions, XG improves the dispersion of powders in water, especially in low proportions (Nsengiyumva & Alexandridis, 2022). In the optimal formulation, a Sol of 90.23% was obtained, higher than estimated, showing that XG did not limit but rather favored the dissolution of the active components.

Conclusions

Xanthan gum proved to be a key component in controlling the physicochemical properties of the powdered dye obtained. Its use in combination with other encapsulants optimized encapsulation efficiency, controlled internal moisture, stabilized hygroscopicity, and promoted high solubility. These effects are directly related to its molecular structure and rheological behavior, which provide stability without compromising product functionality.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization: José A. Arencibia, Alicia C. Casariego. **Data curation:** José A. Arencibia, Dairon I. Iglesias, Ariel A. Vergel. **Formal analysis:** José A. Arencibia, Alicia C. Casariego, Dairon I. Iglesias, Ariel A. Vergel. **Research:** Dairon I. Iglesias, Ariel A. Vergel. **Methodology:** José A. Arencibia, Alicia C. Casariego. **Supervision:** José A. Arencibia, Alicia C. Casariego. **Validation:** Ariel A. Vergel. **Visualization:** Ariel A. Vergel. **Writing – original draft:** José A. Arencibia, Alicia C. Casariego, Dairon I. Iglesias, Ariel A. Vergel. **Writing – review & editing:** José A. Arencibia, Alicia C. Casariego, Dairon I. Iglesias, Ariel A. Vergel.

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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