Journal of the Professional Association for Cactus Development

Optimized Ultrasound-Assisted Extraction of Carminic Acid from *Dactylopius opuntiae*

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Cite: Ochoa-Manzo, G. M., Rodiles-López, J. O., Martínez-Flores, H. E., Portillo, L., García-Pérez, M. E. and Garnica-Romo, M. G. 2025. Optimized Ultrasound-Assisted Extraction of Carminic Acid from *Dactylopius opuntiae*. *Journal of the Professional Association for Cactus Development. 27*: 133-147. https://doi.org/10.56890/jpacd.v27i.583

Associate Editor: Pablo Preciado-Rangel

Technical Editor: Tomás Rivas-García

Received date: 09 August 2025.
Accepted date: 08 September 2025.
Published date: 21 September 2025

Abstract. *Dactylopius opuntiae* is a major pest of prickly pear crops and has therefore been extensively studied with the aim of controlling its proliferation. However, it has largely been disregarded as a potential natural red pigment source, despite producing carminic acid (CA), as does *Dactylopius coccus*, albeit in lower amounts. CA, an anthraquinone compound, is highly valued for its strong coloring capacity and commercial relevance in the food, cosmetic, and pharmaceutical industries. Conventional CA extraction methods are often constrained by long processing times and limited environmental sustainability. In this context, ultrasound-assisted extraction (UAE) has emerged as an efficient and eco-friendly alternative, offering improved extraction yields. This study aimed to optimize the UAE of CA from *D. opuntiae* and maximize extract yield. A Box–Behnken experimental design was applied, testing temperatures of 20, 40, and 60 °C; extraction times of 3, 6, and 9 min; and ultrasound amplitudes of 20, 30, and 40%. The highest CA concentration (14.76 ± 0.32%) was obtained at 60 °C, 3 min of sonication, and 20% amplitude, representing a 7.45% increase compared with the conventional method. These findings demonstrate that UAE enables higher CA yields in significantly shorter processing times, providing a sustainable strategy to transform *D. opuntiae* from an agricultural pest into a valuable natural pigment source.

Keywords: *Anthraquinone, emerging method, natural pigment, wild cochineal*

Introduction

The insect family Dactylopiidae, endemic to America (Paul-Fils *et al*., 2020; El Aalaoui and Sbaghi, 2022), comprises the genus *Dactylopius*, recognized for producing carminic acid (CA) in the hemolymph of females (Mandujano and Mandujano, 2020). This natural red pigment has been exploited since pre-Columbian times, prior to 1492 AD (Bader and Abu-Alloush, 2019), due to its intense coloration and stability. Biomarkers of Mexican cochineal from *Dactylopius* have been identified in aged dyed wool, confirming the long-term stability of these compounds (Cruz *et al*., 2023). Cochineal dye was a resource of prestige and symbolism in pre-Columbian life, used in textiles, art, rituals, and as tribute, anticipating the great value it would later acquire during the colonial period as an export commodity. Chemically, CA is an anthraquinone derivative (Liu *et al*., 2021) used in the production of carmine (E120), a pigment of high commercial relevance in the cosmetics, food, pharmaceutical, and textile industries because of its vivid red hue and exceptional stability against light, temperature variation, and oxidation (Rasmussen *et al*., 2018; Alizadeh *et al*., 2022). Colonies typically establish at the joints of the cladode-trunk, flower-cladode, or fruit-cladode in cacti. These joints are where the cladodes (flattened stems) attach to the trunk or branches, enabling efficient photosynthesis and structural support (Kondo *et al*., 2022). To date, 11 species of *Dactylopius* have been identified (Aragón-Martínez *et al*., 2023), of which only two are of major relevance. *Dactylopius coccus* is an important source of CA due to its high pigment content, ranging from 14 to 26% of dry weight (Rasmussen *et al*., 2018; Mazzeo *et al*., 2019; Mandujano and Mandujano, 2020), whereas in *D. opuntiae* it is significantly lower, ranging from 5 to 8% (Mazzeo *et al*., 2019; Rodríguez-Leyva *et al*., 2024). *Dactylopius opuntiae*, regarded as the most destructive pest, causes severe chlorosis, necrosis, and eventual death of *Opuntia ficus-indica* plants (Torres and Giorgi, 2018; Mazzeo *et al*., 2019). This species has led to substantial economic losses in Mexico and other countries, including Spain, Morocco, South Africa, Ethiopia, and Brazil (Mazzeo *et al*., 2019; López-Rodríguez *et al*., 2021).

Dactylopius opuntiae exhibits a remarkable capacity for propagation and survival, largely due to its ability to produce CA, which functions as a defense mechanism against natural predators (Bader and Abu-Alloush, 2019). In addition, the dense cottony wax covering its body during development enhances its resilience (Torres and Giorgi, 2018). These traits, combined with its high reproductive rate and rapid dispersal capacity, have facilitated its establishment as an invasive pest (El Aalaoui *et al*., 2022). In Tigray, Ethiopia, a rapid spread of *D. coccus* was reported, devastating wild and cultivated *O. ficus-indica* populations because of favorable climatic conditions, prolonged dry spells, abundant cactus vegetation, and the absence of natural predators (Kiros-Meles, 2025). Some authors suggest that controlled harvesting could have balanced cochineal exploitation with cactus pear production (Kiros-Meles, 2025). For this reason, under appropriate management, this species could be considered a potential source of natural pigment, as its prolific reproduction may provide sufficient raw material for CA extraction.

The extraction process is a critical step in the separation and efficient recovery of natural pigments (Masyita *et al*., 2025). Various extraction methods are available, ranging from conventional to emerging technologies. Conventional approaches, such as maceration and thermal processing, have been used since ancient times and are valued for their simplicity (Ngamwonglumlert *et al*., 2017). However, these methods often present drawbacks, including low yields, long processing times, high energy consumption, and consequently elevated production costs (Reyes-Pérez *et al*., 2023). In contrast, emerging extraction techniques offer more cost-effective and sustainable alternatives, achieving higher yields in shorter times while reducing solvent use, energy demand, and environmental impact (Linares and Rojas, 2022; Masyita *et al*., 2025). Reported emerging technologies include high-pressure processing, microwave-assisted extraction, and ultrasound-assisted extraction (UAE). Ultrasound-assisted extraction has been widely applied for the recovery of natural pigments due to its ability to improve extract purity. This enhancement is attributed to its operating mechanism, in which ultrasonic waves generate acoustic cavitation-the formation, growth, and subsequent collapse of microbubbles in a liquid medium. The collapse of these bubbles produces shock waves that disrupt cell walls, facilitating the release of intracellular components, enhancing solvent penetration, and

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improving mass transfer. As a result, pigment diffusion into the solvent is accelerated, and extraction efficiency is increased (Prakash *et al*., 2013; Kutlu *et al*., 2022).

This study aimed to optimize the UAE of CA from *D. opuntiae* as a more efficient and sustainable process. A Box-Behnken design (BBD) combined with response surface methodology (RSM) was employed to determine the optimal temperature, extraction time, and ultrasound amplitude. RSM is a set of statistical and mathematical techniques used to model and analyze processes in which a response variable is influenced by multiple independent factors. Its application allows for the evaluation of main effects and interactions among factors, as well as the determination of optimal experimental conditions through the construction of second-order polynomial models. In this study, the BBD was selected. This second-order response surface design requires fewer experimental runs compared to other designs, such as the Central Composite Design. The BBD distributes experimental points in a spherical manner and avoids combinations at the extreme levels of the factors, making it particularly suitable when extreme conditions are impractical, unstable, or not representative of the system under investigation (Montgomery, 2017). This approach enabled the estimation of linear and quadratic effects with a reduced number of experiments, thereby identifying the conditions that maximize CA yield.

Material and Methods

Material

The experimental material, *D. opuntiae*, was provided by the Universitary Center for Biological and Agricultural Sciences (CUCBA) of the University of Guadalajara, Jalisco, Mexico. The samples were then subjected to a dehydration process at 60 °C, following the methodology recommended by González *et al.* (2002). The process was conducted in an oven (model FE-291-D, Felisa, Guadalajara, Mexico) for 10 h, until constant weight was achieved. Subsequently, the sample was placed in a glass beaker with 99% anhydrous ethyl ether ($C_4H_{10}O$) as the solvent, at a ratio of 70:30 (w/v), to remove as much wax as possible. The dried insects were then ground in a coffee grinder (model 803893, Hamilton Beach, Glen Allen, USA) until a fine powder was obtained, which was sieved through a US No. 120 mesh (125 µm) to homogenize the sample.

Experimental design

To determine the optimal conditions for extracting the highest concentration of CA from *D. opuntiae*, a Box-Behnken (BBD) experimental design was employed, incorporating three independent variables, each at three levels. The independent variables in this study were temperature (20, 40, and 60 °C), ultrasound equipment wave amplitude (20, 30, and 40%), and processing time (3, 6, and 9 min). These variables were selected based on a review of previous studies on pigment extraction by ultrasound (Prakash *et al*., 2013; Masyita *et al*., 2025; Reyes-Pérez *et al*., 2024), as well as the operating specifications of the equipment used. The design consisted of 17 runs, including five central points, as shown in Table 1. The response variables were CA content ($C_{22}H_{20}O_{13}$) and extract yield.

Quantification of carminic acid

The CA content was determined using the Marmión method (1984), as reported by Arroyo-Figueroa *et al*. (2020) and Salazar-Llangarí *et al*. (2023). A quantity of 0.025 g of the powdered *D. opuntiae* extract was dissolved in 7.5 mL of 2 N hydrochloric acid (HCl). The solution was heated in a boiling water bath for 30 min, then allowed to cool to 22 °C and subsequently diluted to 250 mL with distilled water. The solution was filtered through Whatman No. 2 filter paper, and absorbance was measured

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at 494 nm using a spectrophotometer (model VE-5600UV, VELAB, México). The percentage of CA was calculated using Equation 1.

$$Carminic\ acid\ (\%) = \frac{Absorbance \times 100}{1.39} \tag{1}$$

Ultrasound-assisted extraction

Ultrasound equipment coupled with a Sonic Vibra-Cell probe (model VC505, Sonics & Materials Inc., Newtown, CT, USA) operating at a frequency of 20 kHz was used. Intermittent 30-s pulses of ultrasound, followed by 30 s of rest, were applied, subjecting the sample to agitation via direct ultrasonic cavitation. Following Peng *et al*. (2023), who reported that a higher solvent proportion facilitates the diffusion of bioactive compounds during ultrasound extraction, a high sample-to-solvent ratio of 1:100 (w/v) was employed in this study. A quantity of 0.30 g of *D. opuntiae* powder was carefully weighed and dissolved in 30 mL of distilled water to prepare the solution. After extraction, the samples were centrifuged at 1.05 × g for 15 min. The resulting supernatant was collected and transferred to a Felisa oven (model FE-291-D, Felisa, Guadalajara, Mexico) maintained at 60 °C for 18 h to dehydrate the samples.

Extraction of carminic acid using the conventional method

The conventional method for CA extraction was based on the methodology proposed by Salazar-Llangarí *et al*. (2023), with modifications based on preliminary tests, given the limited number of reference procedures in the literature. A mixture of distilled water and *D. opuntiae* was prepared at a ratio of 1:100 (w/v) and boiled for 15 min to maintain the same ratio used in ultrasound extraction. The samples were centrifuged at 1.05 × g for 15 min using a Centrificient centrifuge (model TD5M-WS, CRM Globe Int., Chicago, USA). Thereafter, the supernatant was collected and transferred to an oven (model FE-291-D, Felisa, Guadalajara, Mexico) maintained at 60 °C for 18 h to dehydrate the samples. These conditions were employed to ensure uniformity and allow for comparative purposes.

Determination of extract yield

The powder extracts obtained from UAE were weighed, and the extraction yield was calculated using Equation 2. The initial weight corresponded to the mass of the sample used (0.3 ± 0.0003 g of *D. opuntiae*), and the final weight corresponded to the mass of the powder extract.

$$\%\ Extract\ yield = \frac{Final\ weight}{Initial\ weight} \times 100 \tag{2}$$

Statistical analysis

The data obtained from the Box–Behnken experimental design (BBD) were analyzed using Statgraphics Centurion XVII software (2010, USA). An analysis of variance (ANOVA) was performed at a significance level of $p < 0.05$, and the correlation coefficient (R) was calculated to assess the validity of the model. The corresponding response surface graphs were then generated.

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Results and Discussion

Carminic acid content

The dry powder of *D. opuntiae* contained 9.62 ± 0.36% CA, slightly exceeding the 6–8% range reported by Mazzeo *et al*. (2019). This value also surpasses the 2.91% (w/w) CA content reported by Aragón-Martínez *et al*. (2023), which was quantified using high-performance liquid chromatography (HPLC) with allopurinol as an internal standard.

Extraction of carminic acid and extract yield by ultrasound

Table 1 presents the results of the Box–Behnken design (BBD) experiments assessing CA extraction efficiency and extract yield from *D. opuntiae*. Each experimental combination was performed once, while the center point was repeated five times to estimate experimental error and validate model repeatability. The response surface analysis facilitated visualization of the different treatment conditions, each showing varying percentages of CA extraction and extract yield. It is important to select conditions that balance extract yield and CA concentration. Treatment 14 (60 °C, 20% amplitude, and 9 min) resulted in the highest CA concentration (14.65%), with a corresponding powder extract yield of 52.03%.

Table 1. Box–Behnken design analysis of carminic acid and yield.

Run	T (°C)	A (%)	t (min)	CA (%)	Y (%)
1	20	20	9	11.48	38.94
2	20	30	3	12.11	41.41
3	20	30	15	12.35	51.35
4	20	40	9	12.20	51.05
5	40	20	3	13.78	45.62
6	40	20	15	13.83	50.70
7	40	30	9	13.35	48.33
8	40	30	9	13.16	52.54
9	40	30	9	13.66	52.30
10	40	30	9	13.78	49.03
11	40	30	9	13.76	47.27
12	40	40	3	13.98	48.97
13	40	40	15	14.00	52.53
14	60	20	9	14.65	52.03
15	60	30	3	14.53	53.05
16	60	30	15	13.38	49.38
17	60	40	9	14.24	55.55

T: Temperature. A: Amplitude. t: Time. CA: Carminic acid. Y: yield. All values correspond to a single analysis, except for the center point, which was repeated five times to estimate the experimental error.

Analysis of variance and effect of variables: percentage of carminic acid

The results of the analysis of variance (ANOVA) are shown in Table 2 and allowed identification of significant differences in CA extraction percentages.

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Table 2. Analysis of Variance (ANOVA) for carminic acid content using Box-Behnken ultrasonic extraction design.

	Sum of Squares	F Ratio	*P* Value	Optimization
Constant				+ 8.279290
A	9.36839	81.29	0.0001**	+ 0.243559
B	0.05634	0.49	0.5070	- 0.056649
C	0.08807	0.76	0.4110	+ 0.026472
AA	1.54385	13.4	0.0081**	- 0.001513
AB	0.31758	2.76	0.1409	- 0.001408
AC	0.48365	4.20	0.0797	- 0.002897
BB	0.17492	1.52	0.2577	+ 0.002038
BC	0.00014	0.00	0.9729	- 0.000099
CC	0.09457	0.82	0.3951	+ 0.004163
R	0.97			

A = Temperature in °C; B = % Wave Amplitude; C = Time in minutes. ** = Highly significant.

A strong correlation was observed, with a coefficient of R= 0.97, indicating that the model adequately explains CA extraction. The linear term of temperature had a substantial effect, accounting for 77.25%, while the quadratic term of temperature contributed an additional 12.73%, both statistically significant ($p < 0.05$). The interaction between time and temperature also significantly influenced extraction, contributing 3.99% to CA concentration. In contrast, the linear and quadratic terms of amplitude (%) and time (min) had no substantial impact on CA concentration.

Three-dimensional response surface graphs provide a graphical representation of the main and interactive effects of the independent variables. These effects are illustrated by keeping one variable constant at its central point while varying the others within their respective ranges. There is a clear positive correlation between increasing temperature (°C) and the decrease in amplitude (%) on the increase in CA concentration (Figure 1).

In addition, Figure 2 shows that increasing the temperature and decreasing the sonication time result in more efficient CA extraction, indicating that higher temperatures and shorter sonication times promote the release of CA.

Analysis of variance and effect of variables: percentage of extract yield

The results of the analysis of variance (ANOVA) for extract yield are presented in Table 3. The coefficient of determination (R) for this model is 0.9184, indicating a strong correlation between the model and the experimental data. This suggests that the model adequately explains the extraction process, resulting in a high extract yield. The linear term of temperature had a substantial effect, accounting for 38.27% of the variability. The linear term of amplitude contributed 22.30%, while the interaction between temperature and time accounted for 19.08%, all statistically significant ($p < 0.05$).

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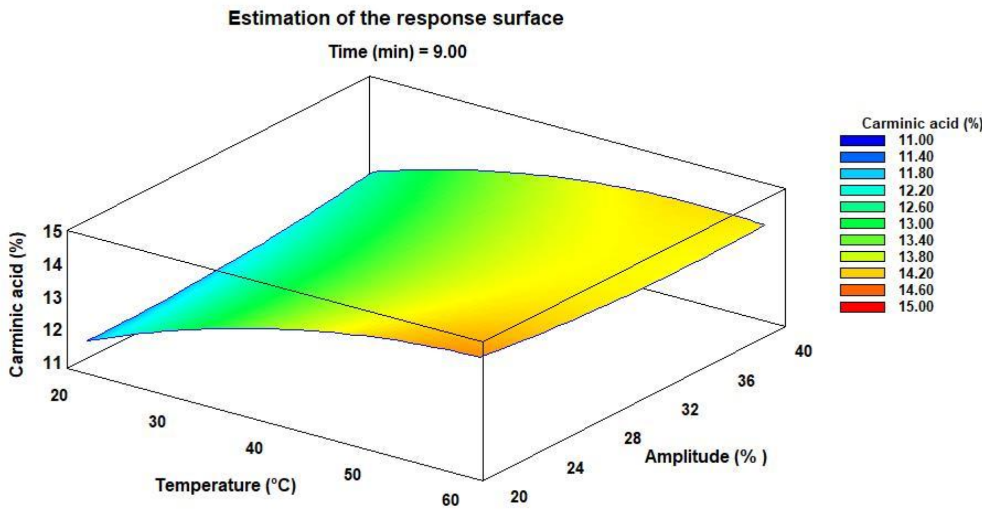

Figure 1. Response surface of carminic acid percentage (%CA) vs. temperature and amplitude in ultrasound extraction (Box–Behnken design, time= 9 min).

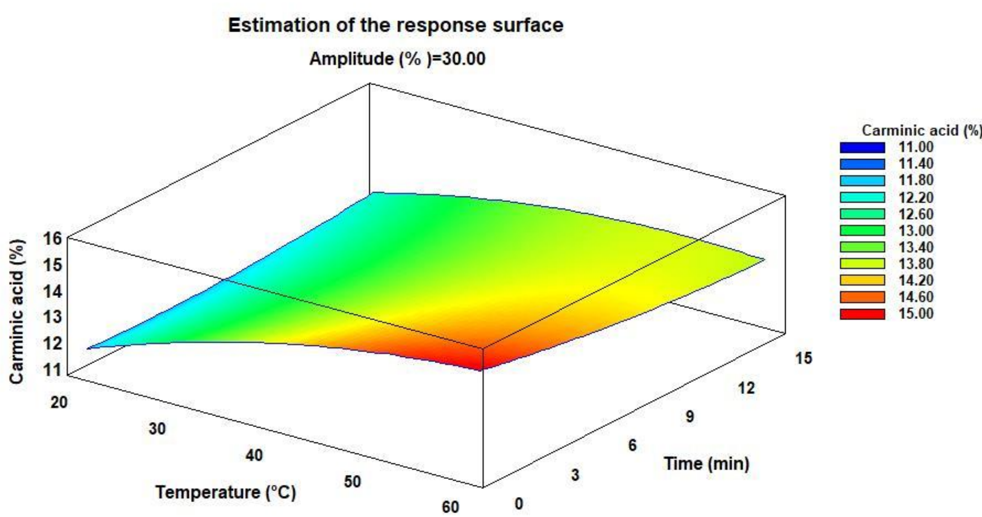

Figure 2. Response surface of carminic acid (%CA) vs. temperature and time in ultrasound extraction (Box–Behnken design, amplitude= 30%).

The linear term of time contributed 11.45% to the increase in extract yield; however, this effect was not statistically significant.

Table 3. Analysis of Variance (ANOVA) of Box-Behnken Design for carminic acid yield using ultrasound extraction.

	Sum of Squares	F Ratio	*P* Value	Optimization
Constant				+ 4.897880
A	92.8884	14.43	0.0067**	+ 0.863587
B	54.1320	8.41	0.0230**	+ 0.699825
C	27.7885	4.32	0.0763	+ 1.893290
AA	1.41398	0.22	0.6535	- 0.001449
AB	18.4470	2.87	0.1343	- 0.010738
AC	46.3080	7.19	0.0314**	- 0.028354
BB	0.02561	0.00	0.9515	+ 0.000780
BC	0.57760	0.09	0.7732	- 0.006333
CC	1.12543	0.17	0.6884	- 0.014361
R	0.9184			

A = Temperature in °C; B = % Wave Amplitude; C = Time in minutes. ** = highly significant.

Analysis of Figure 3 reveals a positive correlation between increases in temperature (°C) and amplitude (%) and extract yield. Conversely, Figure 4 shows that increasing temperature in combination with shorter sonication times results in higher extract yields, indicating that this parameter combination is optimal for maximizing yield.

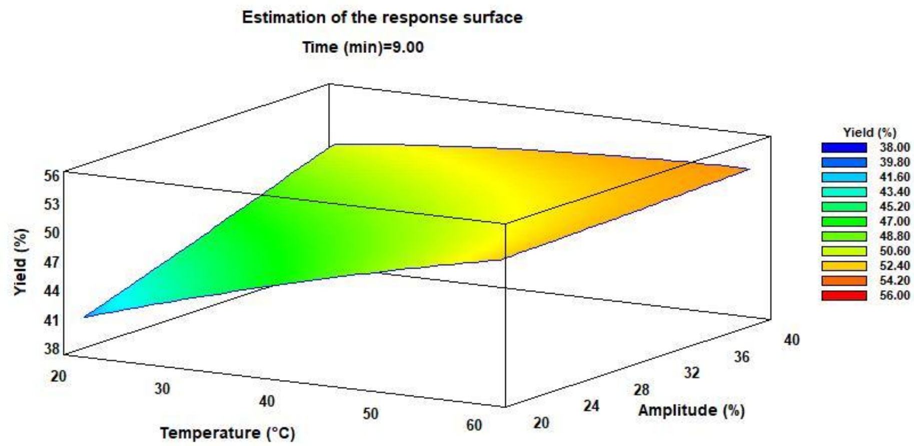

Figure 3. Response surface of carminic acid yield (%CA) vs. temperature and amplitude in ultrasound extraction (Box–Behnken Design, time= 9 min).

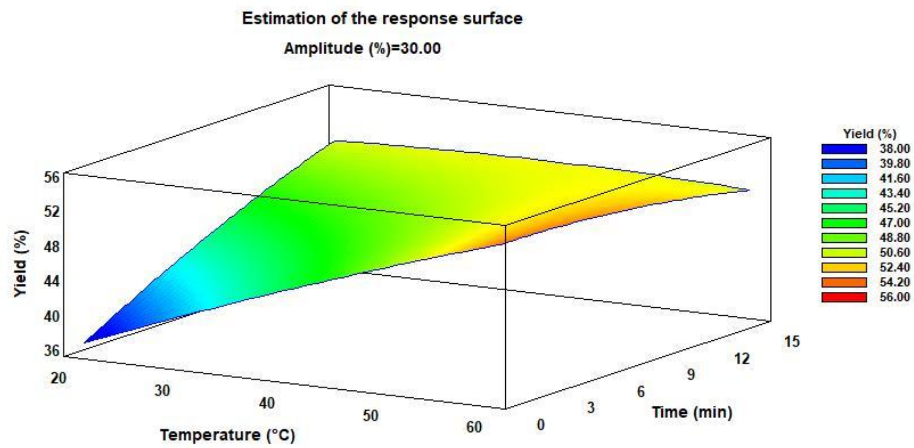

Figure 4. Response surface of carminic acid yield (%CA) vs. temperature and time in ultrasound extraction (Box–Behnken design, amplitude= 30%).

Optimization for carminic acid extraction

The response values obtained from the Box-Behnken design analysis were used to establish a second-order polynomial equation for the model. This equation is presented in Table 2, which shows the optimization values for each linear and quadratic term, as well as their interactions. The optimal conditions for CA extraction were as follows: temperature 59.93 °C, amplitude 20.0%, and extraction time 3.25 min. The estimated theoretical CA concentration was 14.99%. Experimental validation under these conditions yielded 14.76 ± 0.32% CA, representing a relative difference of 1.60% from the predicted value, corresponding to 0.23%. This close agreement supports the predictive capability of the proposed model.

This study is the first to report the application of UAE for recovering CA from *D. opuntiae*. A comparable study by Reyes-Pérez *et al*. (2024) applied UAE to *D. coccus*, identifying optimal extraction conditions of 67.3 °C and 15 min, achieving a CA content of 26.3%. Although their optimal temperature is similar to the 60 °C determined here, our optimized process reduced the extraction time to only 3 min. This difference likely stems from variations in experimental design, particularly regarding ultrasonic amplitude. Reyes-Pérez *et al*. (2024) used equipment operating at 500 W and 20 kHz although did not specify the amplitude, a critical parameter influencing cavitation intensity and mass transfer efficiency. In contrast, the present study explicitly included amplitude as an independent variable, demonstrating its significant interaction with temperature and time in optimizing CA extraction.

The CA content of 14.76 ± 0.32% obtained from *D. opuntiae* in this study may be attributed to interspecific differences, particularly in the composition and structure of the wax coating the insect's body. *D. opuntiae* is covered by a dense, highly adherent wax called coccicerin, which is difficult to remove (Torres and Giorgi, 2018). In contrast, *D. coccus* is coated with a fine, powdery wax that detaches easily. This structural difference likely acts as a physical barrier, impeding solvent penetration and ultrasonic cavitation, thereby limiting the release and extraction efficiency of CA from *D. opuntiae*.

Optimization for extract yield

The response values obtained from the Box-Behnken design were used to derive a second-order polynomial model, presented in Table 3, which details the optimization coefficients for linear, quadratic, and interaction terms. The optimal conditions for extract yield were identified as 59.94 °C, 39.99% amplitude, and 3 min of sonication, predicting a maximum yield of 54.65%. Experimental validation under these conditions resulted in a yield of 60.29 ± 1.53%, representing a 9.35% relative increase over the predicted value and an absolute increase of 5.64%.

The extraction yields obtained in this study exceed those reported by Borges *et al*. (2012), who achieved yields of 42.4% and 39.4% using pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE), respectively, on *D. coccus*. Additionally, our results are consistent with those of Reyes-Pérez *et al*. (2024), who reported a 50.3% yield via UAE of *D. coccus*. Despite using *D. opuntiae* in the present study, the comparable extraction yields suggest that species differences do not substantially affect extraction efficiency. These findings support the hypothesis that *D. opuntiae*, although characterized by a lower CA concentration, can nonetheless provide efficient extraction yields under optimized conditions.

Simultaneous optimization: carminic acid extraction and extract yield

Simultaneous optimization of the extraction conditions for CA content and extract yield was performed using the desirability function, which ranges from 0 (undesirable) to 1 (fully desirable) (Tranquilino-Rodríguez and Martínez-Flores, 2023). The model achieved a high overall desirability of 0.96. Optimal extraction parameters were identified as a temperature of 60 °C, ultrasound amplitude of 37.8%, and a sonication time of 3 min, resulting in 14.60% CA and 54.44% extract yield.

Figure 5 shows the three-dimensional response surface, illustrating that higher temperatures combined with shorter sonication times maximize both CA extraction and extract yield. This trend was consistent across the three optimization models (CA extraction and extract yield, and simultaneous), with ultrasound amplitude being the main variable differing among them. The efficacy of the optimization process was validated experimentally, yielding a CA content of 13.10 ± 0.39% and an extract yield of 59.90 ± 1.07%. These values differed from the model's predicted values by 1.5% for CA content and 5.46% for extract yield, confirming the accuracy and reliability of the optimized extraction conditions. Furthermore, compared to the initial *D. opuntiae* powder sample containing 9.62 ± 0.36% CA, the optimized UAE increased CA concentration by 4.86%.

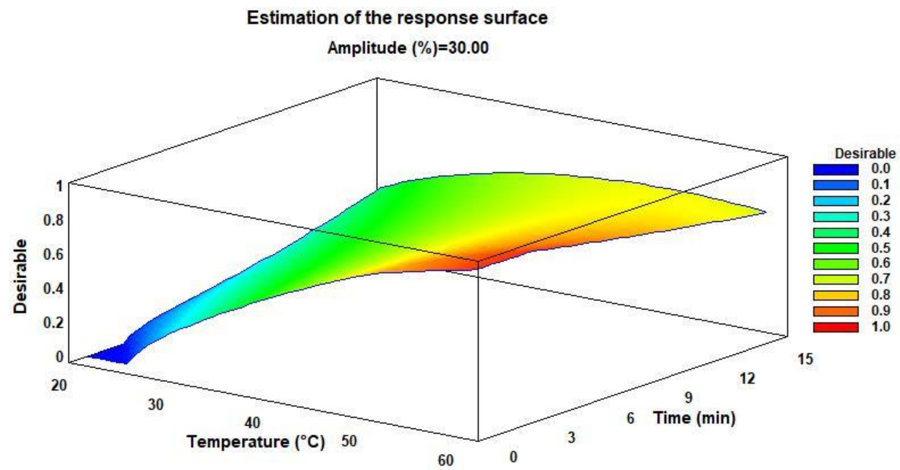

Figure 5. Response surface for simultaneous optimization of carminic acid percentage and yield under ultrasound extraction: Temperature-time relationship at 30% amplitude.

Comparison of the efficiency of ultrasound extraction versus the conventional method

The present study compared the effectiveness of UAE under the optimized conditions established here with the conventional extraction method. As shown in Table 4, the conventional method yielded a CA concentration of 13.66 ± 0.47% and an extract yield of 50.92 ± 2.82%, whereas simultaneous optimization of UAE resulted in 13.10 ± 0.39% CA, 0.56% lower than the conventional method, accompanied by a 59.90 ± 1.07% extract yield, representing an 8.98% improvement. When optimization was targeted at maximizing CA content, UAE achieved 14.76 ± 0.32% CA, exceeding the conventional method by 1.1%. These results confirm that UAE can outperform traditional approaches in extracting the target compound under optimized conditions.

Ultrasound-assisted extraction achieved these results under significantly milder and more sustainable conditions. While the conventional method required 15 min at boiling temperature (~100 °C), UAE required only 3 min at 60 °C, substantially reducing thermal energy input and process duration. This marked reduction in extraction time and temperature not only decreases energy consumption but also minimizes the risk of thermal degradation of bioactive compounds, positioning UAE as an environmentally advantageous and energy-efficient alternative for CA extraction.

Table 4. Experimental comparison between conventional and ultrasound-optimized methods for carminic acid yield.

Parameter	Conventional method	Simultaneous optimization	%CA optimization	%Y optimization
Carminic acid (%)	13.66 ± 0.47	13.10 ± 0.39	14.76 ± 0.32	13.35 ± 0.68
Extract yield (%)	50.92 ± 2.82	59.90 ± 1.07	53.61 ± 1.87	60.29 ± 1.53
Temperature (°C)	Boiling	60.00	59.93	59.94
Wavelength (%)	—	37.78	20.00	39.99
Extraction time (min)	15.00	3.00	3.24	3.00

CA%: Individual optimization based on carminic acid content. **%Y:** Individual optimization based on extract yield. Simultaneous: Joint optimization of both responses (%AC and %Y).

A key factor contributing to extraction efficiency was the optimization of ultrasonic amplitude. Lower amplitude values consistently enhanced CA extraction, likely by promoting controlled cavitation events that minimize thermal hotspots and mechanical stress. This observation is consistent with Linares and Rojas (2022), who reported that exceeding an optimal ultrasonic amplitude or power threshold reduces pigment extraction efficiency due to over-cavitation effects, including localized overheating.

Conclusions

Ultrasound-assisted extraction has proven to be an efficient and sustainable technique for enhancing both CA content and extract yield from *D. opuntiae*. The highest concentration of CA obtained was 14.76%, achieved at 60 °C, 3 min sonication, and 20% amplitude. Temperature was identified as the dominant factor influencing extraction efficiency, with significant interactions observed between temperature and extraction time. Optimal results were obtained under conditions of elevated temperature combined with shorter sonication durations. These findings highlight the potential of *D. opuntiae,* traditionally considered a pest, as a viable and scalable source of natural pigments, owing to its rapid reproduction and abundant biomass. Compared to conventional extraction methods, UAE delivers higher yields under milder conditions, reinforcing its advantages in terms of sustainability and cost-effectiveness.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this scientific paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Not applicable

AUTHOR CONTRIBUTIONS

Conceptualization and Formal Analysis: H.E.M.-F., J.O.R.-L. Methodology: G.M.O.-M. Investigation: G.M.O.-M, M.G.G.-R, M.E.G.-P. Resources: H.E.M.-F, L.P. Data analysis: J.O.R.-L., H.E.M.-F. Writing—original draft preparation: G.M.O.-M. Writing—review and editing: H.E.M.-F, J.O.R.-L., L.P., M.G.G.-R. Project administration: H.E.M.F.

ACKNOWLEDGMENTS

Gabriela M. Ochoa-Manzo thanks to Secretaría de Ciencia, Humanidades, Tecnología e Innovación - SECIHTI of México for the postgraduate scholarship.

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