Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 47 Issue 4 July 2026 pp. 728-735
DOI: http://doi.org/10.22438/jeb/47/4/MRN-5910

J. Environ. Biol., 2026

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

High-shear optimized shade-dried marigold carotenoid extract: process optimization and stability kinetics

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Received: 18 October 2025 **Revised:** 21 January 2026 **Accepted:** 16 April 2026

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Abstract

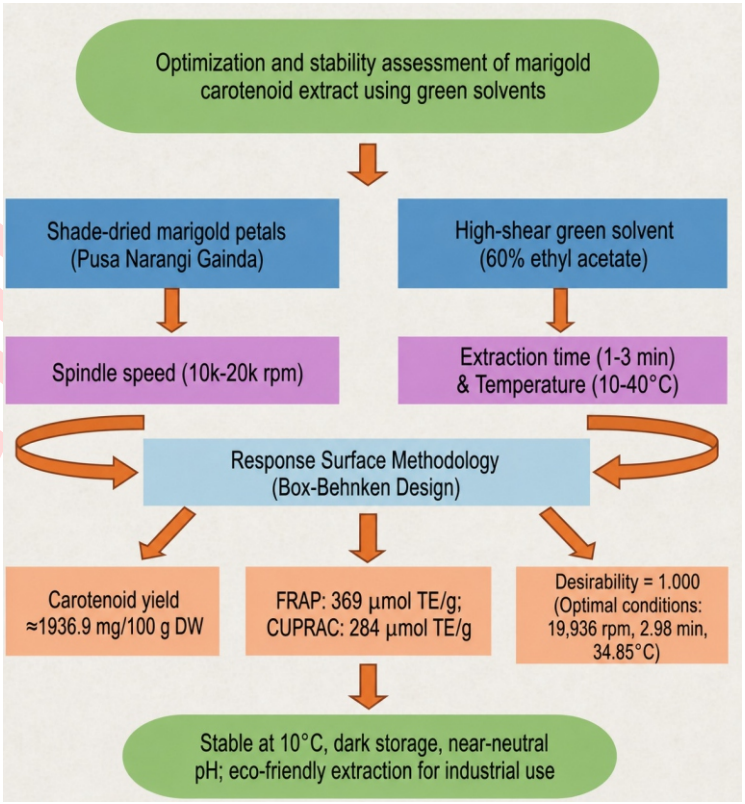
Aim: To optimize a green-solvent, high-shear extraction protocol for carotenoids from shade-dried marigold (*Tagetes erecta* L.) petals and assess extract stability under varying temperature, light, and pH.

Methodology: Petals (cv. 'Pusa Narangi Gainda') were extracted using ethyl acetate 60% (v/v) formulated with acetone. A Box–Behnken Design under Response Surface Methodology (RSM) was employed, with spindle speed (10,000–20,000 rpm), extraction time (1–3 min), and temperature (10–40°C) as independent variables. Responses included total carotenoid content, antioxidant activity (FRAP, CUPRAC), and color parameters. The optimized extract was subjected to stability testing under different storage temperatures (10–40°C), light vs. dark exposure, and pH (2–8) over 7 days and degradation kinetics were fitted to first-order models.

Results: Optimal conditions (19,936.73 rpm, 2.98 min, 34.85°C) predicted total carotenoid content of 1,936.94 mg 100 g^{-1} d.wt., FRAP of 369.08 µmol TE g^{-1}, and CUPRAC of 284.04 µmol TE g^{-1}. The maximum retention occurred at 10°C, in darkness and near-neutral pH (6–7.5), while degradation accelerated under heat, light, and extreme pH.

Interpretation: High-shear ethyl acetate extraction is efficient and eco-friendly for marigold carotenoid recovery. Shade drying combined with low temperature, darkness, and near-neutral pH effectively preserves pigment stability, supporting sustainable industrial utilization.

Key words: Antioxidant activity, Carotenoids, Green solvents, High-shear extraction, Marigold, Stability kinetics



How to cite: Rajeev, A.K., P. Anand, A. Joshi, K.P. Singh and M.C. Singh: High-shear optimized shade-dried marigold carotenoid extract: process optimization and stability kinetics. *J. Environ. Biol.*, **47,** 728-735 (2026).

Introduction

Carotenoids are naturally occurring lipophilic pigments synthesized by plants, algae, fungi, and some microorganisms, while animals obtain them exclusively through dietary sources. Structurally, carotenoids possess a conjugated polyene chain that governs their strong light absorption and antioxidant properties. They are broadly classified into carotenes and xanthophylls, and are valued for their roles in singlet oxygen quenching, free radical scavenging, provitamin A activity, and protective effects against oxidative stress-related disorders (Rao and Rao, 2007; Jaswir *et al.,* 2011; Sandmann, 2015). Due to their functional and coloring properties, carotenoids are widely utilized as natural food colorants and bioactive ingredients in nutraceutical and pharmaceutical formulations. Marigold (*Tagetes erecta* L.) is one of the most important commercial sources of xanthophylls, particularly lutein, which predominantly occurs in esterified forms in petal chromoplasts. High lutein concentration, biomass productivity, and adaptability of marigold to diverse agroclimatic conditions make it a preferred industrial raw material (Jothi, 2008; Del Villar-Martínez *et al.,* 2010).

However, carotenoids are chemically unstable due to their highly unsaturated structure and undergo oxidative degradation and trans–cis isomerization when exposed to heat, oxygen, light, and unfavorable pH, resulting in loss of color intensity and bioactivity. Therefore, extraction strategies must maximize pigment recovery while minimizing degradation during processing and storage. Conventional carotenoid extraction relies heavily on petrochemical solvents such as hexane, which raise concerns regarding toxicity, sustainability, and suitability for food-grade applications, besides promoting pigment loss through prolonged extraction time and high solvent consumption (Mustafa *et al.,* 2012; Singh *et al.,* 2015). Green solvents offer a promising approach to sustainable pigment extraction in floriculture because not only they might simultaneously increase the extractability of polar and nonpolar compounds, they may also improve recovery rates of valuable bioactive compounds (Anand *et al.,* 2025).

In recent years, green solvents such as vegetable oils, ionic liquids, and deep eutectic solvents have emerged as safer alternatives, offering reduced environmental burden and improved compatibility with nutraceutical applications (Viñas-Ospino *et al.,* 2023; Morón-Ortiz *et al.,* 2024;). Additionally, green extraction technologies such as ultrasound, microwave, enzyme-assisted, pressurized liquid, and supercritical CO_2 extraction have been explored to enhance mass transfer and reduce thermal exposure (Singh *et al.,* 2015; Kultys and Kurek, 2022). High-shear dispersion is a promising mechanical approach that enhances extraction efficiency by rapidly disrupting cellular structures and improving pigment release, particularly in viscous green solvents and edible oils (Tiwari *et al.,* 2019; Murugesan *et al.,* 2023). Although marigold carotenoid extraction has been widely studied, limited information is available on the integrated evaluation of process optimization together with storage stability under controlled environmental stressors. Such integration is essential because the commercial applicability of green-solvent extracts depend not only on extraction yield, but also on pigment stability during storage and use. Therefore, the present study aimed to optimize high-shear green-solvent extraction of carotenoids from shade-dried marigold petals and to evaluate stability under varying temperature, light, and pH conditions using first-order kinetic modeling.

Materials and Methods

Flowers of marigold (*Tagetes erecta* L.) cultivar 'Pusa Narangi Gainda' were harvested at full bloom stage during January–February 2025 from the experimental plots of the Division of Floriculture and Landscaping, ICAR–IARI, New Delhi, for this experiment.

Plant material and preliminary characterization: The suitability of marigold (*Tagetes erecta* L.) cultivar 'Pusa Narangi Gainda' for carotenoid extraction was based on pigment content, color intensity, and basic physico-chemical characteristics. Fully opened flowers were collected from the experimental plots of the Division of Floriculture and Landscaping, ICAR–IARI, New Delhi, between January and February 2025. Fresh flowers were wiped with distilled water, shade-dried under ambient conditions (28–32°C) and stored in low-density polyethylene (LDPE) resealable pouches at 10°C until further use.

The moisture content of fresh petals was determined using an infrared moisture analyzer (MS-70, Panacea Instruments, Japan). The pH of the petal extract was determined using a digital pH meter (Model pH 700, EUTECH Instruments, Singapore). Total carotenoid content was estimated following the standard spectrophotometric method (Ranganna, 1986). The absorbance was recorded at 452 nm using a UV–Vis spectrophotometer.

Optimization of carotenoid extraction protocol: Based on initial solvent evaluation, ethyl acetate 60% formulated with acetone was selected as the standardized solvent system for the extraction protocol to maximize carotenoid yield through Response Surface Methodology (RSM) (Myers *et al.*, 2016), using a three-factor, three-level Box–Behnken Design (BBD) (Box and Benken, 1960). This design comprised 17 experimental runs, including all possible combinations of independent variables such as spindle speed (10 000, 15 000, and 20 000 rpm), extraction time (1, 2, and 3 min), and extraction temperature (10, 25, and 40°C). A solid-to-solvent ratio of 1:200 (w/v) was maintained throughout the trials, appropriate for the reduced moisture content and higher density of the shade-dried petals. After each run, carotenoids were extracted using a high-shear homogenizer (IKA T 25 digital ULTRA-TURRAX, IKA, Germany) and stored in amber-colored vials at 10°C for response quantification. The responses measured were total carotenoid content (TCC, spectrophotometrically at 452 nm), antioxidant activity (FRAP and CUPRAC), and color parameters (L*, a*, b*), enabling precise determination of optimum high-shear extraction conditions for shade-dried marigold material.

Total color change (ΔE): The color expression of carotenoid extracts from 17 experimental runs was determined using the RGB color cube (Joshi *et al.*, 2016). The recorded values were

expressed in Hunter's L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) coordinates. The total color variation (ΔE) was determined using the following formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where Δ*L* = *L* sample –*L* extract; Δ*a*= *a* sample –*a* extract; Δ*b*=*b* sample –*b* extract

Antioxidant activity: The antioxidant potential of marigold extracts was evaluated through FRAP (Ferric Reducing Antioxidant Power) and CUPRAC (Cupric Reducing Antioxidant Capacity) assays. For the FRAP assay, extracts prepared in 80% methanol were reacted with FRAP reagent containing acetate buffer (pH 3.6), TPTZ solution, and $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio, incubated at 37°C for 30 min, and the absorbance was read at 593 nm (Benzie and Strain, 1996). CUPRAC activity was determined by mixing the extract with $CuCl_2$, neocuproine, and ammonium acetate buffer (pH 7.0), adjusting the final volume to 4.1 ml, incubating in the dark for 30 min, and recording absorbance at 450 nm (Apak *et al.,* 2004). In both assays, the antioxidant capacity was measured against a Trolox standard curve, with results expressed as µmol Trolox equivalents per gram of dry weight.

Determination of half-life time by general linear model: The degradation kinetics of carotenoids under different storage conditions were found to follow first-order reaction kinetics. The first-order rate constant (k) and half-life (*t½*, time required for 50% degradation) were calculated using the following equations (Song *et al.,* 2017):

$$\ln (C_t/C_0) = -kt$$

Here, C_0 represents the initial carotenoid concentration, C_t denotes the concentration at time *t,* and k is the degradation rate constant (day^{-1}). The half-life ($t_{1/2}$) was calculated as:

$$t_{1/2} = \frac{0.693}{k}$$

Statistical design and analysis of data: For extraction protocol standardization, three-factor three-level Box–Behnken design of response surface methodology (RSM) was used using the Design Expert 7 software. The experimental data were further processed and utilized to estimate the degradation rate constant (k) and corresponding half-life ($t_{1/2}$) values (Song *et al.,* 2017).

Results and Discussion

The effects of extraction and storage conditions on carotenoid yield and antioxidant capacity of shade-dried marigold petals were systematically analyzed using response surface methodology (RSM) and stability models. The results are discussed in light of statistical interactions, model adequacy, and consistency with previously reported studies. Table 1 presents the response of 17 runs of Box–Behnken design for carotenoid extraction from shade dried marigold extract. As depicted in Fig. 1 a–c, the response surface trends clearly demonstrate that the extraction process is primarily driven by mechanically induced shear forces, which accelerate cell disruption and enhance mass transfer. Fig. 1a highlights a steep increase in carotenoid concentration with increasing spindle speed, with the highest yield achieved at 20,000 rpm within approximately 2.0 min. This strong dependence on speed can be attributed to the intense shear stress and localized cavitation produced during high-speed homogenization, which effectively breaks down the rigid cellulose–lignin network of plant cell walls (Chang *et al.,* 2025).

Table 1: Response of 17 runs of Box–Behnken design for carotenoid extraction from shade-dried marigold extract

Independent variable				Dependent variable		
Spindle Speed (RPM)	Time (min)	Temperature (°C)	Carotenoid yield mg $(100g)^{-1}$ DW	Total color change (ΔE)	FRAP (µmol TE g^{-1}) DW	CUPRAC (µmol TE g^{-1}) DW
20000.00	1.00	25.00	1680.28	62.40	137.42	142.50
10000.00	2.00	40.00	1225.25	62.44	256.54	256.50
15000.00	1.00	10.00	1485.38	63.87	128.69	85.24
20000.00	3.00	25.00	1800.15	64.08	363.08	283.95
15000.00	2.00	25.00	1420.58	63.49	225.40	221.76
15000.00	2.00	25.00	1380.75	65.60	230.58	226.36
20000.00	2.00	40.00	1840.79	66.15	279.48	270.95
15000.00	2.00	25.00	1540.69	65.72	250.44	220.65
10000.00	3.00	25.00	1120.43	63.24	325.76	273.82
10000.00	1.00	25.00	1050.34	68.17	151.06	223.51
15000.00	1.00	40.00	1600.76	65.03	138.41	270.85
15000.00	2.00	25.00	1500.18	66.68	219.61	132.28
15000.00	2.00	25.00	1554.65	66.68	237.30	176.96
20000.00	2.00	10.00	1580.65	66.14	162.17	228.76
15000.00	3.00	10.00	1460.93	66.58	269.38	236.36
10000.00	2.00	10.00	980.91	67.72	200.94	174.16
15000.00	3.00	40.00	1750.96	67.85	324.68	283.16

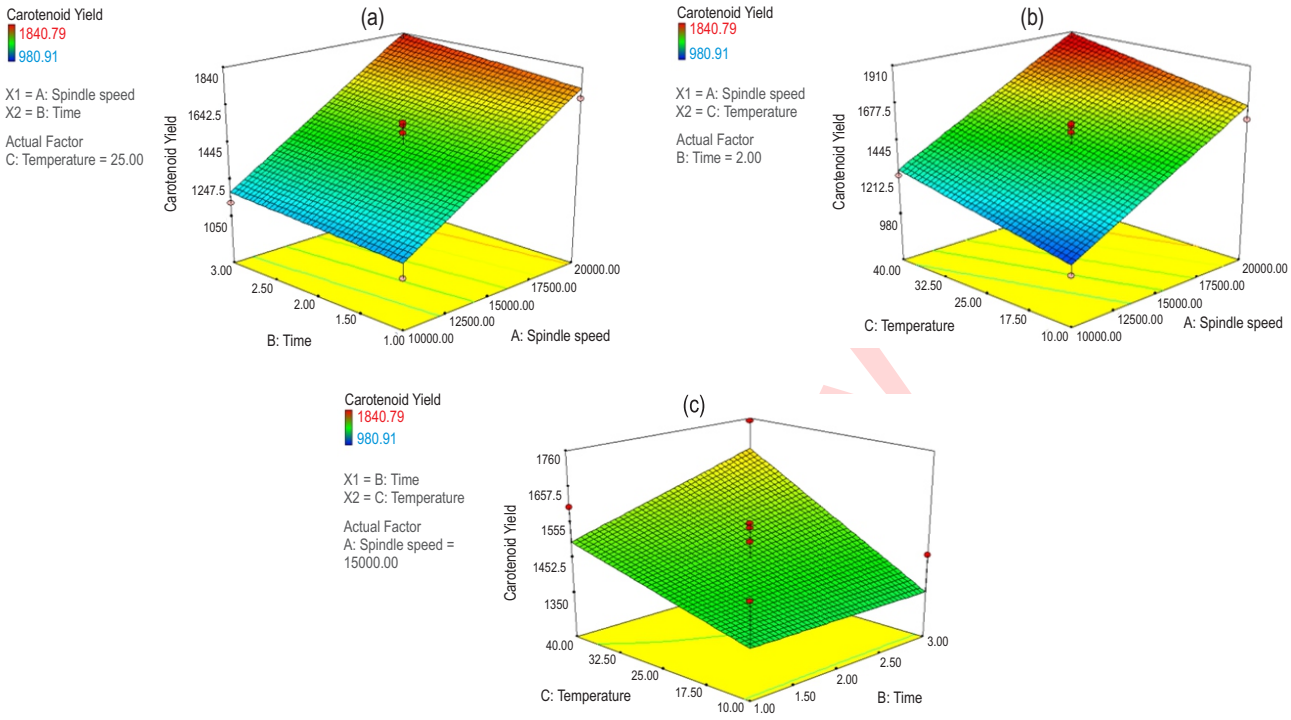


Fig. 1: Impact of (a) spindle speed and time, (b) spindle speed and temperature and (c) time and temperature on carotenoid yield.

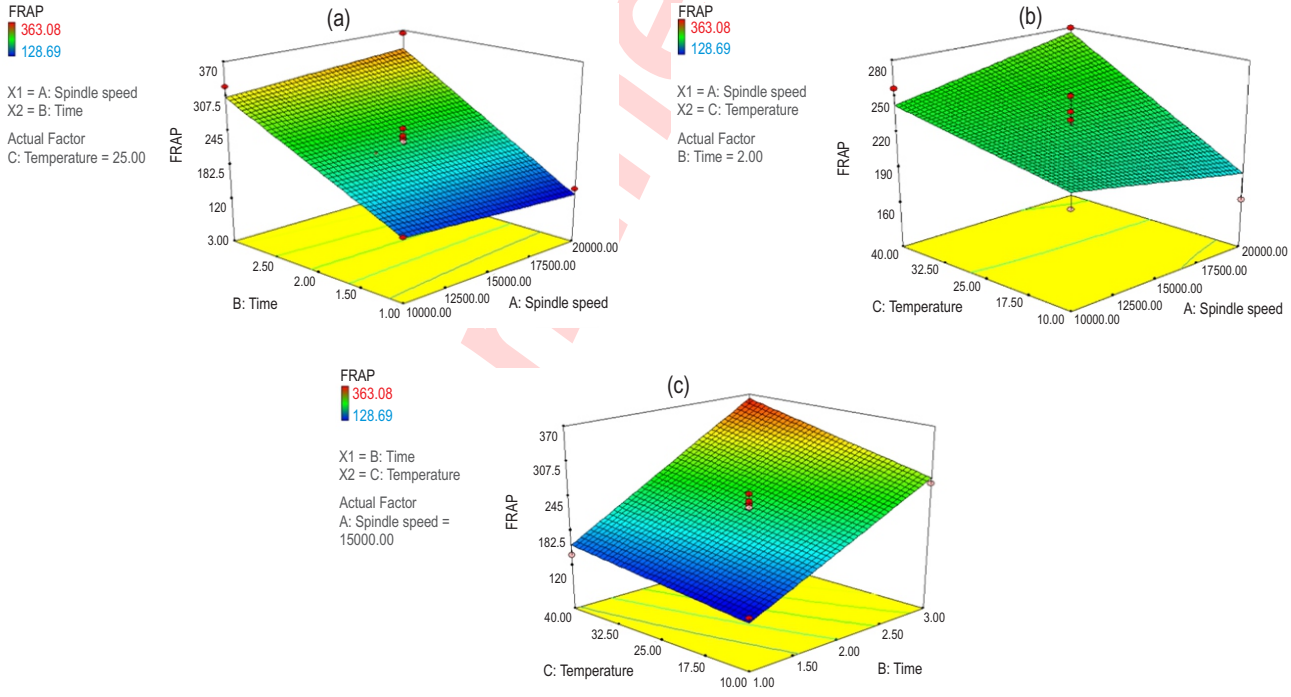


Fig. 2: Impact of (a) spindle speed and time, (b) spindle speed and temperature and (c) time and temperature on FRAP activity.

Unlike conventional maceration-based extraction, high shear processing induces microfractures and structural weakening of the tissue, thereby reducing internal diffusion resistance and enabling the rapid release of intracellular lipophilic pigments (Katsouli *et al.,* 2024). The interaction between spindle speed and temperature (Fig. 1b) revealed a pronounced

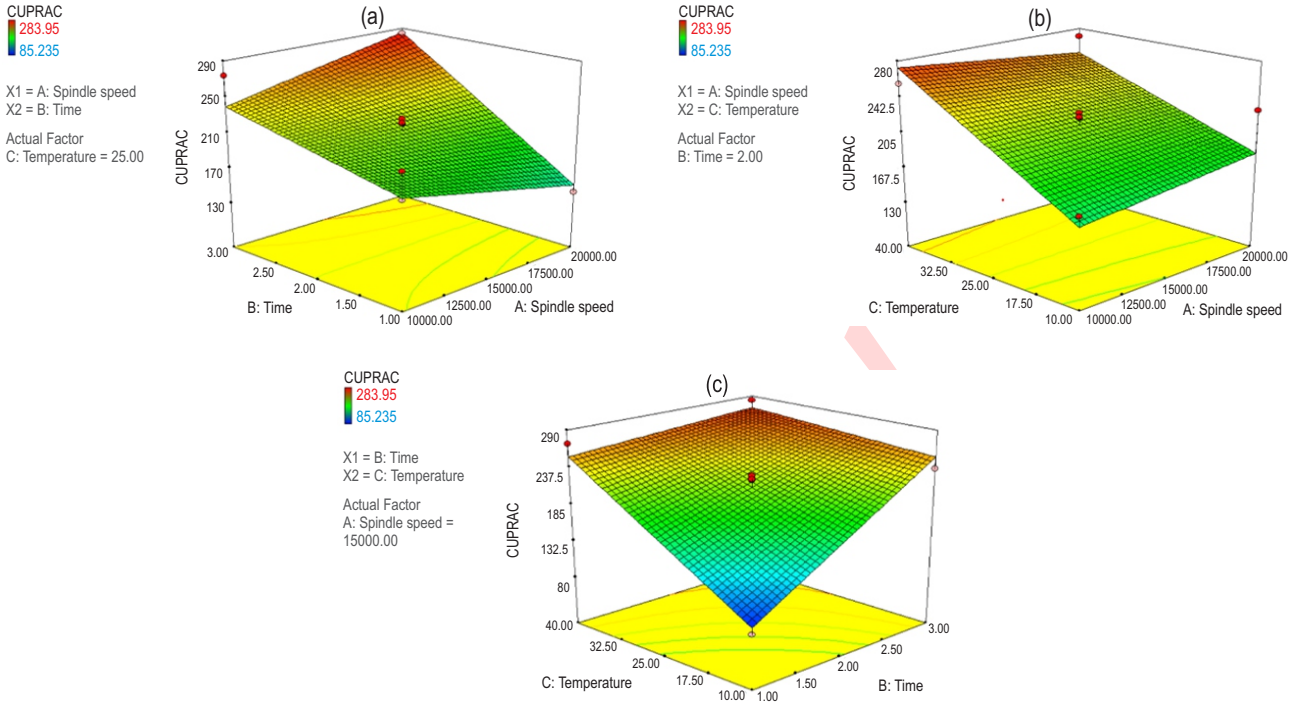


Fig. 3: Impact of (a) spindle speed and time, (b) spindle speed and temperature and (c) time and temperature on CUPRAC activity.

synergistic effect. Carotenoid recovery peaked when high shear conditions (≥15,000 rpm) were combined with moderate temperatures (25–32.5°C). This outcome suggests that mild thermal input enhances extraction efficiency by lowering the viscosity and surface tension of the extraction medium, thereby improving solvent penetration into the disrupted plant matrix, without exposing carotenoids to conditions that promote degradation (Quintero-Quiroz *et al.,* 2026). Recent evidence supports that although elevated temperatures can improve the solubility of hydrophobic pigments, excessive heating may induce undesirable isomerization of trans-carotenoids, particularly under high-energy extraction systems (Travičić *et al.,* 2025).

In contrast, extraction time exhibited a comparatively weaker influence on carotenoid yield (Fig. 1c). The rapid attainment of equilibrium at approximately 2.0 min indicates that once the cellular structure is sufficiently disrupted by high spindle speed, pigment release becomes nearly instantaneous and quickly approaches the solvent saturation limit. This observation aligns with the findings from hydrodynamic cavitation studies, where extending processing time beyond the initial disruption phase produced minimal improvements in yield (Quintero-Quiroz *et al.,* 2026). The fitted two-factor interaction (2FI) model (F = 15.50, p = 0.0002) further supported these experimental trends, confirming the statistical significance of the model and validating that carotenoid extraction is kinetically controlled by mechanical energy input (spindle speed) and thermodynamically supported by temperature-dependent solubility. Overall, the results confirm that optimizing high shear conditions under mild heating enables rapid, efficient, and degradation-minimized recovery of carotenoids from shade-dried marigold petals.

Carotenoid Yield= 1469.04+ 315.62A+ 39.46B+ 113.74C+12.44AB+3.95AC+ 43.66BC

Fig. 2a shows that FRAP antioxidant activity increased clearly as the extraction time was increased, and it reached the highest value (363.08 µmol TE g^{-1} d.wt.) at around 2 min. However, changing the spindle speed from 10,000 to 20,000 rpm did not make much difference, which is also supported by the non-significant p-value (p = 0.9008). This means that once the mixing is strong enough, increasing speed further does not improve the extraction, because of antioxidants the slow movement of antioxidants from inside the plant tissue into the solvent is the limiting factor, which follows a diffusion-based mechanism (Shi *et al.,* 2025). Fig. 2b shows that the FRAP activity was mainly driven by temperature, with optimum values observed between 25°C and 32.5°C. Although the interaction between spindle speed and temperature was non-significant (p=0.1858), temperature emerged as a significant main effect (p = 0.0031). This suggests that mild heating improves the antioxidant solubilization and diffusivity without causing thermal degradation, which is in agreement with the previous reports on *Tagetes erecta* extracts (Oo *et al.,* 2024). Fig. 2c shows that FRAP activity increased with longer extraction times and mild heating, reaching a peak in 2.5 min and 32.5°C. Beyond this point, the levels plateaued, indicating the process reached equilibrium. Across all the tests, FRAP values ranged from 128.69 to 363.08 µmol TE g^{-1} d.wt.

Table 2: Degradation kinetics of carotenoids from shade-dried marigold extract under different storage conditions (temperature, light and pH)

Factor	Level	C_0 mg $(100g)^{-1}$ DW	C_t mg $(100g)^{-1}$ DW	Time	k	t½	Carotenoid retention (%)
Temperature	10 °C	2092.57	1799.88	168 h	$0.0009h^{-1}$	772.86 h	86.01
	25 °C	2051.68	1767.53	48 h	$0.0032h^{-1}$	216.53 h	86.15
	30 °C	1807.61	1529.36	24 h	$0.00792\ h^{-1}$	87.51 h	84.61
	40 °C	1699.55	1554.65	6 h	$0.01498\ h^{-1}$	46.26 h	91.47
Light condition	Dark	1932.64	1843.07	7 d	$0.00721\ d^{-1}$	96.14 d	95.37
	Light	1846.59	1565.28	7 d	$0.0241\ d^{-1}$	28.76 d	84.77
pH	pH 2	1845.75	1506.16	7 d	$0.03074\ d^{-1}$	22.55 d	81.60
	pH 3	1844.54	1591.36	7 d	$0.02243\ d^{-1}$	30.91 d	86.27
	pH 4	1847.32	1653.1	7 d	$0.01711\ d^{-1}$	40.50 d	89.49
	pH 5	1847.96	1714.48	7 d	$0.01126\ d^{-1}$	61.54 d	92.78
	pH 6	1848.36	1736.89	7 d	$0.00929\ d^{-1}$	74.60 d	93.97
	pH 7.5	1848.73	1754.78	7 d	$0.00783\ d^{-1}$	88.57 d	94.92
	pH 8	1848.25	1593.41	7 d	$0.02223d^{-1}$	31.19 d	86.21

The strong impact of time and temperature, rather than mixing speed, confirms that extraction relies on the solvent soaking into the plant material (diffusion) rather than physical force. Moderate heat helps by thinning the solvent, allowing it to penetrate the marigold powder easily and release antioxidants without damaging them (Oo *et al.,* 2024).

FRAP = 229.47 + 0.98A + 90.92B + 29.74C + 12.74AB + 15.43AC + 11.40BC

Fig. 3a illustrates the interaction between spindle speed and extraction duration, revealing that CUPRAC activity is primarily governed by time rather than mechanical shear. Activity peaked at 283.95 µmol TE g^{-1} d.wt. around 2.0 min, while varying spindle speed (10,000–20,000 rpm) showed a negligible effect, evidenced by a flat response surface and non-significant ANOVA results. This suggests that while initial high-speed shearing effectively ruptures the cellular matrix of *Tagetes erecta*, extending the duration is critical for the diffusion-controlled mass transfer of intracellular antioxidants into the solvent. This aligns with the recent findings of Siddiqa *et al.* (2025), who demonstrated that once cell disruption is achieved—often enhanced by techniques like ultrasonication or high-speed blending—the release of phenolics becomes a time-dependent solubility process rather than a force-dependent one.

In Fig. 3b, the interaction between spindle speed and temperature at a fixed duration (2.0 min) highlights temperature as the dominant factor driving antioxidant release. The response surface exhibits a convex trend where CUPRAC activity rises steadily with temperature, peaking at 25–32.5°C. This increase is attributed to reduced solvent viscosity and enhanced diffusivity of bioactive compounds, mechanisms recently validated in Tagetes extraction optimization by Mejía-Resendiz *et al.* (2025). The lack of significant interaction between speed and temperature further confirms that moderate heating is far more effective at improving solubility than increasing mechanical agitation, which offers diminishing returns after the initial homogenization phase. Fig. 3c demonstrates the significant combined influence of extraction duration and temperature, with maximum CUPRAC activity observed at the intersection of 2.0–2.5 min and 25–32.5°C. The surface plateau beyond these points indicates the establishment of equilibrium, where the rate of extraction is balanced by the saturation of the solvent or the potential thermal degradation of sensitive compounds. This observation is consistent with the kinetic studies conducted by Zhang *et al.* (2024), who noted that prolonged exposure to heat even at moderate levels can eventually lead to the breakdown of thermolabile antioxidants like flavonoids and carotenoids. The regression model (F = 4.44) supports this, pinpointing time and temperature as critical variables for maximizing antioxidant yield while minimizing degradation.

CUPRAC = 218.10 − 0.23A + 44.40B + 44.62C + 22.78AB − 10.04AC − 34.70BC

The optimization of carotenoid extraction from shade-dried marigold petals was carried out using Response Surface Methodology (RSM) with a Box–Behnken Design, considering spindle speed, extraction time, and temperature as independent factors. The optimized extraction conditions were identified as a spindle speed of 19,936.73 rpm, an extraction time of 2.98 min, and a temperature of 34.85°C. Under these conditions, the predicted responses were a carotenoid yield of 1936.94 mg $(100g)^{-1}$ d.wt., a FRAP value of 369.08 µmol TEg^{-1} d.wt., and a CUPRAC value of 284.04 µmol Teg^{-1} d.wt., with an overall desirability score of 1.000, indicating a highly refined and reliable optimization outcome. Shade-dried marigold extract showed strong thermal sensitivity, where the carotenoid half-life (t½) declined sharply from 772.86 hr (10°C) to 46.26 hr (40°C), indicating nearly a 94% loss in stability. This trend follows the Arrhenius principle, where degradation accelerates exponentially with increasing temperature, due to disruption of polyene chains and oxidative breakdown of xanthophylls (Su *et al.,* 2023).

Light exposure significantly accelerated carotenoid degradation, whereas storage in darkness maintained higher

stability. Dark-stored extracts retained 1843.07 mg $(100g)^{-1}$ d.wt. carotenoids after seven days (only~4.63% loss), while light-exposed samples showed reduced stability ($t_{1/2}$=28.76 days). This supports previous findings that photo-oxidation causes cleavage of conjugated double bonds and promotes isomerization, reducing pigment integrity (Kim and Eom, 2025). Carotenoid stability showed strong pH dependence and followed first-order kinetics. Rapid degradation occurred at pH 2 ($t_{1/2}$= 22.55 days), while moderate acidity improved stability (pH 3–4; $t_{1/2}$= 30.91–40.50 days). Maximum stability was observed near neutral conditions at pH 7.5 ($t_{1/2}$ = 88.57 days; k = 0.00783 day^{-1}), with ~94.92% retention after seven days whereas mild alkalinity (pH 8) again reduced stability ($t_{1/2}$= 31.19 days). Overall, near-neutral conditions (pH 6–7.5) best preserved carotenoids, while extreme acidic or alkaline environments likely promoted degradation through acid-catalyzed isomerization or alkaline hydrolysis (Yang *et al.,* 2024).

The observed temperature-, light-, and pH-dependent degradation behavior is consistent with broader reports on carotenoid instability in plant-based matrices, where oxidative reactions, isomerization, and cleavage of conjugated double bonds are accelerated under unfavorable storage conditions. Similar conclusions were drawn by Rodriguez-Amaya (2019), who emphasized that controlling thermal exposure, light incidence, and pH is critical for maintaining carotenoid integrity during processing and storage of natural colorants. Thus, the present study successfully established an eco-friendly high-shear extraction method using ethyl acetate for efficient recovery of carotenoids from shade-dried marigold petals. Optimization through Response Surface Methodology (RSM) yielded the highest carotenoid content and antioxidant activity under mild operational conditions. Kinetic analysis confirmed first-order degradation behavior, with maximum pigment stability observed at low temperature, in dark storage, and near-neutral pH. These findings demonstrate that combining shade drying with optimized green extraction provides a sustainable and industrially viable approach for producing stable, food-grade natural colorants from marigold.

Acknowledgment

Research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India.

Authors' contribution: A.K. Rajeev: Execution of the experiments, data collection and analysis of data; **P. Anand:** Conceptualization of research, designing of the experiments, preparation of the manuscript; **A. Joshi:** Designing of the experiments, data interpretation; **K.P. Singh and M.C. Singh:** Contribution of experimental materials.

Funding: Not applicable.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: The authors have followed and complied with the National/ International Guidelines of relevant and appropriate author appropriate authority for the use of plants in the experimental study.

Conflict of interest: The authors declare that is there is no conflict of interest.

Data availability: The data is available with the authors.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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