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Marigold Flower (Tagates erecta L.) as a Natural Yellow Food Colorant: Extraction Optimization and Stability Assessment

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ABSTRAK

Bunga marigold (*Tagetes erecta* L.) sangat kaya akan karotenoid dan secara luas digunakan sebagai pewarna makanan kuning alami. Penelitian-penelitian sebelumnya mengenai ekstraksi karotenoid dari marigold sebagian besar masih mengandalkan metode faktor tunggal, dengan fokus yang terbatas pada interaksi antar variabel maupun stabilitas ekstrak. Penelitian ini bertujuan untuk mengoptimalkan kondisi ekstraksi menggunakan metodologi *response surface methodology* dan untuk menilai stabilitas ekstrak karotenoid selama masa penyimpanan dengan garam dan gula komersial. Bunga marigold segar dikeringkan selama empat hari pada suhu ruang dengan aliran udara konveksi sebelum diekstraksi dengan Soxhlet maupun labu leher tiga. Kandungan total flavonoid, fenolik, dan karotenoid dari bahan segar dianalisis secara kuantitatif menggunakan spektrofotometri visibel. Desain *Central Composite Design* (CCD) dengan model interaksi dua faktor (2FI) digunakan untuk mengoptimalkan kondisi ekstraksi, dengan mempertimbangkan tiga variabel: pH, suhu, dan waktu. Koefisien determinasi (R^2) yang didapatkan adalah 80,25%. Kondisi ekstraksi terbaik berada pada pH 5,9, suhu 62,9 °C, dan waktu 67,1 menit, yang menghasilkan TCC (*Total Carotenoid Content*) sebesar 3490 mg/100 g DW (berat kering) dengan nilai desirability 1,000. Karotenoid menunjukkan stabilitas yang lebih tinggi dengan adanya 2% asam sitrat atau 3% garam, sedangkan keberadaan 3% gula menurunkan stabilitas selama 76 jam masa penyimpanan.

Kata Kunci: Bunga marigold, pewarna makanan kuning alami, karotenoid, Stabilitas, Optimal

ABSTRACT

Marigold flowers (Tagetes erecta L.) are especially rich in carotenoids and are widely used as natural yellow food colorants. Previous studies on extracting carotenoids from marigolds mainly relied on single-factor methods, with limited focus on variable interactions or extract stability. This study aimed to optimize extraction conditions using response surface methodology and to assess the stability of the carotenoid extract during storage with commercial salt and sugar. Following four days drying of fresh marigold flower petals in the laboratory with air convection, extraction was carried out using either a Soxhlet extractor or a three neck round bottom flask (extraction). The total amounts of flavonoids, phenolics, and carotenoids in the fresh flower material were determined initially by using visible spectroscopy. A Central Composite Design (CCD) with a two-factor interaction (2FI) model was used to optimize extraction conditions, considering three variables: pH, temperature, and time. The coefficient of determination (R^2) was 80.25%. The best extraction conditions were pH 5.9, 62.9 °C, and 67.1 minutes, resulting in a TCC of 3490 mg/100 g DW with a desirability of 1.000. Carotenoids showed greater stability in the presence of 2% citric acid or 3% salt, while 3% sugar reduced stability during 76 hours of storage.

Keywords: *Marigold flowers, Yellow food colorant, Carotenoid, Stability, Optimization*

1. Introduction

In the context of the Sustainable Development Goals (SDGs), developing natural food colorants has become increasingly important to support good health and well-being (SDG 3), promote resource efficiency and reduce waste (SDG 12), decrease reliance on petrochemical-based synthetic dyes (SDG 13), and encourage the use of functional food products sourced from natural resources (SDG 15). Recently, the "back to nature" trend has strengthened consumer interest in natural products over synthetic options (Roman et al., 2017). The ongoing use of synthetic food colorants, especially yellow dyes like Tartrazine and Sunset Yellow, has been associated with hypersensitivity reactions, behavioral issues such as hyperactivity, carcinogenic risks, and organ damage, particularly to the kidneys and liver (Kobylewski & Jacobson, 2012; Miller et al., 2022). Conversely, carotenoids (e.g., lutein, β-carotene) are among the most significant groups of natural yellow pigments, commonly found in plants and valued for their coloring as well as their nutritional and functional benefits (Maoka, 2020). Antioxidants and cell protectors (cytoprotectors and photoprotectors) are these types of compounds, which may help mitigate the adverse effects of oxidative stress and subsequently reduce the likelihood of developing chronic diseases (Bufka et al., 2024). Therefore, studying and using natural food colorants is an important part of improving diet quality and advancing sustainability in our food systems. This indicates the need to replace synthetic dyes with safer and more naturally derived ones.

Among other sources of Carotenoids, marigold (*Tagetes erecta L.*) has significantly higher levels of carotenoid content when compared to pumpkin and carrot - both of which are commonly used as food colorants and nutraceutical sources (Zahra et al, 2024).The principal carotenoid in Marigold, Lutein is found at concentrations in the Marigold flower in the range of 0.019 - 2.532g per 100g of dried weight (Manivannan et al., 2021). In addition to serving as a source of pigment, Marigold flowers also have been traditionally used for therapeutic and culinary purposes throughout India, China, and Indonesia (Singh et al.., 2020). Agronomically Marigolds are a low maintenance plant and can be grown successfully across a very wide range of temperatures from 14.5 - 28.6 °C (Zahra et al, 2024). Phytochemical analysis of methanol and ethyl acetate extracts of marigold flowers have shown the presence of alkaloids, tannin/phenolics, carotenoids, and flavonoids (Youssef et al, 2020). The carotenoid compounds may be extracted from Marigolds using various solvent-based methods (Lin et al., 2015). The separation of solids and/or liquids by dissolving them (target or otherwise) into a chosen (target) solvent, which is then referred to as "extraction." The type of solvent, ratio of solids to liquid, amount of moisture in the solids, and stirring speed, are all examples of the parameters that affect how efficiently carotenoids are extracted. In addition, there are process parameters that must be maintained for maximum yield or stability of the carotenoids being extracted, including the pH, temperature, and amount of time that the carotenoids have been in the extraction solution, with regards to the amount of extraction time. Marigold carotenoids are sensitive to pH, temperature and extraction duration, which require optimization to achieve maximum yields and stability. Therefore, optimizing the conditions for the extraction of carotenoids is critical to achieving maximum recovery and stability of carotenoids, as well as improving the efficiency of extraction methodologies and design through advances in extraction technologies and equipment.

Carotenoid extraction studies of marigold have tended to use one-factor extraction optimization protocols and did not consider the interactions between extraction variables. For example, in Manivannan and colleagues (2021), they were able to extract carotenoids from marigold flower material by extracting 1g of marigold powder for 1 hr. using room temperature extraction and 50 °C ultrasonic extraction for the same amount of time. Youssef and colleagues (2020) used methanol and maceration to extract carotenoid in a 24 hr. extraction time with three rinses of fresh solvent to maximize the extraction. Akshaya (2017) used 0.1g of dried marigold petals and acetone for extraction purposes. None of these previous studies have been conducted in such a manner that they evaluated the effect of important extraction parameters such as; extraction time, extraction pH, and extraction temperature on the extraction method. In addition, some of the key parameters needed for the development of optimized processes include processing equipment design and processing speed and efficiency of processing equipment. Furthermore, marigold extracts primarily contain carotenoids and therefore, extraction method parameters should take into consideration the sensitivity of carotenoids to processing conditions.

The extraction of carotenoids from dried marigold flower petals is complicated, and these challenges can hinder the wider application of marigolds in food applications. Response Surface Methodology provides an effective, practical tool for the simultaneous adjustment of several variables involved in the extraction process, which allows researchers to optimize extraction conditions without conducting an unmanageable number of experiments (Bezerra et al. 2016). RSM has already been used successfully in food science applications, including the extraction of green coloring agent from Pleomele angustifolia leaves (Rahayuningsih et al., 2018). Within RSM, Central Composite Design (CCD) presents greater flexibility and a broader range of testing options than Box–Behnken Design (BBD) while still requiring fewer tests than

full factorial design (Myers et al., 2016). The use of CCD to extract carotenoid from marigolds is innovative. However, storing the extracted carotenoids must also be focused on as the storage conditions impact the carotenoids' shelf life. Much of the prior research on carotenoids fails to address this issue. Therefore, both the optimization of the extraction procedure and the evaluation of the stability of the extracted carotenoid when stored are needed to determine whether successful applications of carotenoids will occur in the future.

This research aimed to determine the optimal conditions for extracting natural yellow food colorants from marigold flowers, using ethanol as the solvent. A central composite design was applied within the response surface methodology framework to assess the effects of pH, temperature, and extraction time on total carotenoid content. In addition, the stability of the marigold extract was tested with common food additives, including sugar, salt, and citric acid, to understand how these substances affect the preservation of the colorants.

2. Materials and Methods

2.1. Materials

Fresh marigold flowers (Fig.1a) aged 21 days were taken from RPT umah Atsiri Indonesia in Tawangmangu, Karanganyar, Central Java. The technical grade reagent used in this study included ethanol as a solvent for extraction. Analytical grade reagents from Sigma-Aldrich included quercetin ($C_{15}H_{10}O_7$, ≥95%), β-carotene ($C_{40}H_{56}$, ≥93%), gallic acid ($C_7H_6O_5$, ≥98.5%), sodium nitrite solution ($NaNO_3$ 5%, ≥99%), aluminum chloride solution ($AlCl_3$ 10%, ≥99.9%), sodium hydroxide pellets (NaOH, ≥95%), sodium carbonate (Na_2CO_3, ≥99.5%), hydrochloric acid (HCl, 37%), and Folin–Ciocalteu phenol reagent (2 M). Stabilize materials included citric acid monohydrate ($C_6H_8O_7$, ≥99.5%) from Ensign (China), sodium chloride (NaCl) from Unichem Candi, commercial sucrose ($C_{12}H_{22}O_{11}$, 99%), and food-grade citric acid from locally store in Yogyakarta.

2.2. Methods

Drying of marigold petals

Marigold petals were arranged in a single, even layer on clean trays and left to air-dry indoors at 25–30 °C in a well-ventilated space away from direct sunlight. The petals dried naturally with gentle airflow for four days, until they no longer lost weight.

Soxhlet Extraction of Marigold Petals

Dried marigold petals (3 g) were wrapped in filter cloth and placed in the Soxhlet apparatus. Extraction was conducted with 200 mL of ethanol at a controlled temperature below 80 °C. The process ran for 8 hours per day, with the samples left to stand overnight. This procedure was repeated over five days to ensure thorough carotenoid extraction. It was also used to determine the initial flavonoid, phenolic, and carotenoid contents in marigold flowers.

Total flavonoid content (TFC) analysis

TFC was determined using the aluminum chloride colorimetric method, as described by Siddhuraju and Becker (2003). A 0.5 mL aliquot of the extract was dried in an oven at 50 °C to obtain a solid residue. The mixture was then incubated for 5 min, followed by the addition of 0.3 mL of 5% sodium nitrite and 0.6 mL of 10% aluminum chloride. Subsequently, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the final volume was adjusted to 10 mL with distilled water. The absorbance of the resulting solution was measured at 510 nm to determine the *TFC*, which was calculated according to the following equation:

$$TFC\ \left(\frac{mg\ QE}{1\ gram\ dry\ weight}\right) = \frac{QE \times V \times DF \times 10^{-3}}{W} \quad (1)$$

Where, *QE* is the quercetin equivalent (μg/mL) obtained from the calculation from standard curve in Fig. S2; *V* is the total sample volume (mL); *DF* is the dilution factor; and *W* is the sample mass (g). All measurements were conducted in triplicate.

Total phenolic content (TPC) analysis

TPC was determined using the Folin–Ciocalteu method according to the procedure of Gong et al. (2012). A 0.5 mL aliquot of each standard solution or sample extract was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water), vortexed, and incubated for 5 min. Subsequently, 2 mL of 7% (w/v) Na_2CO_3 solution was added, and the mixture was incubated at room temperature for 2 h. Absorbance was measured at 755 nm using a UV–Vis spectrophotometer. All measurements were conducted in triplicate, and TPC was calculated according to the following equation:

$$TPC\left(\frac{mg\ GAE}{1\ g\ dry\ weight}\right) = \frac{\mathrm{GAE} \times V \times DF \times 10^{-3}}{\mathrm{W}} \quad (2)$$

Where, GAE is gallic acid equivalent, μg/mL obtained from the calibration curve in Fig. S3. *V* is the total sample volume (mL); *DF* is the dilution factor, and *W* is the sample mass (g).

Total carotenoid content (TCC) Analysis

TCC was measured by UV–Vis spectrophotometry using the protocol described by Scott (2001). A 2 mL sample was diluted to a final volume of 10 mL with ethanol. The absorbance of the β-carotene standard solutions and the diluted extract samples was recorded at the peak

wavelength (λ_{max}) of 453 nm. The TCC was then calculated using the following equation:

$$TCC\ (\frac{mg}{100\ g\ dry\ weight}) = \frac{BCE \times V \times DF \times 10^{-1}}{W} \quad (3)$$

Where, BCE is beta carotene equivalent (μg/mL) obtained from the calibration curve. *V* is the total sample volume (mL); *DF* is the dilution factor, and *W* is the sample mass (g).

Optimization of the Extraction Process using RSM

Following the initial screening experiment (Fig. S1), marigold petal extraction was further refined using RSM based on a Central Composite Design (CCD). The independent variables selected for the CCD model were pH, extraction time, and temperature, while total carotenoid content (TCC) served as the response variable. The ranges and coded levels of these independent variables are summarized in Table 1. For each experimental run, 3 g of dried marigold petals were extracted with 250 mL of ethanol under reflux conditions using a three-neck round-bottom flask equipped with mechanical stirring at 150 rpm. The TCC results were then statistically analyzed using Design-Expert software (Version 13, Stat-Ease Inc., Minneapolis, USA).

Table 1. CCD for extraction optimization with variable ranges and levels

Variable	**Units**	**Range and level of actual and coded values**				
		-α	**-1**	**0**	**1**	**α**
pH	N/A	5	5.8	7	8.2	9
Temperature	°C	35	42	52.5	63	70
Time	min	30	39	50	62	70

The experimental data were modeled using a two-factor interaction (2FI) regression. The TCC (Y) was expressed as a function of the independent variables (X) according to equation (4).

$$Y = \beta_0 + \sum \beta_i . X_i + \sum \beta_{ij} . X_i . X_j + \varepsilon \quad (4)$$

where β_0 is the intercept, β_i indicates the linear coefficients, β_{ij} represents the interaction coefficients between variables, and ε is the random error. A two-factor interaction (2FI) model was used because it captures both the linear effects of the factors and their pairwise interactions. This model provides enough flexibility to represent interaction effects while being less complex than higher-order polynomial models (Myers et al., 2016).

Stabilization of marigold extract

Carotenoid stability of marigold extract was tested by the addition of either citric acid, sugar, or salt in fixed amounts. Each additive was dissolved into 180 mL of marigold extract to yield a total of three replicates with 3.6 g citric acid (2% w/v) or 5.4 g each of sugar (3% w/v) and salt (3% w/v) in separate vials. A fourth batch (labeled as control), with no additives, was also tested to determine if there were any differences in the carotenoid levels between the control and the other three batches. There were 20 mL aliquots taken from each batch and placed into vials for storage in the refrigerator. The carotenoid content of the aliquots was determined using a visible spectrophotometer at set times after the initial mixing occurred.

3. Result and discussion

3.1. *Initial TFC, TPC, and TCC of marigold flower*

In order to decrease the quantity of water in the petals, the marigold petals have been dried out, which will also allow for the concentration of carotenoids. Once dried, the petals retained their normal colour (yellow), indicating that the carotenoid pigments essentially remained intact and, therefore, the dried petals will be able to provide suitable material for extracting carotenoids. (Fig. 1b).

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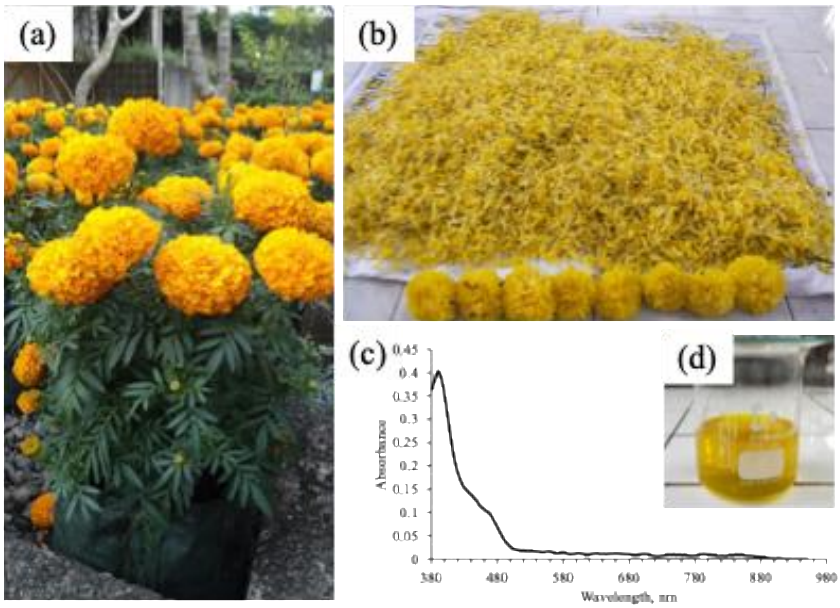


Fig. 1 (a) Fresh marigold flower, (b) marigold flower after drying, (c) chemical structure of carotenoid, and (d) UV spectrum of marigold extract.

To identify the basic phytochemical composition of dried marigold petals used in this research, dried marigold flower petals were extracted using a Soxhlet extractor to determine the phytochemical composition or content of dried petal samples. Flavonoids are well known to have radical scavenging properties. When measured using the spectrophotometric method, the total flavonoid content of the dried marigold petals was measured at approximately 92.7 mg QE/g DW, showing that flavonoids are present in considerable quantity and represent a unique subclass of compounds among the total number of phenolic compounds. The total flavonoid content of the dried marigold petals is similar to the total flavonoid content values reported by Siddiqa and coworkers (2025), which ranged from 72.6 to 130.3 mg QE/g DW when extracted with acetone, ethanol, or hexane. The total phenolic content of the marigold petals was measured at 432.8 mg GAE/g DW, indicating that phenolic compounds are the primary contributors to the antioxidant capacity of marigold. Several studies have reported lower total phenolic content in marigold petals using methanol maceration at room temperature for 24 hours, with total phenolic content values typically between 57.5 and 125.0 mg GAE/g DW (Youssef *et al.*, 2020). A higher yield from this study was observed due to the use of Soxhlet extraction with ethanol under reflux, which is considered a more complete extraction method allowing for the recovery of the total free and bound phenolic content than milder methods of extraction. The TCC of the marigold extract is 3490 mg/100 g DW, indicating the carotenoid-rich characteristics of the petals themselves (see Fig. S5). This is very much in line with the work of Akshaya et al. (2017), indicating total carotenoid content (up to 525.68 mg/100 g FW) of marigold genotypes can be achieved. These values, while presented on a fresh weight basis, would represent a higher concentration in terms of dry weight due to the high percent of water associated with marigold petals. Therefore, even though a direct comparison between the current research and that of Akshaya et al. is not appropriate, both studies indicate the existence of high concentrations of carotenoid pigments in marigold flowers, especially xanthophylls (lutein and zeaxanthin), and yellow carotenoids (β-carotene)(Deineka *et. al*., 2008).

3.2. *CCD experimental design and statistical analysis*

The UV–Vis spectrum of the marigold extract obtained using ethanol as the solvent (20-fold dilution) is shown in Fig. 1c, with the corresponding extract presented in Fig. 1d. The spectrum exhibited a single peak consistent with the absorption pattern of β-carotene from the standard solution. In the experimental design, a solvent-to-solid ratio of 3:250 (w/v) was selected as the control variable. This choice was informed by the findings of Xu et al. (2015), who reported an optimal ratio of 1:50 (w/v) for subcritical water extraction, and was further adjusted based on technical considerations to facilitate separation and prevent clotting of the flower petals. Agitation was also applied as a control variable, set at 150 rpm to promote longer solute–solvent contact and improve mass transfer. In contrast, previous studies often employed ultrasound-assisted extraction (UAE), in which acoustic energy is used to accelerate the extraction process (Manzoor et al., 2022).

A CCD was employed to study the effects of pH, extraction temperature, and extraction time on the TCC of marigold flower extracts. The design consisted of 20 experimental runs, including factorial, axial, and replicated center points (Table 2). The TCC results were calculated using the standard curve shown in Fig. S6. The actual experimental responses ranged from 400 to 3200 mg/100 g DW, while the model-predicted values closely matched the experimental results. The small deviations between actual and predicted values confirm the reliability of the CCD-based RSM model for predicting carotenoid yield under the studied conditions.

The statistical analysis results are summarized in Table 3. The TCC responses were best fitted by a two-factor interaction (2FI) model, which was statistically significant with a Model *F*-value of 13.87 ($p < 0.0001$), indicating that the variation explained by the model greatly exceeded that due to random error. The model also exhibited a significant sequential *p*-value (p=0.0161), an acceptable lack-of-fit (p=0.0589), and good agreement between adjusted R^2 (0.8025) and predicted R^2 (0.6185, difference < 0.2). The adequate precision value of 13.17 confirmed an adequate signal-to-noise ratio, well above the desirable threshold of 4. Furthermore, the 2FI model produced the lowest PRESS value among alternative models, confirming its predictive adequacy. By contrast, the quadratic model was not significant, and the cubic model was aliased, reinforcing the selection of the 2FI model as the most appropriate for this system.

The model terms that were statistically significant were pH (X1), temperature (X2), and their multiplicative interaction (X1X2) because all the p-values associated with each were less than 0.05. However, none of the interaction terms with extraction time (X3), X1X3, or X2X3 were statistically significant. The resulting polynomial equation the TCC was positive in regard to pH (231.85) and temperature (785.85), indicating that increasing either will increase the TCC yield. Conversely, there was a negative coefficient (–465) for the interaction term X1X2, indicating that increasing the pH and temperature simultaneously reduced the ability to recover carotenoid concentrate, producing a reduction in TCC yield at higher levels. The interaction of pH and extraction time (X1X3) also had a negative coefficient (–100), which was consistent with the observation that prolonged time of extraction (>70 minutes) negatively impacted TCC yield. These results were consistent with both the initial experiments and existing theory that demonstrate that carotenoids are more stable at lower pH levels (acidic) and that carotenoids are less stable at higher extraction time and therefore degrade with extended time of extraction.

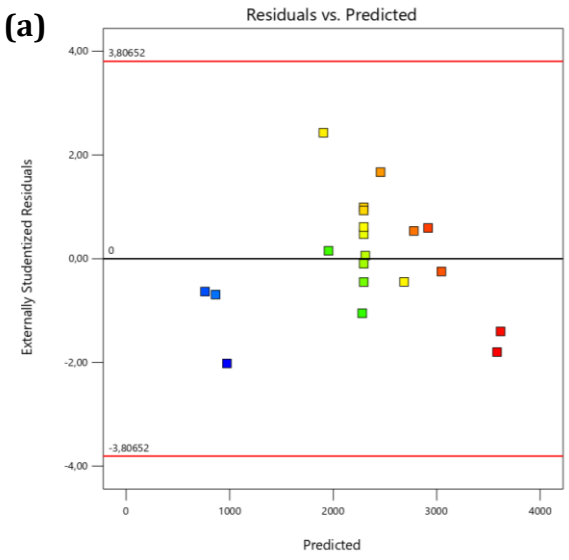
Table 2. Experimental design using CCD and the total carotenoid content (TCC) results

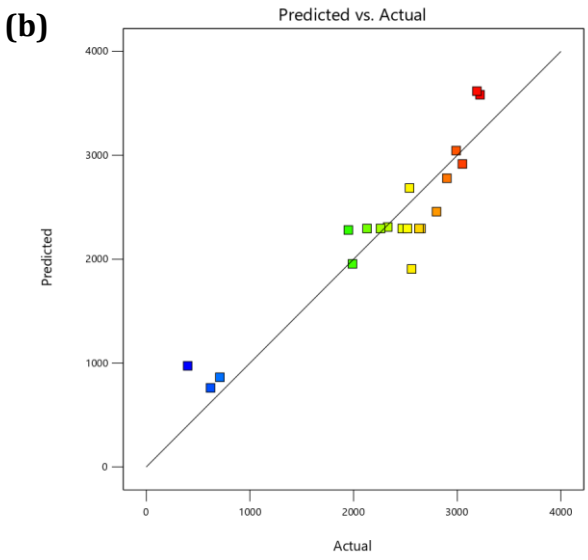
Run	Coded factor			Actual Factor			TCC Actual (mg/100g DW)	TCC Predicted (mg/100g DW)
	X_1	X_2	X_3	pH	T (°C)	Time (min)		
1	0	0	0	7	52.5	50	2130	2300
2	0	0	0	7	52.5	50	2630	2300
3	-α	0	0	5	52.5	50	2560	1910
4	0	0	+α	7	52.5	70	2330	2310
5	-1	-1	-1	5.8	42.1	38.1	710	860
6	0	0	0	7	52.5	50	2470	2300
7	+1	+1	+1	8.2	62.9	61.9	3050	2920
8	-1	+1	-1	5.8	62.9	38.1	2990	3050
9	-1	+1	+1	5.8	62.9	61.9	3220	3580
10	-1	-1	+1	5.8	42.1	61.9	620	760
11	+1	+1	-1	8.2	62.9	38.1	2900	2780
12	+1	-1	+1	8.2	42.1	61.9	1990	1960
13	0	0	0	7	52.5	50	2260	2300
14	0	0	-α	7	52.5	30	1950	2280

Run	Coded factor			Actual Factor			TCC Actual (mg/100g DW)	TCC Predicted (mg/100g DW)
	X_1	X_2	X_3	pH	T (°C)	Time (min)		
15	0	0	0	7	52.5	50	2520	2300
16	0	-α	0	7	35	50	400	970
17	+α	0	0	9	52.5	50	2540	2690
18	0	0	0	7	52.5	50	2650	2300
19	+1	-1	-1	8.2	42.1	38.1	2800	2460
20	0	+α	0	7	70	50	3190	3620

Table 3. Statistical analysis results from the parameters of the 2FI

Parameter	Value	Parameter	*p*-value
sequential *p*-value	0.0161	ANOVA coefficient	
Model *F*-value	13.87	X_1 - pH	0.0361
Model *p*-value	< 0.0001	X_2 - Temperature	< 0.0001
R^2	0.8649	X_3 - Time	0.9313
Lack of fit *p*-value	0.0589	$X_1 X_2$ - pH*Temperature	0.0033
Adjusted R^2	0.8025	$X_1 X_3$ - pH*Time	0.4542
Predicted R^2	0.6185	$X_2 X_3$ - Temperature*Time	0.2389
PRESS	4.93E+06	Adequate precision	13.1654
Equation (codded factor)	$TCC = 2295.5 + 231.85X_1 + 785.85X_2 + 8.72X_3 - 465\ X_1 X_2 - 100X_1X_3 + 160\ X_2X_3$		





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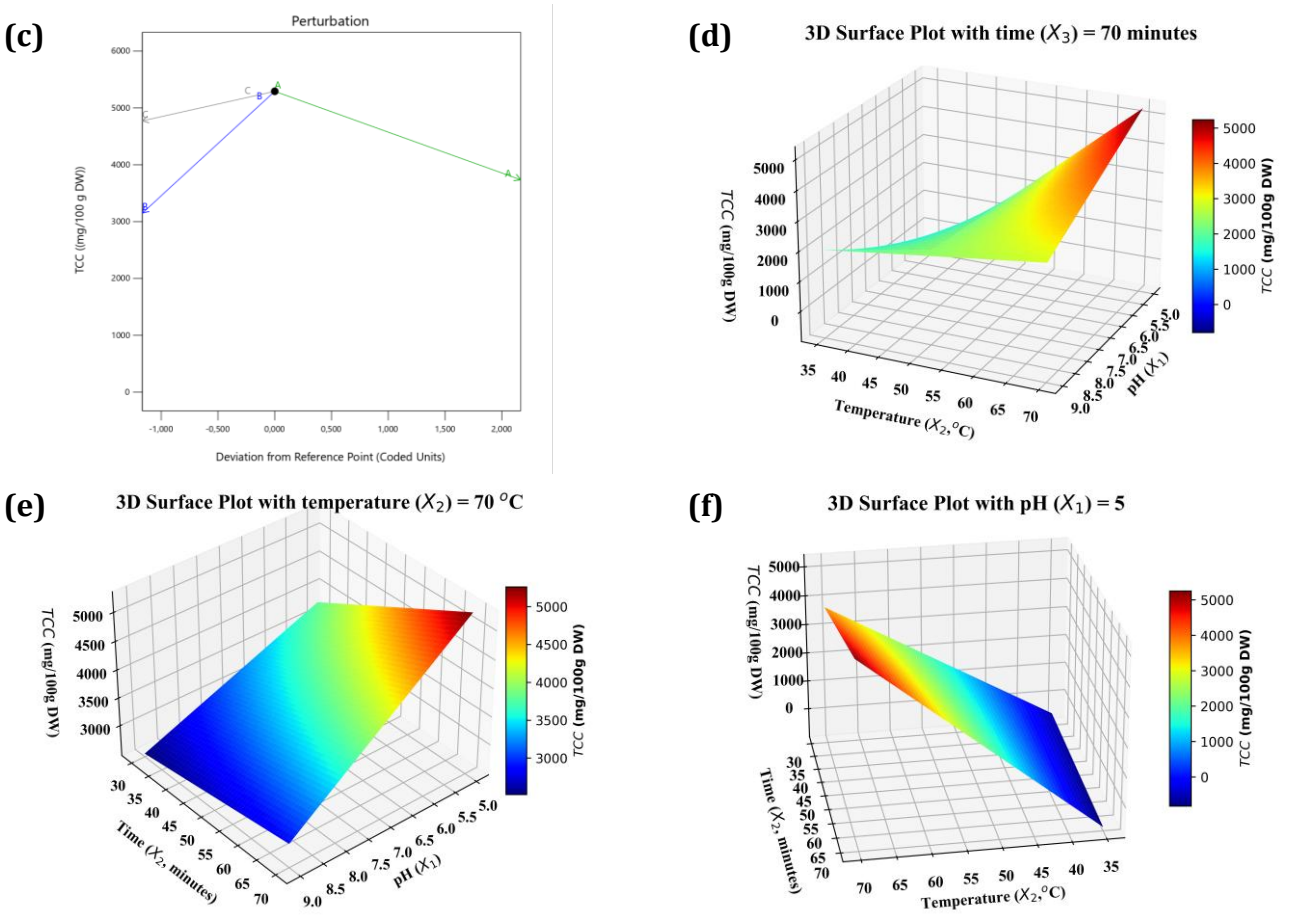

Fig. 2. Diagnostic plots and response surface analysis of the CCD model for total carotenoid content (TCC): (a) residuals versus predicted values, (b) predicted versus actual values (linear fitting), (c) perturbation plot showing the relative effects of pH (A), temperature (B), and time (C), (d) response surface of TCC as a function of pH and temperature, (e) response surface of TCC as a function of pH and time, and (f) response surface of TCC as a function of time and temperature.

3.3. *Diagnostic plots and response surface evaluation*

Fig. 2 illustrates the predictive performance of the CCD-based RSM model, showing how pH, extraction temperature, and extraction time interact to influence TCC. The residuals-versus-predicted plot (Fig. 2a) indicated that errors were small and evenly distributed at low carotenoid concentrations, whereas the residual spread increased at higher expected values. This suggests slight heteroscedasticity, with the model performing better at lower carotenoid levels. Markedly, all the residuals stayed within the allowed ±3.81 range. In Fig. 2b, the predicted-versus-actual plot shows that The TCC data points are very close to the 1:1 line, indicating that the experimental TCC yield and the model TCC yield closely match and that the model accurately predicts the effects of pH, temperature, and time on TCC yield.

Furthermore, the close correlation of the residuals-versus-predicted and predicted-versus-actual plots provides additional evidence that the CCD-RSM model accurately predicts TCC yield. The perturbation plot (Fig. 2c) clearly illustrates the influence of each independent variable on carotenoid yield at the reference conditions of pH = 5, temperature = 70 °C, and time = 70 min. Both time and pH had significant positive and negative relationships with carotenoid yield (higher temperature and longer time correlated with higher carotenoid yield), whereas pH had a strong negative relationship (higher pH correlated with lower carrot yield). The slope of each curve indicates the relative strength of each factor's effect; time had the most significant impact (B), followed by pH (A) and lastly time (C).

Fig. 2. Diagnostic plots and response surface analysis of the CCD model for total carotenoid content (TCC): (a) residuals versus predicted values, (b) predicted versus actual values (linear fitting), (c) perturbation plot showing the relative effects of pH (A), temperature (B), and time (C),

(d) response surface of TCC as a function of pH and temperature, (e) response surface of TCC as a function of pH and time, and (f) response surface of TCC as a function of time and temperature.

Fig. 2d–f presents the response surface plots showing the interaction between two independent variables and their effect on TCC, providing a three-dimensional visualization for interpreting these correlations. Fig. 2d (pH versus temperature) showed that TCC increased markedly with rising temperature, reaching its maximum under high-temperature conditions (70 °C). The pH significantly affected carotenoid yield, with higher values at acidic conditions (pH 5–6), moderate yields at neutral pH, and markedly lower yields at alkaline pH (>8). The findings suggest that the most efficient method for extracting carotenoids from carrot tissue is at higher temperatures and slightly acidic conditions while alkaline conditions can inhibit the ability of carotenoids to be extracted and degrade their quality. This is consistent with prior research which indicated that carotenoids are more stable when stored in acidic rather than neutral and alkaline environments Bell and Graf (2016). Further evidence exists supporting thermal degradation by demonstrating that 1.4% degradation was identified at 70 °C after 30 minutes (Kardile et al., 2017). The plot of extraction Time VS pH shows no areas of low returns when using the test conditions (70°C) providing further evidence that all tested conditions produced moderate to high return rates of total carotenoids.

Extended time of extraction produced increasing amounts of carotenoid (as found in many different types of fruit and vegetables). This indicates that the appropriate amount of time should be allowed for both diffusion and the contact of the solvent (ethanol) to the solid material. The pH of the extraction material was also very important. The maximum yield was achieved in a slightly acidic pH (5–6), while a gradual decrease in recovery was seen at more alkaline pH values (>8). Therefore, it appears that a longer extraction time at a mildly acidic pH would provide the best means of extracting carotenoid material. Figure 2f shows an interaction between extraction time and temperature on total carotenoid content (TCC) at constant pH. Because there was no prominent peak, the optimal extraction conditions might extend beyond the temperatures that were tested, as seen by the curved times indicating that there may also be a synergistic relationship between temperature and time. Although calculated to improve recovery of carotenoid materials, a higher temperature of extraction or longer duration of extraction needs to be balanced with the practical limitations of the boiling point of ethanol and the potential degradation of carotenoid materials. The analytical procedure used for optimizing extraction conditions found the best results for extraction using ethanol as the solvent at 5.9 pH, extracting at a temperature of 62.9 °C and for a period of 67.1 minutes. Under these conditions the total carotenoid content was found to be 3490 mg/100 g DW.

3.4. *Stabilization of marigold extract*

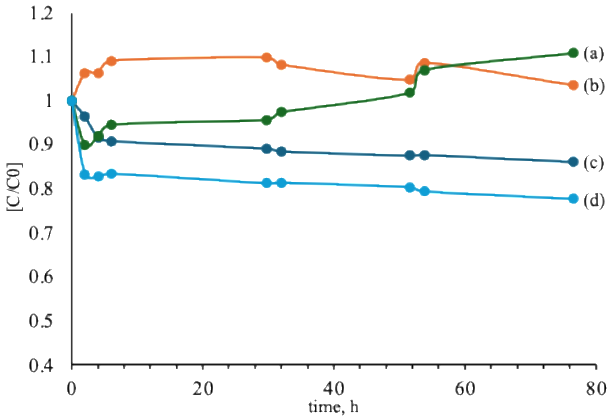

Fig. 3 Time vs ratio of carotenoid concentration to the initial concentration (C/C_0) by adding (a) 3% salt, (b) 2% citric acid, (c) no addition, (d) 3% sugar.

The degree of stability of carotenoids within marigold extract over the 76 hour storage test is dependent on whether sodium chloride, citric acid or sugar was included in the formulation. The carotenoid concentration during the storage interval remained relatively unchanged in both the 3% NaCl and 2% citric acid formulations as evidenced by Figs 3a and 3b, and therefore provided a greater amount of stabilization than did a control formulation (Fig 3c). Increased stabilization of carotenoids when sodium chloride is included may occur due to the action of the Na^+ and Cl^- ions which create an alternate physical environment for carotenoids making them less susceptible to oxidative degradation when exposed to atmospheric oxygen at elevated temperature and/or humidity. Ren et al., 2021, documented that the addition of salt stress results in the accumulation of carotenoids in plants, and within the food industry when Pickering emulsion systems contain high concentrations of salt, carotenoids are more effectively protected through an increase in spatial repulsion and barrier thickness around the encapsulated droplet. Conversely, citric acid functions as a stabilizer by reducing pH, thus suppressing oxidation of carotenoids and preventing isomerism of carotenoids, thereby enabling the continued presence of carotenoids during storage (Plaza et al., 2016). The studies conducted previously are in agreement with the current study showing that acidification effectively reduces the rate of degradation of β-carotene and lutein in both water and emulsion systems (Davidov et al., 2016). A storage period of 76 hours was chosen for comparison purposes as this was the longest

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period during which both 3% salt and 2% citric acid treatments maintained carotenoid levels at or near average stability compared to control. This provides an opportunity for comparison between treatments, despite their different kinetic behaviour: salt showed a steady increase; whereas a slight decrease was seen with citric acid but both treatments demonstrated an overall increase in stability.

Figures 3c and 3d show that the carotenoid concentration decreased steadily during storage, demonstrating that carotenoids continuously degraded. Furthermore, the control (no additive) sample retained carotenoids better than the 3% sugar treated sample. Therefore, sugar added to a food system promotes degradation instead of acting as a protective agent. This degradation can be attributed to the hygroscopic nature of sugars, which creates higher local water activity in the extract. The increased water activity will increase moisture absorption and promote sugar crystallization, all of which will encourage degradation processes such as oxidation and isomerization of carotenoids. Similar findings have been reported previously; for example, higher sugar activity in fruit & vegetable-based food systems increases degradation of carotenoids, as higher sugar activity increases molecular mobility (Gonzales et al., 2021; Harnkarnsujarit & Charoenrein, 2011). Therefore, the present results further support the conclusion that sugars accelerate the degradation of carotenoids compared to untreated controls.

4. Conclusions

To quantify the total amount of flavonoids, phenolics and carotenoids found in whole fresh marigold flowers (TCK = 3490 mg carotenoids/100 g dried) a Soxhlet extraction was utilized. The Central Composite Design (CCD) experiment to optimize extraction of carotenoids, demonstrated that temperature and pH were the two primary factors influencing extraction yield. The three optimum values determined to extract the highest amount of total carotenoids from marigold flowers were: pH 5.9, Temperature 62.9 °C, Time 67.1 min. The extraction also featured constant stirring during the extraction process and a constant flower:ethanol ratio for all extraction trials. The accuracy of the CCD method was confirmed using diagnostic analysis of the CCD experimental data, which indicated that the predicted values matched the observed values quite closely, particularly with regard to temperature and pH importance. Testing the extract for carotenoid stability showed that the addition of 2 percent citric acid or 3 percent salt aided in preserving carotenoids over three days, while adding 3 percent sugar caused more rapid breakdown. These results indicate that an acid or a salt can help to preserve carotenoids, while sugar does not, likely making them less stable due to its nature. This study gives practical recommendations for the extraction, formulation, and storage of marigold yellow colorants to preserve their natural pigments.

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