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RESEARCH ARTICLE

Chemical composition and bioactive compounds of *Opuntia ficus-indica* fruits in Ninh Thuan province, Vietnam

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Abstract

This study presents the first comprehensive analysis of the chemical composition and bioactive compounds of *Opuntia ficus-indica* fruits cultivated in Ninh Thuan province, Vietnam in 2024. Key findings include a free acid content of 136.7 ± 5.77 mEq/kg, mineral content of 21.8 ± 0.05 %, total sugar content of 10.7 ± 0.06 %, reducing sugar content of 10.48 ± 0.1 %, lipid content of 1.624 ± 0.02 %, protein content of 1.619 ± 0.04 % and crude fiber content of 2.53 ± 0.06 %. These values indicate their potential as a nutrient-dense food source. In addition, the extraction of bioactive compounds was optimized using response surface methodology (RSM). Under the optimal conditions of 59.13 °C, 96.43 min and a liquid-to-material ratio of 46.5 mL/g, the total phenolic content (TPC) and total flavonoid content (TFC) reached 15.355 ± 0.028 mg gallic acid equivalents (GAE)/g dry weight (DW) and 2.404 ± 0.0428 mg quercetin equivalents (QE)/g DW, respectively. The polynomial models applied were shown to be effective in predicting and maximizing yields. Overall, the findings underscore the nutritional richness and bioactive potential of *O. ficus-indica* fruits, offering a scientific basis for their development into functional foods and nutraceuticals. This work provides novel insights into the value-added utilization of cactus fruits from Vietnam, contributing to both human health promotion and sustainable agricultural practices.

Keywords: extraction; *Opuntia ficus-indica*; response surface methodology; total flavonoid content; total phenolic content

Introduction

Opuntia spp. is one of the most important fruit plants of the Cactaceae family that thrive in various environments, especially in dry and semiarid regions (1). Nopal cactus or prickly pears, scientifically known as *Opuntia ficus-indica* (OFI) and endemic to Mexico. This plant is also found in numerous locations across America and is present globally, including in regions of Australia, Africa and the Mediterranean (2, 3). *O. ficus-indica* fruit grows in stem tissues from a lower ovary. It matures about 110 to 120 days after flowering and can vary in weight from 80 to 200 g. The prickly pear fruit is cylindrical or ovoid in shape, measuring 5 to 10 cm long and 4 to 8 cm wide (4).

O. ficus-indica is a highly significant cactus crop species for agriculture and economy and it is grown in various parts of the world for its fruits. The prickly pear fruits comprise three parts that can be utilized commercially including seeds, skin and flesh. This fruit is made up of around 85 % water, 15 % sugar, 0.3 % ash and under 1 % protein (5). With a significant water content, this fruit has a caloric value of 50 kcal/100 g, comparable to many fresher fruits such as pears, oranges and apricots (6). Antioxidants (betacyanin, betaxanthin, flavonoids and phenols), ascorbic acid, vitamin E, carotenoids, fiber and amino acids are all found in high concentrations in cactus fruit (7).Flavonoids and phenolic acids are abundant in all parts of the cactus, both belonging to the polyphenol family. The glycoside isorhamnetin has a high content (50.6 mg/100g) compared to other flavonoids, while the total phenol content in the fruit pulp is 218.8 mg/100g (8). The antioxidant and free radical scavenging activities of polyphenols in cacti may influence their health-promoting abilities (9). Natural antioxidants have received a lot of attention recently due to their association with health benefits (10). They produce various antioxidant molecules to fight reactive oxygen species (ROS) (11). Prickly pear fruit extract is a natural source of antioxidants. Its flesh is an excellent source of minerals (calcium, magnesium, potassium and phosphorous), amino acids (alanine, arginine and asparagine), vitamins (vitamin C, vitamin E and vitamin K) and beta-carotene. *O. ficus-indica* is also a valuable source of natural antioxidants for food due to its content of flavonoids, ascorbic acid and carotenoids. Therefore, the nutritional and chemical characteristics of the prickly pear might not provide a fully balanced diet for human consumption, unlike primary staple foods such as cereals and legumes. However, prickly pear fruits and their derivatives can play an important role in enhancing human nutritional intake. There is a growing trend in the consumption of natural nutritional supplements and health-promoting food products. Consequently, the various functional characteristics of cactus pear, combined with its antioxidant properties, align with growing consumer demand for plant-based functional foods (6). *O. ficus-indica* is recognized for its ability to enhance diet quality by providing essential nutrients and a wide range of bioactive compounds that may help to

prevent several diseases. Prickly pear fruits can be consumed fresh, sun-dried or processed into marmalade and in different regions of Mexico, they are commonly used as an ingredient in salads.

O. ficus-indica is gaining popularity as an unique and nutritious meal in several nations such as Mexico, China and America on the everyday diet. Additionally, *O. ficus-indica* possesses diverse qualities that allow its utilization across multiple sectors. In health-related applications, it is employed in pharmaceuticals, cosmetics and animal care. In agriculture, it serves as human food, livestock feed and a resource for bee honey (9). From an industrial perspective, it can be used in the production of alternative fuels, civil construction materials and for soil erosion prevention.

This study aimed to evaluate the chemical composition, total polyphenol content (TPC) and total flavonoid content of *O. ficus-indica* fruits from Ninh Thuan province, Vietnam. Ninh Thuan is characterized by its arid climate and unique ecological conditions, yet the nutritional and bioactive properties of *O. ficus-indica* fruits from this region remain largely underexplored. The findings of this research will serve as a valuable reference for assessing the quality of *O. ficus-indica* fruits from other regions.

Materials and Methods

Sample collection and preparation

Fully ripened fruits of *O. ficus-indica* were harvested in December 2023 from Ninh Thuan Province, Vietnam (11°28′27.6″N, 109°00′ 28.2″E). The botanical identification of the collected material was performed by Assoc. Prof. Dr. Dam Duc Tien (Vietnam Academy of Science and Technology). The fruits were immediately freeze-dried for 48 hr to preserve bioactive compounds and then finely ground using a high-speed grinder. The powdered sample was packed in polyethylene (PE) vacuum-sealed bags with a moisture content of 8-10 % and stored at 4-6 °C until analysis. A representative image of the sample is provided in Fig. 1.

Chemicals and reagents

All reagents used in the study, including sodium carbonate, quercetin, aluminum chloride, ethanol, Folin–Ciocalteu reagent and gallic acid, were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA).

Physicochemical analysis

The composition of the *O. ficus-indica* fruit powder was assessed using standardized methods to determine total sugars, reducing sugars, protein, lipid, fiber, acid and mineral contents.

Total and reducing sugar contents

The total sugar content was determined via the phenol-sulfuric acid method, using glucose as the standard. A 0.5 g sample was diluted in distilled water, filtered and reacted with phenol and sulfuric acid. Absorbance was measured at 490 nm. Reducing sugar content was analyzed using the dinitrosalicylic acid (DNS) method, with absorbance recorded at 540 nm (12). Both analyses were conducted in triplicate and standard curves were constructed using glucose concentrations ranging from 0-100 ppm. The total sugar content in the *O. ficus-indica* fruits was calculated based on the glucose standard curve using the corresponding formula:

$$\text{Total sugar content (\%)} = C \frac{V_1 \times V_2 \times 100}{10^6 \times V \times W} \quad (1)$$

Where, C: total sugar concentration from the calibration curve (ppm); *W*: weight of sample (g); V_1: 1st titration volume (mL); V_2: second titration volume (mL); 10^6: convert ppm concentration to g/mL; *V*: analytical sample volume (mL).

The reducing sugar content of *O. ficus-indica* fruits was calculated based on the glucose standard curve using the appropriate formula:

$$\text{Glucose (\%)} = C \frac{V_1 \times V_2 \times 10 \times 100}{10^6 \times V \times W} \quad (2)$$

Where, C: concentration from the calibration curve (ppm); V_1: volumetric first time (mL); V_2: second titration volume (mL); 10^6: convert concentration (ppm) to (g/mL); *V*: analytical sample volume (mL); *W*: weight of sample (g).

Free acid contents

Free acid was quantified by titration. Briefly, 1 g of sample was ultrasonically extracted with 75 mL distilled water for 60 min using an ultrasonic bath (FALC, Model LBS 2, 40 kHz, Italy). The extract was titrated with 0.1 N NaOH until reaching pH 8.30 (12). The free acid content was then calculated according to standard titrimetric equations.

Fig. 1. The portrait of *Opuntia ficus-indica* fruits.

$$\text{Free acid contents (mEq/kg)} = \frac{0.1 \times V_{NaOH} \times 1000}{W} \quad (3)$$

Where, V_{NaOH}: volume of NaOH (mL); W: sample weight (g).

Mineral content

Mineral content was estimated using the dry ashing method. Approximately 3 g of the sample was incinerated in a muffle furnace at 550 °C for 4 hr (12). The residual ash was weighed and expressed as a percentage of total sample mass.

Total lipid content

Crude lipid was determined using Soxhlet extraction. A 2 g dried sample, wrapped in filter paper, was extracted with ether for 6-8 hr. Post-extraction, the sample was dried at 105 °C, cooled and weighed (13). Lipid content was expressed as a percentage of dry weight.

$$\% \text{ Crude fat} = \frac{(\text{weight of cup with fat} - \text{Weight of empty cup}) \times 100}{\text{Sample weight}} \quad (4)$$

Crude protein content

Protein content was measured via the Kjeldahl method. Digestion was carried out with concentrated sulfuric acid and a catalyst mixture, followed by distillation and titration with 0.1 N sulfuric acid. Nitrogen content was calculated and converted to protein using a conversion factor of 6.25 (14). The crude protein content is calculated as:

$$\text{Crude protein content} = \frac{0.0014 \times (V_1 - V_2) \times 100 \times 6.25}{m} \quad (5)$$

Where, V_1: volume of H_2SO_4 added during sample digestion; V_2: volume of 0.1N H_2SO_4 used for titration; m: initial sample mass; 100: conversion to percentage; 0.0014: the mass of 1 milliequivalent of nitrogen (N). 6.25: conversion factor from % nitrogen to protein. This factor is based on the assumption that crude protein contains 16 % nitrogen (100/16 = 6.25). This factor varies depending on the sample source (e.g., 6.39 for milk), but for simplicity in food analysis, 6.25 is used for all samples.

Crude fiber content

Fiber content was determined following AOAC procedures. A 1.5 g sample was sequentially treated with dilute sulfuric acid and potassium hydroxide under reflux. The residue was filtered, dried, incinerated at 500 °C and weighed (15). Fiber percentage was calculated from the mass difference before and after ashing.

$$\% \text{ Fiber} = \frac{\text{Fiber weight} \times 100}{\text{Sample weight}} \quad (6)$$

Experimental design for bioactive compound extraction

The optimization of extraction parameters for TPC and total flavonoid content (TFC) was conducted using Response Surface Methodology (RSM) with a Central Composite Design (CCD). The Design-Expert® software (v13.0, Stat-Ease Inc., Minneapolis, USA) was used for statistical analysis. Independent variables included extraction temperature (°C), time (min) and liquid-to-material ratio (mL/g), while the response variables were TPC (Y_1) and TFC (Y_2). Seventeen experiments, including five replicates at the center point, were performed to model and optimize the responses.

Determination of total polyphenol content (TPC)

TPC was quantified using the Folin–Ciocalteu assay (16-18). One milliliter of the diluted extract was mixed with 5 mL of 10 % Folin –Ciocalteu reagent and incubated for 5 min. Subsequently, 4 mL of sodium carbonate solution (75 g/L) was added and the mixture was incubated at room temperature for 30 min. Absorbance was recorded at 765 nm. Gallic acid standards (0–50 ppm) were used for calibration and results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW). All measurements were conducted in triplicate.

$$A = 0.0057 \times C + 0.0455 \ (R^2 = 0.972) \quad (7)$$

Where, A: absorbance; C: concentration.

Determination of total flavonoid content (TFC)

TFC was assessed using the aluminum chloride colorimetric method (18, 19). One milliliter of extract was mixed with 3 mL of ethanol, 0.2 mL of 10 % aluminum chloride and 0.2 mL of potassium acetate. The mixture was diluted with 5.6 mL of distilled water and incubated for 30 min. Absorbance was measured at 415 nm. Quercetin standards (0–50 ppm) were used to generate the calibration curve and results were reported as mg quercetin equivalents per gram dry weight (mg QE/g DW). Analyses were performed in triplicate.

$$A = 0.0038 \times C + 0.0462 \ (R^2 = 0.9618) \quad (8)$$

Where, A: absorbance; C: concentration.

Results and Discussion

Chemical composition

This study presents a detailed characterization of the chemical composition of *O. ficus-indica* fruits harvested in Ninh Thuan province, Vietnam (Table 1). As observed in previous research, the composition of *Opuntia* fruits varies significantly across species and growing regions, which may account for the differences reported in literature. The protein content of *O. ficus-indica* fruits in this study was 1.619 ± 0.04 %, which is relatively high compared to the typical range of 0.2 % to 1.6 % reported in

Table 1. Results of chemical composition of *O. ficus-indica* fruits

Chemical composition	Ninh Thuan	Mexico (23)	Range (20)
Free acid content (mEq/kg)	136.7 ± 5.77	-	-
Mineral content (%)	21.8 ± 0.05	-	-
Total sugar content (%)	10.7 ± 0.06	-	8 – 7
Total lipid content (%)	1.624 ± 0.02	0.1 ± 0.0	0.09 – 1.7
Free reducing sugar content (%)	10.48 ± 0.1	-	-
Crude protein content (%)	1.619 ± 0.04	1.5 ± 0.0	0.2 – 1.6
Crude fiber content (%)	2.53 ± 0.06	2.3 ± 0.0	0.02 – 3.16

The values are mean values of four replicate samples ± standard error of mean and the values with different alphabetical letters represents values significantly different at the 0.05 level of probability according to the ANOVA.

prior studies. Notably, prickly pear fruits have been reported to contain free amino acids, including essential ones such as proline, taurine and glutamine, contributing to their nutritional value (20).

The total sugar content of the Ninh Thuan samples was 10.7 ± 0.06 %, which is higher than the commonly reported range of 7–8 % in other *Opuntia* fruits but lower than that observed in fruits from Saudi Arabia, which reach up to 12.8 %. The reducing sugar content comprised approximately 98 % of the total sugars, in agreement with previous findings by Sawaya et al., who emphasized that glucose and fructose are the predominant sugars in *Opuntia* fruits, while sucrose is largely absent (21). Lipid content was measured at 1.624 ± 0.02 %, placing it at the higher end of the reported range for *Opuntia* fruits (0.09–1.7 %). However, much higher values have been observed in Egyptian varieties, with lipid content reaching as high as 16.8 %, nearly ten times greater than in Ninh Thuan fruits (22). This finding underscores the influence of geographic and environmental factors on fruit composition.

Other key parameters include a free acid content of 136.7 ± 5.77 mEq/kg, a mineral content of 21.8 ± 0.05 % and a crude fiber content of 2.53 ± 0.06 %. The observed free acid level is consistent with earlier studies reporting low titratable acidity in *Opuntia* pulp (0.01–0.2 %), classifying it as a low-acid food despite a pH range of 5.3 to 7.1, which paradoxically indicates moderately high acidity (20). Although specific mineral concentrations were not quantified in this study, the high total mineral content suggests significant levels of calcium, magnesium and potassium, as supported by previous research. These minerals play an essential role in maintaining electrolyte balance and reducing fatigue during physical exertion (20). The crude fiber content of 2.53 ± 0.06 % was higher than values reported by Valero-Galván et al. (2020) for red (2.3 %) and green (1.5 %) *O. ficus-indica* fruits but lower than the 5.37 % reported by Medina et al. (2007) (23, 24). Literature shows that fiber content varies widely depending on species and maturity stage, typically ranging from 0.02 % to 3.16 %. As previously reported, dietary fiber from cactus fruits has been associated with hypoglycemic and hypocholesterolemic effects, enhancing their nutritional relevance (20).

Fitting the response surface models

This study applied CCD within the framework of RSM to optimize the extraction conditions and model the relationships between response variables and process factors. The three selected response variables TPC, TFC and extraction yield were analyzed under varying conditions of temperature, extraction time and liquid-to-material ratio, as presented in Table 2.

To determine the best-fitting model for the experimental data, sequential sum of squares analysis was performed. Following the recommendations provided by the Design-Expert software, a second-degree polynomial regression model was selected for its superior fit across all response variables. This model effectively represented the relationships between independent variables and their respective responses.

The second-degree polynomial regression equations describing the relationship between the independent variables and the response variables (Y_1 = TPC and Y_2 = TFC) are presented in Eqs. (9) and (10):

$$Y1 = 15.16 + 0.6713A + 0.7050B + 0.3463C + 0.3425AB - 0.3550AC - 0.3175BC - 1.08A^2 - 1.31B^2 - 0.4400C^2 \quad (9)$$

$$Y2 = 2.43 + 0.0240A + 0.0076B - 0.0378A2 - 0.0210B2 - 0.0157C2 \quad (10)$$

Here, A is the liquid-to-material ratio (mL/g), B is the extraction time (minutes), C is the extraction temperature (°C), Y_1 is the total phenolic content (mg GAE/g DW) and Y_2 is the total flavonoid content (mg QE/g DW). The presence of positive and negative coefficients within the equations reflects synergistic and antagonistic effects, respectively, of the individual and interactive variables on the responses.

Analysis of variance (ANOVA) was conducted to assess the significance and adequacy of the second-degree polynomial model. Key statistical parameters for each model are summarized in Table 3. The high F-values and low p-values for the main and interaction terms confirmed their significant effects on the response variables ($p < 0.05$). Moreover, the lack-of-fit tests were non-significant ($p > 0.05$), indicating that the model provided a good fit to the experimental data.

The model's accuracy was further confirmed by the high coefficients of determination (R^2) 0.9924 for TPC and 0.9721 for TFC demonstrating strong predictive capability and goodness-of-fit. R^2 reflects the proportion of variability in the response explained by the model. The coefficients of variation (C.V.) for

Table 2. Experimental of Box-Behnken design

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
		A:Liquid/material ratio	B:Time extraction	C:Temperature extraction	TPC	TFC
		mL/g	Min	°C	mgGAE/g	mgQE/g
1	15	20.0	60.0	60.0	11.84	2.33
2	7	60.0	60.0	60.0	12.46	2.38
3	1	20.0	120.0	60.0	12.39	2.35
4	9	60.0	120.0	60.0	14.38	2.41
5	14	20.0	90.0	40.0	12.28	2.36
6	16	60.0	90.0	40.0	14.37	2.39
7	3	20.0	90.0	80.0	13.62	2.34
8	17	60.0	90.0	80.0	14.29	2.40
9	5	40.0	60.0	40.0	11.92	2.38
10	6	40.0	120.0	40.0	14.14	2.40
11	4	40.0	60.0	80.0	13.31	2.39
12	8	40.0	120.0	80.0	14.26	2.39
13	11	40.0	90.0	60.0	15.35	2.42
14	2	40.0	90.0	60.0	14.93	2.42
15	12	40.0	90.0	60.0	15.06	2.43
16	10	40.0	90.0	60.0	15.18	2.44
17	13	40.0	90.0	60.0	15.28	2.43

Table 3. The projected second-order polynomial models' regression coefficients for the TPC and TFC

Source	Y_1 – TPC			Y_2 – TFC		
	Mean square	F-value	p-value	Mean square	F-value	p-value
Model	2.68	101.10	<0.0001^S	0.0017	27.09	<0.0001^S
A	3.60	135.89	<0.0001^S	0.0046	74.35	<0.0001^S
B	3.98	149.90	<0.0001^S	0.0005	7.54	0.0286^S
C	0.9591	36.16	0.0005^S	<0.0001	0.3906	>0.05^{NS}
AB	0.4692	17.69	0.0040^S	<0.0001	0.7961	>0.05^{NS}
AC	0.5041	19.00	0.0033^S	0.0001	1.16	>0.05^{NS}
BC	0.4032	15.20	0.0059^S	0.0001	1.42	>0.05^{NS}
A^2	4.91	185.15	<0.0001^S	0.0060	97.34	<0.0001^S
B^2	7.25	273.45	<0.0001^S	0.0019	30.17	0.0009^S
C^2	0.8152	30.73	0.0009^S	0.0010	16.73	0.0046^S
Lack of fit	0.0240	0.8421	>0.05^{NS}	0.0001	1.05	>0.05^{NS}
R^2		0.9924			0.9721	
Adjusted R^2		0.9825			0.9362	
C.V%		1.18			0.3286	

S: significant ($p < 0.05$); NS: non-significant.

TPC and TFC were 1.18 % and 0.33 %, respectively. These low C.V. values suggest minimal experimental error and confirm model reliability.

Three-dimensional surface response plots were generated to visually interpret the interaction effects between variables and to identify optimal extraction conditions. These plots illustrated how each process variable influenced phenolic and flavonoid yields individually and in combination.

In conclusion, the RSM-based CCD model effectively optimized the extraction process and revealed key interactions among process variables that significantly impact the recovery of phenolic and flavonoid compounds from *O. ficus-indica* fruit powder.

Response surface analysis of extraction parameters

The extraction efficiency for TPC and TFC was significantly influenced by three main process variables: extraction time, temperature and the liquid-to-material ratio. To visualize their combined effects, three-dimensional surface plots were generated. These plots illustrate the interactions between two variables at a time, while the third variable was held constant at its central (coded 0) level

Effects of process parameters on TPC

The measured TPC, expressed in milligrams of gallic acid equivalent per gram dry weight (mg GAE/g DW), varied between 11.84 and 15.35 across the experimental runs. The highest TPC was recorded in run 13, while the lowest occurred in run 1. Statistical analysis using ANOVA confirmed that the fitted model was highly significant, with an F-value of 101.10 and a p-value < 0.0001. This low p-value indicates that the model's predictive power is not due to random chance. Table 3 indicates all linear terms (A: liquid/material ratio, B: time, C: temperature), interaction terms (AC, BC) and quadratic terms (A^2, B^2) had significant effects on TPC ($p < 0.05$).

As shown in Fig. 2A, the surface plot demonstrates the combined effect of the liquid/material ratio and extraction time on TPC, with temperature fixed at 60 °C. The plot shows that increasing both the liquid/material ratio and extraction time generally improved TPC up to a point. Specifically, optimal TPC was observed within the ratio range of 1:20 to 1:50 and an extraction time of 60–80 min. Beyond this range particularly at the highest liquid/material ratio (1:60) and longest extraction time (120 min) TPC began to decline. This suggests that overly diluted extraction conditions or extended durations may reduce phenolic yield, likely due to degradation or oxidation of polyphenolic compounds. In the short term, an increase in temperature will elevate the phenolic content. However, over longer periods, phenolic content decreased. This aligns with previous reports indicating that polyphenols are thermally sensitive and prone to oxidative degradation (25). Studies by Vajić and Chi support this behavior, attributing initial increases to enhanced solubility and mass transfer as per Fick's second law of diffusion, followed by stabilization once extraction equilibrium is reached (26, 27).

In Fig. 2B, the surface plot shows the interaction between temperature and liquid/material ratio, with extraction time fixed at 90 min. The highest TPC values were observed at the upper end of the temperature range (60 °C), confirming that moderate heat improves polyphenol solubility. The polar nature of water (used as a solvent) likely facilitated this improvement, as heat softened plant tissue and accelerated phenolic release (28). However, excessive temperature or overly prolonged extraction times could lead to polyphenol degradation, highlighting the need to balance intensity and duration.

Fig. 2C examines the interaction between extraction time and temperature at a fixed liquid/material ratio of 1:40. The surface plot illustrates that phenolic content increased with temperature especially up to 60 °C when extraction time was 90 min. However, at prolonged times beyond this point, TPC decreased, likely due to compound degradation. This confirms that 60 °C and 90 min represent near-optimal extraction conditions for maximizing phenolic yield in *O. ficus-indica* fruit.

Effects of process variables on total flavonoid content (TFC)

TFC of *O. ficus-indica* fruit extracts, expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g), ranged between 2.33 and 2.44 across the experimental conditions, with an average yield of 2.39 mg QE/g. The lowest TFC was observed in experimental run 1, whereas run 16 yielded the highest. Analysis of variance (ANOVA) revealed a highly significant model with an F-value of 27.09 and a p-value < 0.0001, suggesting that the probability of this outcome being due to random chance is less than 0.01 %.

Table 3 indicates all linear (A: liquid/material ratio, B: time, C: temperature), interaction (AB, AC, BC) and quadratic (A^2, B^2, C^2) terms significantly influenced TFC ($p < 0.05$). This comprehensive significance indicates a strong model capable of accurately describing the experimental data. The response surface plots (Fig. 3A -C) provide a visual representation of the variable interactions and their effects on TFC.

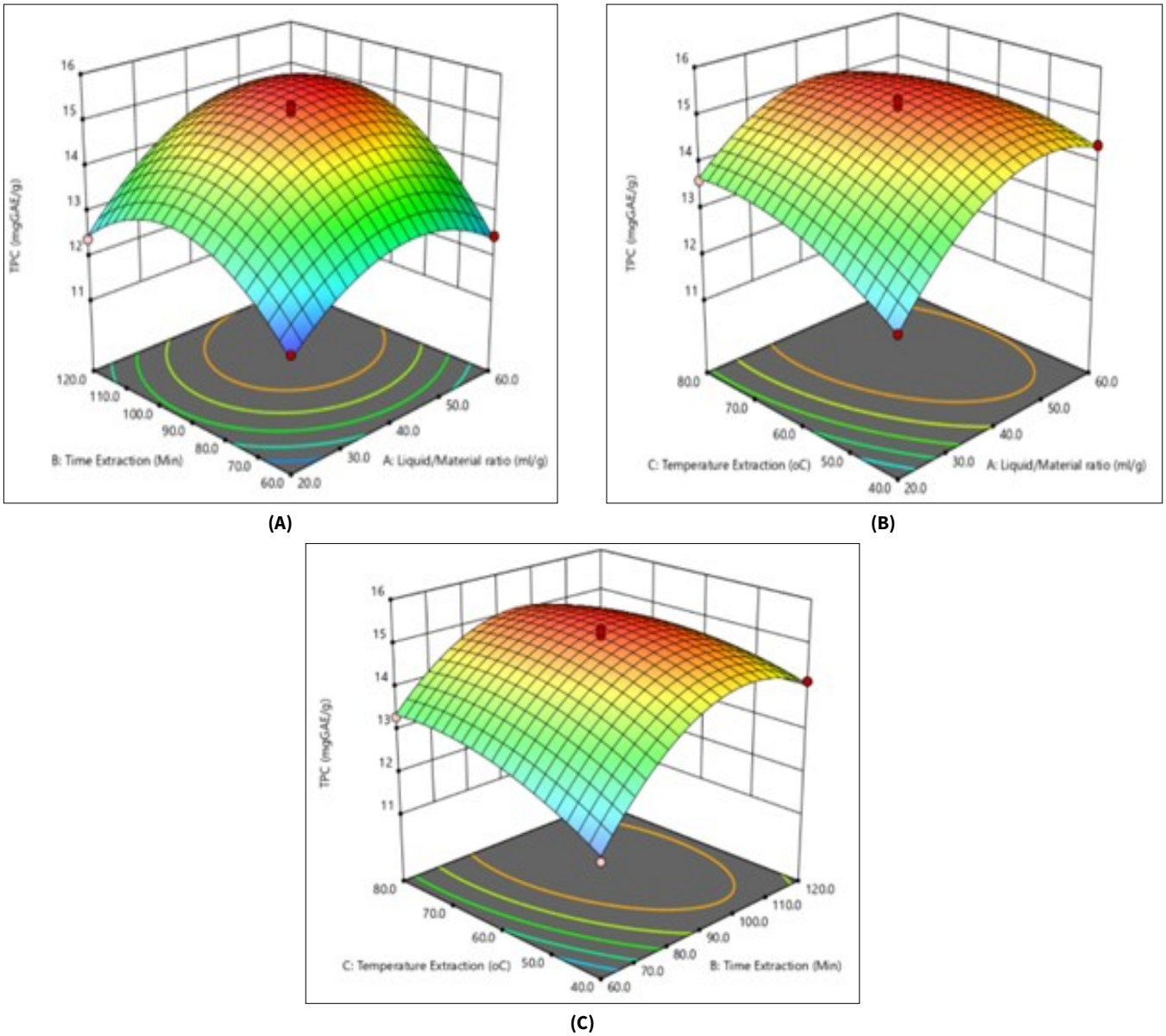

Fig. 2. The response surface plot of TPC.

As shown in Fig. 3A, which holds temperature constant at 60 °C, the interaction between the liquid/material ratio and extraction time is illustrated. The surface plot suggests that TFC is more sensitive to changes in the liquid/material ratio than to extraction time. Nevertheless, prolonging extraction time at any fixed liquid/material ratio contributed positively to TFC yield. Maximum TFC was obtained when the liquid/material ratio ranged between 1:40 and 1:50 and the extraction time extended from 90 to 100 min.

In Fig. 3B, the influence of temperature and liquid/material ratio was examined while holding extraction time constant at 90 min. The response surface reveals that at lower liquid/material ratios, increasing temperature led to a rise in TFC up to a certain point after which the TFC declined. This indicates a non-linear relationship and suggests that thermal degradation may occur beyond the optimal temperature threshold. The ideal range for maximizing TFC was again observed around a temperature of 60 °C and a liquid/material ratio of 1:40 to 1:50.

Fig. 3C displays the interaction between time and temperature at a constant liquid/material ratio of 1:40. Interestingly, under these conditions, temperature had little observable effect on TFC, with the most pronounced increase occurring due to longer extraction times. The data showed that at 60 °C, TFC reached its peak between 90 and 100 min of extraction. This indicates that flavonoid stability under moderate thermal conditions may be less vulnerable than that of phenolics, provided extraction is not prolonged excessively.

Optimization of extracting parameters and model validation

The primary objective of this study was to optimize the extraction conditions to maximize both TPC and TFC from *O. ficus-indica* fruits. Using Design-Expert software and CCD, the optimal extraction parameters were identified as follows: a liquid/material ratio of 1:46.5 g/mL, an extraction time of 96.43 min and a temperature of 59.13 °C.

The response surface and contour plots were instrumental in visualizing the relationship between each process parameter and the responses. These plots were used in conjunction to identify conditions that maximize both TPC and TFC simultaneously. The reliability of the optimization was validated experimentally: the predicted values closely matched the observed experimental results, as summarized in Table 4. This strong correlation, confirmed at the 95 % confidence level, underscores the robustness and predictive accuracy of the fitted polynomial models.

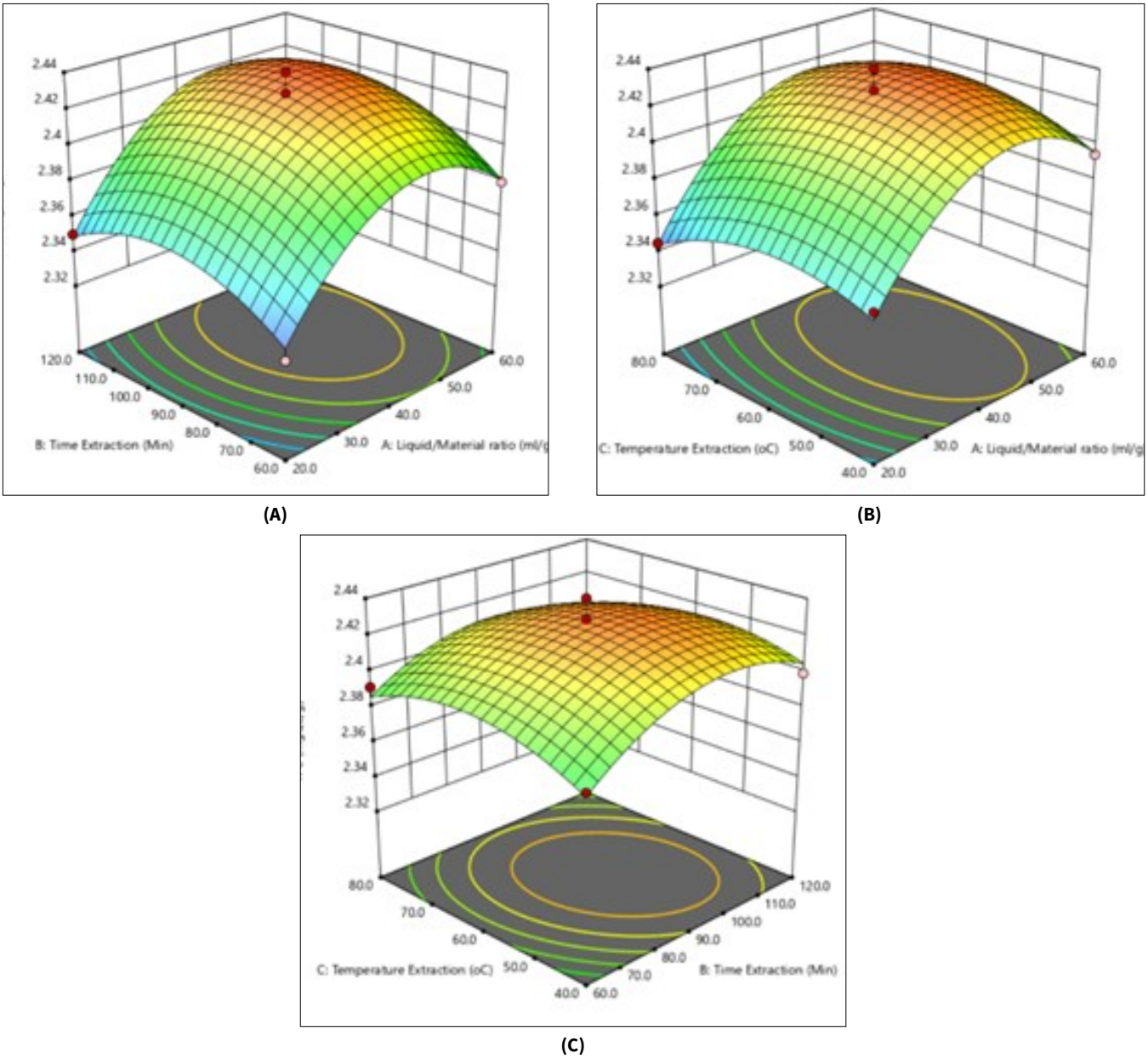

Fig. 3. The response surface plot of TFC.

Table 4. Predicted and experimental values of responses under optimal conditions

Responses	Optimum extraction conditions			Maximum value		% difference (CV)
	A	B	C	Experimental[a]	Predicted	
TPC (mgGAE/g)	46.5 g/mL	96.43 min	59.13 °C	15.355 ± 0.028	15.37	0.097
TFC (mgQE/g)				2.404 ± 0.0428	2.43	1.08

A: liquid/material ratio (ml/g); B: time extraction (min); C: temperature extraction (°C); Y_1: TPC (mgGAE/g); Y_2: TFC (mgQE/g).

[a]Responses are the means ± SD (n = 3).

Conclusion

This study successfully optimized the extraction conditions for maximizing TPC and TFC from Vietnamese *O. ficus-indica* fruits using RSM combined with CCD. The optimal parameters 59.13 °C, 96.43 min and a liquid-to-material ratio of 1:46.5 yielded a TPC of 15.355 ± 0.028 mg gallic acid equivalents (GAE)/g dry weight and a TFC of 2.404 ± 0.043 mg quercetin equivalents (QE)/g dry weight. These values highlight the strong antioxidant potential of *O. ficus-indica* fruits and reinforce their suitability as functional food ingredients.

The findings also emphasize the effectiveness of water-based extraction methods, particularly under carefully controlled temperature and time conditions, for achieving high yields of bioactive compounds. *O. ficus-indica* fruits thus represent a nutrient-rich natural resource with promising applications in both the nutraceutical and food industries.

Future research should focus on validating these antioxidant effects through in vivo studies, as well as exploring the incorporation of *O. ficus-indica* extracts into functional food and beverage formulations. Such efforts would further substantiate the health-promoting potential of *O. ficus-indica* and support its broader utilization in value-added product development.

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Authors' contributions

NKK was responsible for conceptualization, formal analysis, investigation, methodology, software, writing – original draft, writing – review and editing. VTH and NNT contributed to the conceptualization, data curation, investigation, methodology, writing – original draft, writing – review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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