Supercritical carbon dioxide extraction of lutein from yellow maize (*Zea mays*) kernels: process optimization based on lutein content, antioxidant activity, and ω -6/ ω -3 fatty acid ratio

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Abstract Yellow maize kernels were subjected to supercritical carbon dioxide (SC-CO₂) extraction to obtain a lutein-rich extract with potential nutraceutical properties. SC-CO₂ extraction parameters (pressure and temperature) were optimized by employing a full-factorial (3²) design of experiments and response surface methodology, based on yield of lutein, antioxidant activity, and ω -6/ ω -3 fatty acid ratio of the extracts. A Chrastil equation was also developed for predicting the solubility of lutein in SC-CO₂ under different extraction conditions. The optimized extraction condition was obtained at 500 bar, 70 °C for 90 min, at which the extract was found to possess a unique combination of the highest lutein yield (275.00 ± 3.50 μ g/g of dry weight), along with a well-balanced ω -6/ ω -3 fatty acid ratio (3:1). Moreover, the total phenol content and antioxidant activity were also found to be the highest at this condition. This lutein-rich extract is a promising nutraceutical or dietary supplement in the food industry.

Keywords Supercritical carbon dioxide extraction · Yellow maize · Lutein · Antioxidant activity · ω -6/ ω -3 fatty acid

Introduction

Lutein (C₄₀H₅₆O₂), a yellow plant pigment of the xanthophyll family, is commonly used as a natural colorant in foods and feeds and as an antioxidant in nutraceuticals

[1–3]. It is a therapeutically important biomolecule which is reportedly known to reduce risks of cataract, age related macular degeneration (AMD), coronary heart disease, and certain types of cancer [4, 5].

Yellow maize (*Zea mays*), the third most important crop after rice and wheat, owing to its widespread acceptability as a staple food in many populations [6], is considered to be one of the major dietary sources of lutein [7]. The current study endeavors to obtain a lutein-rich extract from yellow maize kernels by employing supercritical carbon dioxide (SC-CO₂) extraction technology. This is a green extraction technique that exerts several advantages over the conventional techniques of solvent extraction and steam distillation. This technique is environment-friendly, the extracts are devoid of organic solvent residues, and there is minimal thermal degradation of phytochemicals during extraction. This process is therefore highly amenable to extraction of phytochemicals from natural (food) matrices [8].

It is opined that this high-pressure extraction process would most likely rupture the oil glands of maize kernels; therefore, ω -6 and ω -3 polyunsaturated fatty acids (PUFAs) could possibly be co-extracted with lutein. It is known that the ratio of ω -6/ ω -3 PUFAs is an important factor for human health, wherein an imbalance may lead to adverse health consequences [9]. An excessive amount of ω -6 PUFA and a very high ω -6/ ω -3 fatty acid ratio may trigger the onset of cardiovascular diseases, cancer, and inflammatory diseases; whereas increased levels of ω -3 PUFAs may exert suppressive effects on the same [10].

This study therefore focuses on the optimization of the SC-CO₂ extraction parameters (temperature and pressure) using a full-factorial (3²) design and response surface methodology (RSM) to obtain an extract with the best combination of phytochemical properties (yield of lutein,

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total phenol content, and antioxidant activity) along with a balanced ω -6/ ω -3 fatty acid ratio. This investigation also aims at determining the solubility of lutein in SC-CO₂ under different temperature and pressure conditions using the Chrastil approach. The Chrastil equation would allow the prediction of lutein yield at different extraction conditions and would certainly be useful for scaling up of the SC-CO₂ extraction process for this bioactive pigment.

To the best of our knowledge, there is no available literature on SC-CO₂ extraction of lutein from yellow maize kernels till date. The current investigation reports for the first time on SC-CO₂ extraction of lutein from yellow maize kernels of West Bengal (in East India) origin. We envisage that this lutein-rich extract could have promising usage as a nutraceutical in the food and pharmaceutical industries.

Materials and methods

Materials

Fresh yellow maize kernels (*Zea mays*, var: HKI 193-1F) were procured from Zonal Adaptive Research Station (ZARS), located at Krishnagar (88°33'N, 23°24'E; 13 m above sea level), West Bengal, India. The plants were grown in well-drained, deep, loamy soil (pH: 7.5–8.5) and in mild climate (18–27 °C, 60–80% relative humidity) [11].

Chemicals such as lutein (xanthophyll, X6250), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and gallic acid were procured from M/s Sigma (Bengaluru, Karnataka, India), and fatty acid methyl esters (FAME) mix [butyric acid methyl ester (C4:0)—nervonic acid methyl ester (C24:1n9)] was procured from M/s Supelco Analytical (St. Louis, MO, USA). Food grade CO₂ was purchased from M/s BOC India Ltd. (Kolkata, West Bengal, India), and the SPE-ED matrix for supercritical fluid extraction (SFE) vessel packing was procured from M/s Applied Separations (Allentown, PA, USA). All other chemicals were purchased from M/s E-Merck (Mumbai, Maharashtra, India). All chemicals, solvents, and buffers used in this work were of analytical reagent (AR) grade.

Preparation of powder samples from maize kernels

Maize kernels were pulverized using an electric grinder (HL 1618, M/s Philips, Kolkata, West Bengal, India), and their particle diameters were determined using the sieve analysis method. The maize samples were screened through a set of standard sieves in a sieve shaker (AS 200, M/s Retsch, Haan, North Rhine-Westphalia, Germany) and the mean particle diameter (d_p) was determined to be

0.42 ± 0.02 mm in accordance with the method reported by Bhattacharjee et al. [12].

Optimization of SC-CO₂ extraction conditions for extraction of lutein from raw maize kernels

SC-CO₂ extractions were performed in a laboratory scale SCF Green Technology SPE-ED SFE 2 model (M/s Applied Separations, Allentown, PA, USA). The extraction vessel was a 50 mL SS 316 cylinder of length 304.79 mm and internal diameter 14.22 mm. The extraction process parameters such as sample batch size, particle diameter, extraction time (= static time + dynamic time), and flow rate of gaseous CO₂ were fixed through several preliminary trials.

From the preliminary extractions, it was observed that a diameter (d_p) greater than 0.42 ± 0.02 mm decreased the surface to volume ratio of the ground maize kernels, which decreased yield of lutein in the extracts; whereas a lower d_p resulted in tight packing of the sample matrix in the extraction vessel, restricting free channeling of SC-CO₂ through the sample matrix. Therefore, the particle diameter of 0.42 ± 0.02 mm was used in all extraction runs. A batch size of 35 g of ground maize powder was required to allow reliable quantification of yields of lutein in the extracts. A batch size greater than 35 g restricted loading the filler material (SPE-ED matrix) in the vessel, which is necessary to control moisture content and minimize voidage in the packed bed. Higher batch size also impeded placement of polypropylene frits and end fittings, essential for high-pressure vessels. Hence a batch size of 35 g was employed in each extraction. Moreover, preliminary trials conducted at three different extraction time (60, 90, and 150 min) established that yield of lutein (the amount of lutein extracted/recovered per 100 g mass of dried maize kernels under varying SC-CO₂ extraction conditions [13]) was significantly higher at 90 min (static time: 60 min, dynamic time: 30 min, after which no extract was obtained) than those obtained at 60 and 150 min of extraction. Therefore the extraction time was kept constant at 90 min. The flow rate of gaseous CO₂ (food grade) was set at 2 L/min because a flow rate above this resulted in sputtering of the extract in the wall of the collection vial and carryover of the same in the outlet tubing leading to a loss in the extract yield. Preliminary trials also suggested that significant yields of lutein with appreciable phytochemical properties of the extracts were obtained in a temperature range of 60–80 °C and a pressure zone of 400–500 bar, beyond which yields of lutein in the extracts were found to be significantly ($p < 0.05$) low. Hence, the upper and lower limits of extraction pressure were fixed at 500 and 400 bar, respectively; whereas for temperature, these limits were set to 80 and 60 °C, respectively.

Based on the results of preliminary trials, the SC-CO₂ extraction parameters (pressure and temperature) were optimized using a 3² full-factorial design, where extraction pressure (400, 450, and 500 bar) and temperature (60, 70, and 80 °C) were varied at three levels. The extracts were collected in screw-capped collection vials (previously wrapped in Al foil) and placed in an ice bath in the dark. They were subsequently weighed, dissolved in high performance liquid chromatography (HPLC) grade dichloromethane (DCM), and stored in nitrogen flushed screw-capped amber colored glass vials at 4 °C until further analyses.

Solvent extraction of lutein from yellow maize kernels was also performed using the classical Soxhlet extraction assembly with 5 g ground, sieved maize kernels ($d_p = 0.42 \pm 0.02$ mm) to quantify the amount of lutein present. The extraction was conducted for 8 h using *n*-hexane as the extracting solvent, in accordance with AOAC official method 920.39A [14]. The extract was then concentrated using a rotary vacuum evaporator (M/s Eyela Corp., Tokyo, Japan) at 40–45 °C and 50 mbar and stored under conditions similar to those mentioned above.

Quantification of lutein in SC-CO₂ and solvent extracts of maize kernels

Lutein-rich extracts (SC-CO₂ and solvent extracts) were subjected to HPLC analyses for quantification of lutein contents, in accordance with the method described by Bhattacharyya et al. [15].

20 µL of lutein-rich extracts (dissolved in DCM) was injected into a JASCO HPLC system, which was equipped with an LC-NET (LC-NET-2/ADC), an isocratic pump (PU-2080 Plus), a degasser (DG-2080-54), and a detector (MD-2015 Plus). A C18 reversed phase column (l = 250 mm and i.d. = 4.6 mm) was used for the separation of lutein from the mixture of compounds present in the extracts, and the flow rate of the mobile phase [acetonitrile/methanol/ethyl acetate (9:1:2)] was maintained at 1 mL/min. The eluents were continuously monitored in a photo diode array (PDA) detector at 447 nm. Identification of peak of lutein was based on its retention time in comparison with standard lutein (M/s Sigma, St. Louis, MO, USA).

Evaluation of phytochemical properties of SC-CO₂ extracts of maize kernels

Antioxidant activities of the extracts were determined by assaying the radical scavenging activities of DPPH [16], and expressed as IC₅₀ values (mg/mL), and also by ferric reducing antioxidant power (FRAP) assay, and expressed as mM FeSO₄ equivalent/g dry weight (DW)

[17]. The total phenolic content in the extracts was also estimated using Folin–Ciocalteu's reagent [18] and was expressed as µg gallic acid equivalent (GAE)/g D.W. The total phenolic content and FRAP values were determined from their respective standard curves of gallic acid and ferrous sulfate heptahydrate (FeSO₄·7H₂O).

Analysis of the morphology of yellow maize grit matrix using a field emission scanning electron microscope (FESEM)

To fully understand the mechanism of SC-CO₂ extraction of lutein from the matrix of yellow maize kernels, the microstructure of the unprocessed and SC-CO₂ processed sample matrices were subjected to microscopic studies by FE SEM. The samples were coated with platinum using an Autofine Coater (JFC-1600, JEOL Company Ltd., Japan) and analyzed using a FESEM (JSM-6700F, JEOL Company Ltd., Japan) operated at 5 kV with a working distance of 8 mm.

Analysis of fatty acid composition of maize grit extracts

Samples of FAME derivatives of fatty acids were prepared using SC-CO₂ extracts of maize kernels at different extraction conditions, in accordance with the method described by Kaushik and Agnihotri [19]. A gas chromatograph (GC) (Trace GC 700, M/s Thermo Fisher Scientific, Waltham, MA, USA) attached to TR-1 capillary column (30 m × 0.32 mm, i.d. 0.25 µm) and equipped with a flame ionization detector (FID) was used to determine the fatty acid profile of the individual FAME sample. The carrier gas used was N₂ at a flow rate of 1 mL/min. The injector and detector temperatures were 250 and 260 °C, respectively. The oven temperature was increased from 60 °C (2 min hold with an increasing rate of 10 °C/min) to 200 °C (with an increasing rate of 3 °C/min) and further increased to 260 °C (8 min hold). 1 µL of each FAME sample (dissolved in *n*-hexane) was injected into the GC injector port in split-less mode for analyses. Identification of fatty acids was conducted using the standard 37-component FAME mix [butyric acid methyl ester (C4:0)—nervonic acid methyl ester (C24:1n9)] of M/s Supelco Analytical (St. Louis, MO, USA).

Determination of solubility of lutein in SC-CO₂ using Chrastil equation

Determination of solubility of lutein in SC-CO₂ at different extracting pressure–temperature conditions is crucial for validation of varying lutein yields, and therefore for the optimization of the extraction process. The solubilities of

lutein were evaluated under varying extraction conditions in accordance with the method of Chatterjee and Bhattacharjee [20]. The solubility was expressed as the ratio of the total mass of extracted lutein (g) and the mass of carbon dioxide consumed in the extraction process:

$$Y = \text{mass of lutein} / \text{mass of CO}_2 = M_o / \rho V \quad (1)$$

where Y is the solubility (mass fraction) of lutein in SC-CO₂ extracts, M_o is the total mass of lutein recovered (g) in the total amount of the extract, V is the volume of carbon dioxide (mL) used for extraction, and ρ is the density of SC-CO₂ (kg/m³) under different extraction conditions. The densities of SC-CO₂ under different conditions (temperature and pressure) were calculated using the empirical Peng–Robinson cubic equation of state [21] in accordance with the method reported by Bhattacharjee et al. [12].

In order to correlate the solubility data of solids and liquids in dense gases, Chrastil equation which gives a linear relationship between the logarithm of solubility of a solute and the logarithm of SC-CO₂ density has been used by several researchers [22–24]. The Chrastil equation [23, 24] of Catchpole and Von-Kamp [25], is as follows:

$$\ln S = k \ln \rho + F + G/T \quad (2)$$

where S is the solubility of lutein in the gas phase (g/kg), k is the association constant related to the total number of molecules in the complex [26], ρ is the density of CO₂ (kg/m³), F and G are empirical constants in density correlation, and T is the temperature (K).

The values of k , F , and G of Eq. (2) were determined and a linear equation was developed which allowed the prediction of solubility of lutein in SC-CO₂ under varying extraction conditions [20].

Statistical analyses

All experiments were performed in triplicate and the data were expressed as mean $\pm$ SD of three independent experimental runs. One-way ANOVA was performed to study the effects of extraction parameters (pressure and temperature) on the response variables i.e., yield of lutein, DPPH radical scavenging activity, FRAP, and ω -6/ ω -3 fatty acid ratio of the extracts. Significant differences between means were determined by Duncan's multiple-range test, using p values ≤ 0.05 . RSM and regression modeling were performed to explore the relation among the explanatory variables (pressure and temperature) and the four different response variables mentioned above. STATISTICA 8.0 software (Statsoft, Oklahoma, USA) was used to test the experimental results.

Results and discussion

Optimization of SC-CO₂ extraction parameters using RSM

Fitting the model

Table 1 represents lutein yields of SC-CO₂ extracts of maize kernels under different extraction conditions. The maximum lutein yield (275 ± 4.65 μ g/100 g D.W.) was obtained at 500 bar, 70 °C after 90 min of extraction. This could be due to the maximum solubility of lutein at this extraction condition (discussed later). This extract also showed maximum phytochemical potency in terms of highest phenol content, minimum IC₅₀ value, and maximum FRAP value, along with a well-balanced ω -6/ ω -3 fatty acid ratio (Table 1).

From the ANOVA tables of regression models, it was established that yields of lutein (Table 2), and FRAP values (ANOVA tables) increased significantly ($p = 0.000$), and IC₅₀ values decreased significantly ($p = 0.001$) with the increase in extraction temperature (in linear terms) from 60 to 80 °C, at constant pressure (400, 450, and 500 bar); and with increasing extraction pressure from 400 to 500 bar, at constant temperature (60, 70, and 80 °C). Similar results of significantly enhanced yields of lutein ($p < 0.05$) with increasing pressure (350–450 bar) and temperature (from 70 to 80 °C) were also obtained in our previous investigation on SC-CO₂ extraction of lutein from African marigold flowers [27]. In another study, Ma et al. [28] reported that pressure and temperature in their linear and quadratic forms had significant effects on yield of lutein extracted from marigold flowers.

Moreover, the ratio of ω -6/ ω -3 fatty acids was also found to increase significantly ($p = 0.000$) with increasing pressure under isothermal conditions; however under isobaric conditions, an opposite trend of significant ($p = 0.000$) decrease in the ratio was observed with increasing temperature (ANOVA tables).

Thus pressure and temperature in their linear and quadratic forms had significant effects on the yields of lutein and on antioxidant activities of the extracts; while pressure in its linear and quadratic forms and temperature in its linear forms significantly affected ω -6/ ω -3 fatty acid ratio of the extracts.

Estimation of lutein content in the solvent extract of maize

The amount of lutein obtained by the Soxhlet extraction process was found to be 168.35 ± 3.22 μ g/100 g dry

Table 1 Experimental yields of SC-CO₂ extracts of maize kernels, yields of lutein and phytochemical properties of the extracts

Pressure (bar)	Temperature (°C)	Yields of maize grit extract (mg/g dry maize grits) ¹	Yields of lutein (μg/100 g dry maize grits) ¹	Total phenolic contents (μg gallic acid equivalent/g dry maize grits) ¹	IC ₅₀ values of DPPH radical scavenging activities (mg/mL) ¹	FRAP values (mM FeSO ₄ equivalent/g dry maize grits) ¹	ω-6:ω-3 PUFAs ¹
400	60	17.16 ± 0.53 ^a	77 ± 2.13 ^a	0.81 ± 0.02 ^a	9.34 ± 0.58 ^a	130 ± 1.25 ^a	1.57
400	70	28.33 ± 1.78 ^b	138 ± 3.65 ^b	2.05 ± 0.12 ^c	7.56 ± 0.39 ^b	232 ± 2.74 ^b	0.20
400	80	70.33 ± 1.74 ^c	153 ± 3.74 ^c	0.92 ± 0.06 ^d	7.12 ± 0.45 ^c	252 ± 3.87 ^c	0.09
450	60	78.34 ± 1.34 ^{cd}	179 ± 4.17 ^d	1.72 ± 0.03 ^a	6.35 ± 0.31 ^d	278 ± 2.19 ^d	9.83
450	70	91.43 ± 1.19 ^d	218 ± 3.49 ^e	2.00 ± 0.05 ^e	5.12 ± 0.29 ^e	300 ± 2.65 ^e	2.89
450	80	135.2 ± 2.17 ^f	239 ± 2.97 ^f	1.12 ± 0.04 ^b	4.89 ± 0.33 ^f	320 ± 5.48 ^f	0.16
500	60	105.5 ± 3.59 ^e	253 ± 2.11 ^g	1.23 ± 0.07 ^b	4.51 ± 0.26 ^g	375 ± 4.38 ^g	28.65
500	70	112.56 ± 2.02 ^{ef}	275 ± 4.65 ^h	2.55 ± 0.14 ^f	3.85 ± 0.17 ^h	402 ± 3.99 ^h	3.00
500	80	156 ± 2.76 ^g	207 ± 3.29 ⁱ	1.42 ± 0.05 ^c	5.96 ± 0.41 ⁱ	289 ± 4.28 ⁱ	0.63

¹ Yields of maize grit extracts, yields of lutein and total phenol contents, IC₅₀ values of of DPPH radical scavenging activities, and FRAP values of maize seed extracts are mean ± SD of three independent extraction runs of three batches of yellow maize kernels

Different letters within a column indicate significant differences at $p < 0.05$

Table 2 ANOVA study to investigate the effect of extraction parameters (pressure and temperature) on yields of lutein of the extracts

Effect	Degree of freedom	Yields of lutein (μg/100 g) of dried maize grits SS	Yields of lutein (μg/100 g) of dried maize grits MS	Yields of lutein (μg/100 g) of dried maize grits F	Yields of lutein (μg/100 g) of dried maize grits P ^c
X ₁ ^a	1	67,344.50	67,344.50	420.86	0.000000
X ₁ ²	1	4760.17	4760.17	29.74	0.000021
X ₂ ^b	1	4050.00	4050.00	25.31	0.000056
X ₂ ²	1	3952.67	3952.67	24.70	0.000064
X ₁ X ₂	1	11,163.00	11,163.00	69.76	0.000000
Error	21	3360.33	160.02	—	—
Total	26	94,630.67	—	—	—

^aX₁-extraction temperature

^bX₂-extraction pressure

^cSignificant at $p < 0.05$

maize kernels. This value was significantly ($p < 0.05$) lower than the maximum value of lutein (Table 1) obtained by the SC-CO₂ extraction process, indicating the superiority of the latter in selective extraction of lutein from yellow maize kernels.

Generation of response curves

The effects of extraction pressure and temperature on lutein yield, IC₅₀ values, FRAP values, and the ratio of ω-6/ω-3 PUFAs are shown in Fig. 1(A–D), respectively. Regression modeling was used to characterize the response surfaces.

Regression modeling

Regression modeling was conducted by generating a second-order polynomial equation for each response as a function of extraction temperature and pressure. The

second-order polynomial equation that fitted our experimental variables is stated below.

$$Y = B_0 + \sum B_i X_i + \sum B_{ii} X_i^2 + \sum B_{ij} X_i X_j \quad (3)$$

where Y represents the experimental responses [yield of lutein (Eq. 4), IC₅₀ value (Eq. 5), FRAP value (Eq. 6), and ω-6/ω-3 fatty acid ratio (Eq. 7)]. B₀, B_i, B_{ii}, and B_{ij} are constants and regression coefficients of the model; X_i and X_j are two independent variables in coded forms. The expanded model includes linear, quadratic, and cross-product terms as shown below (with intercept):

Fig. 1 Plots of response surfaces indicating (A) yields of lutein; (B) IC_{50} values of DPPH radical scavenging activity; (C) FRAP values and (D) ratios of ω -6/ ω -3 fatty acids; as functions of extraction

pressure (400, 450, and 500 bar) and temperature (60, 70, and 80 °C) after 90 min of extraction at a flow rate of 2 L/min with 35 g batch size

$$Y = -5887.06 + 15.63X_1 - 0.01X_1^2 + 64.88X_2 - 0.26X_2^2 - 0.06X_1X_2 \quad (4)$$

$$Y = 197.50 - 0.49X_1 + 0.000X_1^2 - 2.05X_2 + 0.00X_2^2 - 0.00X_1X_2 \quad (5)$$

$$Y = -7116.11 + 15.75X_1 - 0.01X_1^2 + 100.37X_2 - 0.37X_2^2 - 0.10X_1X_2 \quad (6)$$

$$Y = -69.32 + 0.52X_1 + 0.00X_1^2 - 1.34X_2 + 0.04X_2^2 - 0.01X_1X_2 \quad (7)$$

where X_1 and X_2 denote the extraction pressure and temperature, respectively. These equations explain the effect of the two variables, i.e., pressure and temperature on the response Y .

The implication of the above-mentioned extraction parameters and their interactions were investigated. From the ANOVA tables, it was observed that yields of lutein ($p = 0.000$ for X_1 and X_2); IC_{50} values ($p = 0.000$ for X_1 and X_2), FRAP values ($p = 0.000$ for X_1 and $p = 0.002$ for X_2), and ratios of ω -6/ ω -3 fatty acids ($p = 0.000$ for X_1 and X_2) are significantly dependent on linear terms (X_1 and X_2) of both extraction pressure and temperature. All the second-order terms of pressure (X_1^2) and temperature (X_2^2) also showed significant ($p = 0.000$) effects on lutein yields, IC_{50} and FRAP values; only temperature had a significant ($p = 0.000$) effect in its quadratic form on ω -6/ ω -3 ratio. Further, pressure and temperature in combination showed significant ($p = 0.000$) effects on all four responses, i.e.,

yield of lutein, IC_{50} value, FRAP value, and ratio of ω -6/ ω -3 PUFAs of the extracts.

ANOVA of each of the four models showed good F values (Fisher's variance ratio) (Table 2 for yield of lutein, for other parameters data not shown) indicating significant interactions among the variables. The plots of observed values against the predicted values for yield of lutein, IC_{50} value, FRAP value, and ω -6/ ω -3 PUFAs ratio showed a close fit in each case (ANOVA tables). Thus a statistically significant multiple regression relationship ($r = 0.982$ for yields of lutein, $r = 0.985$ for IC_{50} values, $r = 0.982$ for FRAP, and $r = 0.958$ for ω -6/ ω -3 PUFAs) among the independent variables and the responding variables could be established. The complete quadratic model showed a very good fit.

Analysis of response surfaces

The response surfaces are shown in the stereoscopic figures (Fig. 1 A–D). From the test statistics for the regression models as discussed above, it was observed that the extraction pressure and temperature had significant effects on the yields of lutein, IC_{50} values, FRAP values, and ω -6/ ω -3 fatty acid ratio of SC- CO_2 extracts of yellow maize.

Optimal processing condition

For optimization of the SC- CO_2 process conditions, i.e., to determine the optimal values of X_1 and X_2 , the first partial derivatives of the regression equation was conducted with

respect to X_1 and X_2 and set to zero. This was done by putting the second-order regression equation into the matrix form [29, 30]. The point thus obtained is known as the stationary point, X_S ($X_{1S} = 518.37$ bar, $X_{2S} = 67.79$ °C; obtained from the model of yield of lutein). The predicted values of yield of lutein, IC_{50} value, FRAP value, and ω -6/ ω -3 fatty acid ratio of the extract at this stationary point were found to be 267.03 μ g/g of dry maize kernels, 4.11 mg/mL, 380.22 mM FeSO₄ equivalent/g D.W. of maize kernels and 1.5:1.0 respectively (ANOVA tables), suggesting a close fit of the model.

Characterization of response surfaces

The eigen values [29] obtained in the case of yield of lutein (-0.007533 and -0.260401) and the FRAP values of the extracts (-0.000481 and -0.380586) were negative because the X_S of each of these models was a point of maxima. The eigen values for IC_{50} values were 0.008613 and 0.000273. Since these values were positive, X_S was a point of minima. However, for ω -6/ ω -3 fatty acids ratios, X_S was found to be a saddle point with eigen values of 0.048502 and -0.000353 .

The extraction condition of 500 bar, 70 °C, and 90 min, closest to the stationary point (discussed in section ‘optimal processing condition’ under Results and discussion) was considered to be the optimized extraction condition because at this condition, the extract was found to have the best combination of highest yield of lutein, maximum antioxidant activity, and a balanced ratio of ω -6/ ω -3 PUFAs.

Determination of solubility of lutein in SC-CO₂ using the Chrastil equation

The solubilities of lutein in SC-CO₂ in this current study was found to increase significantly with elevation of temperature from 60 to 70 °C in the high-pressure range (400–500 bar) (Fig. 2). A similar finding of enhanced solute concentration with enhanced temperature during SC-CO₂ extraction of lutein from spearmint has been reported by Prieto et al. [31]. However, the temperature regime (40–60 °C) and the sample matrix investigated in their study are markedly different from those of the current investigation.

From the values of the solubility of lutein in SC-CO₂, a linear regression equation was also developed according to the Chrastil equation. The equation generated was as follows:

$$\ln S = 8.91 \ln \rho - 56.13 - 6271.99/T \quad (8)$$

The regression coefficient r obtained using this equation was 0.81, and the standard error of the equation was 0.25.

From this Chrastil equation, the logarithm of the calculated solubility was plotted against logarithm of SC-CO₂ density [20] and presented in Fig. 2. It was found that the plots were linear and the isotherms at 60, 70, and 80 °C were parallel to each other, which confirmed the suitability of the Chrastil equation in this work. The solubility values of lutein calculated at 60, 70, and 80 °C were in fair agreement with the corresponding solubility values predicted by the Chrastil equation, only with an exception at 500 bar, 80 °C; where the experimental solubility of lutein was found to be significantly ($p < 0.05$) lower than that at 500 bar, 70 °C. It could be attributed to the fact that at high pressure (500 bar), temperature higher than 70 °C could have possibly led to co-extraction of waxy materials, impeding extraction of lutein, thereby lowering yields of lutein in the extracts.

Therefore, Eq. (8) can certainly be used to predict the solubility of lutein in SC-CO₂ under different extraction conditions up to 70 °C. This equation clearly stated that the solubility of lutein in SC-CO₂ was the maximum (2.87×10^{-6} g/kg CO₂) under the optimized extraction condition (500 bar, 70 °C, and 90 min).

Evaluation of morphology of maize grit matrix by FE SEM analyses

Figure 3 shows the micrographs obtained using FE SEM of the sample matrices before extraction (Fig. 3A, B) and those of samples post extraction (Fig. 3C, D). In the unprocessed sample matrix, i.e., prior to extraction, it could be observed that the surface pores of the maize grit matrix were closed, whereas in the SC-CO₂ processed sample, the grit tissues were found to be cracked revealing open pores at the kernel surface, which could be due to the rupture of oil bearing glands of the kernels under high pressure–temperature conditions. These morphological changes of the maize kernel matrices would have resulted in the release of oil (along with lutein) from the damaged cells. The oil then diffuses out onto the surface of the maize

Fig. 2 Solubilities of lutein in SC-CO₂, predicted by the Chrastil equation as a function of solvent densities

Fig. 3 Field emission scanning electron micrographs of ground and sieved maize kernel powder matrices: (A, B) pre-extraction matrix and (C, D) post-extraction matrix; at magnifications of 3000 $\times$ and 10,000 $\times$

kernel particles and forms a film that eventually solubilizes into the bulk phase of SC-CO₂, thus rendering the extracts oily in nature. This phenomenon has also been reported by Ghosh and Bhattacharjee [32] in their study on SC-CO₂ extraction of methyl eugenol from tuberose flowers.

Phytochemical analyses of the SC-CO₂ extracts of maize

Analyses of phytochemical properties such as total phenolic contents, DPPH radical scavenging activities, and FRAP values of the lutein-rich SC-CO₂ extracts are presented in Table 1. The highest amount of lutein was obtained in the extract at 500 bar, 70 °C, and 90 min. This extract also possessed the highest amount of phenolic compounds, along with maximum FRAP value and minimum IC₅₀ value. This possibly established the fact that maximum co-extraction of phenolic compounds also occurred at 500 bar, 70 °C, and 90 min; and thus a strong correlation was found between these bioactive components and the antioxidant activity of the extract, as indicated by the IC₅₀ and FRAP values.

Fatty acid composition of SC-CO₂ extracts of yellow maize kernels

The complete fatty acid profiles of lutein-rich SC-CO₂ extracts at nine different extraction conditions are shown in Table 3. These data revealed that the SC-CO₂ extract at optimized condition (based on yield of lutein and phytochemical properties), i.e., at 500 bar, 70 °C, and 90 min also had a well-balanced fatty acid profile- 35.38% saturated fatty acid (SFA), 6.89% monounsaturated fatty acid (MUFA), 55.35% polyunsaturated fatty acid (PUFA), and a desirable ratio of ω -6/ ω -3 PUFAs (3:1), which is within the optimal range of 1:1–4:1. This range is reportedly known to reduce the risks of several chronic diseases (Simopoulos and Cleland [33]). Besides the above extract, two other extracts obtained at 400 bar, 60 °C, 90 min and 450 bar, 70 °C, and 90 min extraction conditions also possessed desirable ω -6/ ω -3 fatty acids ratio, although yields of lutein and antioxidant activities of these extracts were significantly ($p < 0.05$) lower than those obtained at 500 bar, 70 °C, and 90 min (Table 1).

Table 3 Fatty acid (g/100 g of fatty acids) contents in lutein-rich SC-CO₂ extracts of yellow maize kernels at different extraction conditions

Fatty acid carbon no.	Name of fatty acids	400 bar, 60 °C	400 bar, 70 °C	400 bar, 80 °C	450 bar, 60 °C	450 bar, 70 °C	450 bar, 80 °C	500 bar, 60 °C	500 bar, 70 °C	500 bar, 80 °C
C6:0	Caproic acid	-	-	-	0.88 ± 0.02	7.0 ± 0.37	1.37 ± 0.05	1.00 ± 0.04	-	-
C8:0	Caprylic acid	-	4.00 ± 0.29	-	-	-	-	-	-	-
C10:0	Capric acid	2.48 ± 0.16	-	-	0.07 ± 0.00	-	-	-	0.44 ± 0.01	-
C11:0	Undecanoic acid	0.20 ± 0.14	0.6 ± 0.04	-	-	-	0.08 ± 0.00	0.2 ± 0.01	0.09 ± 0.00	-
C12:0	Lauric acid	-	0.4 ± 0.02	-	-	-	-	-	-	-
C13:0	Tridecanoic acid	2.76 ± 0.17	5.0 ± 0.03	0.23 ± 0.01	1.67 ± 0.08	0.69 ± 0.04	0.96 ± 0.07	-	0.01 ± 0.00	0.5 ± 0.02
C14:1	Myristoleic acid	0.17 ± 0.00	-	0.16 ± 0.00	0.08 ± 0.00	0.27 ± 0.01	0.18 ± 0.00	-	0.05 ± 0.00	0.01 ± 0.00
C15:0	Pentadeca-noic acid	-	-	-	0.06 ± 0.00	-	0.07 ± 0.00	-	-	-
C15:1	Pentadeca-noic acid	-	0.08 ± 0.00	-	-	-	0.16 ± 0.01	-	-	-
C16:0	Palmitic acid	-	-	-	0.05 ± 0.00	1.41 ± 0.07	0.17 ± 0.00	-	1.96 ± 0.06	-
C16:1	Palmitoleic acid	4.37 ± 0.31	3.00 ± 0.18	8.24 ± 0.47	2.82 ± 0.13	3.13 ± 0.27	4.32 ± 0.29	1.0 ± 0.07	-	3.5 ± 0.15
C17:0	Heptadecanoic acid	0.20 ± 0.01	0.20 ± 0.01	0.55 ± 0.04	-	0.82 ± 0.05	0.26 ± 0.00	0.10 ± 0.05	0.07 ± 0.00	0.45 ± 0.02
C17:1	Heptadecenoic acid	0.21 ± 0.00	0.10 ± 0.00	-	0.11 ± 0.00	-	0.18 ± 0.00	-	-	-
C18:0	Stearic acid	-	-	-	-	-	-	0.2 ± 0.01	0.06 ± 0.00	-
C18:1 N9c	Oleic acid	2.75 ± 0.12	0.09 ± 0.00	1.56 ± 0.10	1.57 ± 0.08	0.93 ± 0.01	-	1.00 ± 0.08	-	0.9 ± 0.05
C18:1 N9t	Elaidic acid	-	1.00 ± 0.07	4.48 ± 0.31	-	-	0.18 ± 0.00	-	0.98 ± 0.06	-
C18:2 N6	Linoleic acid	0.05 ± 0.00	2.05 ± 0.00	1.31 ± 0.04	0.84 ± 0.05	0.25 ± 0.01	0.11 ± 0.00	2.45 ± 0.14	0.08 ± 0.00	0.55 ± 0.02
C18:3 N6	γ-Linolenic acid	-	-	-	-	-	0.15 ± 0.00	31.36 ± 1.28	-	-
C20:0	Arachidic acid	-	-	-	-	0.38 ± 0.02	0.13 ± 0.00	-	-	-
C20:1 N9	Cis-11-Ei cosenoic acid	0.61 ± 0.03	0.3 ± 0.02	1.56 ± 0.14	0.41 ± 0.03	1.11 ± 0.07	1.12 ± 0.05	1.00 ± 0.00	0.40 ± 0.03	0.30 ± 0.01
C20:2	Eicosadienoic acid	-	-	19.51 ± 1.27	17.00 ± 1.24	-	13.6 ± 1.17	-	-	20.00 ± 1.59
C20:3 N6	Eicosatrien-oic acid	23.36 ± 2.11	20.3 ± 1.58	8.28 ± 0.48	3.31 ± 0.25	24.5 ± 2.16	3.00 ± 0.24	-	41.43 ± 2.63	5.00 ± 0.44
C20:3 N3	Eicosatrien-oic acid	3.47 ± 0.27	-	-	2.36 ± 0.18	5.56 ± 0.38	4.47 ± 0.37	-	8.78 ± 0.39	4.5 ± 0.37
C20:4 N6	Arachidonic acid	-	5.12 ± 0.47	-	2.26 ± 0.14	-	1.00 ± 0.04	-	-	-
C20:5	Eicosapent-enoic acid	0.25 ± 0.01	10.3 ± 1.01	1.17 ± 0.06	-	1.00 ± 0.08	3.58 ± 2.15	1.18 ± 1.06	3.64 ± 0.22	-
C21:0	Henicosanoic acid	33.05 ± 2.53	30.00 ± 1.99	16.56 ± 1.22	35 ± 2.28	30.00 ± 2.53	28.05 ± 2.29	25.00 ± 1.94	25.22 ± 1.66	41.64 ± 2.64
C22:0	Behenic acid	5.17 ± 0.41	12.00 ± 1.16	6.37 ± 0.04	5.00 ± 0.38	6.82 ± 0.57	2.66 ± 0.14	5.00 ± 0.38	7.53 ± 0.05	3.2 ± 0.26
C22:1N9	Erucic acid	14.54 ± 1.19	-	17.33 ± 1.32	3.00 ± 0.18	11.01 ± 1.00	8.74 ± 0.68	20.00 ± 1.43	5.44 ± 0.65	10 ± 0.78
C22:2	Docosadienoic acid	3.43 ± 0.21	-	8.33 ± 0.53	-	1.46 ± 0.05	7.7 ± 0.53	10.00 ± 0.76	1.38 ± 0.11	2.00 ± 0.08
C22:6	Docosahexa enoic acid	-	-	4.85 ± 0.38	22.11 ± 2.08	2.0 ± 0.16	20.06 ± 2.01	-	1.41 ± 0.10	4.3 ± 0.23
-	Other fatty acids	0.70	0.51	1.01	3.51	1.69	0.39	0.51	2.36	3.15
-	SFA	43.88	52.2	23.73	40.05	47.13	33.79	31.5	35.38	45.79
-	MUFA	19.26	4.57	31.78	7.99	16.46	11.90	23	6.89	14.71
-	PUFA	36.15	42.72	43.46	48.45	34.77	53.90	44.99	55.35	36.35
-	ω-6/ω-3 ratio	1.57	0.20	0.09	9.83	2.89	0.16	28.65	3.00	0.63

Data are mean ± SD of three independent experimental runs

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

Therefore, SC-CO₂ extraction condition at 500 bar, 70 °C, and 90 min is the best condition for the extraction of lutein-rich therapeutic fraction from yellow maize kernels.

Lutein content of this extract was also higher than the conventional solvent extract, attesting the superiority of SFE in selective extraction. A correlated Chrastil equation developed on the solubility of lutein in SC-CO₂ under different extraction conditions further validated the highest yield of lutein at the said condition. This extract also possessed a desirable fatty acid profile with a balanced ω -6/ ω -3 ratio of 3:1. We envisage that this lutein-rich extract could have potential applications in food and pharmaceutical industries.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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