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Supercritical fluid extraction of β -carotene from ripe bitter melon pericarp

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Study ascertained the recovery of β -carotene from enzyme-treated (enzyme load of 167 U/g) pericarp of ripe bitter melon using supercritical fluid extraction (SFE) technique. Effect of different pressure (ranged from 150–450 bar), carbon dioxide (CO₂) flow rates (ranged from 15 to 55 ml/min), temperatures (from 50 to 90 °C), and extraction periods (from 45–225 minutes) were observed on the extraction efficiency of β -carotene. Results showed that extraction pressure (X₁) among extraction parameters had the most significant ($p < 0.05$) effect on extraction efficiency of the β -carotene followed by allowed extraction time (X₄), CO₂ flow rate (X₂) and the temperature of the extraction (X₃). The maximum yield of 90.12% of β -carotene from lyophilized enzymatic pretreated ripe bitter melon pericarp was achieved at the pressure of approx. 390 bar, flow rate of 35 mL/min, temperature at 70 °C and extraction time of 190 min, respectively. Based on the accelerated storage study the 70% retention shelf life of the β -carotene into extract was estimated up to 2.27 months at 10 °C and up to 3.21 months at 5 °C.

Increasing the demand for natural colorants, concerning the derivation of researchers has rewarded the attention towards new biological resources instead of attention to chemical synthesis^{1–6}. Several natural colors such as anthocyanin, carotenoids, chlorophyll, betalains, iridoids, phycobiliproteins, etc. are extensively have been studied for their potential as a natural food colorant^{2,6–9}. Moreover, these natural colorants have nutraceutical properties, which helps to fight against several diseases such as cancer, cardiac, inflammation, diabetes, neural problems, etc.^{1,6}. Carotenoids are the yellowish-red pigments found in many plants, algae, and phototropic bacteria and have attracted vast research attention in the global market due to their potent antioxidant properties^{8,10,11}. Beta-carotene (β -carotene) is one of the extensively used carotenoids as either additives or dietary supplements since years and helps in preventing several types of cancer including, lung, stomach and skin^{12–15}. It is rehabilitated in the human body as a precursor of vitamin A (retinol) that is indispensable for appropriate function of the retina, epidermis and mucous membranes¹⁶ and providing other health benefits, including the possible prevention and treatment of cardiovascular disease^{15,17}.

At present the commercial production of the β -carotene is done by either chemical synthesis using β -ionone or from limited selective natural resources^{2,6,10,18}. Among the natural sources of the beta-carotene viz., *Dunaliella* a green microalgae contains 2–3 g of beta-carotene per litre¹⁹, carrot contains 110 μ g per 100 g of fresh weight²⁰ and *Flavobacterium multivorum* a bacteria contains 7.85 μ g per milliliter¹⁰ are the most commercial and widely used.

Bitter melon (*Momordica charantia* L.) is a climbing plant of *Momordica* genus mostly grown in Asian, African and Caribbean countries that have been used for various curative purposes²¹. The outer layer of the fruit is rough, known as pericarp, inner smooth tissue is an appendage or covering of seed called as aril. After ripening bitter melon, fruits turn to yellow due to their rich in carotenoids. Cultivation of ripe fruits at an industrial scale as well as farmer level is only for seed production however the waste and by-products generated during seed processing constitute a great source of β -carotene (967 μ g/100 g fresh weight), can be a potential for the commercialization for the beta-carotene production^{22,23}.

Traditionally the extraction process of valuable compounds from agricultural produces is usually performed by using organic solvents; however, this chemical method may be toxic and has some pollution concerns²⁴. One alternative to traditional extraction by organic solvents is to accomplish the extraction by CO₂ based supercritical

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fluid extraction (SFE) method, which is used to extract numerous bioactive compounds due to its inflammable, protective solvent in nature that responsible for an anaerobic extraction results better stability than the organic solvent extraction^{25,26}. The application of SFE technique to recover the essential compounds is more effective than other techniques, especially considering environmental protection. Moreover, eliminate the process of after separation from extracts resulting extensively pure²⁷. β -Carotene extraction with SFE from tray dried carrots is an excellent technique suitable to replace the use of harmful organic solvents and satisfy the increasing demand for biological solvent-free β -carotene²⁰. The extraction efficiency of targeted compounds depends upon the applied extraction parameters such as extraction pressure and temperature that both are significant SFE equipped constraint. In both indispensable characteristics of supercritical fluid, other factors such as the interaction between targeted compounds and ecological aspects are also important to extraction efficiency²⁸. Due to these multi-faceted relations, a solitary condition of SFE cannot give sufficient information. To defeat this complexity, response surface methodology (RSM) a statistical investigational design has frequently been implemented to assist seems to be the optimum process factors^{29,30}. Except for instrumental parameter, other factors such as enzymatic digestion, heat treatment, particle size, moisture content matrices play a key role in the extraction efficiency of targeted compounds^{31,32}. These pretreatments may humiliate the complex structure of matrixes with targeted compounds and make it easily available and improve the content also by conversion process^{33,34}. Since the cell wall of plants is composed of main polysaccharides (cellulose, hemicelluloses) and heteropolysaccharide (pectin), most of the bioactive compounds are contained in these saccharides and bind to gather tightly. Enzymes mainly cellulase, pectinase, protease, and α -amylase are used to make extraction efficiency of these compounds^{11,34}.

Enzymatically pretreated and solvent added SFE of bioactive compounds were performed by many researchers^{34,35}. SFE of β -carotene from different bio-resources were also studied^{20,36}. However, very little or no reports are available on SFE of β -carotene from the ripe bitter melon pericarp. Hence, an attempt was made to investigate systematically the effects of SFE parameters on the percent yields of β -carotene from pretreated followed by lyophilized pericarp of ripe bitter melon.

In particular, interest lies in determining the effect of different SFE parameters on the extraction efficiency of beta-carotene from ripe bitter melon pericarp. A response surface methodology (RSM) tool is applied to optimize best set of combination of pressure, temperature, time and flow rate. Extracted beta-carotene is quantified using supercritical fluid chromatography (SFC) technique.

Materials and Methods

Materials. Ripe bitter melon was procured from the orchard of Indian Agricultural Research Institute, New Delhi. Chemicals and reagents in this experiment were purchased from Merck, KGaA-64271, Darmstadt, Germany. β -Carotene standard was purchased from Sigma-Aldrich (Chemie GmbH, Taufkirchen, Germany). Pectinase (456 U/g, *Aspergillus* sp.) was purchased from Sigma-Aldrich (Japan) and deep tube liquid CO₂ (99.98%) from Amit labs, New Delhi, India.

Sample preparation. Ripe bitter melon was washed in tap water to remove dust and foreign materials. The top and bottom portion of washed fruit were removed, then the whole fruit was cut one-sided lengthwise with help of knife. The aril with the seed of fruits was removed manually and collected pericarp was kept at -20°C temperature.

Enzymatic digestion of pericarp. For the enzymatic pretreatment of the ripe bitter melon pericarp a method described by Ranveer *et al.*³⁵ was used. A working enzyme solution of 167 U/g was prepared by diluting the stock enzyme solution into citrate buffer (pH 5.0)³⁵. Reaction mixture was prepared in the ratio of 3:1 (enzymatic citrate buffer solution: pericarp). The reaction mixture was continuously stirred at 25°C for 4 hours. This mixture was filtered through Whatman No. 42, and the residue was kept in a deep freezer (-80°C) for 6 hours. The pericarp was lyophilized by Labconco lyophilizer (Kansas, USA) at the temperature of -55°C with a vacuum of 0.1 mbar for 72 hours. The lyophilizing jars were wrapped with aluminum foil (11 μm thickness) to avoid degradation.

Supercritical fluid extraction (SFE) of β -carotene. Before feeding the sample in 1 litre steel vessel of SFE, the lyophilized pericarp was powdered and sieved with standard 35 BSS mesh (500 μm pore size). Extraction of β -carotene from powdered pericarp matrixes was carried out at each of the experimental combinations through an automated SFE system (Model 7100, Thar Technologies Inc., USA). For each extraction run, the extraction vessel was loaded with 50 g of powdered pericarp matrix. Ethanol (5%, w/w) was used as a co-solvent to enhance the extraction yield³⁰. SFE parameters during the study were controlled by software (SuperChrom SFC Suite v5.9, Thar Technologies Inc., USA). The systematic representation of the SFE system for extraction of β -carotene from powdered matrix shown in Fig. 1, which comprises two high-speed pumps, one is CO₂ pump (280 mL/min) and another is modifier pump (150 mL/min). The extracted β -carotene at the end of each experiment was collected, vacuum concentrated and stored at -20°C until quantitative analysis.

Analysis of total β -carotene (TBC). The extracted β -carotene was characterized quantitatively using supercritical fluid chromatography based ultra-performance conversance chromatography (Acquity UPC² system, Waters Technologies, USA) equipped with a reverse-phase analytical polymeric High Strength Silica C18 (HSS C18 SB), 3×100 mm with particle size 1.8 μm , as reported by Runco *et al.*³⁷. Empower³ software was used to operate the system during the quantitative analysis of samples.

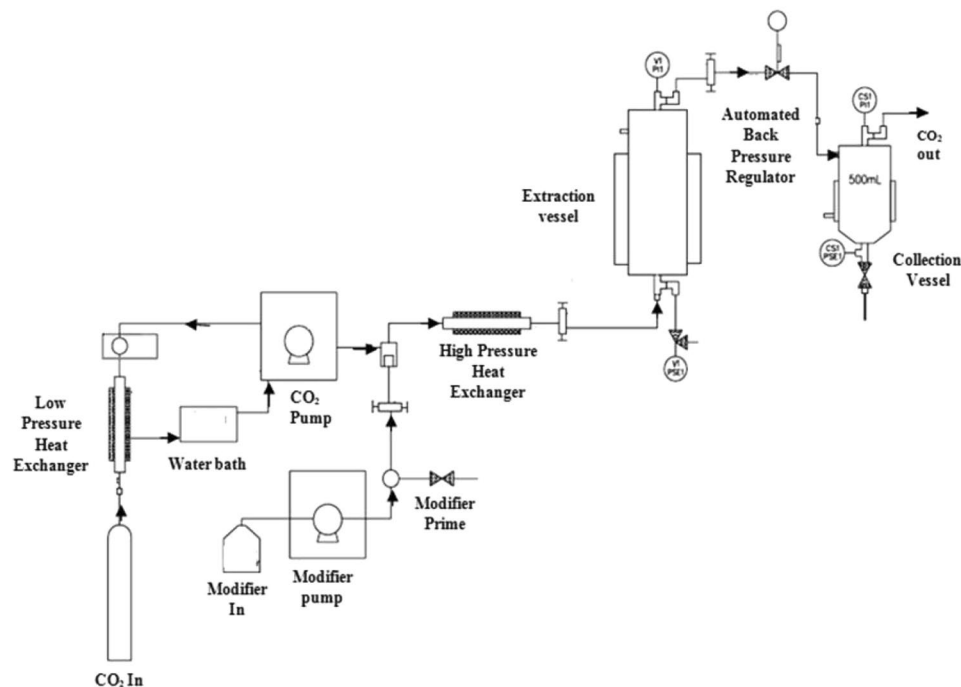


Figure 1. SFE process flowchart used in the extraction of β -carotene from the ripe bitter melon pericarp.

Independent variables	Symbols	Coded variable levels				
		-2	-1	0	1	2
Pressure (bar)	X_1	150	225	300	375	450
Flow rate (mL/min)	X_2	15	25	35	45	55
Temperature ($^{\circ}$ C)	X_3	50	60	70	80	90
Time (min)	X_4	45	90	135	180	225

Table 1. Central composite rotatable design (CCRD) showing independent variables, their levels and responses.

Experimental layout. For optimization of the extraction parameters, the independent variables were coded as X_1 (pressure), X_2 (flow rate), X_3 (temperature), and X_4 (time) for maximum recovery of β -carotene using supercritical CO_2 based SFE technique from ripe bitter melon pericarp matrices (Table 1). The whole experiment was designed to use the central composite design (CCD) of response surface methodology (RSM) resulted in thirty experiments and each was conducted for the optimization studies (Table 2). Data pertaining to five independent and one response variable were analysed to get a multiple regression equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 \quad (1)$$

where Y refers to the measured predicted % yield, b_0 is the intercept; b_1 , b_2 , b_3 , and b_4 are the linear terms; b_{12} , b_{13} , b_{14} , b_{23} , b_{24} , and b_{34} are interaction coefficients and b_{11} , b_{22} , b_{33} , and b_{44} are quadratic terms, respectively.

Kinetics of accelerated storage study. The shelf-life prediction of foodstuff is based on environmental circumstance viz., temperature, humidity, microbes, etc., and its reaction kinetics. Under these environmental circumstances, the temperature is decisive to influence the storage kinetics. At optimum condition storage kinetics of SFE extract was conducted as are in the model suggested by Dien *et al.*³⁸. The extract was stored in transparent and amber-colored 30 mL airtight vial at 45 $^{\circ}$ C and 55 $^{\circ}$ C in incubator until it degraded up to 80%. The frequency of analytical testing is the next important decision. The higher the storage temperature, the more frequent should be the testing.

Results and Discussion

During SFE, the moisture content of lyophilized pericarp powder was 6.45% (d.b.). The quantitative analysis of β -carotene content was studied by supercritical CO_2 based UPC² system using the standard calibration curve of 0, 25, 50, 75, 100, 125 and 150 ppm (Fig. 2). Initially, β -carotene in ripe fresh pericarp was 7.63 mg/100 g, however, enzymatic digestion increased it up to 21.05% (9.72 mg/100 g) and 85.54 mg/100 g in digested dried powder. Enzymatic treatment improved the β -carotene content due to degradation of their interfacial tension,

Run	Independent variables levels (coded)				Yield (%)	
	X ₁	X ₂	X ₃	X ₄	Experimental	Predicted
1	-1	-1	-1	2	30.13	33.51
2	-1	1	-1	1	45.44	42.50
3	1	-1	-1	1	76.12	75.67
4	-1	-1	1	1	48.84	43.14
5	-1	-2	1	-1	35.46	36.02
6	1	-1	1	1	51.60	54.63
7	1	1	-1	-1	45.33	51.13
8	1	1	1	1	84.77	81.51
9	0	0	0	0	76.51	76.53
10	0	0	0	0	78.48	76.53
11	0	0	0	0	77.53	76.53
12	0	0	0	0	75.69	76.53
13	0	0	0	0	74.91	76.53
14	0	0	0	0	76.12	76.53
15	-1	-1	-1	1	43.64	42.21
16	-1	1	-1	-1	31.00	27.62
17	1	-1	-1	-1	50.11	52.90
18	-1	-1	1	-1	37.43	38.33
19	1	1	-1	1	81.29	80.08
20	-1	-1	1	1	50.11	47.01
21	1	-1	1	1	70.44	73.51
22	1	1	1	-1	55.34	56.45
23	0	0	2	0	68.26	69.81
24	0	0	-2	0	64.96	63.56
25	2	0	0	0	80.02	74.43
26	-2	0	0	0	14.81	20.54
27	0	2	0	0	37.79	40.87
28	0	-2	0	0	41.71	38.77
29	0	0	0	-2	45.41	38.19
30	0	0	0	2	64.57	71.95

Table 2. Central composite arrangement for independent variables.

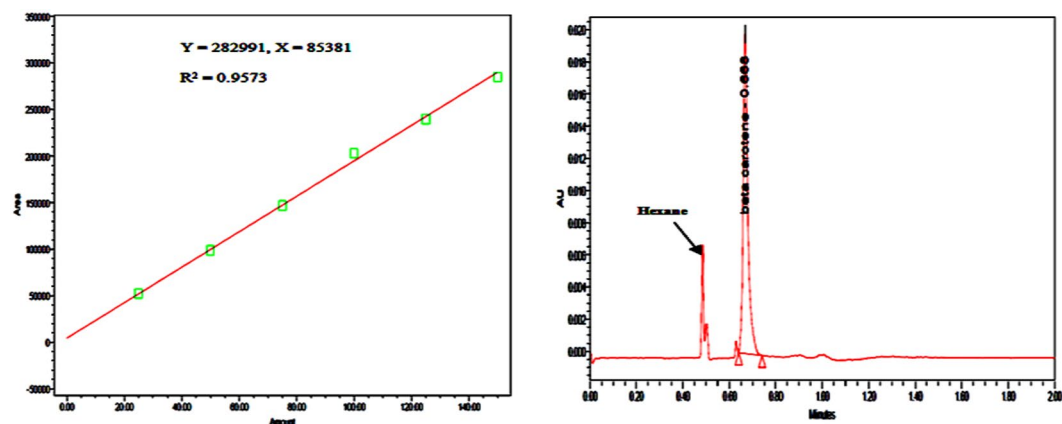


Figure 2. Supercritical fluid chromatography based UPC² calibration curve (A) used in quantification of β -carotene extracted at the optimized condition and its chromatogram (B).

which increases its availability^{11,34}. The reported results were in agreement with Lenucci *et al.*³⁴ for tomato processing waste in which enzymatic treatment increased lycopene recovery up to ~150%. Vuong and King³⁹ also reported the similar content of β -carotene in fresh *Momordica* genus ripe fruit (gac fruit) was 8.3 to 76.9 mg/100 g. However, Tran *et al.*⁴⁰ reported 37.9 mg/100 g in gac powder.

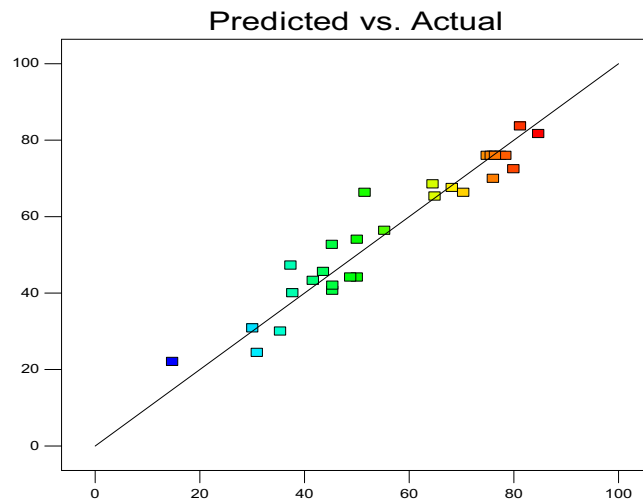


Figure 3. A plot of predicted and experimental value for the % yield of β -carotene extracted from ripe bitter melon pericarp using SFE technique.

Regression	Yield			
	DF	Sum of Squares	F-Value	Pr > F
Linear	4	6132.400179	65.36	<0.0001
Quadratic	4	3613.201070	38.51	<0.0001
Cross product	6	290.457584	2.06	0.1195
Total Model	14	10036	30.56	<0.0001
Lack of Fit	10	343.518284	20.66	0.0019
Pure Error	5	8.315508	—	—
Total Error	15	351.833792	—	—

Table 3. Analysis of variance of (ANOVA) independent variables for the extraction of β -carotene from the ripe bitter melon pericarp. Note: R-Squares 0.9661, Degree of freedom (DF).

Extraction optimization. The data pertaining to the independent and response variables were analysed to get a regression equation with linear, square and interaction coefficients as follows:

$$\begin{aligned}
 Y = & -360.262743 + 0.837487X_1 + 4.973891X_2 + 3.890651X_3 + 0.621753X_4 \\
 & + 0.001374X_1X_2 - 0.001029X_1X_3 + 0.001042X_1X_4 + 0.008958X_2X_3 \\
 & + 0.003432X_2X_4 - 0.002163X_3X_4 - 0.001291X_1^2 - 0.09177X_2^2 \\
 & - 0.024613X_3^2 - 0.002650X_4^2
 \end{aligned} \quad (2)$$

The predicted values of β -carotene content were calculated using the regression model and compared with experimental values. The value for the coefficient of determination (R^2) was 0.966 which indicates the adequacy of the applied model. The statistical analysis of data revealed that linear, quadratic and model were significant (Table 3). The ANOVA also showed that there was a non-significant ($p > 0.0019$) lack of fit which further validates the model. The scattered plot between the experimental values and difference between the experimental and predicted values did not show a pattern that further indicated the adequacy of the model (Fig. 3). The levels of independent variables for optimal extraction conditions of β -carotene content were determined using response surface graphs plotted between each independent variable (Table 3). Variation in extraction pressure (X_1) showed the most significant effect results in an increase in β -carotene recovery. Maximum β -carotene content was obtained at 393.32 bar; however, further increase in pressure up to 450 bar showed negative effect in % yield (Fig. 4A). Increase in pressure beyond a critical limit decreases the diffusion ability of supercritical CO_2 mainly because of the enhanced compaction of the samples at higher pressure leading to chainaling of the supercritical CO_2 around it rather than diffusing through it^{41,42}. Kaur *et al.*²⁰ reported similar trends for SFE of β -carotene from tray dried carrot. Results in case of flow rate (X_2) was observed as list significant change in β -carotene yield; however, at a fixed flow rate of 35 mL/min was found to be the best. The increase in flow rate beyond 35 mL/min reduces yield significantly. This may be either the enhanced rate of dissolution of solute into the solvent or solvent might have passed touching the sample rather than penetrating inside it, because of the reduced solute-solvent interaction and dwell time of the sample in the extraction vessel as described by Topal *et al.*⁴³. Temperature is an important consideration in any extraction on SFE. As an expected increase in temperature up to about 70 °C

Response Surfaces

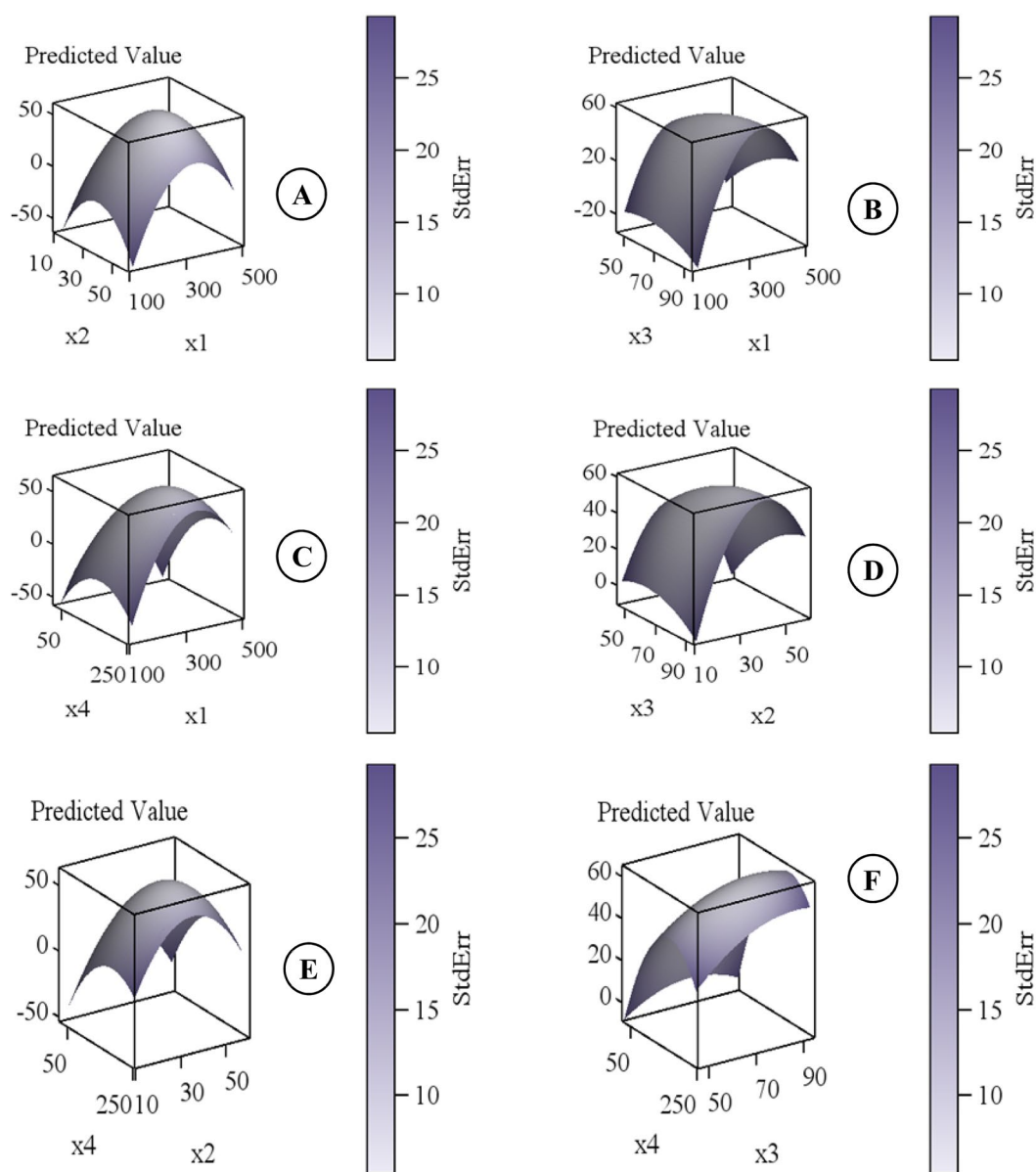


Figure 4. Response surface plot showing effects of independent variables on % yield (A–F) from ripe bitter melon pericarp while the remaining were kept at the central point (pressure: X_1 - 300 bar; flow rate: X_2 - 35 mL/min; temperature: X_3 - 70 °C; and time: X_4 - 135 min).

Factor	Critical Value		Coding Coefficients	
	Coded	Un-coded	Subtracted off	Divided by
X_1	0.62	393.32	300.00	150.00
X_2	0.099	36.98	35.00	20.00
X_3	-0.04	69.15	70.00	20.00
X_4	0.62	190.36	135.00	90.00

Table 4. Canonical analysis of response surface based on coded data for TBC and yield. Predicted value at the stationary point for % yield = 90.11.

increases β -carotene yield at any given pressure since higher temperatures promote the solubility of solute and increase the % yield by the high mass transfer of solute in the matrix⁴⁴. However, the same reduces drastically with any further increase in temperature beyond 70 °C. This can be explained by the loss of balance between

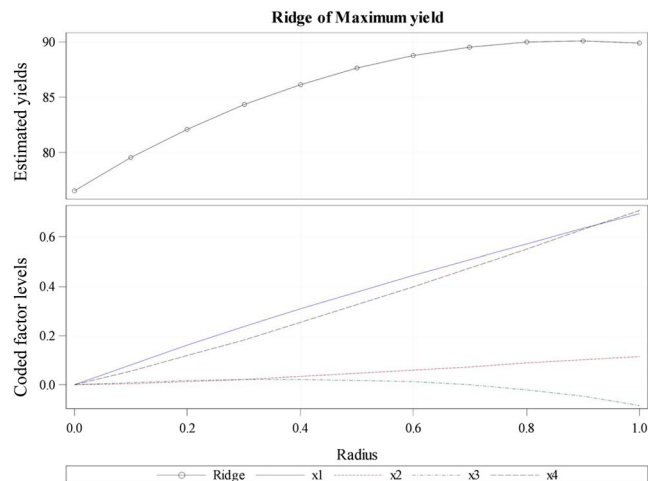


Figure 5. An estimated ridge of maximum response for variable % yield.

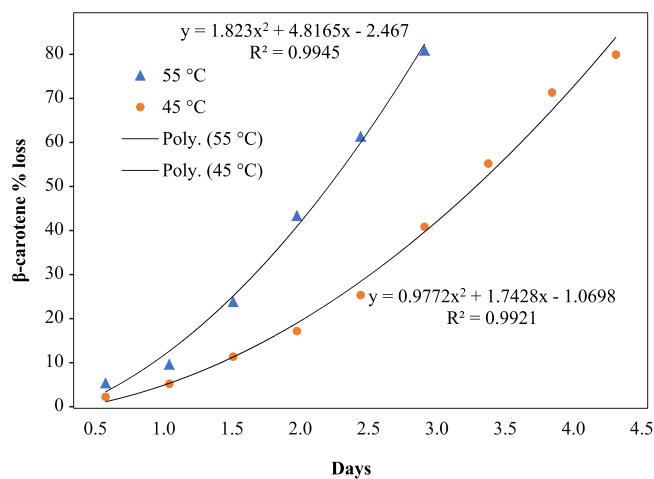


Figure 6. Effect of temperature 55 °C (A) and 45 °C (B) percentage loss and TBC extracted from ripe bitter melon pericarp at optimum condition.

the supercritical CO₂ density and the solute vapor pressure. The effect of temperature at any given time period also remains the same up to a threshold value beyond which the extraction yields have significantly reduced (Fig. 4B). This could be due to the adverse effect of temperature leading to β-carotene degradation and isomerization as suggested by Gomez-Prieto *et al.*⁴⁵ and Nobre *et al.*⁴⁶. Extraction time, in fact, decides the amount of supercritical CO₂ available for the extraction process. In case the available supercritical CO₂ is a limiting factor, the completeness of extraction is adversely affected⁴⁷. However, the increase in time period beyond a point where the available supercritical CO₂ suffices the completeness of extraction could lead to detrimental effect because of other controlling parameters like temperature. Pressure level as described earlier could play a significant role either in aiding extraction by solvent densification or limit it because of sample compaction^{45,47}. Hence, an appropriate balance between the two factors is essential for maximization of β-carotene yields (Fig. 4C). This is further strengthened by the surface plot between the temperature of extraction and the flow rate of supercritical CO₂ (Fig. 4D). It can be clearly seen that increase in extraction time significantly enhances the β-carotene yields since it leads to enhance the time of the solvent with the solutes thereby enhancing the penetration and subsequent extraction of β-carotene from the sample matrix; however, increasing inflow rate of supercritical CO₂ resulted in no significant effect (Fig. 4E). The interface between extraction time and temperature as shown in Fig. 4F was found to be significantly influenced by these independent variables. At initial extraction temperature increasing the time, resulting in an advantageous effect on β-carotene yield, however, a further increase in the temperature beyond 70 °C lead to extensive degradation of thermo-sensible β-carotene resulted in the loss of yield^{41,47}. Based on a statistical analysis of data using PROCRSREG of SAS, it was found that a maximum extraction efficiency of 90.12% of β-carotene could be achieved using 69.15 °C temperature, 393.31 bar pressure, 36.98 mL/min flow rate for 190.36 min (Table 4).

Confirmatory studies. Additionally, three experimental runs were conducted at the optimum combination of independent variables to validate the same. The extraction yield obtained was 91.61%, 88.92% and 87.56% (mean value of $89.36 \pm 0.68\%$) indicating good agreement with the results using statistical modeling. For justification of the above independent variables, the estimated ridge of maximum response for the dependent variable (% yield) shown in Fig. 5, revealed that the maximum yield was 90.099% at stationary point X1 = 395.002 bar, X2 = 37.03 mL/min, X3 = 69.03 °C and X4 = 191.66 min.

Effect of storage temperature on storage stability. In our experiments, we recognized quite clearly that total carotene decrease day by days when preserving β -carotene at 55 °C than 45 °C. Although all sample is kept in an incubator in airtight amber color vials, β -carotene owing to decomposition at high temperature, its bound energy goes from basic energy to excitation energy so molecule breakdown. At higher storage temperature the storage stability was 2.5 days with 90.48% loss than lower temperature (45 °C) 5 days with 89.41% loss (Fig. 6). Calculating from the above figure using polynomial equations, in order to get carotene 30%, it should keep within 3.09 days (55 °C) and 6.16 days (45 °C).

$$Q_{10} = \frac{6.16}{3.09} = 1.99 \quad (3)$$

where Q_{10} is increase in the rate of the reaction when the temperature is increased by 10 °C during storage. Storage duration at of β -carotene at 10 °C (Eq. (4)) and 5 °C (Eq. (5)) (carotene 30% reduction) will be:

$$F_2 = f_1 \times Q_{10}^{\frac{\Delta}{10}} = 3.09(199)^{\frac{55-10}{10}} = 68.36 \text{ days} \approx 2.27 \text{ months} \quad (4)$$

$$F_2 = f_1 \times Q_{10}^{\frac{\Delta}{10}} = 3.09(199)^{\frac{55-5}{10}} = 96.43 \text{ days} \approx 3.21 \text{ months} \quad (5)$$

where f_1 - time between tests at the higher temperature, F_2 - storage life at the lower temperature, Δ - difference in degrees centigrade between the two.

Therefore, we can keep β -carotene within 2.27 months at 10 °C or 3.21 months at 5 °C to maintain 70% TBC carotene. Retention of extracted β -carotene from gac fruit (*Momordica cochinchinensis* Spreng) stored at the same storage temperature was also agreeable with this study³⁸.

Conclusion

SFE of β -carotene from the ripe pericarp of *Momordica* genus has gained great attention in the current year. The study reviewed show that ripe bitter melon pericarp SFE-CO₂ extracts are interesting, innovative, and high-quality products rich with β -carotene. Optimization of experimental parameters, such as pressure, CO₂ flow rate, temperature and extraction period of enzymatically treated lyophilized ripe bitter melon pericarp matrix was done. The experimental values of β -carotene yield were varied from 14.81% to 84.77%. The statistical model revealed the thirty experiment to optimize the best extraction condition of SFE. The second-order model developed for β -carotene yield exhibited non-significant lack of fit and a high value for the coefficient of determination (0.9661). The surface graph indicated that maximum β -carotene % yield was obtained by extracting ripe bitter melon pericarp at 69.15 °C temperature, 393.31 bar pressure, 36.98 mL/min flow rate for 190.36 min. The expected storage stability of extracted β -carotene in the amber-colored vial to strictly restrict oxygen and light was 2.27 months at 10 °C or 3.21 months at 5 °C can maintain 70% of β -carotene.

Ethical approval. Informed consent: This article does not contain any studies with either animals or human participants performed by any of the authors.

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References

- Delgado-Vargas, F. & Paredes-Lopez, O. *Natural colorants for food and nutraceutical uses*. CRC press. pp. 344, (2002).
- Aberomand, A. A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World J. Dairy Food Sci.* **6**(1), 71–78 (2011).
- Ghosh, P., Pradhan, R. C., Mishra, S., Patel, A. S. & Kar, A. Physicochemical and nutritional characterization of jamun (*Syzygium cumini*). *Cur. Res. Nutr. Food Sci. J.* **5**(1), 25–35 (2017).
- Mishra, B. B., Patel, A. S. & Kar, A. Storage Stability of Encapsulated Anthocyanin-Rich Extract from Black Carrot (*Daucus carota* ssp. *Sativus*) using different Coating Materials. *Cur. Agric. Res.* **7**(1), 51–63, <https://doi.org/10.12944/CARJ.7.1.07> (2019).
- Murali, S., Kar, A. & Patel, A. S. Storage stability of encapsulated black carrot powder prepared using spray and freeze-drying techniques. *Cur. Agric. Res.* **7**(2), 261–267 (2019).
- Rodriguez-Amaya, D. B. Natural food pigments and colorants. *Bioact. Mol. Food* **8**, 867–901 (2019).
- Dhakane, J. P., Kar, A., Patel, A. S. & Khan, I. Effect of soy proteins and emulsification- evaporation process on physical stability of lycopene emulsions. *Int. J. Chem. Studies* **5**(5), 1354–1358 (2017).
- Patel, A. S., Kar, A. & Khan, I. Process for development of β -carotene Nanocomposites with ω -fatty acids. (VBRI press), <https://scholar.google.com/scholar?cluster=8793510290280437556&hl=en&oi=scholar>, Accessed on 08.15.2019 (2017).
- Kar, A., Mahato, D. K., Patel, A. S. & Bal, L. M. The Encapsulation Efficiency and Physicochemical Characteristics of Anthocyanin from Black Carrot (*Daucus carota* ssp. *Sativus*) as Affected by Encapsulating Materials. *Cur. Agric. Res.* **7**(1), 26–36 (2019).
- Bhosale, P. & Bernstein, P. S. β -Carotene production by *Flavobacterium multivorum* in the presence of inorganic salts and urea. *J. Ind. Microb. Biotechnol.* **31**(12), 565–571 (2004).

11. Mai, H. C., Truong, V. & Debaste, F. Optimization of enzyme-aided extraction of oil rich in carotenoids from gac fruit (*Momordica cochinchinensis* Spreng.). *Food Technol. Biotechnol.* **51**(4), 488–499 (2013).
12. Omenn, G. S. Chemoprevention of lung cancer: the rise and demise of beta-carotene. *Annu. Rev. Pub. Health* **19**(1), 73–99 (1998).
13. Ray, A. Cancer preventive role of selected dietary factors. *Indian J. Cancer* **42**(1), 15–24 (2005).
14. Borek, C. Dietary Antioxidants and Human Cancer. *J. Restorative Med.* **6**(1), 53–61 (2017).
15. Meléndez-Martínez, A. J. An Overview of Carotenoids, Apocarotenoids and Vitamin A in Agro-Food, Nutrition, Health and Disease. *Mol. Nutri. Food Res.* 1801045, <https://doi.org/10.1002/mnfr.201801045> (2019)
16. Dawson, M. I. The importance of vitamin A in nutrition. *Cur. Pharma Des.* **6**(3), 311–325 (2000).
17. Jha, P., Flather, M., Lonn, E., Farkouh, M. & Yusuf, S. The Antioxidant Vitamins and Cardiovascular Disease: A Critical Review of Epidemiologic and Clinical Trial Data. *Anal. Internal Med.* **123**(11), 860–872 (1995).
18. Ribeiro, B. D., Barreto, D. W. & Coelho, M. A. Z. Technological aspects of β -carotene production. *Food Bioprocess Technol.* **4**(5), 693–701 (2011).
19. Emeish, S. Production of natural β -carotene from Dunaliella living in the Dead Sea. *Jordan J. Earth Environ. Sci.* **4**(2), 23–27 (2012).
20. Kaur, K., Shivhare, U. S., Basu, S. & Raghavan, G. V. Kinetics of Extraction of β -Carotene from Tray Dried Carrots by Using Supercritical Fluid Extraction Technique. *Food Nutr. Sci.* **3**(5), 591–595 (2012).
21. Lee-Huang, S. *et al.* Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. *Gene* **161**(2), 151–156 (1995).
22. Rodriguez, D. B., Raymundo, L. C., Lee, T. C., Simpson, K. L. & Chichester, C. O. Carotenoid pigment changes in ripening *Momordica charantia* fruits. *Anal. Bot.* **40**(3), 615–624 (1976).
23. Kandlakunta, B., Rajendran, A. & Thingnganing, L. Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. *Food Chem.* **106**(1), 85–89 (2008).
24. Shams, K. A. *et al.* Green technology: economically and environmentally innovative methods for extraction of medicinal & aromatic plants (MAP) in Egypt. *J. Chem. Pharm. Res.* **7**(5), 1050–1074 (2015).
25. Lang, Q. & Wai, C. M. Supercritical fluid extraction in herbal and natural product studies—a practical review. *Talanta* **53**(4), 771–782 (2001).
26. Joana Gil-Chávez, G. *et al.* Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. *Compr. Rev. Food Sci. F.* **12**(1), 5–23 (2013).
27. Brunner, G. (2005). Supercritical fluids: technology and application to food processing. *J. Food Eng.* **124**, 105–116 (2014).
28. Sharif, K. M. *et al.* Experimental design of supercritical fluid extraction—A review. *J. Food Eng.* **124**, 105–116 (2014).
29. Patil, P. D. *et al.* Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Bioresour. Technol.* **102**(1), 118–122 (2011).
30. Şanal, İ. S., Bayraktar, E., Mehmetoğlu, Ü. & Çalimli, A. Determination of optimum conditions for SC-(CO₂+ethanol) extraction of β -carotene from apricot pomace using response surface methodology. *J. Supercrit. Fluids* **34**(3), 331–338 (2005).
31. Sowbhagya, H. B. & Chitra, V. N. Enzyme-assisted extraction of flavorings and colorants from plant materials. *Critic. Rev. Food Sci. Nutri.* **50**(2), 146–161 (2010).
32. Durante, M., Lenucci, M. & Mita, G. Supercritical carbon dioxide extraction of carotenoids from pumpkin (*Cucurbita* spp.): a review. *Int. J. Mol. Sci.* **15**(4), 6725–6740 (2014).
33. Dominguez, H., Nunez, M. J. & Lema, J. M. Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. *Food Chem.* **49**(3), 271–286 (1994).
34. Lenucci, M. S. *et al.* Enzyme-aided extraction of lycopene from high-pigment tomato cultivars by supercritical carbon dioxide. *Food Chem.* **170**, 193–202 (2015).
35. Ranveer, R. C., Patil, S. N. & Sahoo, A. K. Effect of different parameters on enzyme-assisted extraction of lycopene from tomato processing waste. *Food Bioprod. Process.* **91**(4), 370–375 (2013).
36. Singh, D., Barrow, C. J., Mathur, A. S., Tuli, D. K. & Puri, M. Optimization of zeaxanthin and β -carotene extraction from *Chlorella saccharophila* isolated from New Zealand marine waters. *Biocatal. Agric. Biotechnol.* **4**(2), 166–173 (2015).
37. Runco, J., Subbarao, L. & Chen, R. Qualitative and Quantitative Analysis of β -carotene Using UPC2. APNT134719455, *Water, Mississauga, ON* (2013).
38. Dien, L. K. L., Minh, N. P. & Dao, D. T. A. The changes of total carotenoid content of gac (*Momordica cochinchinensis* Spreng.) powder product in accelerated temperature to the appropriate temperature and shelf-life of product storage. *Int. Res. J. Natur. Sci.* **2**, 31–37 (2014).
39. Vuong, L. T. & King, J. C. A method for preserving gac fruit oil, a rich source of beta-carotene and essential fatty acids in North Vietnam. *Food Nutr. Bull.* **24**, 372–373 (2003).
40. Tran, T. H., Nguyen, M. H., Zabarar, D. & Vu, L. T. Process development of Gac powder by using different enzymes and drying techniques. *J. Food Eng.* **85**(3), 359–365 (2008).
41. Rozzi, N. L., Singh, R. K., Vierling, R. A. & Watkins, B. A. Supercritical fluid extraction of lycopene from tomato processing byproducts. *J. Agric. Food Chem.* **50**(9), 2638–2643 (2002).
42. Reverchon, E. & De Marco, I. Supercritical fluid extraction and fractionation of natural matter. *J. Supercrit. Fluid.* **38**(2), 146–166 (2006).
43. Topal, U., Sasaki, M., Goto, M. & Hayakawa, K. Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. *J. Agric. Food Chem.* **54**(15), 5604–5610 (2006).
44. Marsili, R. & Callahan, D. Comparison of a liquid solvent extraction technique and supercritical fluid extraction for the determination of α - and β -carotene in vegetables. *J. Chromatogr. Sci.* **31**(10), 422–428 (1993).
45. Gómez-Prieto, M. S., Caja, M. M., Herraiz, M. & Santa-Maria, G. Supercritical fluid extraction of all-trans-lycopene from tomato. *J. Agric. Food Chem.* **51**(1), 3–7 (2003).
46. Nobre, B. P., Palavra, A. F., Pessoa, F. L. & Mendes, R. L. Supercritical CO₂ extraction of trans-lycopene from Portuguese tomato industrial waste. *Food Chem.* **116**(3), 680–685 (2009).
47. Abbas, K. A., Mohamed, A., Abdulmir, A. S. & Abas, H. A. A review on supercritical fluid extraction as new analytical method. *Am. J. Biochem. Biotechnol.* **4**(4), 345–353 (2008).

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Author contributions

Avinash Singh Patel initiated the study, executed the experiments, collected, prepared the figures and wrote the manuscript. Abhijit Kar designed the experiments, supervised the study and reviewed the manuscript. Sukanta Das analyzed the data, Sanjaya K Dash reviewed the manuscript and contributed in data interpretation. All authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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