Supercritical fluid extraction of \(\beta\)-carotene from ripe bitter melon pericarp

Avinash Singh Patel\(^1\), Abhijit Kar\(^1\)*, Sukanta Dash\(^2\) & Sanjaya K. Dash\(^3\)

Study ascertained the recovery of \(\beta\)-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 (CO\(_2\)) 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 \(\beta\)-carotene. Results showed that extraction pressure (\(X_1\)) among extraction parameters had the most significant (\(p < 0.05\)) effect on extraction efficiency of the \(\beta\)-carotene followed by allowed extraction time (\(X_4\)), CO\(_2\) flow rate (\(X_2\)) and the temperature of the extraction (\(X_3\)). The maximum yield of 90.12% of \(\beta\)-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 \(\beta\)-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 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 phototrophic bacteria and have attracted vast research attention in the global market due to their potent antioxidant properties\(^2\),\(^8\),\(^10\),\(^11\). Beta-carotene (\(\beta\)-carotene) is one of the extensively used carotenoids as either additives or dietary supplements since years and help 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 membranes\(^16\) and providing other health benefits, including the possible prevention and treatment of cardiovascular disease\(^15\),\(^17\).

At present the commercial production of the \(\beta\)-carotene is done by either chemical synthesis using \(\beta\)-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 litre\(^19\), carrot contains 110 µg per 100 g of fresh weight\(^20\) and Flavobacterium multivorum a bacteria contains 7.85 µg per milliliter\(^20\) 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 purposes\(^21\). 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 \(\beta\)-carotene (967 µg/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 produce is usually performed by using organic solvents; however, this chemical method may be toxic and has some pollution concerns\(^24\). One alternative to traditional extraction by organic solvents is to accomplish the extraction by CO\(_2\) based supercritical...
Pectinase (456 U/g, Aspergillus sp.) was purchased from Sigma-Aldrich (Japan) and deep tube liquid CO₂ powdered matrix shown in Fig. 1, which comprises two high-speed pumps, one is CO₂ pump (280 mL/min) and Thar Technologies Inc., USA). The systematic representation of the SFE system for extraction of β-carotene from degradation.

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.

For the enzymatic pretreatment of the ripe bitter melon pericarp a method described by Ranveer et al. was used. A working enzyme solution of 167 U/g was prepared by diluting the stock enzyme solution into citrate buffer (pH 5.0). 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.

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 matrices 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 yield. 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.

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. Empower³ software was used to operate the system during the quantitative analysis of samples.
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 $\beta$-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:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} + b_{23}X_{23} + b_{24}X_{24} + b_{34}X_{34} + b_{123}X_{123} + b_{124}X_{124} + b_{134}X_{134} + b_{234}X_{234} + b_{1234}X_{1234}$$

(1)

where $Y$ refers to the measured predicted % yield, $b_0$ is the intercept; $b_1$, $b_2$, and $b_3$ and $b_4$ are the linear terms; $b_{ij}$, $b_{ik}$, $b_{jk}$, $b_{jl}$ and $b_{kl}$ are interaction coefficients and $b_{ii}$, $b_{jj}$, $b_{kk}$ and $b_{ll}$ 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°C and 55°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 $\beta$-carotene content was studied by supercritical CO$_2$ based UPC$_2$ system using the standard calibration curve of 0, 25, 50, 75, 100, 125 and 150 ppm (Fig. 2). Initially, $\beta$-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 $\beta$-carotene content due to degradation of their interfacial tension.
which increases its availability.\textsuperscript{11,34} The reported results were in agreement with Lenucci \textit{et al.}\textsuperscript{34} for tomato processing waste in which enzymatic treatment increased lycopene recovery up to ~150%. Vuong and King \textsuperscript{39} also reported the similar content of \(\beta\)-carotene in fresh \textit{Momordica} genus ripe fruit (gac fruit) was 8.3 to 76.9 mg/100 g. However, Tran \textit{et al.}\textsuperscript{40} reported 37.9 mg/100 g in gac powder.

| Run | Independent variables levels (coded) | Yield (%) | 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 UPC\textsuperscript{2} calibration curve (A) used in quantification of \(\beta\)-carotene extracted at the optimized condition and its chromatogram (B).
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:

\[ Y = -360.262743 + 0.837487X_1 + 4.973891X_2 + 3.890651X_3 + 0.621753X_4 \\
+ 0.001374X_1^2 - 0.001029X_2X_3 + 0.001042X_2X_4 + 0.008958X_3X_4 \\
+ 0.003432X_2^2 - 0.002163X_3X_4 - 0.001291X_1^2 - 0.09177X_2^2 \\
- 0.024613X_3^2 - 0.002650X_4^2 \]

The predicted values of \(\beta\)-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 \(\beta\)-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 \(\beta\)-carotene recovery. Maximum \(\beta\)-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 channeling of the supercritical CO\(_2\) around it rather than diffusing through it\(^{1,42}\). Kaur et al.\(^{30}\) reported similar trends for SFE of \(\beta\)-carotene from tray dried carrot. Results in case of flow rate \((X_2)\) was observed as list significant change in \(\beta\)-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

| Regression  | 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 \(\beta\)-carotene from the ripe bitter melon pericarp. Note: R-Squares 0.9661, Degree of freedom (DF).
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 matrix. 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 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: X₁ - 300 bar; flow rate: X₂ - 35 mL/min; temperature: X₃ – 70 °C; and time: X₄ - 135 min).

| Factor | Critical Value | Coding Coefficients |
|--------|----------------|---------------------|
|        | Coded | Un-coded | Subtracted off | Divided by |
| X₁     | 0.62  | 393.32    | 300.00         | 150.00     |
| X₂     | 0.099 | 36.98     | 35.00          | 20.00      |
| X₃     | -0.04 | 69.15     | 70.00          | 20.00      |
| X₄     | 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.
the supercritical CO$_2$ 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 CO$_2$ available for the extraction process. In case the available supercritical CO$_2$ is a limiting factor, the completeness of extraction is adversely affected.$^{47}$ However, the increase in time period beyond a point where the available supercritical CO$_2$ 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 CO$_2$ (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 CO$_2$ 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 ± 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 day 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).

\[
Q_{10} = \frac{6.16}{3.09} = 1.99
\]

(3)

where \(Q_{10}\) is increase in the rate of the reaction when the temperature is increased by 10 °C during storage.

Storage duration of β-carotene at 10 °C (Eq. (4)) and 5 °C (Eq. (5)) (carotene 30% reduction) will be:

\[
F_2 = f_1 \times Q_{10}^{55-10} = 3.09(199)^{55-10} = 68.36 \text{ days} \approx 2.27 \text{ months}
\]

(4)

\[
F_2 = f_1 \times Q_{10}^{30-5} = 3.09(199)^{30-5} = 96.43 \text{ days} \approx 3.21 \text{ months}
\]

(5)

where \(f_1\) - time between tests at the higher temperature, \(F_2\) - storage life at the lower temperature, \(\Delta\) - 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 study.

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.

Received: 3 October 2019; Accepted: 25 November 2019;
Published online: 17 December 2019

References

1. Delgado-Vargas, F. & Paredes-Lopez, O. Natural colorants for food and nutraceutical uses. CRC press. pp. 344, (2002).
2. Aberoumand, 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).
3. 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. 1(1), 25–35 (2017).
4. 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/CARR.7.1.07 (2019).
5. 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).
6. Rodriguez-Amaya, D. B. Natural food pigments and colorants. Bioact. Mol. Food 8, 867–901 (2019).
7. 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 8(5), 1354–1358 (2017).
8. Patel, A. S., Kar, A. & Khan, I. Process for development of β-carotene Nanocomposites with ω-fatty acids. (VBR Press), https://scholar.google.com/scholar?cluster=8793510290280437566&hl=en&oi=scholarr, Accessed on 08.15.2019 (2017).
9. 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).
10. 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. & Debase, 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. Publ. 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. Nutr. Food Res. 1810145, https://doi.org/10.1002/mnfr.201810145 (2019).

16. Dawson, M. I. The importance of vitamin A in nutrition. Car. 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. Ann. 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. Emesh, 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., Shrivare, U. S., Basu, S. & Raghavan, G. K. Kinetics of Extraction of β-Carotene from Tray Dried Carrots by Using Supercritical Fluid Extraction Technique. Nutri. Food Sci. 3(5), 391–395 (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. Rodríguez, D. B., Raymundo, L. C., Lee, T. C., Simpson, K. L. & Chishcker, C. O. Carotenoid pigment changes in ripening Momordica charantia fruits. Anal. Biothem. 40(3), 615–624 (1976).

23. Kandlakunta, B., Rajendran, A. & Thiangnaging, L. Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and un conventional 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. 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. Şanel, İ. S., Bayraktar, E., Mehmetoğlu, Ü. & Çalış, 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. Crit. Rev. Food Sci. Nutri. 50(2), 146–161 (2010).

32. Durante, M., Lenucci, M. & Mitra, 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. Runcio, J., Subbarao, L. & Chen, R. Qualitative and Quantitative Analysis of β-carotene Using UPLC. APNT134719455, Water, Mississauga, ON (2013).

38. Djen, 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., Zaharas, 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, L. 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. Sánchez-Prieto, M. S., Caja, M. M., Herranz, 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., Abdulamir, A. S. & Abas, H. A. A review on supercritical fluid extraction as new analytical method. Am. J. Biochem. Biotechnol. 4(4), 345–353 (2008).
Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to A.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019