TO OPTIMIZE THE EXTRACTION OF CAROTENOIDS FROM PASSION FRUIT PEEL USING NOVEL AND GREEN TECHNIQUES AND STUDY ITS KINETICS FOR INDUSTRIAL APPLICATION.

CHAPTER 3

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OPTIMIZATION OF THE EXTRACTION OF CAROTENOIDS FROM PASSION FRUIT PEEL USING NOVEL AND GREEN TECHNIQUES AND STUDY OF ITS KINETICS FOR INDUSTRIAL APPLICATION

3.1. Introduction

Passion fruit is rich in bioactive compounds [50]. In India, the passion fruits are mostly used as juice so passion fruit juice processing industries generate a huge amount of waste portion, comprises of peels and seeds. This waste that constitutes more than half of the whole fruit [41] causes environmental deterioration and the loss of beneficial bioactive substances. Besides the pulp, peel and seeds of passion fruit are very rich in bioactive compounds, specially carotenoids– predominated by β-carotene, anthocyanins, phenolic groups, etc [11,40,41].

The peel has properties to reduce wheeze and cough, improve shortness of breath in adults with asthma, control hypertension, treat anxiety, insomnia, etc. [52,54]. One of the most useful options for management of fruit waste is the recovery of its health benefitting compounds from the wastes, which could be used in food processing, pharmaceutical, and cosmetic related industries. Passion fruit waste can be a source of beneficial health promoting compounds like carotenoids, which are useful in the food and pharmaceutical industries.

Conventional extraction (CE) methods with common petrochemical solvents have been useful for extraction and recovery of the valuable compounds from plant food matrices, but such extraction processes have many disadvantages [49,55]. Therefore, green chemistry principle based on high-energy extraction techniques are gaining wide acceptance in recent years and find extensive use in the extraction of phytochemicals [18,25,44].

Vegetable oils are regarded as environmentally friendly and substitute solvents for the extraction of lipophilic chemicals such as carotenoids [12,18,25]. Various vegetable oils such as mustard oil, soy oil, sunflower oil, coconut oil, groundnut oil, rice bran oil and gingelly oil were studied [43] for the extraction of carotenoids. Although oil shows environment friendly nature and several desirable characteristics, there are limitations of using vegetable oils in conventional extraction process [12,17,18], as the high viscosity is one of the major drawbacks. Although high temperature of oil for a long time may

decrease the viscosity i.e. improve the diffusivity, it adversely causes degradation of the carotenoids [18].

Recently, many advanced techniques have been investigated for improving the extraction efficiency of bioactive compounds and overcoming the drawbacks of CE. Among the recent techniques, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) techniques have got wide acceptability as the techniques are adaptable in industries, are easy to operate, costs less, have high extraction efficiency and also has less impact on environment [12,18]. A few studies have been reported on the application of UAE [18,25,35] and MAE [12,14] using oil as an alternative solvent for the extraction of bioactive compounds from food matrix [12,14].

The green extraction of carotenoids from PFP waste using UAE and MAE treatments and vegetable oils as solvent has not been reported. Further, there are limited studies that have compared UAE, MAE and CE processes for extraction of bioactive compounds using vegetables oils as solvent. The primary purpose of this chapter was to study the application of UAE and MAE techniques for the extraction of carotenoids from passion fruit peel (PFP) in alternate solvents like vegetable oils and make a comparative analysis with conventional methods. The process parameters were optimized for the extraction of carotenoids from PFP, and the extraction techniques in terms of energy efficiency was compared and the effective diffusivity, kinetics study of mass transfer process, and thermodynamics of extraction of carotenoids for the optimized process were studied to get an insight into the feasibility of industrial application of the green technologies.

3.2. Materials and Methods

3.2.1. Chemicals

Standards of β-carotene with more than 95% purity of HPLC assay and gallic acid were obtained from Merck. All other analytical grade chemicals were purchased from SRL and Sigma-Aldrich. All the experiments were performed in triplicates.

3.2.2. Preparation of sample

The passion fruit (yellow) sample was collected from Bishnupur District, Manipur, India. The fully ripened fruits were immediately transported to the laboratory. The fruits were washed, cut into halves and the pulp, peel, and seeds were separated. Seeds and pulp were packed separately in plastics bags and glass bottle, respectively and stored at -18 ℃ for futher study. A freeze dryer (Lyolab, India) was used to dry the PFP and

processed into a powder that can pass through a 60-mesh sieve, and packed in a tightly capped Borosil glass container and refrigerated until analysis.

3.2.3. Ultrasonic assisted extraction (UAE) of PFP using vegetables oils

There are two methods for applying UAE to samples: (A) the direct method involves directly submerging the ultrasonic probe into the sample solution; (B) the indirect method involves applying ultrasonic power to the solution containing the sample through the wall of the container that is placed in the water bath. It is known that an ultrasonic probe may produce ultrasonic power that is at least 100 times stronger than that produced by a bath [18]. In this study, an ultrasonic probe (U500, Takashi, Japan) at 100 W (power) was used to extract carotenoids and bioactive compounds from the PFP. PFP and solvent in the required solid to liquid (S/L) ratios were taken in beakers. Olive oil (OO) (Aar Gee Formulations) and sunflower (SO) oil were used as solvents for carotenoids extraction from the PFP. Thermostat control helped to control beaker fluid temperature. The temperature range taken for extraction was 30-60 ℃. The independent parameters selected in this study were ultrasonic treatment time (t) between 10 and 50 min, extraction temperature (T) between 30 and 60 ℃, and S/L ratio between 10 and 30 g/100 mL.

3.2.4. Microwave assisted extraction (MAE) of PFP using olive oil

A microwave system (Twin Engineers, TW/MWEX/2/18, India) was used for the extraction process. The weighed amount of PFP was taken in an extraction vessel (borosilicate glass with a cap made of silicone) and mixed with the oil in an S/L ratio which had the highest extractability of carotenoids in UAE process and kept for microwave extraction. The experimental parameters are presented in **Table 3.1**. For all extraction runs, the chamber was cooled by circulating air in ice bath and precautionary measures were taken to keep the temperature below 110 ℃. After the extraction, the oil enriched with carotenoids was filtered through glass microfiber paper to remove the powder residue and stored in tightly capped amber glass bottles in a deep freezer (-18 ℃) until further analyses.

3.2.5. Energy efficiency of UAE and MAE

The principle of UAE and MAE extraction processes are different and although the input energy of the system are same there is a chance of changes in supply energy delivered to the sample, so it will be more efficient if power from the device was

converted to heat, which gets dissipated in the medium [46]. This way, comparison is based on actual power/energy deliverd to the process.

Energy density is the amount of thermal energy that systems like UAE and MAE disperse per unit volume, and it has long been recognised as a useful metric for comparing how effective various treatments are [37]. Calorimetric measurements were performed to assess actual power P (W), power per unit mass P_m (W/g), and energy density and calculated by **Eq. 3.1, 3.2** and **3.3** [10,37,46].

$$P = mC_p \frac{dT}{dt} \quad (3.1)$$

$$P_m = mC_p \frac{dT}{dt} \quad (3.2)$$

$$E_v = \frac{P*t}{V*S} \quad (3.3)$$

Where, m is the sample mass (g), C_p is the specific heat of the solvent at constant pressure (J/g/℃), dT/dt is the rate of increasing heat (℃/s), V is the volume of the solution (mL), t is the treatment time (s), S is the solid in g, E_v is the energy density (J/mL).

3.2.6. Conventional extraction (CE) of PFP using OO

The optimized S/L ratio of UAE process was taken for conventional extraction using OO as solvent. Same amount of S/L ratio as taken for UAE process was taken and heated to 30, 45 and 60 ℃ in a mixer incubator for 600, 360 and 240 min, respectively. The samples were taken out at every 30 min intervals for the first 120 min and after that at every 60 min interval up to 600 min and analysed for carotenoid content.

3.2.7. Soxhlet extraction of PFP

The method of Elik et al. [12] with slight modifications was followed. In brief, 5 g PFP powder was mixed with 150 mL of n-hexane, acetone, ethyl acetate and ethanol (2:1:1:1 v:v:v:v) in extraction thimble and extracted for 5-6 h in the Soxhlet apparatus. Following the extraction, the carotenoids-enriched solvent was vacuum-vaporized at 35 to 40 ℃ in a rotary vacuum evaporator.

After the extraction, the solvent enriched with carotenoids was evaporated in a rotary vacuum evaporator under vacuum at 35-40 ℃ and carotenoids extract was obtained. Carotenoids extract was diluted in n-hexane just before the analysis and total carotenoids and β-carotene content were estimated.

3.2.8. Total carotenoids content (TCC) and β-carotene content

TCC of PFP was estimated using the diluted extract obtained from Soxhlet extraction [12]. TCC of oil extracted samples was determined spectrophotometrically, using UV-visible spectrophotometer (Cary 60 UV-Vis, Agilent), reading the absorbance at 450 nm against the used oil as blank [12,18] with some modification. The amount was expressed in terms of the content of μg carotenoids per 100 g oil. Briefly, 3 g of oil samples was precisely measured and dissolved in hexane to obtain a final amount of 10 mL and absorbance was taken.

TCC was investigated using the following formula (**Eq. 3.4**) [7]**.**

$$\text{TCC (μg/100 g oil)} = \frac{A \times v \times 10^4 \times D}{E_{1\,cm}^{1\%} \times V} \times 100 \quad (3.4)$$

where V and v are volume of the sample (mL) and extract used for analysis respectively, $E_{1\,cm}^{1\%}$ and A are the extinction coefficient (2592) and absorbance at 450 nm for for β-carotene in hexane [7], respectively, and D is the dilution factor.

β-carotene in oil was investigated by the same procedure as adopted by Hsu et al. [22], and the details of the procedure was mentioned in **Chapter 6.**

3.2.9. Total phenolic content

Folin–Ciocalteu Reagent (FCR) colorimetric procedure was adopted for the determination of phenolic content of treated and untreated oil [12]. Gallic acid was chosen as the benchmark equivalent. Briefly, 2.5 mL of 7.5 % Na_2HCO_3 and 0.5 mL of 10 % FCR reagent were mixed with 0.5 mL extracted solution. The combination was then incubated for 45 min, and after that, using a spectrophotometer (CECIL 7400, 700 series, Aquarius), absorbance was recorded at λ_{max} = 765 nm against the blank. For blank, the whole solution but without the peel extract was taken. Total phenolic content was expressed as gallic acid equivalents (GAE)/g of sample.

3.2.10. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity as antioxidant activity

Antioxidant activity (DPPH activity) of sample was investigated by using the method adopted by Chutia et al. [9]. Briefly, 0.2 mL of the sample was mixed vigorously with DPPH solution (2.8 mL, 0.8 nM), then the combination was placed in a dark room and left for 30 min. The sample extract was substituted with 0.2 ml of methanol as a control, and the mixture of the control and DPPH served as a blank. The absorbance of the mixture was calculated at 517 nm in a UV–Vis spectrophotometer (CECIL 7400, 700

series, Aquarius). Antioxidant activity in terms of DPPH free radical scavenging activity was expressed by the following equation (**Eq. 3.5**).

$$\text{Antioxidant (DPPH) activity (\%)} = \left(1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right) \times 100 \quad (3.5)$$

3.2.11. Experimental design

Designing of experiments will help in a systematic study to collect a lot of data with a limited number of experiments. Response surface methodology (RSM) was used in this study for designing the experiments, which provides large amount information as compared to traditional experimental designs and also gives the idea about the effect of the dependent variables on the independent variables and gives the optimal conditions. For the UAE, three independent parameters namely, treatment time (A), processing temperature (B), and ratio of solid to liquid (C) were studied with the carotenoids yield as response. Similarly for MAE, microwave power (A), treatment time (B), and solid to liquid ratio (C) were used as the three independent parameters and extraction yield of carotenoids was taken as the response for designing the experiments and optimization process. The range of the independent parameters of this study was selected to maintain the same amount of energy input of the system and the optimum condition of the equipment was also taken into consideration, which was decided previously by single factor analysis. Although various kinds of design are there, in this study for both UAE and MAE, face centred central composite design (FCCD) was used. In FCCD, less number of experiments are required due its three levels ($\alpha = \pm 1,0$) and provides good predictions over the entire design range [9]. The experimental design is given in **Table 3.1.**

Table 3.1. Real and coded values of the variables of the UAE and MAE processes.

Extraction process	Experimental variables	Code	Coded levels		
			-1	0	+1
UAE	Treatment time (min)	A	10	30	50
	Treatment temperature (℃)	B	30	45	60
	Solid to liquid ratio (S/L) (g/100 mL)	C	10	20	30
MAE	Microwave power (W)	A	100	150	200
	Treatment time (min)	B	10	17.5	25
	Solid to liquid ratio (S/L) (g/100 mL)	C	10	20	30

The independent variables, given in **Table 3.1**, were coded according to **Eq. 3.6**. [46]

$$x_i = \frac{X_i - X_{i0}}{\Delta X_i} \quad (3.6)$$

where X_i, x_i and X_{i0} are the actual, coded and central point value of the 'i^{th}' input parameters, respectively, and ΔX_i is the incremental change in the dimensionless value. FCCD design for the both UAE and MAE were fitted to a second-order quadratic model. The experiment was designed using Design-Expert (Stat-Ease, Inc. MN). In all, 20 experimental runs for both UAE and MAE were performed as per the design, which had eight factorial points (2^3), six axial points ($\alpha = \pm 1$) and six center points ($\alpha = 0$) for replications. The suitability of the model was judged based on the experimental results. **Table 3.1.** gives the real and coded values of the variables used for UAE and MAE processes, respectively.

3.2.12. Optimization

The output parameter of yield of carotenoids for the UAE and MAE processes was optimized based on higher desirability value. Optimization of processes were performed by setting the desired goal of output parameters and putting the actual value of variables of the possible goal. A second order polynomial equation, presented in **Eq. 3.7** predicted the responses.

$$y = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 BC + \beta_6 CA + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2 + \in \quad (3.7)$$

Where y represents the output parameter, which is a combination of linear, quadratic and interaction components, βo represents intercept of the graph, $\beta_1, \beta_2, \ldots . \text{and } \beta_9$ represents regression coefficients, A, B, C represent the input parameters, $A^2, B^2 \text{ and } C^2$ are the nonlinear (quadratic) effect of the input parameters and $AB, BC, \text{and } CA$ represent the interaction effects of the input parameters. $\in$ represents the error.

Subsequent to regression analysis, output parameter (y_i) was transformed into desirability function $d_i(y_i)$, a value that varies in the range $0 \leq d_i(y_i) \leq 1$ and changes proportionally to desirability of the corresponding output. The output parameter of yield of carotenoids was desirable and so desirability was set at maximum. The overall desirability (OD), was determined by **Eq. 3.8.**

$$OD = (d_1 y_1 \times d_2 y_2 \times \ldots\ldots \times d_m y_m)^{\frac{1}{m}} \quad (3.8)$$

where $y_{i=1\ldots m}$ denotes the output parameters and m represents the number of output parameters. For the present study, m =1.

3.2.13. Quality parameters of Oil

The untreated olive oil was coded as UOO and the UAE treated carotenoids-enriched olive oil at the optimum conditions was coded as CEOO.

The treated and untreated oil were compared to determine how treatments affected the quality of the oil and samples were analysed for acid value (AV) [13], peroxide value (PV) [13,18] and conjugated diene value (CDV) [13] with some modification in procedures.

Briefly for AV, weighted 10 g of oil sample was mixed in 50 mL of neutralised chloroform ethanol solution (1:1 v/v), and phenolphthalein was used as an indicator to titrate against 0.1 N ethanolic potassium hydroxide (KOH). The amount of KOH needed (in mg) to neutralise the free fatty acids present per g of the oil sample was used to express AV (mg/g) [13].

For PV value, 0.4 g of oil sample was mixed with 19.6 mL chloroform–methanol solution (70:30 v/v ratio) and vortexed for 10 s. Then, 100 mL of ammonium thiocyanate solution (30% w/v) was mixed with the sample's solution and vortexed for 5 s. To this, iron (II) chloride solution (100 mL) was added and mixed properly using a vortex mixer for 10 s. The samples were then incubated for 5 min at room temperature, and the absorbance was measured at 500 nm against a blank that included all the reagents except the sample using a UV spectrophotometer (CECIL 7400, 700 series, Aquarius). The entire process took 10 min to complete and was done in low light. The results were expressed in milliequivalents of oxygen (O_2) per kg of oil [13].

Similarly, CDV values were determined spectrophotometrically by measuring the absorbance at 234 nm and using HPLC grade hexane as blank. Treated and untreated oil samples were dissolved in hexane in 1:600 ratio. By utilising the extinction coefficient value (29,000 mol/L), amounts of conjugated dienes produced during the process were determined [13].

3.2.14. Colour parameters

Colour is one of the consumer acceptance parameters of a food product. The colour of sample was determined with the help of a Hunter-Lab Color Flex (Hunter Lab, VA), and the parameters of L* (lightness valve, 0-indicates compete dark and 100-indicates complete white), a* (negative indicates green and positive indicates red) and b* (negative indicates blue and positive indicates yellow) were recorded.

3.2.15. Pseudo second-order model carotenoids extraction kinetics

Extraction kinetics of bio-compounds from the plant matrices into the extracted solvent can be considered as the reverse of an adsorption process. Pseudo first and second order models have been widely applied to investigate the rate of adsorption in extraction of solids using liquid process, but pseudo first order model has been demonstrated to be efficiently predicted and fitted only at the starting stage of the adsorption process [31], so pseudo seconder order model was used to evaluate the experimental rate of the extraction process [18,39]. Second-order polynomial model was already developed by RSM and its prediction efficiency analysed.

In this chapter, kinetics parameters of UAE and CE treatment that were investigated by pseudo second order model were compared. The model has been used by various authors to understand the extraction kinetics of bioactive compounds from dragon fruits by UAE [39], carotenoids extraction from pomegranate using UAE [18], and flavonoids extraction using MAE from Terminalia [56].

The equation used to determine the extraction rate was represented by the following equation (**Eq. 3.9**).

$$\frac{dC_t}{dt} = k_s(C_{sa} - C_t)^2 \quad (3.9)$$

Where, C_t is the carotenoids concentration in the solvent at a particular treatment time period 't', C_{sa} is the concentration of carotenoids that the liquid extraction has reached saturation at, and k_s is extraction rate constant of the Pseudo second-order model.

For both extraction processes,

Boundary condition, $C_t = 0$, when t=0, and $C_t = C_t$, when t=t

By integrating the **Eq. 3.9**, it can be transformed into the **Eq. 3.10** with respect to the boundary conditions.

$$C_t = \frac{C_{sa}^2 k_s t}{1 + C_{sa} k_s t} \quad (3.10)$$

Eq. 3.10 can be simplified into linear form using **Eq. 3.11** and **3.12.**

$$\frac{t}{C_t} = \frac{1}{C_{sa}^2 k_s} + \frac{t}{C_{sa}} \quad (3.11)$$

$$\frac{t}{C_t} = \frac{1}{h_0} + \frac{t}{C_{sa}} \quad (3.12)$$

where 'h_0' is the initial carotenoids extraction rate and can be defined as $h_0 = C_{sa}^2 k_s$

Correlation between temperature and k_s, can be expianed by Arrhenius equation (**Eq. 3.13).**

$$k_s = k_0\left(\frac{E_a}{RT}\right) \quad (3.13)$$

where E_a is the energy required to activate the cartotenoids extraction process from PFP , R is the universal gas constant (8.314 J $mol^{-1}K^{-1}$). The **Eq. 3.13** can be rearranged into linear form (**Eq. 3.14).**

$$\ln(k_{s)} = \ln(k_0) - \left(\frac{E_a}{R}\right)\frac{1}{T} \quad (3.14)$$

3.2.16. Phenomenological extraction kinetics model

Modelling can be used for efficient prediction of extraction process. Physical extraction kinetic models are the most popular models, which are based on the mass transfer phenomena from surface into the bulk extracting liquid and via solid matrice up to the surface [26]. These models are very complex, but can be simplified by using the concept of film theory and unsteady diffusion [29]. Various kinds of mathematical modelling have been used in food engineering, but among them the so-called phenomenological or thermodynamic models are of special interest, which are based on Irreversible Thermodynamics [36].

The mathematical model was based on some assumptions [26,29], (which are described in details in **Chapter 5**). Briefly, the model can be represented by **Eq. 3.15.**

$$w = w_\infty[1 - f1 \times \exp(-k_1 t) - (1 - f1) \times \exp(-k_2 t)] \quad (3.15)$$

w_∞ = carotenoids yield at saturation stage, w is the carotenoids yield, t = carotenoids extraction process time, f1 denotes the fragment/fraction of carotenoids washed away from damage cell, k_1 and k_2 represent the washing and diffusion rate constant, respectively.

When, washing surpasses diffusion ($k_1 \gg k_2$), **Eq. 3.15** can be expressed (**Eq. 3.16**).

$$w = w_\infty[1 - (1 - f1)\exp(-k_2 t)] \quad (3.16)$$

when, f1= 0, implies washing does not take place, then **Eq. 3.17** is followed.

$$w = w_\infty[1 - \exp(-k_2 t)] \quad (3.17)$$

3.2.17. Determination of effective diffusion coefficient during extraction

Effective diffusion coefficient of carotenoids extraction during UAE and CE treatments from PFP was calculated according to Fick's second law [39]. Before applying the law, following assumptions were made for studying effective diffusion coefficient using UAE and CE extraction methods: (i) Mass transfer resistance generated by external factors was considered as negligible; (ii) PFP powder particles were considered to be uniform in size and spherical; and (iii) Difference in concentration was

only significant in the radial direction. 66×10^{-6} m was the average particle diameter of dried PFP powder as determined by sieving method.

Based on the above assumption, the mass transfer for unsteady state can be expressed by **Eq. 3.18.**

$$\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r} \tag{3.18}$$

Replacing v = Cr, **Eq. 3.18** can be transformed to **Eq. 3.19.**

$$\frac{\partial v}{\partial t} = D\frac{\partial^2 v}{\partial r^2} \tag{3.19}$$

Boundary conditions are

$v = 0$ when $x = 0$ and $t>0$ (3.20.1)

$v = aC_{sa}$ when, $x = r$ and $t>0$ (3.20.2)

$v=f(x)$ when $t = 0$ and $0<x<r$ (3.20.3)

The spherical particles initially have a homogeneous concentration (C_i) and Consistent concentration (C_{sa}) is maintained at the surface. Fick's second law in **Eq. 3.19** under the boundary conditions given in **Eqs. 3.20.1-3.20.3** can be expressed by **Eq. 3.21.**

$$\frac{C_t - C_i}{C_{sa} - C_i} = 1 + \frac{2r}{\pi x}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\frac{n\pi x}{r}\exp\left(-\frac{D_e n^2\pi^2 t}{r^2}\right) \tag{3.21}$$

The total concentration of carotenoids outflowing or inflowing the sphere can be determined from **Eq. 3.22**.

$$\frac{C_t}{C_{sa}} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D_e n^2\pi^2 t}{r^2}\right) \tag{3.22}$$

Where, 'C' denotes carotenoids content in PFP, 'r' denotes radius of PFP spherical particle, D_e denotes effective-diffusion-coefficient of carotenoids and 't' is the extraction time.

The above **Eq. 3.18** can be transformed into simplified form presented in **Eq. 3.23.**

$$CC_r = \frac{C_{sa} - C_t}{C_{sa}} = \frac{6}{\pi^2}\exp\left(-\frac{D_e\pi^2 t}{r^2}\right) \tag{3.23}$$

Where 'CC_r' is the ratio of carotenoids content that did not diffuse out of PFP at time 't'. 'C_t' is the amount of extractable carotenoids in PFP at time 't', 'C_{sa}' denotes total carotenoids content in the extract at saturation stage.

3.2.18. Biot number

Biot number *(*B_i) gives information about mass behaviour. The mass behaviour measures the relative magnitude of the external and internal resistances of the mass

transportation process (extraction process). It is dimensionless and for the extraction process was calculated from **Eq. 3.24.**

$$\mathrm{Bi} = \frac{\mathrm{k_{mt}} \mathrm{D_p}}{\mathrm{D_e}} \tag{3.24}$$

where $\mathrm{k_{mt}}$ is the coefficient for mass transfer (m/s) and $\mathrm{D_p}$ is the size of the particles (PFP) (m). $\mathrm{k_{mt}}$ value can be calculated by using **Eq. 3.25** [39,48].

$$\ln\left(\frac{\mathrm{C_{sa}}}{\mathrm{C_{sa}} - \mathrm{C_t}}\right) = \frac{\mathrm{k_{mt}}}{\mathrm{L_s}}\ \mathrm{t} \tag{3.25}$$

where $\mathrm{L_s}$ is the characteristic length of the sphere particles, $\mathrm{C_{sa}}$ is the concentration of carotenoids at saturated state with respective temperature, $\mathrm{C_t}$ represents the extractable carotenoids content of PFP at time t with respective temperature.

3.2.19. Thermodynamics of UAE and CE

Thermodynamic properties of a chemical process can be used to investigate the state of the chemical reaction. In the extraction process, mainly in extraction of solid compounds using liquid solvent, diffusion of solute molecules into the solvent is the major phase of extraction process and the extent of diffusion determines the extraction efficiency. Gibbs free energy (ΔG) gives the idea about energy available from a system to do work at isotherm and isobaric conditions. In this study, UAE and CE of PFP samples in OO were thermodynamically analysed and the three parameters namely, Enthalpy change (ΔH), Gibbs free energy (ΔG), and change in entropy (ΔS) were calculated to get information about the spontaneity of extraction process, associated heat energy of the process and reversibility of the chemical changes, respectively.

The relationship between formation of free energy, enthalpy of formation and entropy of the particles in the standard form can be presented as in **Eq. 3.26**.

$$\Delta \mathrm{G^o} = \Delta \mathrm{H^o} - \mathrm{T}\Delta \mathrm{S^o} \tag{3.26}$$

The ΔG value at constant temperature can be expressed by **Eq. 3.27.**

$$\Delta \mathrm{G^o} = -\mathrm{RT}\ln \mathrm{K_{eq}} \tag{3.27}$$

where, 'T' implies the extraction temperature (K), and '$\mathrm{K_{eq}}$' is the carotenoids extraction process equilibrium constant.

By combining the **Eq. 3.26** and **3.27**, the Van't Hoff equation is obtained as expressed by **Eq. 3.28.**

$$\ln \mathrm{K_{eq}} = -\frac{\Delta \mathrm{H^o}}{\mathrm{RT}} + \frac{\Delta \mathrm{S^o}}{\mathrm{R}} \tag{3.28}$$

and $\mathrm{K_{eq}}$ can be expressed by **Eq. 3.29.**

$$K_{eq} = \frac{Y_{ts}}{Y_{max} - Y_{ts}} \quad (3.29)$$

Where, Y_{ts} is the carotenoids extracted after 40 min of UAE at a temperature T (K) and saturation time for the CE process, and Y_{max} represents the maximum amount of carotenoids extracted after an exhaustive extraction.

3.2.20. Statistical Analysis

Analysis of Variance (ANOVA) test was used to statistically examine all the data in SPSS 24.0 (SPPS, USA). MATLAB 7.14 (Release 2012a) and Microsoft Excel Solver (Microsoft Office, USA) were both used to estimate the model parameters for all equations. Various statistical parameters, including coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), coefficient of variance (CV%), root mean squared error (RMSE) and sum square error (SSE) were calculated using **Eq. 3.30 – 3.33**, respectively.

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(Z_{pre} - Z_{exp})}{\sum_{i=1}^{N}(Z_{mean} - Z_{exp})} \quad (3.30)$$

$$R^2_{adj} = \frac{(N-1)}{(N-N_p)}\frac{SSR}{SSTO} \quad (3.31)$$

$$CV\% = \frac{Std.Dev}{Mean} X100 \quad (3.32)$$

$$RMSE = \sqrt{\frac{\sum_{n=1}^{N}(Z_{pre} - Z_{exp}}{N}} \quad (3.33)$$

Where, Z_{Pre}, Z_{Exp} ,and Z_{mean} represents predicted, experimental, and mean values respectively, N is total data sets number (n = 1, 2, 3,. . . ,N). SSTO is the total sum square errors ($\sum_{i=1}^{N}(Z_{Exp} - Z_{mean})^2$) , SSR is the sum square of regressions ($\sum_{i=1}^{N}(Z_{Exp} - Z_{Pred})^2$), N_p is the number of predictors, std. dev indicates the standard deviation.

3.3. Results and Discussion

3.3.1. Extraction yield of carotenoids

Using the optimized conditions designed by RSM, the highest extraction of carotenoids in olive oil and sunflower oil was 1207.93 and 1185.45 µg /100 g d.w. of PFP, respectively. The efficiency of carotenoids extraction process using various oil as a solvent depends on several factors including type of oil, their polarity, amount of phospholipid present in the oil, and chain length of fatty acids [4,5,33,45]. Oils with low amount of phospholipids and high quantity of short chain fatty acids give better results [4,33]. Olive oil contains higher amount of short chain fatty acids [24,33], acceptable

polarity and phospholipid content [3] as compared to sunflower oil that helps in enhanced extraction of carotenoids [33].

Following the experimental conditions, OO and SO were able to extract 89.07 and 87.13 %, respectively of carotenoids present in PFP. UAE process extracted 93.8 % of carotenoids from pomegranate waste against 85.7 % using vegetables oils only [18]. Carotenoids are lipophilic bioactive compounds and show better solubility in oil [1,25]. Large amplitude waves with high frequency move through a liquid medium, which results in compression and rarefaction of molecules in the liquid that consequently alter the elastic modulus and density [18]. The sudden drop of pressure at the periphery of the ultrasonic wave during rarefaction generates small bubbles that expand and implode. This turbulent flow generates localised high temperature (upto 5000 K), pressure (1000 atm) with high heating, and cooling rate ($\geq 10^{10}$ K/s) [35], which enhances the diffusion, disruption, and leaching out of the inside cell materials. As the particle size reduction is enhanced by the waves, the number of cells exposed to the extraction solvent directly has increased [18,51]. Isomerization of carotenoids occurs during ultrasonic treatment and the quantity of *cis* isomers increase depending upon treatment time [49]; and *cis* isomers have higher solubility.

3.3.2. Effect of treatment time on carotenoids extraction of UAE

For both solvents, the extraction yield of carotenoids (Y) was time dependent. For OO solvent, yield increased as ultrasonic time increased from 10 to 40 min (**Fig. 3.1a** and **3.1b**) followed by a slight decrease with further increase in time, where other parameters were kept constant.

But for the SO solvent, yield increased with ultrasonic time for the entire range used in this study i.e. from 10 to 50 min **(Fig. 3.2a** and **3.2b**). Thus, the desirable ultrasonication time was 40 min and 50 min for maximising carotenoids yield from PFP using OO and SO solvents, respectively. The deviation in overall parameters from mean for OO and SO are presented in **Fig. 3.1d** and **3.2d,** respectively.

Goula et al. [18] and Li et al. [25] also found similar trends while extracting carotenoids in vegetables oils (solvent) from pomegranate and carrot wastes, respectively. On the contrary, Ahmad-Qasem et al. [2] reported 84 % extraction of total phenolic content after first 5 min of the UAE treatment.

UAE extraction process comprises of two stages (mentioned in detail in the phenomenological section in **Chapter 5). I**n the first stage known as washing process,

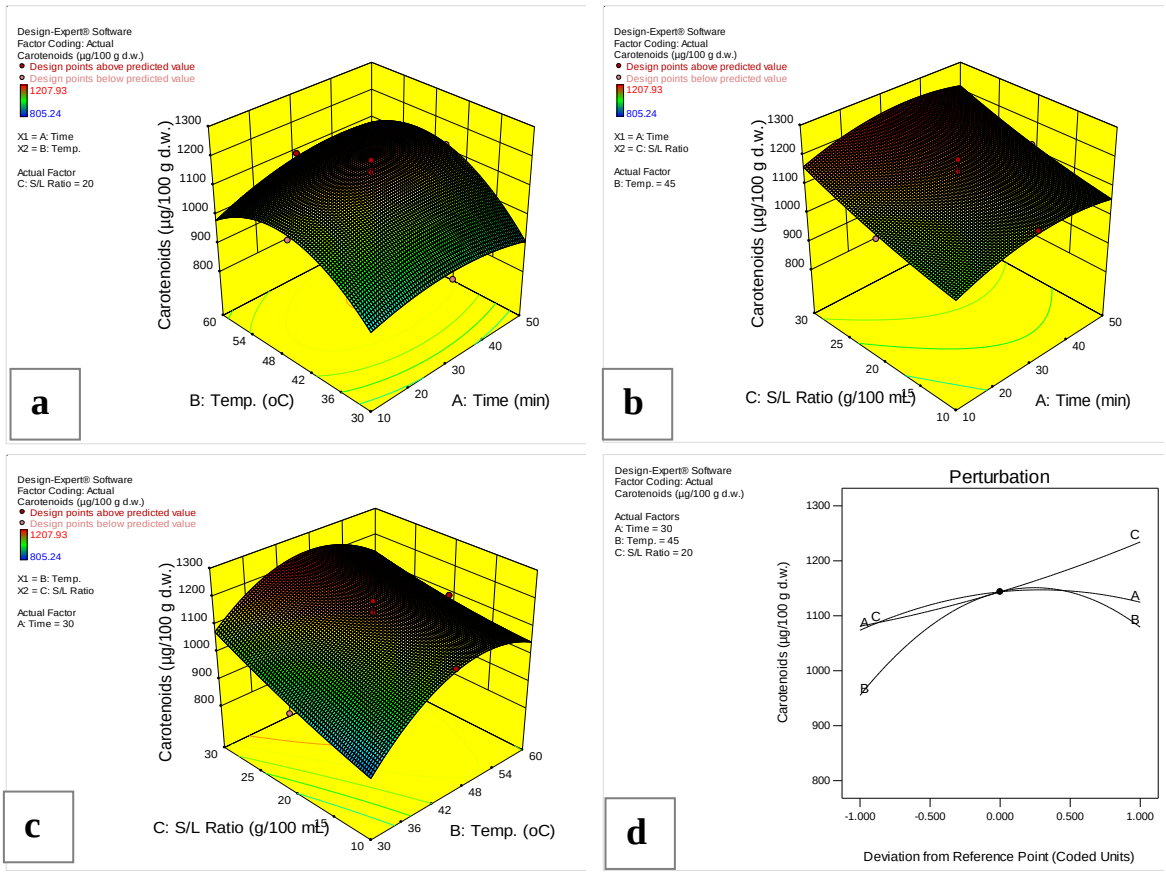

Fig. 3.1. Response surface plots showing the effects of parameters on extraction of carotenoids in UAE process using OO as solvent. Parameters in (a) Time and temperature, (b) Time and solid to oil ratio, (c) Temperature and solid to oil ratio, and (d) Deviation from reference point (coded point).

rapid penetration of the liquid molecules into the solid matrices occur as soon as disruption of cells take place and in the second stage known as diffusion process there is slow mass transfer of extractable materials from the solid matrices to the liquid by external diffusion and osmosis [29,35].

3.3.3. Effect of temperature on carotenoids extraction of UAE

The impact of temperature on carotene extraction from PFP was significant for OO and non-significant for SO (**Table 3.2**) that were used as solvents ($p < 0.05$). Extraction yield was found to increase with an increase in temperature up to 50 ℃ and thereafter reduce with further increase in temperature for both OO (**Fig. 3.1a** and **3.1c**) and SO (**Fig. 3.2a** and **3.2c**). Increase in temperature causes viscosity of oil to decrease, which enhances the fluidity [21] and facilitates its passage through the solid matrices and increases the diffusion coefficients of the extractable lipophilic compounds. But at higher temperatures (>50 ℃), dissolution of cell impurities and decomposition of some constituents may increase [18,35].

The impact of temperature on carotenoids extraction from PFP using OO was more as compared to SO, which may be attributed to the lesser effect of temperature on the changes in oil composition and decomposition of thermolabile compounds in OO [3,21].

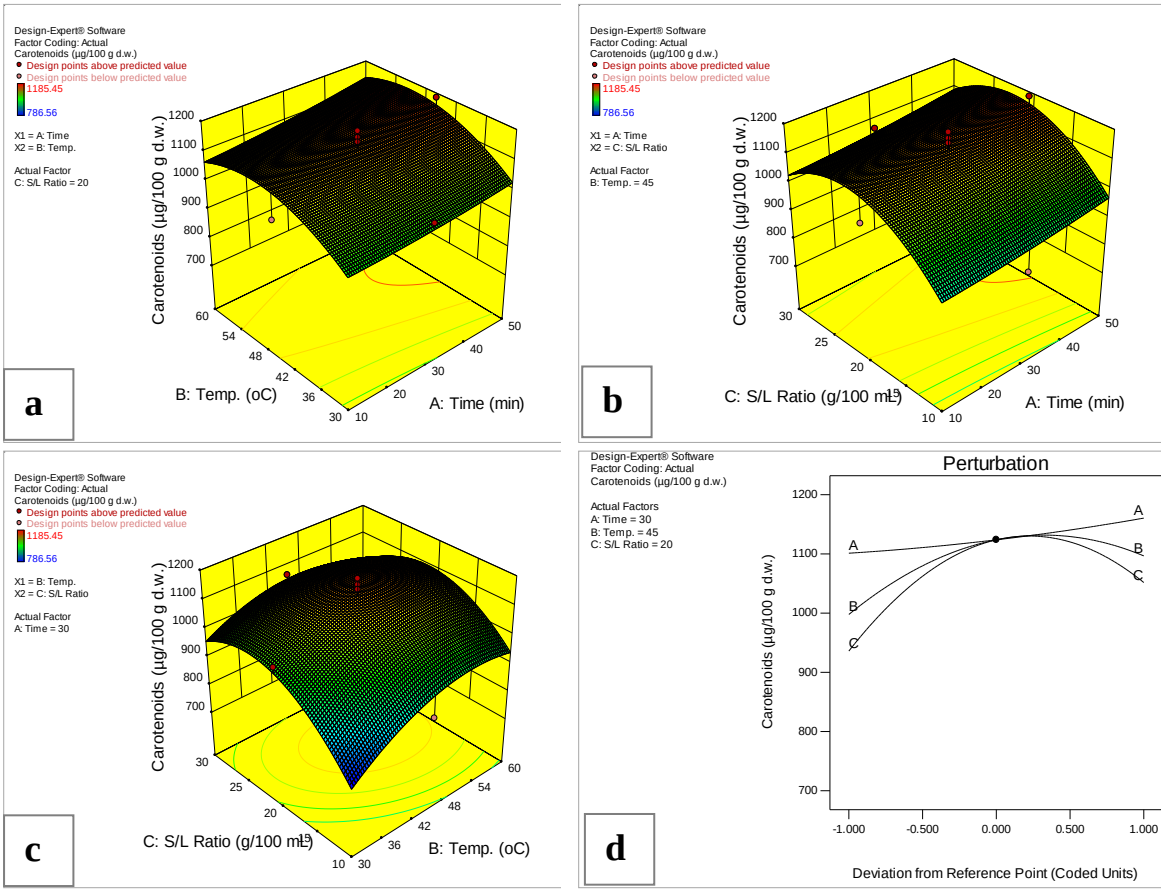

Fig. 3.2. Response surface plots representing the effects of input parameters on extraction of carotenoids in UAE process using sunflower oil as solvent. Parameters in (A) Time and Temperature, (B) Time and Solid to oil ratio, (C) Temperature and Solid to oil ratio, and (D) Deviation from reference point.

3.3.4. Effect of solid to oil ratio on carotenoids extraction of UAE

For OO, the extraction yield of carotenoids was seen to increase as the S/L ratio rises from 10 to 30 g/100 mL (**Fig. 3.1b** and **3.1c**), which is in accordance with the principles of mass transfer. However, for SO (**Fig. 3.2b** and **3.2c**), the yield increased up to 20 g/100 mL only. Li et al. [25] and Goula et al. [18] also observed similar trends. The impact of S/L ratio was significant for OO but insignificant for SO (**Table 3.2**). Qu et al. [38] noticed a significant impact of water/sample ratio on the phenolics extraction. Goula et al. [18] extracted the carotenoids from pomegranate peel wastes using two oils (sunflower oil and soy oil); the effects of S/L ratio on extraction using one oil were found to be significant, while those using the other oil were found to be non-significant.

Table 3.2. ANOVA table for UAE of carotenoids using oils as solvent.

Source	dF	Olive oil			Sunflower oil		
		Mean square	F value	p value	Mean square	F value	p value
Model	9	25174.61	41.40	<0.0001	26754.61	7.45	0.0021
Time- A	1	6489.76	10.67	0.0085	8782.27	2.45	0.1489
Temperature-B	1	38444.96	63.23	<0.0001	24460.15	6.81	0.0260
S/L ratio-C	1	58709.31	96.55	<0.0001	33517.15	9.34	0.0121
AB	1	5183.36	8.53	0.0153	661.90	0.18	0.6767
AC	1	21.19	0.035	0.8556	37.89	0.011	0.9202
BC	1	7916.59	13.02	0.0048	8250.47	2.30	0.1605
A^2	1	5414.36	8.90	0.0137	136.45	0.038	0.8493
B^2	1	43913.58	72.22	<0.0001	16134.26	4.49	0.0600
C^2	1	553.20	0.91	0.3627	46468.83	12.94	0.0049
Residual	10	608.05			3590.00		
Lack of fit	5	719.06	1.45	0.3476	5925.96	4.73	0.0567
Pure error	5	497.03			1254.04		
Cor total	19						
R^2			0.98			0.87	
Adj R^2			0.95			0.78	
Press			44760.32			$2.04*10^5$	
CV%			2.31			5.85	
Std dev			24.66			59.92	
Adequate precision			24.033			9.301	

3.3.5. Modelling and validation

All the experimental data calculated according to FCCD design were statistically analysed using the design-expert (Design-Expert 13 software, Stat-Ease). The quadratic polynomial equations fitted with all the experimental data explained the effect of parameters on carotenoids extraction and are presented in **Eq. 3.34** and **3.35.**

$$Y_1 = 1143.54 + 25.47\times A + 62.00\times B + 76.62\times C + 25.46\times AB + 1.63\times AC - 31.46\times BC - 44.37\times A^2 - 126.37\times B^2 + 14.18\times C^2 \quad (3.34)$$

$$Y_2 = 1123.54 + 29.63\times A + 49.46\times B + 57.89\times C + 9.10\times AB + 2.18\times AC - 32.11\times BC + 7.04\times A^2 - 76.60\times B^2 - 129.99\times C^2 \quad (3.35)$$

R^2 (high regression coefficient) values obtained were 0.98 and 0.87 for yield of carotenoids extracted by UAE process using OO (Y_1) and SO (Y_2) as solvents, respectively **(Table 3.2**). Higher R^2 of the quadratic model indicated the good correlation between the input parameters and response variables. CV% value less than 10 % indicated good fitting of the model. Models were validated by the non-significant lack of fit for responses. The model was highly significant (<0.0001 for OO and <0.0021 for SO), which indicated that the model can explain the experimental data with high efficiency.

3.3.6. Optimization

As shown in ANOVA table (**Table 3.2**), all the UAE input parameters of time (A), temperature (B), and S/L ratio (C), with the p-value = 0.0085, <0.0001 and <0.0001 respectively, have significant influence on the carotenoids extraction yield ($p < 0.05$) for OO solvent. Regarding the SO solvent, the extraction parameters of temperature and S/L ratio, with a probability value of 0.026 and 0.012 were found to be the significant factors.

The regression equation was obtained by setting the maximum carotenoids yield to be within the range of input parameters. Desirability profile for the optimization process of OO is given in **Table 3.3.** For OO as solvent, 39.062 min extraction time, 46.590 ℃ temperature, and 29.9 g/100 mL S/L ratio gives the maximum desirability level of 1.0 (**Table 3.3**).

Table 3.3. Optimization table for carotenoids extraction by UAE using oils as solvent.

Solvent	Time (min)	Temperature (℃)	S/L (g/100 mL)	Carotenoids (µg/100 g d.w)	Desirability
OO	39.062	46.590	29.904	1239.580	1.000
	37.942	49.359	29.953	1238.802	1.000
	30.755	46.765	29.889	1236.148	1.000
	34.601	48.607	29.523	1235.047	1.000
SO	50.000	50.140	21.884	1176.195	0.977
	50.000	50.065	21.949	1176.188	0.977
	50.000	50.150	21.960	1176.187	0.977
	50.000	49.986	21.856	1176.184	0.977

OO- Olive oil, SO- Sunflower oil

Using these conditions (39.1 min time, 47 ℃ extraction temperature, and 29.9 g/100 mL solid to liquid ratio), the model-based predicted value for carotenoids

extraction yield was 1239.58 µg/100 g of dry PFP, whereas the experimental value was 1241.95 µg carotenoids/100 g d.w. of PFP. For extraction of carotenoids using SO as solvent, the optimized values of treatment time, temperature and S/L ratio was 50 min, 50.14 ℃, and 21.88 g/100 mL, respectively with 0.977 desirability, which gave an extraction of 1176.195 µg carotenoids/100 g of dry PFP (experimental result) The optimized conditions taken therefore were 50 min, 50 ℃, and 29.9 g/100 mL (**Table 3.3**). Thus, at the optimum conditions, 91.38 % and 86.7 % of carotenoids present in PFP could be extracted by OO and SO, respectively. Results implied that oil can be used for green UAE extraction of carotenoids from PFP and that olive oil was a better solvent than sunflower oil for UAE process.

3.3.7. The effect of input parameters on recovery of carotenoids using MAE treatment with OO

The extraction yield of carotenoids as per FCCD design (**Table 3.4**) ranged from

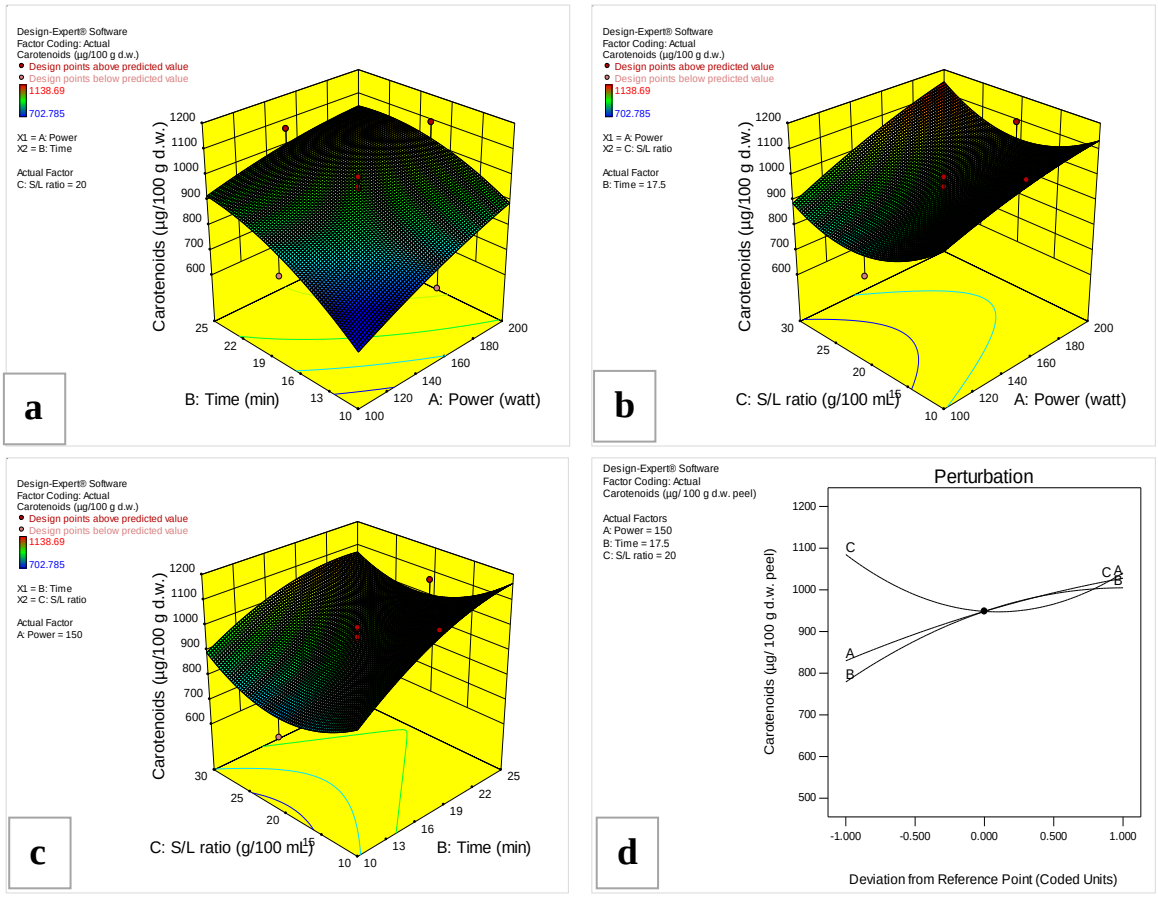

Fig. 3.3. Response surface graphs showing the effects of extraction of carotenoids of MAE treatment, using olive oil as solvent by the parameters: (a) microwave power and treatment time, (b) microwave power and solid to oil ratio, (c) treatment time and solid to oil ratio, and (d) Deviation from reference point (coded point).

702.76 to 1138.69 µg/ 100 g PFP d.w. With an increase in the power of MAE (**Fig. 3.3a** and **3.3b**), the extraction yield of carotenoids increased, which may be due to the increase in the dipolar interaction that rapidly increases the temperature of the mixture and decreases the viscosity of oil with resultant enhanced extraction efficiency [20]. Chuyen et al. [10] also reported an increase in carotenoids extraction using vegetables oil as soon as the microwave power was increased from 240 W to 360 W. With an increase in MAE process treatment time, the amount of carotenoids extracted increased (**Fig. 3.3a** and **3.3c**), which may be due to prolonged exposure of the PFP to the solvent [12]. On increasing the S/L ratio, the extraction yield of carotenoids decreased. In other words, low S/L ratio (**Fig. 3.3b** and **3.3c**), longer treatment time and higher microwave power are suitable for the extraction of carotenoids from PFP. The perturbation graph (**Fig. 3d**) clearly indicated it.

Table 3.4. ANOVA table for MAE of carotenoids using OO as solvent.

Source	**dF**	**Mean square**	**F value**	**p value**
Model	9	32228.54	9.24	0.0009
Microwave power-A	1	98163.46	28.15	0.0003
Treatment time- B	1	$1.27*10^5$	36.60	0.0001
S/L ratio- C	1	4867.08	1.40	0.2647
AB	1	9658.45	2.77	0.1270
AC	1	7126.91	2.04	0.1833
BC	1	4868.96	1.40	0.2647
A^2	1	1045.85	0.30	0.5959
B^2	1	8753.49	2.51	0.1442
C^2	1	36005.87	10.33	0.0093
Residual	10	3486.70		
Lack of fit	5	5694.62	4.45	0.0634
Pure error	5	1278.77		
Cor total	19			
R^2		0.90		
Adj R^2		0.80		
Press		$2.27*10^5$		
CV%		6.10		
Std dev		59.05		
Adequate precision		11.214		

Design-Expert software was used to statistically assess the experimental data, and the data were then fitted to the quadratic equations. R^2 value of 0.90 was obtained for yield of carotenoids (Y_1) using OO as solvent and the model equation obtained from the data is presented in **Eq. 3.36.**

$$Y_1 = 948.29 + 99.08\times A + 112.97\times B - 22.06\times C - 34.75\times AB + 29.85\times AC - 24.67\times BC - 19.50\times A^2 - 56.42\times B^2 + 114.42\times C^2 \quad (3.36)$$

From the ANOVA table for MAE (**Table 3.4),** it can be seen that for OO as solvent, microwave power (A) and treatment time (B) with probability value of 0.0003 and 0.0001, respectively, had significant effect on the extraction of carotenoids from PFP, while S/L ratio (C) (p=0.2647) was non-significant (p>0.05). The interactive effects of A, B, and C were non-significant (p>0.05). Quadratic effect of 'C' was significant but effect of A and B was non-significant.

The optimized conditions with highest desirability for maximum carotenoids yield were 200 W microwave power, 25 min treatment time and 10 g/100 mL S/L ratio. Using the optimized conditions, extraction yield was predicted to be 1180.98 µg carotenoids/100 g d.w. of PFP. The observed experimental value was 1178.54 µg carotenoids/100 g d.w. of PFP that was similar to the predicted value. Elik et al. [12] reported 78 % recovery of carotenoids using optimized condition of microwave power, extraction time and oil to peel ratio used was 8.06:1 g/g oil to peel.

3.3.8. Conventional extraction

Fig. 3.4b. clearly shows that with increase in temperature at constant time periods, the yield of PFP carotenoids increased up to the saturation point, similarly for the increase in the treatment time at constant temperature also, the extraction yield of carotenoids increased. Viscosity reduction of the oil, enhanced fluidity and solubility of the carotenoids, enhanced diffusivity and mass transfer are the factors for higher yield [18,21,55]. Further, higher treatment time means prolonged contact or exposure to the solvent [29].

At 30 ℃, to extract 830.25 µg carotenoids/100 g of PFP, it required approximately 8 h. At 45 ℃, to extract 990 µg carotenoids/100 g d.w. of PFP, it required approximately 5 h. But at 60 ℃, within 30 min of extraction, carotenoids yield was 739.456 µg/100 g d.w. of peel, which calculates to approximately 54 % extraction yield, and with further increase in extraction time up to 180 min, approximately 80 % extraction took place. The results are in agreement with Elik et al. [12] and Milic´ et al. [29].

3.3.9. Comparison of UAE, MAE, and conventional extraction methods

UAE, MAE, and CE methods for the carotenoids extraction from PFP using OO were comparatively analysed. Although in this study same input energy (1000W-5000W) were used for UAE and MAE treatments, the dissipating energy may vary.

The energy consumption in an extraction process has significant effects on the power dissipated to the sample medium, which is related to factors like type of the system, input power, treatment time, the total mass of the sample, the viscosity of the solvent, and treatment temperature [6,16]. Energy density (E_v) that was calculated for UAE and MAE at optimized conditions (process conditions optimized by RSM) was 48.9 J/mL and 185.82 J/mL, respectively. As E_v is a widely used parameter for efficacy determination, E_v delivered by MAE is approximately three times of that developed by UAE. These results imply that a lower energy was sufficient to attain almost similar extraction performance. Similarly, the power per unit mass for UAE and MAE was 0.094 W/g and 0.2178 W/g respectively, which suggested that UAE was more efficient than MAE. Similar trends were observed by Chuyen et al. [10], Meullemiestre et al. [28], and Plazzotta et al. [37] on extraction from Gac peel carotenoids, *Yarrowia lypolitica* yeast lipids and peach waste antioxidant compounds, respectively. Meullemiestre et al. [28] reported that extraction yield of UAE was higher than MAE, which may be due to the volumetric heating during microwave treatment as against concentratic energy near the probe [37] and also the UAE optimum treatment was distinguished by an extraction time greater than 1.5 times that of the MAE optimal treatment.

At the optimized conditions of UAE treatment, 91.4 % carotenoids present in PFP could be extracted, whereas at the optimized conditions for MAE, 86.9 % of carotenoids present in PFP were extracted, within a short period of time. These results showed that both UAE and MAE with OO as solvent have the potential to enhance the extraction efficiency of carotenoids and can be used as novel green extraction techniques [10,12,18,25]. UAE extracted considerably more carotenoids than MAE. In MAE, absorption of microwave energy depends on the dielectric properties of the materials [27] that cause volumetric heating [12]. Oils have much less mobility and less response to oscillating microwave radiation due to their long-chain non polar fatty acids [12]. During the MAE treatment, when the solvent molecules absorb the microwave due to the dipolar interactions, friction is generated which generates high internal temperature resulting in disruption of cell structure and release of target bioactive compounds [37].

Also, the consequent rise in temperature lowers the viscosity that promotes the diffusion rate and extraction of desired compounds [20]. But in UAE process, due to cavitation phenomenon, significantly high pressure and temperature gets generated within a very short time (heating and cooling rate above 10^{10} K/s). There is very less chance of degradation of carotenoids by UAE with oil as solvent [18] as cellular structure collapses at the surface of the cell [35] allowing for easier extraction with minimum degradation of carotenoids. Further, as UAE requires longer treatment time (39.06 min) as compared to MAE (25 min), more bioactive compounds diffuse into the solvent and increase the yield [10].

Chuyen et al. [10] also observed that UAE was better than MAE in terms of energy consumption/ efficiency for the extraction of carotenoids from Gac peel. Garcia-vaquero et al. [15] were able to extract 2340.00 mg gallic acid equivalent and 1179.93 mg gallic acid equivalent/100 g dry matter of phenolic compounds using the optimized conditions of UAE and MAE, respectively.

Among all three extraction processes, namely, UAE, MAE, and CE, it was observed that for UAE process, 91.4 % carotenoids present in PFP were extracted within the first 39.06 min at temperature of 46.6 ℃, while 86.9 % carotenoids were able to be extracted within 25 min in MAE, and 85.5 % of carotenoids were extracted in 180 min at 60 ℃ treatment temperature by conventional extraction method. Conventional method of extraction consumes considerable energy.

In addition, use of vegetable oils as solvent has many benefits like its biodegradable and non-toxic nature, no emission of volatile organic compounds, and also no need for separation of carotenoids from the oil, since carotenoids-enriched oil can have direct use in food products [18] as a source of bioactive compound or colorant. Further, UAE with OO as solvent requires small capital investment for industrial use.

3.3.10. Physical and chemical properties of UOO and CEOO

In UAE treatment, the cavitation phenomena may cause flavour deterioration, oxidation, and composition changes of some edible oils [18,47]. Quality parameters of optimally extracted CEOO was compared with UOO. **Table 3.5** gives a comparative analysis of quality parameters of CEOO and UOO oil.

PV is the primary product of oil oxidation reactions [12,18,47]. CDV gives an idea about the oxidative stability [13,47], while AV is a good indicator for hydrolytic reactions [12,47] of edible fats and oils.

Table 3.5. Quality parameters of UOO and CEOO in UAE.

Parameters	**UOO**	**CEOO**
Peroxide value (meq O_2/kg)	7.6 ± 0.50	8.5 ± 0.80
Total phenolic content (mg GAE/ g oil)	0.185 ± 8	2.820 + 29
DPPH-scavenging activity assay (%)	35.2 ± 1.4	69.4 ± 1.2
Carotenoids (µg/100 g oil)	189 ± 35	595 ± 42
β-carotene (µg/100 g oil)	72 ± 26	298 ± 37
Acid value (mg KOH/g oil)	0.31 ± 0.04	0.41 ± 0.3
Conjugated diene (µmol/g oil)	7.05 ± 0.04	7.96 ± 0.11
L*	95.71 ± 1.57	82.30 ± 2.15
a*	-5.90 ± 1.85	11.30 ± 3.50
b*	32.94 ± 4.5	26.29 05 ± 5.95

UOO - Untreated olive oil, CEOO - Carotenoids enriched olive oil

The PV value for UOO oil was 7.6 meq O_2/kg, whereas immediately after UAE treatment it increased to 8.5 meq O_2/kg. Similarly, the AV of UOO and CEOO was found to range from 0.3 to 0.41 mg KOH/g oil and CDV from 7.05 to 7.96 mmol/L (**Table 3.5**). However, the Codex Alimentarius standard states that the AV and PV values of edible oil should be, respectively, less than 4.0 mg KOH/g oil and 15 meq O_2/kg oil. Thus, although the PV and AV increased after treatment, the observed values were within the acceptable range. PV value was reported to increase after ultrasonication treatment [47] and microwave treatment [12].

Among the carotenoids, β-carotene is the major one followed by lutein in the peel of purple coloured passion fruits, comprising approximately 58 % and 30 % of the whole carotenoids [41], respectively. There was no carotenoids degradation in sonicated oil, as evidenced by HPLC chromatograms of the carotenoids extracted by ultrasound under optimal conditions [18,25]. So, β-carotene, the major form of carotenoids was studied. PFP had β-carotene content of 746.45 ± 35.6 µg/100 g and TCC of 1356 ± 81.5 µg/100 g PFP, which showed that β-carotene fraction of the total carotenoids in PFP was 55.6 %. In CEOO, β-carotene and total carotenoids contents was 2.98 and 5.59 µg/g oil, respectively, indicating that after the UAE treatment, carotenoids and β-carotene levels increased to 4.06 and 2.26 µg/g oil, respectively and in the oil β-carotene content increased from 38 % to 51 % of total carotenoids **(Table 3.5)**. These results indicated the good extraction of carotenoids and β-carotene from PFP without any significant

degradation of carotenoids and the carotenoids content in the extraction oil was more than three times of the value before the treatment.

Table 3.5 shows that UAE process for carotenoid extraction increased the antioxidant activity and the total phenolic content in oil. Total phenolic content in CEOO increased by more than fifteen times (2.820 mg GAE/g oil) than that in UOO (0.185 mg GAE/g oil). For the antioxidant activity of the extracted DPPH, free radical scavenging activity (%) was calculated. Its value for UOO was 35.2 %, reached up to 69.4 % i.e., increased by 34.2 % and became approximately double. The increase in activity may be due to the strong antioxidant activity of carotenoids like β-carotene [12,20] and also the other compounds like phenols, anthocyanins etc, [41]. Similar results of increase in antioxidant activity were reported by Goula et al. [18] and Elik et al. [12] for carotenoids extraction using oil as solvent.

Comparisons were made between the color values (CIELAB L*, a*, and b* parameters) of CEOO and UOO. Higher a* value and slightly lower b* and L* (**Table 3.5**) indicated that CEOO was darker, more reddish and slightly moving towards blue colour than UOO. Silva et al. [47] and Elik et al. [12] have observed that the development of blue-red colour of CEOO is due to the extraction of higher amount of anthocyanins in oil during UAE treatment because besides β-carotene, anthocyanin is an important compound in passion fruit.

3.3.11. Pseudo second order kinetics

The extraction kinetics of carotenoids for the UAE and CE treatment using OO were analysed at various combinations of temperature and treatment time. For UAE, temperature ranged from 30 ℃ to 45 ℃ (optimum temperature level obtained from RSM optimization) and the responses were measured up to 35 min of UAE treatment time at every 5 min intervals; and for CE, temperature of 30 ℃, 45 ℃ and 60 ℃ were used for extraction time of 4 h, 6 h and 10 h (**Fig. 3.4a**). S/L ratio was kept constant at optimized condition obtained by UAE using OO throughout the extraction process for its kinetic study. Based on the calculated parameters, kinetics of UAE and CE for carotenoids extraction from PFP using OO was compared.

The model characteristics of carotenoids extraction from PFP by UAE and CE methods were studied using a pseudo second order model **(Eq. 3.9**). To perform the kinetics analysis for both UAE and CE treatments, the model was converted to a linear form by plotting the graphs 't/C_t' versus 't' expressed by **Eq. 3.11**. Saturation

concentration (C_{sa}) and extraction rate constant (k_s) were determined from the slopes and intercepts of the graphs (**Table 3.6**).

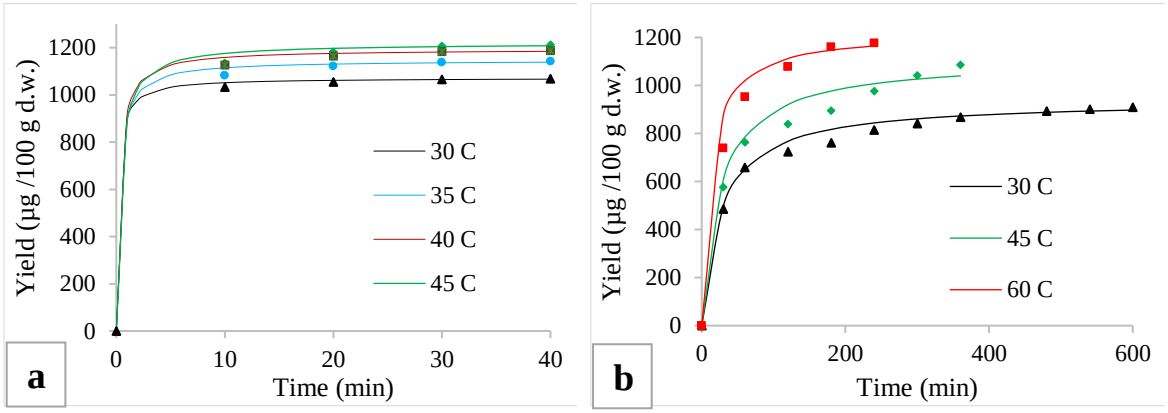

Fig. 3.4. Pseudo second order kinetics and experimental graphs of extraction of carotenoids by (a) UAE, and (b) CE extraction.

Saturation concentration (C_{sa}) of carotenoid compounds for both UAE and CE process in OO extraction increased with rise in temperature and values were observed in the range between 1071.81 to 1219.51 µg carotenoids/100g of PFP for UAE and 937.21-1222.49 µg carotenoids/ 100g of d.w. for CE (**Table 3.6**). The k_s value was observed in the range between 0.0022 to 0.0048 100g/µg carotenoids × min for UAE and 4.02×10^{-5} to 6.85×10^{-5} 100g PFP/ µg carotenoids × min for CE (**Table 3.6**).

Table 3.6. Pseudo-second-order model kinetics parameters for the UAE and CE of carotenoids from PFP at different extraction temperatures.

Method	Temp(°C)	C_{sa}	k_s	h_0	R^2	RMSE
	30	1071.8113 ± 40.5212	0.0048±0.0005	5555.5554 ± 303.5	0.9996	2.0849
UAE	35	1146.7889 ± 56.2542	0.0030 ± 0.0003	3968.2539 ± 290.5	0.9992	2.6399
	40	1193.3174 ± 35.5885	0.0027 ± 0.0001	3984.0637 ± 220.2	0.9993	2.6819
	45	1219.5121 ± 36.2785	0.0022 ± 0.0001	3257.3289 ± 185.4	0.9988	3.0514
	30	937.2071 ± 27.2619	4.02 x10^{-05} ± 0000	35.3545 ± 1.8845	0.9931	3.4141
CE	45	1112.3475 ± 58.2145	4.19 x10^{-05} ± 0000	51.5488 ± 1.2541	0.9846	4.7521
	60	1222.4944 ± 68.8954	6.85 x10^{-05} ± 0000	102.4451 ± 9.0024	0.9833	5.4719

Both C_{sa} and k values for UAE were higher as compared to CE for the same extraction temperature which indicated the positive effect of ultrasound and successful extraction by UAE. Very high temperature and pressure generated for very short time during UAE resulted in higher solubility and k_s value [35]. In this work, with increase in the temperature, for both the processes, the C_{sa} value also increased, this may be due to

the unsteady interactions of oil and solid molecules and positive impact of temperature on solubility of compounds [34,39]. The rate constant (k_s) for the CE treatment increased with increase in the extraction temperature, which may be due to the higher thermal energy required for diffusion of solute and also to the reciprocal effect of temperature on viscosity of solvent that resulted in improved extraction rate [29,39]. But for UAE, the rate constant (k_s) decreased with increase in the extraction temperature, because at higher temperature, cavitation bubbles generated by ultrasound probably collapses easily, which results in reducing the intensity of bubbles and decreases the cell damage as well as intensity of micro turbulent flow, so overall reduction in mass transfer occurs [8,17]. Goula et al. [18] reported decreased rate constant of carotenoids with increase in temperature from 20 to 40 ℃, from waste portion of pomegranate using SO. Goula [17] reported the highest rate constant at the lowest temperature (20 ℃) in the range of 0.0045 - 0.0075 min^{-1}. For the ultrasonic treatment, Charpe and Rathod [8] observed increment of saturation concentration value but reduction of rate constant with increase in treatment temperature. For the initial extraction rate, h_0 values for the UAE and CE were found to range from 3257.3289 to 5555.5554 and 35.3545 to 102.4451 100g/ µg carotenoids × min. h_0 value increased with increasing temperature for CE but decreased for UAE, as also reported by Goula [17].

3.3.12. Phenomenological kinetic modelling of extraction

Fig. 3.5 represents the change in the yield of carotenoids due to the techniques used for extraction from PFP by OO at various temperatures. For both processes, carotenoids yield increased with independent parameters of extraction time and temperature independently (**Fig. 3.5A** and **3.5B**).

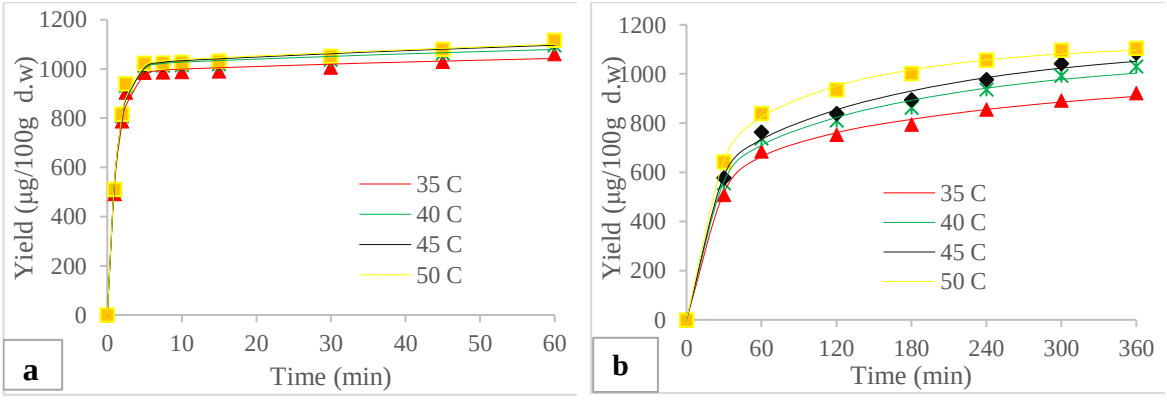

Fig. 3.5. Phenomenological kinetics of carotenoids extraction from PFP by SO at different temperatures: (A) UAE, (B) CE.

Table 3.7. Parameters of phenomenological model for extraction of carotenoids.

Method	Temp (℃)	w_∞ (µg/100 g, d.w)	f1	k_1 $(min)^{-1}$	k_2 $(min)^{-1}$	R^2	MRPD (%)
CE	35	981.0 ± 5.6	0.612154 ± 0.06	0.050543 ± 0.004	0.004619 ± 0.0008	0.998115	± 1.339884
	40	1068.5 ± 7.6	0.555923 ± 0.04	0.058559 ± 0.003	0.005509 ± 0.0005	0.996348	± 1.899916
	45	1120.1± 11.1	0.533535 ± 0.08	0.062241 ± 0.007	0.005637 ± 0.0007	0.995465	± 2.118118
	50	1122.5 ± 10.25	0.602532 ± 0.07	0.056978 ± 0.004	0.008062 ± 0.0009	0.998889	± 1.032693
UAE	35	1108.2 ± 9.54	0.891787 ± 0.07	0.783036 ± 0.002	0.010155 ± 0.0005	0.99889	± 0.893609
	40	1182.1 ± 8.47	0.858738 ± 0.05	0.783006 ± 0.005	0.008113 ± 0.0005	0.998961	± 0.874011
	45	1219.5 ± 12.2	0.834917 ± 0.09	0.782178 ± 0.008	0.008292 ± 0.0007	0.999018	± 0.878479
	50	1278.6 ± 9.85	0.801574 ± 0.08	0.783569 ± 0.007	0.005962 ± 0.0005	0.998992	± 0.867865

Table 3.8. Parameters of pseudo-first order model and model based on instantaneous washing followed by diffusion for extraction of carotenoids from PFP.

Method	Temp (℃)	Pseudo-first order model			Model based on instantaneous washing followed by diffusion			
		$k_1\ (min)^{-1}$	R^2	MRPD (%)	f1	$k_2\ (min)^{-1}$	R^2	MRPD (%)
CE	35	0.016952	0.952085	±11.33376	0.080711	0.014614	0.935184	±10.49522
	40	0.0166	0.949076	±10.86277	0.084688	0.01415	0.933881	±9.909175
	45	0.01594	0.944801	±11.15481	0.091923	0.013415	0.929053	±9.93391
	50	0.022986	0.979194	±5.580781	0.029899	0.022089	0.976992	±5.519963
UAE	35	0.59892	0.989402	±7.296316	0.004482	0.595976	0.98913	±7.293148
	40	0.542932	0.982802	±10.16086	0.015571	0.532419	0.981233	±10.13764
	45	0.503084	0.976522	±11.98093	0.026795	0.484567	0.972905	±11.91576
	50	0.445634	0.962797	±15.59492	0.052566	0.407653	0.950672	±15.33493

The carotenoids extraction mechanism from PFP comprised of both rapid washing process and slow diffusion process. In the initial stage, rapid extraction of the extractable materials located at the surfaces of the plant matrices and present within the cells took place, while the extractable materials that were intact with cells slowly diffused to solid matrices surface. This phenomenon was the base for subsequent modelling of PFP carotenoids extraction kinetics.

Table 3.7 presents the values of **Eq. 3.15** for CE and UAE extraction techniques. As shown in **Fig. 3.5**, **Eq. 3.15** was well fitted with the experimental data. This was supported by higher R^2 value (~1) (**Table 3.7)**. The values of the PFP carotenoids yield obtained from experiment and model (predicted) agreed with each other (MRPD = ± 0.867865% to 2.118118 %), which can be visually observed in **Fig. 3.5**. At saturation, the carotenoids extraction yield increased as the extraction temperature was increased. The fraction of washable compounds was reduced with increasing extraction temperature for both extraction processes. The "f1" values were higher for the UAE (0.801574 - 0.891787) at lower process temperatures than the conventional one (0.533535 - 0.612154), which implied the greater impact of ultrasound on the washing extraction than the conventional process [29]. For the comparison with phenomenological model, pseudo-first order model where washing process was neglected and instantaneous washing followed by diffusion model, where diffusion process was neglected, was developed and can explained by **Eq. 3.16** and **3.17** respectively. For the evaluation purpose, **Eq. 3.16** and **3.17** were compared with **Eq. 3.15** (phenomenological model). The parameter values of two simpler models (**Eq. 3.16** and **3.17**) are listed in **Table 3.8**. Both the models had small R^2 value and relatively high MRPD (%) value. However, the instantaneous washing followed by diffusion model (**Eq. 3.17)** had relatively high R^2 and low MRPD (%) values (**Table 3.8**). Therefore, combination of both processes (in phenomenological model) showed better prediction and describe well the extraction kinetics of carotenoids from PFP compared to individual methods. So, the phenomenological model could be recommended for modelling the extraction kinetics of bioactive compounds.

3.3.13. Effective diffusion coefficient (D_e), mass transfer coefficient (k_{mt}), and Biot number (B_i) estimation of carotenoids extraction processs

The D_e, k_{mt} and B_i of carotenoids extracted by both UAE and CE methods as influenced by temperature are presented in **Table 3.9.** For UAE, the D_e value decreased

with increasing temperature (2.3740×10^{-13} - 2.8260×10^{-13} m^2/s) but the effect was opposite for CE (0.997×10^{-14}- 2.336×10^{-14}). Similar trend was seen for k_{mt} and values were observed in the range of 1.625×10^{-7}- 1.8731×10^{-13} m/s and 0.0728×10^{-8} - 0.1714×10^{-8} m/s for UAE and CE, respectively. Higher D_e and k_{mt} values observed for UAE treatment may be due to the accelerated mass transfer because of micro-turbulence and high-velocity particle collisions of the cellular material caused by the cavitation phenomenon. But for B_i, with increase in temperature, the value also increased from 43.7454 to 45.1798 for UAE and from 48.1925 to 48.4264 for CE process.

Table 3.9. Effective diffusion coefficient, mass transfer coefficient, and mass transfer at different temperatures during UAE and CE of carotenoids from PFP

Method	Temp. (°C)	D_e x 10^{-13} (m^2/s)	K_{mt} x 10^{-7} (m/s)	B_i
UAE	30	2.8260 ± 0.7621	1.8731 ± 0.5480	43.7454
	35	2.5661 ± 0.6553	1.7172 ± 0.4785	44.1663
	40	2.5443 ± 0.6180	1.7194 ± 0.4641	44.6018
	45	2.3740 ± 0.5450	1.6251 ± 0.4200	45.1798
CE	30	0.0997 ± 0.0103	0.0728 ± 0.0108	48.1925
	45	0.1448± 0.0263	0.1081 ± 0.0145	49.2722
	60	0.2336 ± 0.0452	0.1714 ± 0.0355	48.4264

As shown in **Table 3.9,** for same parameter conditions, the mass transfer coefficient (k_{mt}) is much higher than effective diffusion (D_e) for both UAE and CE. For CE process, k_{mt} and D_e were positively impacted by an increase in temperature, probably due to positive effect of thermal energy and reciprocal effect of temperature on viscosity. But for UAE treatment, with increase in temperature, a decrease in both D_e and k_{mt} were observed that can be attributed to changes in vapour pressure and surface tension. Vapour pressure has a greater impact on the generation of bubbles and intensity of cavitation phenomena and increases with temperature. At low temperature, due to low vapour pressure very few cavitation bubbles are generated but these bubbles explode with high intensity that enhances the disruption of cell tissues. On the other hand, at higher temperature, large number of bubbles is generated under the influence of high vapour pressure, but due to low pressure difference of the inside and outside of bubbles, they collapse at low intensity causing less impact on cell damage [8,17]. Another reason may be the decrease in surface tension with increasing temperature which gives negative

impact on bubble formation and collapse and as a result the mass transfer rate reduces. Raj and Dash [39] found slightly lower effective diffusivity and mass transfer rate than Norena and Meireles [32] and Ruiz et al. [42], probably because of the use of oil as a solvent which has higher viscosity than ethanol.

Biot number (B_i) gives an idea about the mass transfer of the extraction process and tells whether the process is dominated by external or internal diffusion. When the B_i value is ≤ 1, external mass transfer is what limits the process. However, with $1 \leq B_i \leq (30 \div 40)$, a mixed mass transfer mechanism is shown, and diffusion is internal when $B_i \geq (30 \div 40)$ [48]. The B_i values for UAE and CE extraction processes were found to be in the range of 43.7454 - 45.1798 and 48.1925- 49.2722, respectively (**Table 3.9**), which indicate that internal diffusion dominated the extraction rate (for all, $B_i \geq (30 \div 40)$). For CE, B_i values were found to be higher as compared to UAE for the same temperature. For both UAE and CE, with increase in temperature the value of B_i increased. Simeonov, Yaneva, and Chilev [48] and Jo and Kim [23] reported B_i values to be in the range of 0.3-13000 and 3.927-8.959, respectively for solid-liquid extraction.

3.3.14. Thermodynamics properties of UAE and CE

Table 3.10 shows the thermodynamic characteristics for the UAE and CE of the carotenoids extracted from PFP. The ΔH value was found to be 106.0866 kJ/mol and 70.6152 kJ/mol for UAE and CE, respectively. For both treatments, positive ΔH was observed, this demonstrates that the extraction process was endothermic [53]. Compared to CE, the ΔH value for the UAE was higher, probably due to the absorption of large quantity of ultrasonic energy and its conversion to heat [30]. Change in entropy (ΔS) for the two extraction methods were found to be positive (364.9846 and 237.0114 J/mol K for UAE and CE, respectively) (**Table 3.10**), which indicates the irreversibility of both processes for extraction of carotenoids from PFP. The disorder in the extraction system can be attributed to the shift of solute from the highly ordered structure in the peel to less ordered structure in the oil phase [39]. The ΔS value was higher for UAE process due to effects of the cavitation process [35]. In this study, for UAE and CE, Gibbs free energy (ΔG) values ranged from -10.0866 to -4.6284 KJ/mol and -8.9691 to -1.7515 KJ/mol, respectively (**Table 3.10**). Negative value of ΔG implies the spontaneous nature of the reaction/ extraction process and at the same temperature UAE was more spontaneous as compared to CE.

Table 3.10. Thermodynamic parameters for UAE and CE of total carotenoids content from PFP.

Methods	Temp. (℃)	ΔH (KJ/mol)	ΔS (J/mol)	ΔG (KJ/mol)
UAE	30	106.0866	364.9846	-4.6284
	35			-6.2962
	40			-8.1850
	45			-10.0866
CE	30	70.6152	237.0114	-1.7515
	45			-3.6521
	60			-8.9691

For both extraction processes, the values remained negative with increase in temperature. As a result, when the extraction was done at a higher temperature, it was more spontaneous and highly possible [19]. The results indicated that both CE and UAE processes using oil as solvent for recovery of carotenoids from passion peel were endothermic, irreversible, and spontaneous but UAE was more feasible as compared to CE at the same temperature.

3.4. Conclusion

A novel green approach based on UAE and MAE of carotenoids using vegetable oils as solvents for the utilization of passion fruit peels is suggested. Both extraction processes have the potential to enhance the extraction efficiency. Among UAE, MAE, and CE, UAE with olive oil as a solvent was found best for the extraction of carotenoids from PFP. There is no need for separating carotenoids from the oil, because the coloured oil can be used as a source of carotenoids for foods. Both pseudo second order model and phenomenological model showed the potential to be used for explaining the changes during the extraction of carotenoids. D_e, k_{mt} and B_i of carotenoids extracted by both UAE and CE methods were explained which could be used to understand the extraction behaviour. The thermodynamics results showed that CE and UAE process for recovery of carotenoids from passion fruit peel using oil as solvent were endothermic, irreversible, and spontaneous but UAE was more feasible as compared to CE at the same temperature. The kinetics data from the ultrasonic-assisted green extraction process, particularly the rate constant, effective diffusivity, and mass transfer coefficient, would enable the prediction of operational parameters for industrial use.

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