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A Study for Extraction Kinetics of *Β*-Carotene from Oven Dried Carrot by Solvent Extraction

Sudipta Das*, Saikat Mazumder**, Soumitra Banerjee***
* (Department of Food Technology, Techno India, Salt Lake, Kolkata-91, India)
** (Department of Food Technology, Techno India, Salt Lake, Kolkata-91, India)
*** (Department of Food Technology, Techno India, Salt Lake, Kolkata-91, India)

ABSTRACT
The main aim of this work was to study the kinetics of the extraction of β-carotene by solvent (ethanol) extraction from carrots dried at 50ºC in an oven. The moisture content after drying was 11±1.5% (dry weight basis). Extraction time was 5 hours and extraction process was carried out at 30ºC. The ratio of Carrot (gm): ethanol (ml) of 1:60 was maintained throughout the extraction process. The experimental data of extracted *β*-Carotene yield during solvent extraction were modeled with seven different mathematical models, including Power law, Peleg, Weinbull's equation, Pseudo 2nd order, Intraparticle diffusion, Logarithmic, Weinbull's distribution and one RSM model developed in this study. Pseudo 2nd order model was best fitted model (R^2- 0.99, χ^2- 0.0091, RMSE- 0.1566, MAE- 0.0115) to describe extraction kinetics of β-carotene from carrot. The extraction time has significant effect on extraction process at $p<0.05$ level.
***Keywords* -** *β*-Carotene, carrot, extraction kinetics, mathematical model, RSM modeling

I. Introduction

Carotenoids are one of the major groups of natural pigments that find widespread utilization in the food industry due to their nutritional and colorant properties [1]. These pigments are fat-soluble and present in wide variety of fruits and vegetables like carrots, peaches, banana skins, tomatoes, red pepper, paprika, sweet potatoes, etc. as well as other parts of plants e.g. in yellow, orange and red flowers. The carotene content of carrots ranges from 600 - 1200 μg/g, while some varieties may contain up to 3000 μg/g [2]. Carotenoids play an important role as food ingredients due to important role as food ingredients due to their provitamin A activity and antioxidant function. This antioxidant activity is associated with the reduced risk of lung and colon cancers. β -carotene is a terpene and is most common form of carotenoid. It is a red-orange pigment abundant in plants and fruits, peculiarly in carrot. β-carotene is converted to retinol, which is essential for vision and is subsequently converted to retinoic acid, which is used for processes involving growth and cell differentiation. The β-carotene content is about 80% of total carotenoids present in the carrots.

Extraction of carotenoids may be accomplished by traditional solvent extraction (TSE) and supercritical fluid extraction (SCFE). Conventional methods are usually carried out at high temperatures, at which destruction of valuable substances may be significant. Additionally, use of organic solvents can also lead to product contamination with solvent residues [3]. Traditional extraction methods used to obtain the products have several drawbacks; they are time consuming, laborious, have low selectivity and low extraction yields. Although having some problem, (TSE) is most cost effective process for extraction for third world. In (TSE) we have to choose food grade organic solvent for extraction. Sometimes vegetable oils (sunflower oil, peanut oil, soy oil, coconut oil) are used for extraction. The objective of the work is to study the kinetics of extraction of β- carotene from

II. Material and Methods

2.1 Collection and Preparation of Raw Material

Carrots were procured from local market of Kolkata, West Bengal. They were cleaned under running tap water to remove adhering soil and impurities. Cleaned fresh carrots were peeled, sliced into small pieces, thoroughly mixed, and then one part of the Carrot slices were dried at 50°C in an oven dryer for 24 hrs and followed by grinding using a laboratory grinder. The raw and dried sample was used for further experiment. Ethanol as food grade solvent selected for the purpose of solvent extraction on the basis of literature survey [4].

2.2 Determination of Moisture Content

Moisture content of the raw and oven dried carrot sample was determined by gravimetric method, drying the sample in hot air oven at 103°C ± 2°C for 3±0.5 hrs and then to constant weight.

2.3 Solvent Extraction

Solvent extraction was performed in rotary water bath shaker under selected operating condition. About 2 gm grinded sample and 120 ml ethanol was transferred into conical flask. The conical flask was then placed in water bath shaker and temperature was set at 30ºC. The sample was picked up at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min. Extraction was continued for the desired period for the kinetic study.

2.4 Estimation of β-Carotene

Estimation of β-carotene was determined by spectrophotometric method at 450nm (pc based double beam spectrophotometer 2202). The concentration of Carotenes expressed as β-carotene (mg/100ml) was calculated using the response factors as follows:

β- Carotene = $(A \times d \times V) / (E^{1\%}_{1cm} \times w)$ [5] ….. (1)

Where:
A – Absorbance
d – Dilution
$E^{1\%}_{1cm}$ – coefficient of absorbency (2620 for ethanol)
w – Weight of sample (g)
V – Volume (ml)

2.4 Hunter Lab Colorimetric Analysis

The colour analysis was also conducted in this study to obtain some knowledge about the quality of the extracted product. The visual appearance of raw, extracted carrot cubes is evaluated by a color-difference meter technique using a hunter Lab (color flex 45/0 spec photometer). Three parameters, L (lightness), a* (redness), and b* (yellowness), were used to study the colour changes. The L refers to the lightness of the samples and ranges from black = 0 to white = 100. The negative value of a* indicates green, while the positive a* indicates red colour and positive b* indicates yellow and the negative b* indicates blue colour [6]. The total colour difference (ΔE) was also calculated. In addition to the parameters a*, b*, and L, the colour density C and the hue angle H^o were also determined. These parameters depend on the colour space coordinates and are defined as follows:

$C^* = (a^{*2} + b^{*2})^{0.5}$ ……………….……………….. (2)

$H^o = \tan^{-1} (b^*/a^*)$ ……………….……….... (3)

$\Delta E = [(L^*-L_{standard})^2 + (a^*-a_{standard})^2 + (b^*-b_{standard})^2]^{0.5}$ ……………………………………… (4)

$\Delta C^* = C_0 - C^*$ ……………….…………. (5)

$\Delta H^o = (\Delta a^{*2} + \Delta b^{*2} - \Delta c^{*2})^{0.5}$ …………... (6)

2.6 Kinetics Study of *β*-Carotene by different Model

Various mathematical models have been used to study of kinetics of extraction. These include 1) Power law, 2) Peleg, 3) Weinbull's equation, 4) Weinbull's distribution 5) Intraparticle diffusion, 6) Pseudo 2nd order and 7) Logarithmic model. Fick's law was not used in this study due to considerable variation in particle size of the ground sample.

Power law model equation expressed as:

$q = B.\, t^n$ …………….……………….. (7)

Where B is a constant which is related with the characteristics of the carrier–active agent system and n is the diffusion exponent. In case of extraction from vegetable component n is always less than 1. Power law model is used for the diffusion of an active agent where diffusion is carried out through non swelling devices. [7]

B - Parameter of power low model (min^{-n})
n - Diffusional exponent of the power law model
t - Time (min), q - Extraction yield

Peleg model also called hyperbolic model. In 1988 this model was proposed to describe the moisture sorption curves [8]. The mathematical expression of this model is given by equation:

$q = (K_1.\, t)/ (1+K_2.\, t)$ ……………………. (8)

Where K_1 and K_2 are parameters of the hyperbolic model
K_1 - parameter of the hyperbolic model; extraction rate at the very beginning (min^{-1})
K_2 - parameter of the hyperbolic model; constant related to the maximum extraction yield (min^{-1}).

Weinbull's equation can be also applied for plant extraction in the following form:

$q = 1-\exp(-(t/\delta)^m$ ……………….……... (9)

This model is also used for extraction of different component from vegetables. Where δ is the scale parameter of the extraction process and m is the shape parameter of extraction curve, δ is related to the reciprocal of the extraction rate constant. If it is found that m<1 for extraction process, parabolic curve is generated with a high initial slope [9].

q - Extraction yield; $q = q_t / q_e$ ……….…….... (10)

The pseudo second-order equation is based on the desorption capacity of the solid phase [10]. This equation can be present at two forms, such as integrated form and linear form. The integrated form of the equation is:

$1/(q_e-q_t) = 1/q_e + k_2 t$ …………………… (11)

Where, k_2 is the second–order rate constant.
The linear form of the equation is

$t / q_t = 1/h + (1 / q_e)\, t$ ………………….. (12)

Where, $h = k_2.q_e^2$ and h is the initial sorption rate of the extraction process.

q - Extraction yield ($q = q_t / q_e$)

The Intraparticle diffusion model is used to determine the participation of this process in the desorption of carotene from carrot**.** According to this model, graph should be plotted of desorped amount (q_t), versus the square root of time ($t^{0.5}$). Graph should be linear if intraparticle diffusion is involved in the overall desorption mechanism of carotene from carrot. Further-more, if this line passes through the origin then the intra particle diffusion is the rate-controlling step of the process [11, 12]. The initial rate of intra particle diffusion, K_d, can be calculated in the following way:

$q_t = K_d t^{0.5} + C$ ………..……..………… (13)

Where, q_t (mgg^{-1}) is the amount of carotene on the surface of the carrot at time t, K_d is the intra-particle rate constant and t is the time (min).

Logarithmic model is expressed as:

$(q_e - q_t)/ q_t = \exp(-kt)$ …………………….. (14)

Where, q_t is concentration of β-carotene in the extract (mg/g) at different time t, q_e is the concentration of β-carotene when equilibrium reached. k is extraction rate (min^{-1}); and, t is the time (min) [13].

Weibull distribution model is expressed as:

$(q_e - q_t)/ (q_o - q_t) = \exp(-kt^n)$ …………… (15)

Where, n is dimensionless coefficient [13].

Four parameters, coefficient of determination (R^2), reduced chi-square (χ^2), and root mean square error (RMSE), Mean absolute error (MAE) were used to evaluate the goodness of fit of tested models to the experimental data. The lowest χ^2 and RMSE values, Mean absolute error (MAE) values and the highest R^2 value describe the fitness of model.

The root-mean-square error (RMSE) is used measure of the differences between values predicted by a model or an estimator and the values actually observed. The RMSE represents the sample standard deviation of the differences between predicted values and observed values.

The mean absolute error (MAE) is a quantity that is used to measure how close forecasts or predictions are to the eventual outcomes or experimental value.

Statistical values were defined with equations following:

$$\chi^2 = \sum_{i=1}^{N} (q_{exp,i} - q_{pre,i})^2 / (N-n) \quad \text{…………….....} (16)$$

$$RMSE = \sqrt{\sum_{i=1}^{N} (q_{pre,i} - q_{exp,i})^2 / N} \quad \text{…………} (17)$$

$$MAE = 1/n \sum_{i=1}^{N} \left| (q_{exp,i} - q_{pre,i}) \right| \quad \text{…………….....} (18)$$

Where $q_{exp,i}$ and $q_{pre,i}$ are experimental and predicted values of amount of β-carotene respectively. N is the number of observations, and n is the number of model constants.

2.7 Determination of Regression Equation (RSM Modeling)

Functional relationships between the independent variables (extraction time) and dependent variables (yield of β carotene) were determined using multiple regression technique by fitting second order regression equation [14] of the following type:

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n-1} \beta_{ij} X_i X_j + e \quad \text{……………………………………………….} (19)$$

Where β_0, β_i, β_{ii}, β_{ij} are regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively, X_i, X_j are the independent variables, Y is the dependent variables n is number of independent variables.. The significance or P-value was decided at a probability level of 0.05. The relationships between the responses were judged by correlation coefficients of determination (R^2).

III. Results and Discussion

3.1 Determination of Moisture Content

Moisture content of fresh, oven dried and extracted carrot was found to be 86±1%, 11±1.5% and 9.5±0.5% (dry weight basis) respectively.

3.2 Determination of Extracted β-carotene Amount

Concentration of β-carotene (q_e) was determined at 450nm by spectrophotometric method. Fig 1 shows the amount of extracted β-carotene from oven dried carrot at different time. After 5 hrs the extraction process almost reach to the equilibrium condition, amount of extracted β-carotene at 300 minutes was 10.442mg/100ml.

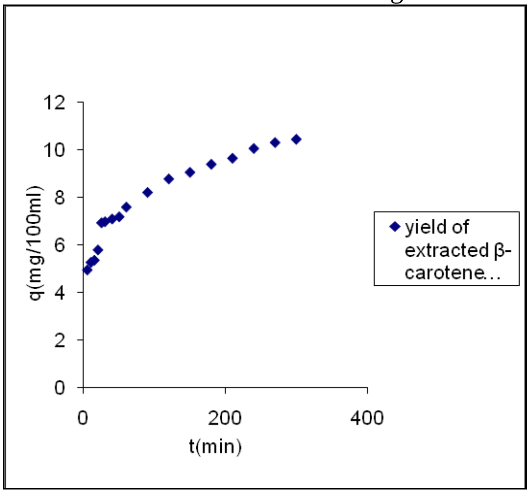

Fig 1: Yield of β- carotene w. r. t time

3.3 Mathematical modeling of extraction kinetics

It was found that extraction process almost reached to equilibrium after 5hrs from starting. Kinetics study was therefore conducted for up to 5 hrs. It was observed power law model was good fitted at selected temperature. Value of diffusion exponent (n) <1(n always less than 1 in case of vegetables) and B was 3.412 $min^{-0.2}$ was found for power law model. Sorption rate of process from Psuedo 2nd order model was 0.577 and 2nd order rate constant was (k_2) 0.0048 min^{-1}. Graph of Intraparticle diffusion model was linear with 4.4971 intercept. In this graph line does not pass through origin, so it can be conclude that intra particle diffusion is not the rate-controlling step of the process. The initial rate of intra particle diffusion was 0.3632.For Weibull;s equation extraction rate constant of the process was 0.4 and shape parameter(m)was not greater than1. Extraction rate of Weinbull's distribution model was found 0.235. Both Weibull's model was also good fitted for the extraction process.

Peleg model described two model constant for extraction process. As per Peleg model extraction rate during washing stage was 1.688 min^{-n} and 0.194 $min^{-1}$was when β-carotene extracted from core of the carrot. Extraction rate of logarithmic model was 0.00025(min^{-1}). Peleg model and logarithmic model were not well filled for extraction process.

Parameters of all model is shown in Table1. The statistical analysis of the better fitted models is summarized in Table 2.

Table 1: Values of model parameter of extraction at 30°C

Model name	Model equation	Model constants		
Power law	$q = 0.1955\ln t + 1.2269$	B (min^{-n})	n	
		3.412	0.1955	
Peleg	$1/q = (0.5924)1/t + 0.115$	$K_1(min^{-1})$	$K_2(min^{-1})$	
		1.688	0.194	
Weinbull's equation	$\ln[\ln\{1-(q_t/q_e)\}] = 0.4499\ln t - 1.4508$	δ[min^{-1}]	m	
		25.15	0.4499	
Intraparticle diffusion	$q_t = 0.3632t^{0.5} + 4.4971$	K_d	C	
		0.3632	4.4971	
Pseudo 2nd order	$t/q_t = 0.0933t + 1.7319$	h	q_e	k_2
		0.577	10.718	0.0048
Logarithmic	$1-(q_t/q_e) = -0.00002t$	K(min^{-1})		
		0.00025		
Weinbull's distribution	$\ln[-\ln(q_t-q_e/-q_t)] = 0.4494 \ln t - 1.4484$	K	n	
		0.235	0.4494	

Table 2: Values of statistical parameters of better fitted models

Model name	R^2	χ^2	RMSE	MAE
Power law	0.98	0.0564	0.2233	0.1597
Intraparticle diffusion	0.98	0.1222	0.3283	0.2824
Pseudo 2nd order	0.99	0.0091	0.1566	0.0115
Weinbull's equation	0.95	0.1953	0.4151	0.3590
Weinbull's distribution	0.95	0.1956	0.4153	0.3594

3.4 RSM modeling

The second order model was fitted to response data of extracted yield of β-carotene, It has been found that extraction time and square term of extraction time have significant effect at $p<0.05$ level on yield of β-carotene.

The student's t-test was performed to determine the significance of the regression co-efficient. The results of statistical analysis including the regression co-efficient, t and p values for linear, quadratic of the variables were given in the Table 3. The larger the magnitude of the t-value and the smaller the p-value, indicate more significant of the corresponding coefficient and its effect on extracted yield of β-carotene. The p-values are used as a tool to check the significance of each of the coefficients.

Table 3: Estimation of Regression Parameters, p value, and t value

Effect	parameter	p	t
Intercept	5.329116	0.000000a	26.49387
Extraction time	0.036361	0.000001a	8.68347
Extraction time^2	-0.000068	0.000364a	-4.66628

Table 4: Multiple R, Multiple R^2, and Adjusted R^2 of Regression Equation

Multiple R	Multiple R^2	Adjusted R^2
0.977382	0.955275	0.948886

Joglekar and May (1987) [15] have suggested for a good fit of a model, regression coefficient (R^2) should be at least 80%. All the R^2 values are the proportion of variation in the response attributed to the model was > 0.80 (Table 4), this means that this model fitted well with the experimental data. The regression model equation from the parameters of Table 3 for responses of yield of β-carotene has used to predict the data for different responses. Correlation of experimental and predicted data

(Table 4) provided an observation that a good relationship exist between experimental and calculated data.

3.5 Hunter lab Colorimeter Analysis of Carrot

The fresh carrot cubes are taken as the reference and a higher ΔE stands for greater color change from the reference material. The hunter Lab was calibrated automatically before each color measurements with the standard white plate having "L", "a" and "b" values of 92.65, -0.82 and 1.31 respectively.

Table 5: Hunter lab Colorimeter analysis of carrot

Colour parameter	Original/Fresh	Residue after extraction
L	56.22	43.13
a*	26.72	2.58
b*	25.25	5.32
a*/b*	1.06	0.49
C*	36.76	±5.91
ΔC*	-	±30.85
H°	43.38	64.13
ΔH°	-	5.31
ΔE	-	±33.93

After solvent extraction Lab value was measured. L, a*and b* value of residue decreased from original. Total colour difference (ΔE) of the residue was ±33.93 which lead to huge colour difference occurred during extraction. The hue angel (H°) was between 0°-90° which indicates that the residue belonged within red portion of Lab chart. Other colour parameter also indicates good extraction. From above table it can be conclude that extraction process was very effective at selected parameter and also conclude that good amount of carotene was extracted from oven dried carrot.

IV. Conclusion

Extraction yield of β-carotene was increased with respect to different time interval. Extraction process was saturated after 5 hrs from the beginning of the process. β- Carotene is very sensitive to heat treatment. Drying may decrease the yield of β-carotene. But further study is required to confirm the above statement, such as optimization of drying temperature and drying time. Pseudo 2nd order was the best model to describe the extraction process.

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