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CHARACTERIZATION AND OPTIMIZATION OF CAROTENOID EXTRACTED FROM THE PEELS OF TOMATO *LYCOPERSICON ESCULENTUM* GROWN IN NIGERIA

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ABSTRACT

The application of synthetic colourants to foods, snacks, and beverages has increased within the past 50 years, and up to a 500% increase has been reported in Nigeria. Consumers of coloured foods and beverages have been showing worries about the possible health hazards of such products over time. Hence, researchers have shifted attention to alternative and natural colourants that are harmless. Extraction of carotenoid from tomato peel by-product will not only solve the problem associated with consuming synthetic colourant but solve the pollution problem connected with tomato processing. This research was conducted to study the kinetics and determine the thermodynamics of carotenoid extraction from tomato peel (*Lycopersicon esculentum*) using ethanol. Tomato peel by-product was collected, cleaned, oven-dried, and ground. It was characterized to determine the proximate and phytochemicals composition. The carotenoid extraction process parameters were optimized using response surface methodology, and the carotenoid extracted was analysed using a gas chromatograph-mass spectrophotometer. The kinetics and thermodynamics of extraction were studied using a first-order mass transfer model and thermodynamics to determine energy changes involved in the extraction. The result of the proximate analysis showed the following values; crude protein (0.69%), Fibre (20.63%), Ash (17.40%), Fat (8.53%), Moisture (8.13%), and Carbohydrate (44.62%). The phytochemical analysis showed that the tomato peel contains terpenoid, alkaloid, saponin, and flavonoid. The optimal conditions for carotenoid extraction were 0.306 mL/g solvent/solid ratio, 20 minutes extraction time, and temperature of 36 °C with the optimal yield of 82.35%. The result indicates that tomatoes contain 82.35 g of carotenoid. It is observed that the carotenoid contains lutein 1.89%, lycopene 88.11%, β- carotene 2.25%, cis-ζ-carotene 2.41%, γ-carotene 1.23%, cis-lycopene 0.89%, phytofluene 0.62%,ζ-carotene 1.2% and phytoene 1.52%. Therefore, among the components, lycopene is the most dominant with the composition of 88.11% yield, and the extraction was endothermic, spontaneous, and feasible. Ethanol is a good solvent for the extraction of carotenoid from tomatoes peel.

Key words: Carotenoid, optimization, extraction, phytochemicals, lycopene, ethanol, flavonoid, saponin, alkaloid

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INTRODUCTION

The use of artificial colouring in food industries has started generating negative perceptions by consumers in recent times. Some of these colourants, such as sunset yellow, tartrazine, brilliant blue FC, and Allura red, are harmful to human health [1]. They can cause health challenges such as cancer and oxidative anxiety [2]. Researchers have shifted attention to alternative and natural colourants that are harmless. The sources of the natural colourants are β-carotene from orange, lutein from yellowish-green lutein, chlorophyll from green, lycopene from tomatoes, and anthocyanin from blue-purple colour [3]. Among these, lycopene has gained more focus. Lycopene is one of the carotenoid constituents responsible for their attractive colours that it frames the light retaining chromospheres [4]. The presence of lycopene in carotenoids makes it most important in the food industry as a nutritional substance for its medical advantages [5]. It has more importance because of its broad utilization in food, makeup, and pharmaceuticals. The utilization of carotenoids has gained more attention due to their ability to decrease the danger of atherosclerosis, coronary heart disorders, and a few sorts of tumours [6]. It is an antioxidant produced by plants, soluble in lipid, and mostly not made by humans, animals, and microorganisms [7]. The primary source of lycopene in carotenoids is tomatoes.

Tomato is the fruit of the plant *Solanum Lycopersicon* from the family of the *Solanaceae* (nightshade). It is an annual crop that can grow to 70 – 200 cm [8]. Fresh tomatoes are well-known and adaptable organic vegetables that make considerable commitments to human nutrition worldwide for their content of acids, sugars, minerals, vitamins, lycopene, and other carotenoids, among different constituents [9]. Tomatoes originated in North America and were spread worldwide following the Spanish colonization of the Americans, and its many varieties are now widely grown, often in greenhouses in cooler climates [10].

In Nigeria, tomatoes are grown mainly in the Northern region, except for few varieties. They are processed into Catsup, sauces, and salsa; 10–30 % of their weight becomes waste or pomace [11]. Generally, processed fruits and vegetables have long been considered lower dietary value than their fresh counterparts because of losing vitamin C and other heat-labile nutrients during processing, especially during thermal processing [12]. Harvesting the crop at the best phase of maturity will increase the storage life of the tomatoes fruit, and it is the initial step required for making sure successful marketing is achieved. Extending the shelf-life of tomatoes is very important for domestic and export purposes through the knowledge of crop physiology and proper storage conditions [13]. Although plant food by-products are remarkable from a nutritional point of view, only a few have been effectively developed from the vast amount of plant residues produced by the food processing industry. The storage of tomatoes in developing countries has been a significant challenge due to insufficient technology to create efficient storage facilities. There is a need to convert them for industrial use to avoid wastages. The peel of tomato waste contains more carotenoid than its pulp [7]. Food by-products typically represent an environmental problem for the industry. Many studies have been conducted on the possible use of several vegetable-origin by-products to add to the human diet, thereby decreasing processing

costs and pollution problems related to food processing [14]. Therefore, the present study tends to extract carotenoids from tomato peels using ethanol. To maximize the carotenoid extraction yield, the extraction process was optimized using a central composite design with varied extraction temperatures, solvent/solute ratios, and extraction times.

This study aimed to optimize the process of the extraction of carotenoids from tomato peels using response surface methodology, thus increasing the tomato industrial bio-economy and solve the pollution problem connected with tomato processing.

MATERIALS AND METHODS

Sample preparation

Tomato peels were obtained from the by-product of Somzel Agro Enterprise, Abuja, Nigeria. The peel was cleaned, dried under sunlight to a moisture content of 8%, finely ground using a house grinder, and stored at −18 °C until further use.

Characterization of the sample

The tomato peel was characterized to determine the proximate composition according to the Association of Official Analytical Chemists [15], and the phytochemicals were determined by the standard method described by Miranda *et al*. [16].

Extraction of Carotenoids

Carotenoids were extracted by adopting the procedure described by Hackett *et al*. [17]. A quantity (100 g) of tomato peels was oven-dried at 40 °C to reduce the moisture content to 6%. Then it was milled using a dice attrition mill and sieved through 0.15 mm strainers. Fifty grams of tomato peel powder and 250 ml of ethanol were added into a 4-L beaker and mixed for 20 minutes. The blend was homogenized for one minute and filtered with filter papers (Whatman no 1). The filtrate was blended with 250 ml of ethanol solution (1:1, v/v) and homogenized for one minute. The mixture was allowed to settle in a separation funnel. The non-polar ethanol layer containing lipid material was separated from the water/soluble fraction, and then the solvents were evacuated. The extracted crude carotenoid was stored at a temperature of -18 °C until analysis. The extraction was done by varying the process variables as stated in the range of values, Table 1.

Experimental design

The optimization of the carotenoid extraction was done using Rotatable Central Composite Design of Response Surface Methodology of Design-Expert version 9.0.6. The experimental design employed in this work was a five-level-three factor complete factorial design involving 15 experiments. Extraction temperature, solvent/solute ratio, and extraction time were chosen as independent variables. The response determined was the carotenoid yield obtained from solvent extraction. Six repeated center points were utilized to predict with minimum errors, and experiments were carried out in a randomized order. The actual and coded values are presented in Table 1, and the ranges were selected based on the previous experiment performed by Gonabad *et al*. [18]. The responses obtained using the experimental design matrix are presented in Table 2. The

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software employs the concept of the coded values for the examination of the essential terms. Therefore, an Equation in coded values is used to investigate the effect of the variables on the response. The empirical Equation is given below:

$$Y_i = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad 1)$$

Where Y_i is the response for carotenoid (yield of carotenoid), β_o is the coefficient of the constant term. $\sum_{i=1}^{3} \beta_i$ is the coefficient of the linear term, $\sum_{i=1}^{3} \beta_{ii}$ is the coefficient of the interactive term while $\sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij}$ is the coefficient of quadratic term.

The generated model was solved by the Design Expert using numerical optimization. The variables were kept at range while the yield of carotenoid was maximized. The solution with the highest desirability was selected as the optimal conditions.

Determination of Carotenoids Profile

The profile of the carotenoids separated from the peel of tomato was determined using Knauer GC-MS pump 64 as reported by Gaylek *et al*. [19] utilizing octadecylsilane C 18, 3.9 × 150 mm. For both GC-MS columns, two elution solvents were used: (1) methanol (2) ethyl acetate. The flow rate of 1.8 ml/min and 475 nm absorbance was used. A mixture of methanol and ethyl acetic acids (54:46) as mobile phase (sample amount: 20 µL, flow, 1.8 Ml/min) was identified as 475 nm.

Kinetics of Carotenoid extraction

The analysis and design of an extraction process for industrial-scale require relevant kinetic data. The carotenoid concentration gradient in the solid particle is the drag force involved in the extraction and is controlled by diffusion. The kinetics of carotene extraction was modeled with mass transfer occurring at the solid-liquid interface where mass flow by diffusion is equivalent to mass flow by convection [20]. Because extraction was carried out at non-steady-state without chemical reactions, a mass transfer kinetic model was adopted to study carotenoid extraction from tomatoes using ethanol. The rate of change of carotenoid concentration in the liquid phase, $\frac{d[Ca]}{dt}$ ($gL^{-1}min^{-1}$) is written as follows:

$$\frac{d[Ca]}{dt} = k([Ca]_e - [Ca]_t) \quad (2)$$

Where $[Ca]_t$ and $[Ca]_e$ are carotenoids (gL^{-1}) in the liquid phase at time t (minutes) and equilibrium, respectively, and k is the mass transfer coefficient (min^{-1}).

Boundary conditions applied to solve Equation (2) were:

(i) At the start of the extraction (t=0), the concentration of the carotenoid in the liquid phase is equal to zero ($[Ca]_t = 0$).

(ii) At time t, the concentration of carotenoid in the liquid phase is $[Ca]_t$. Integrating Equation (2) using the boundary conditions gives Equation (3).

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$$[Ca]_t = [Ca]_e\,(1 - e^{-kt}) \quad (3)$$

Rewriting Equation (3) in terms of carotenoid yield, gives Equation (4)

$$Y_t = Y_e\,(1 - e^{-kt}) \quad (4)$$

Linearizing Equation (4) by taking the natural logarithm gives Equation (5)

$$\ln Y_t = \ln Y_e + kt \quad (5)$$

Where Y_t is the carotene yield in the liquid phase at a time, t; Y_e is the carotenoid content of the tomato in the liquid phase at equilibrium. The equilibrium yield of carotenoid in the liquid phase, Y_e and mass transfer coefficient, k was obtained from the intercept and slope of the plot of $\ln Y_t$ against t, respectively.

The activation energy was calculated with the Arrhenius equation:

$$k = Ae^{-\frac{E_a}{RT}} \quad (6)$$

Linearizing Equation (6) yields Equation (7)

$$\ln k = \ln A - \frac{E_a}{R}\frac{1}{T} \quad (7)$$

K is the mass transfer coefficient, A is the Arrhenius constant or frequency factor; Ea is the activation energy; T is the absolute temperature, and R is the universal gas constant. The activation energy and Arrhenius constant, A, will be determined from the plot of lnk vs. 1/T.

Thermodynamics of Carotenoid extraction

The thermodynamics parameters enthalpy change (ΔH) and entropy change (ΔS) for the carotenoid extraction process were determined using the Van't Hoff equation.

$$\ln K_{eq} = -\frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R} \quad (8)$$

$$K_{eq} = \frac{Y_e}{Y_{se}} \quad (9)$$

$$\Delta G = \Delta H - T\Delta S \quad (10)$$

Where K_{eq} = equilibrium constant of the extraction process, Y_e is the average yield of carotenoid at temperature T, Y_{se} is the total carotenoid present in the tomato, T = temperature used in the extraction process (K), and R is the universal gas constant (8.314 J $mol^{-1}K^{-1}$).

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The changes in enthalpy and entropy were determined from the slope and intercept of the plot of In K_{eq} against 1/T respectively while ΔG was calculated using Equation (10).

RESULTS AND DISCUSSION

Proximate and phytochemical analysis

The proximate and phytochemical analysis of the tomato peel is presented in Table 3. The sample had a low moisture content, shallow protein content, and high fiber, ash, and carbohydrate content. The present study agrees with the finding of Monika *et al*. [21], who recorded high values of fiber and ash in the peel of tomato. This shows that tomato peel is not a proteinous food and has high energy (calorific) value. The phytochemical analysis shows that the tomato contains terpenoid, alkaloid, saponin, and flavonoid without glycoside, tannin, and phenol. Alkaloids have a wide range of pharmacological activities, including antimalarial (example; quinine), anti-cancer (example; homoharringtonine),anti-asthma (example; ephedrine) [22]. Flavonoids are also attributed to a reduced risk of cancer, heart disease, asthma, and stroke [22]. Terpenoids found in high quantity when added to proteins enhance their attachment to the cell membrane; this is known as isoprenylation [10]. Therefore, the consumption of tomatoes as a colourant in food will improve the consumers' health and enhance the attachment of the protein to the cell membrane.

Fitting Models to Data Obtained from the Yield of Carotenoid Extracted from Tomato Peels

The experimental data of the design plan was used to generate multiple regression equations between the yield of carotenoid (Y_e) and process variables and statistically analyse the significance of the models. The yield of carotenoids extracted with ethanol is presented in Table 3 and shows that the yield of carotenoids extracted from tomatoes significantly ($p < 0.0001$) increased. The coefficient of determination (R^2) for the model was very high. This shows that the model adequately predicted the experimental data. The inability of a model to predict the experimental data at regions not included in the model is measured with lack-of-fit. From Table 4, it is observed that the lack of fit for each of the models was non-significant ($p > 0.05$). Therefore, the quadratic model adequately predicted the data for carotenoid yield using the solvent.

The empirical relationship between the yield of carotenoid extracted from tomatoes using ethanol (Y_e) and the three variables in coded values is given by the equations (11).

$$Y_e = 82.05 + 0.75A + 0.75B + 2.38C - 0.75AB + 0.50AC - 2.5BC - 6.98A^2 - 7.98B^2 - 4.48C^2 \quad (11)$$

Analysis of variance was carried out to compare the significance of the model terms at a 5% significance level. A model is considered significant if the p-value (significance probability value) is less than 0.05. From Table 3, it can be concluded that all the linear terms A, B, and C and interaction term BC and AB with all the quadratic terms A^2, $B^2$and C^2 are significant model terms. Based on this, the non-significant terms of the

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model, excluding the term that supports hierarchy, were removed, and the reduced model is given below:

$$Y_e = 82.05 + 0.75A + 0.75B + 2.38C - 0.75AB - 2.5BC - 6.98A^2 - 7.98B^2 - 4.48C^2 \quad (12)$$

The experimental data were also analyzed to check the correlation between the observed and expected carotene yield. The average probability and residual plot and actual and predicted plots are shown in Figure 1. It was observed from Figure 1 that the reasonable distribution of the data points on the straight line indicates a good relationship between the experimental and predicted values of the response. The result also suggests that the selected quadratic model accurately predicted the response variables for the experimental data.

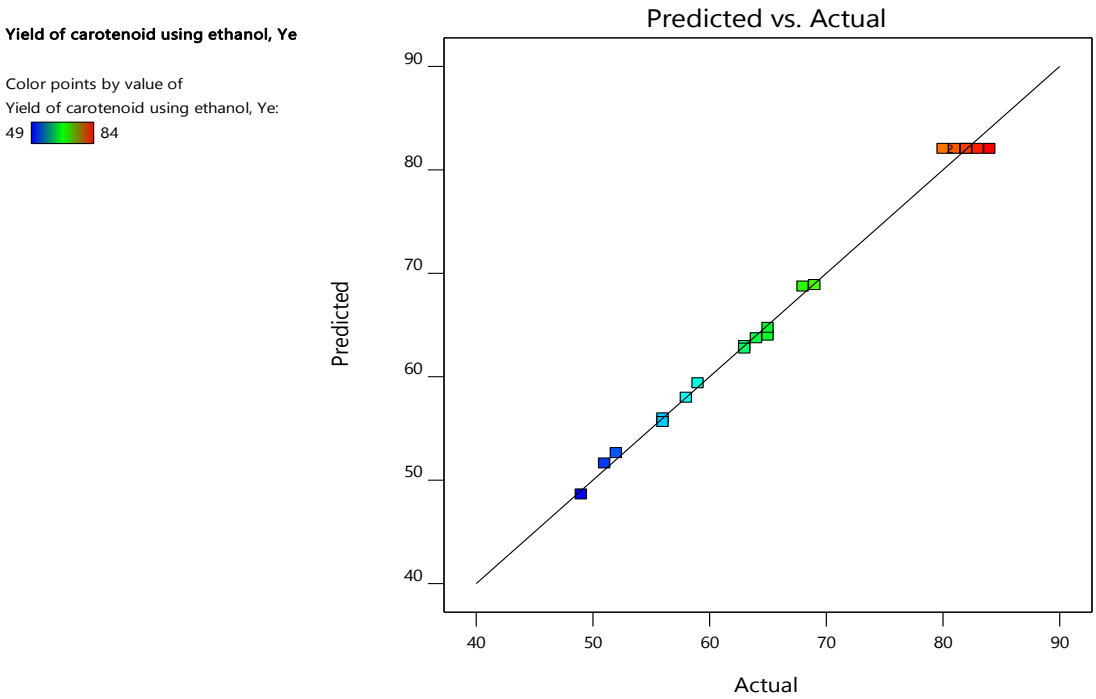

Figure 1: Plot of predicted and actual value for carotenoid extraction using ethanol

Response surface plotting for carotenoid yield

The yield of carotenoid extracted from tomato peels using ethanol was affected by solvent-to-solid ratio, extraction time, and extraction temperature. The linear effect was positive and significant ($p < 0.05$), and the interaction between solvent-to-solid ratio and extraction time; extraction time and extraction temperature for carotenoid ethanol yield were negative and significant ($p < 0.05$). Figure 2 depicts the interaction effects of solvent-to-solid ratio and extraction time on the yield of carotenoid using ethanol. The ethanol carotenoid yields had a significant increase with increasing both solvent-to-solid ratio and extraction time, but beyond 20 minutes and 0.3 mL/g, the yield decreased. The decrease in output may be attributed to the reduction of driving force due to the effectiveness of the solvent to extract the carotenoid in a short period.

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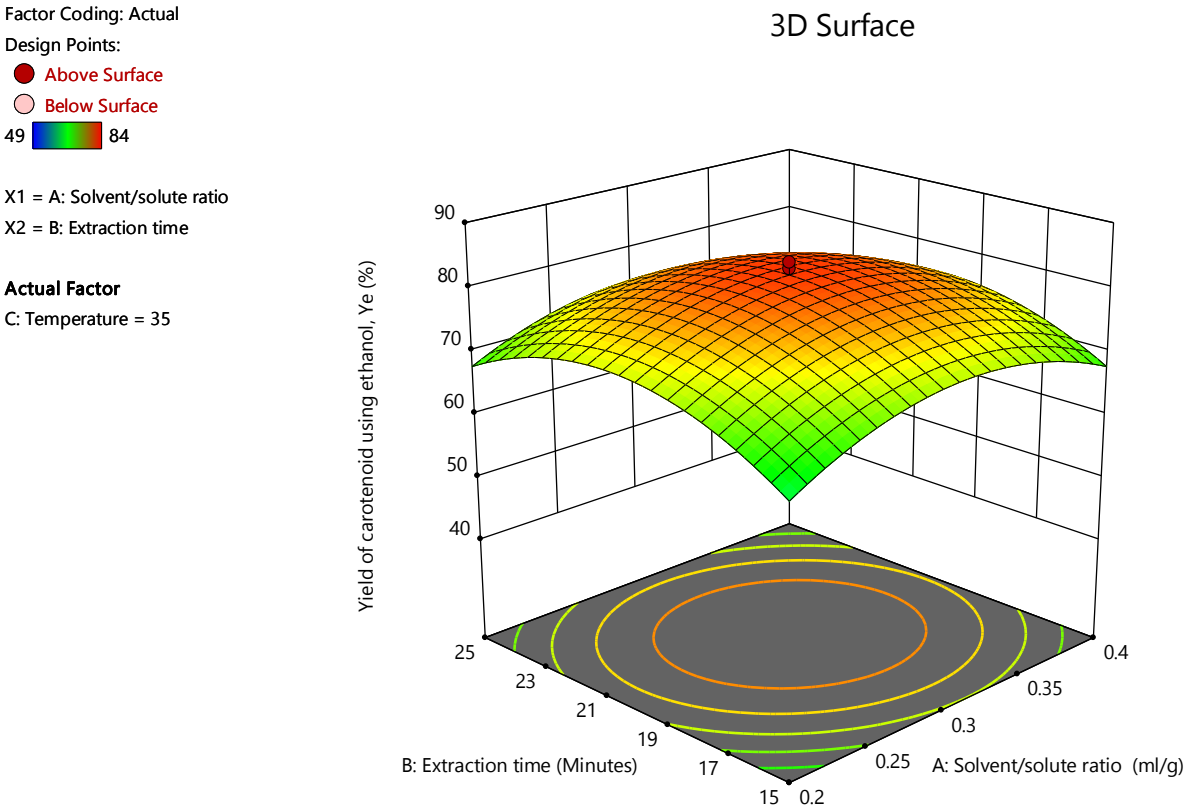

Figure 2: Surface plot of the interactive effect of solvent/solute ratio and time on ethanol carotenoid extract

Figure 3 shows the interactive effect of solvent-to-solid ratio and extraction temperature on the yield of carotenoid extracted using ethanol. The ethanol carotenoid extracts increased with increasing both solvent-to-solid ratio and extraction temperature and then decreased, which may be due to evaporation of the solvent.

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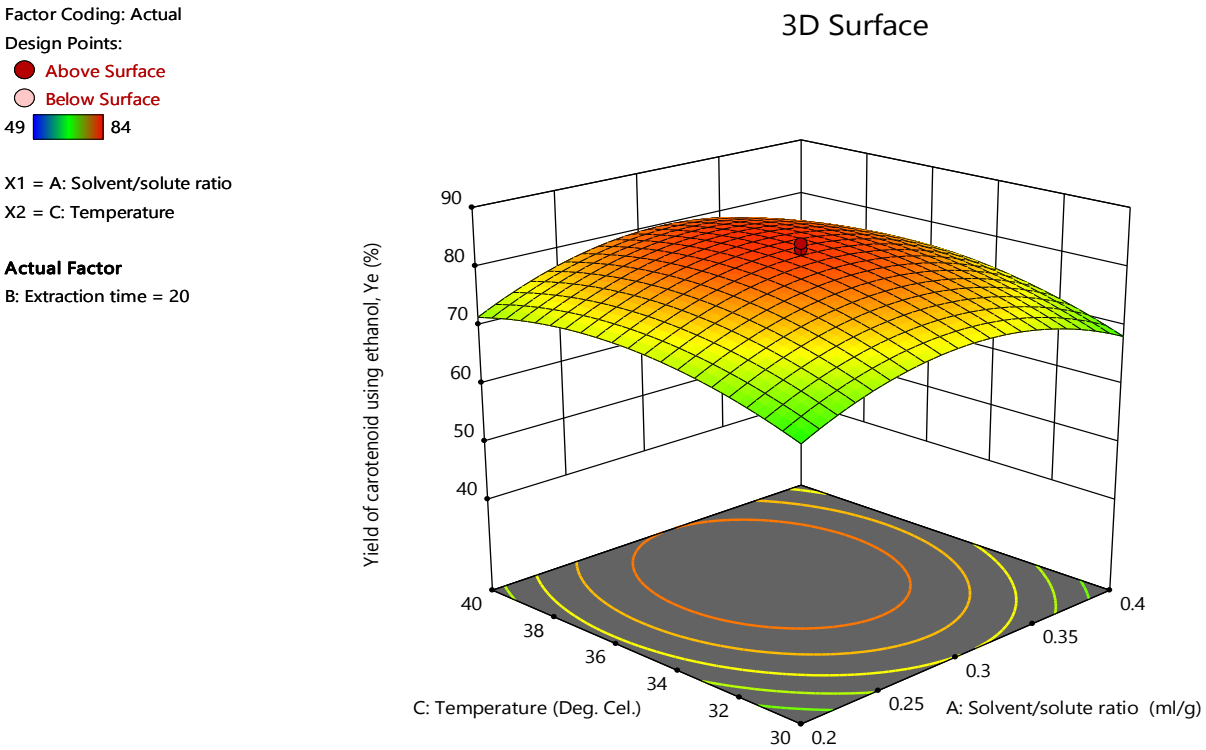

Figure 3: Surface plot of the interactive effect of solvent/solute ratio and temperature on ethanol carotenoid extract

Optimization of carotenoid extract

The optimum conditions for actual values obtained were: solvent/solid ratio of 0.306 mL/g, extraction time of 20 minutes, and extraction temperature of 36 °C with an optimal yield of ethanol carotenoid extract of 82.35%. The experimental values of 82.11% ethanol carotenoid obtained with their optimal conditions align with the values predicted by the technique, which suggested that RSM can accurately, reliably, and practically expect the extraction [23]. The percentage error for the yield using the solvent was less than one percent, also confirming the accuracy of the prediction.

Carotenoid profile of tomato peels extracted using ethanol

The profile is presented below and it is observed that the carotenoid contains lutein 1.89%, lycopene 88.11%, β- carotene 2.25%, cis-ζ-carotene 2.41%, γ-carotene 1.23%, cis-lycopene 0.89%, phytofluene 0.62%,ζ-carotene 1.2% and phytoene 1.52%. These results agree with Aghel *et al*. [24] and Waqas *et al*. [2]. Therefore, among the components, lycopene is the most dominant with the composition of 88.11%.

Kinetics of the extraction

The kinetics of the extraction of carotenoid from tomato peels was studied, and the experimental data were fitted into a first-order kinetic model with a good coefficient of determination of 0.92 and mass transfer coefficient of 0.063, which shows that temperature stimulates the extraction process. The low activation energy of 26.29 kJ/mol obtained shows that the extraction occurred faster.

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Thermodynamics of the extraction process

The thermodynamics study was carried out to determine the energy changes involved in the extraction. The enthalpy and entropy were determined from Figure 4. The enthalpy of -105.4 kJ/mol, the entropy of -0.315 kJ/mol, and free Gibbs energy -9.96 kJ/mol showed that the reaction was exothermic, spontaneous, and feasible.

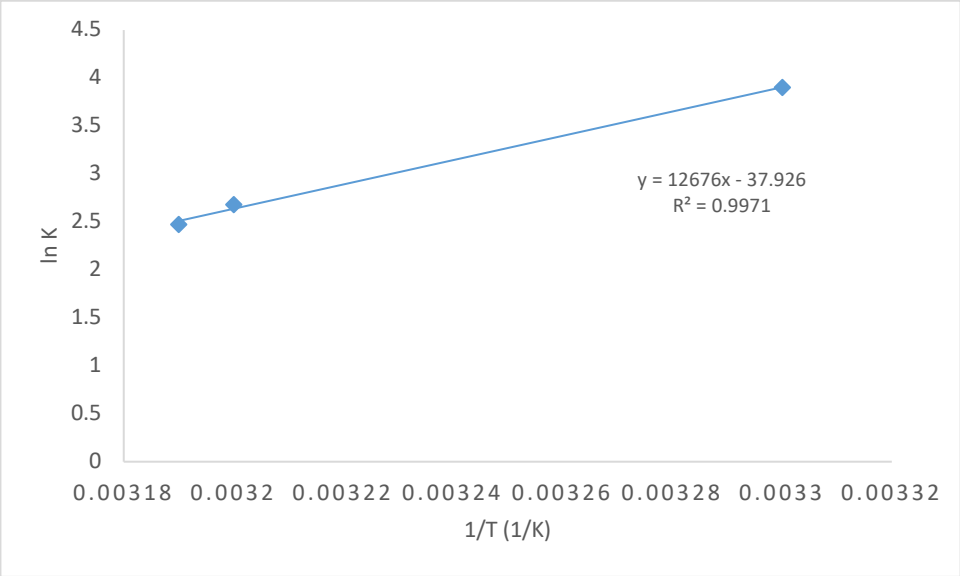

Figure 4: Determination of thermodynamic parameters.

CONCLUSION

The optimization of carotenoid extraction parameters and their kinetics were studied. It was concluded that ethanol is a suitable solvent for extraction of carotenoid from tomato peels and the extracted carotenoid contains lycopene as the principal constituent. The response surface methodology was adequate in predicting carotenoid extraction from tomato peel with an optimal yield of 82.35%. The first-order mass transfer model kinetically guided the carotenoid extraction, and the extraction was exothermic, spontaneous and feasible.

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Table 1: Range of independent variable in actual and coded form

Independent variables	Symbols	Range and levels				
		-2	-1	+1	+2	0
Solvent/solute ratio (mL/g)	A	0.1	0.2	0.4	0.5	0.3
Extraction Time (Minutes)	B	10	15	25	30	20
Extraction temperature (°C)	C	25	30	40	45	35

Table 2: Responses of design expert

Std	A: Solvent/solute ratio	B: Extraction time	C: Temperature	Yield of carotene using ethanol, Ye
	ml/g	Minutes	Deg. Cel.	%
1	0.2	15	30	56
2	0.4	15	30	58
3	0.2	25	30	65
4	0.4	25	30	63
5	0.2	15	40	65
6	0.4	15	40	68
7	0.2	25	40	63
8	0.4	25	40	64
9	0.1	20	35	52
10	0.5	20	35	56
11	0.3	10	35	49
12	0.3	30	35	51
13	0.3	20	25	59
14	0.3	20	45	69
15	0.3	20	35	82
16	0.3	20	35	80
17	0.3	20	35	82
18	0.3	20	35	83
19	0.3	20	35	84
20	0.3	20	35	81

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Table 3: Proximate Composition and phytochemicals of the Tomato Peel

S/N	**Parameters**	**Quantity (%)**
1	Crude protein	0.69 ± 0.02
2	Fibre	20.63 ± 0.02
3	Ash	17.40 ± 0.01
4	Fat	8.53 ± 0.03
5	Moisture	8.13 ± 0.01
6	Carbohydrate	44.62 ± 0.47
7	Terpenoid	17.09 ± 0.02
8	Alkaloid	5.22 ± 0.01
9	Saponin	3.30 ± 0.03
10	Flavonoid	0.70 ± 0.02
11	Crude protein	0.69 ± 0.02
12	Fibre	20.63 ± 0.02
13	Ash	17.40 ± 0.01

Values are the means ± SD duplicate

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Table 4: Analysis of variance of carotenoid using ethanol, Ye

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	2587.98	9	287.55	220.81	< 0.0001
A-Solvent/solute ratio	9.00	1	9.00	6.91	0.0252
B- Extraction Time	9.00	1	9.00	6.91	0.0252
C- Temperature	90.25	1	90.25	69.30	< 0.0001
AB	4.50	1	4.50	3.46	0.0327
AC	2.00	1	2.00	1.54	0.2435
BC	50.00	1	50.00	38.39	0.0001
A^2	1224.01	1	1224.01	939.91	< 0.0001
B^2	1600.01	1	1600.01	1228.63	< 0.0001
C^2	504.01	1	504.01	387.03	< 0.0001
Residual	13.02	10	1.30		
Lack of Fit	3.02	5	0.6045	0.3023	0.8924
Pure Error	10.00	5	2.00		
Cor Total	2601.00	19			

Key: AB-Solvent/solute ratio-extraction time, AC- Solvent/solute ratio-temperature

BC- Extraction time-temperature, Df-degree of freedom

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