Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2024, Volume 15, Issue 2, Page 203-213

http://www.jmaterenvironsci.com

Optimization of Beta Carotene Production from Maize and Moringa Leaves

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Received *27 Sept 2023,*
Revised *28 Jan 2024,*
Accepted *05 Feb 2024*

Citation*: Mbah J. C., Nnanwube I. A., Ekweze, A. M. (2024) Optimization of Beta Carotene Production from Maize and Moringa Leaves, J. Mater. Environ. Sci., 15(2), 203-213*

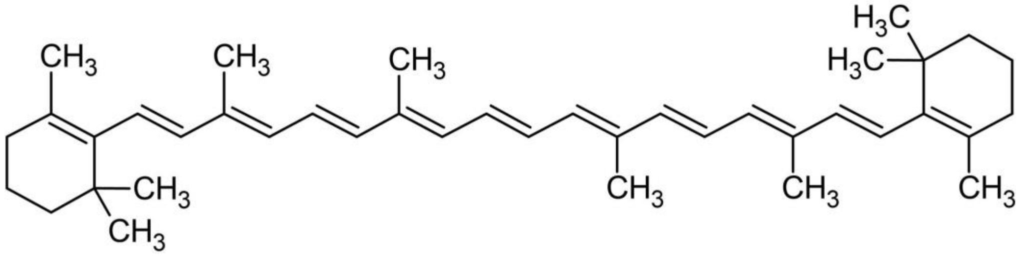
Abstract: This work aimed at producing beta carotene, a vitamin supplement that supplies nutritional benefit to supplement the fruits and vegetables. In the study, the beta carotene was extracted from maize and moringa leaves through a process of solvent extraction using ethanol and ethyl acetate. The optimization was done using response surface methodology and the result showed that all factors considered interacted significantly with $P<0.05$. The results showed that maize using ethanol as solvent produced more carotene than moringa leaves. About 0.134 mg/ml and 0.103 mg/ml carotene were produced using ethanol and ethylacetatate, respectively, at the optimum conditions. The produced beta carotene was characterized using FTIR and it showed that the cycle of the frequencies proves the presence of beta carotene which is an aromatic unsaturated compound.

Keywords: *Ethanol; Optimization; Maize; Moringa leaves; Carotene*

1. Introduction

Beta Carotene alone has more quantity and quality in some fruits and vegetables than others. For example, there might be more quantity of beta carotene in palm oil and carrot but more quality of beta carotene in Dark-leafy vegetables due to its medicinal nature (Gebregziabher *et al.*, 2023; Crupi *et al.*, 2023; Haddou *et al.*, 2023;). Beta carotene's high concentration acts as a pro-antioxidant that induces apoptosis of colon cancer cells, leukemia cells, thus rendering potent preventive effect (Jang *et al.*, 2009).

It is very well known that Vitamin A deficiency (VAD) is primarily due to dietary inadequacy. The most concentrated sources of vitamin A are provided by animal foods but plant foods are less costly (Nankumbi *et al.*, 2023; Lahlou *et al.*, 2019, Bailey *et al.*, 2015). This is an important consideration, especially for the poverty-stricken living children. VAD is one of the most prevalent and major nutritional problems in developing countries especially in Africa, VAD consequences include night blindness, reduced growth in children and increased morbidity and sometimes mortality (Matthew *et* *al.*, 2012). Due to the fact that VAD has become a huge problem, the consumption of these fruits and vegetables might not be sufficient or accurate to revive the already deficient body but with the extraction of carotene from these sources, it can be made in lenitive amounts in medicines and supplements to help meet the needs of the deficient swiftly (Neeld and Pearson, 1963; Kultys *et al.*, 2022; Lockyer *et al.*, 2018). Scheme 1 shows beta carotene molecule.



Scheme 1. beta carotene molecule ($C_{40}H_{56}$)

A study of carotenoid content of current cultivars and resources of the major staples and green vegetables is needed to assess the potential to deliver health-related carotenoids to resource poor populations who are isolated either geographically or socio-economically and heavily dependent on staples and uncontrolled growing leaves for food. This work addresses the situation in the cereals and green-leafy plants.

2. Materials and methods

The vegetable was washed, allowed to dry for three days and ground to a fine pulp using pestle and mortar while the Maize was blended in a grinding machine. The work was done under vague light to reduce the rate of carotene oxidation contained in them.

Maize leaves

Moringa leaves

2.1 Proximate analysis of maize and moringa leaves

The analysis was performed according to the standard methods of analysis (AOAC, 2000).

2.2 Experiment design for optimization processes

In RSM, the first step is the required approximation being introduced to find true relationship between the dependent variable and the set of independent variables. The second-order polynomial is a mathematical model formed to predict the response as a function of independent variables of their interactions. Generally, the behaviour of the system is explained by the following quadratic equation (Eqn. 1).

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_{ii}^2 + \sum b_{ij} x_i x_j + \varepsilon \qquad (1)$$

where Y is the predicted response, b_0 the offset term, b_i the linear effect, b_{ii} the squared effect, b_{ij} the interaction effect and x_i and x_j represent the coded independent variables, ε represents the error term.

Optimization of extraction process involved three numeric factors of dosage, temperature and time used for saturation and one categoric factor of type of solvent with maize.

2.3 Fourier transform infrared spectroscopy (FTIR)

The functional groups and structure were studied using Fourier transform infrared spectroscopy [Buck 530 IR] England. The FTIR spectra of the raw materials, carotene traits and amino acids were scanned at a wavelength of 600–4000 nm.

3. Results and discussion

3.1 Proximate evaluation of maize and moringa

Analysis done and calculated according to AOAC (2000), showed that maize has relatively high moisture when compared to moringa but low content when compared to other breeds of maize from other countries, hence, the extraction of carotene cannot be difficult after much drying. The crude fibre of both maize and moringa shows abundant health effects in the carotene quality. The moderate amount of its fat makes its carotene desorbable in extraction. Hence the low lipids amount of both Maize and Moringa makes saponification unnecessary in the process. The proximate analysis of maize and moringa is shown in Table 1.

Table 1. Proximate analysis of maize and moringa

Proximate	Maize (%)	Moringa (%)
Moisture content	6.92	6.51
Ash content	1.72	7.41
Crude fibre	1.65	9.23
Crude protein	8.81	27.3
Crude fat	3.12	2.7

3.2 Optimization results

The design matrix showing the experimental and predicted values of beta carotene and experimental factors is shown in Table 2.

3.3 ANOVA results

The ANOVA results for beta carotene production is shown in Table 3. Othe regression values are displayed in Table 4. The F-value model of 11.66 implies the model is significant and there is only a 0.01% chance that a "Model F-Value" this large could occur due to error. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, C^2 are significant model terms, values greater than 0.1000 indicate the model terms are not significant. The F-value" of 1.48 implies the Lack of Fit is not significant relative to the pure error.

Table 2. Design structure for carotene production

Run Order	Temper-ature (°C)	Dosage (g)	Time (minutes)	Type of solvent	Beta carotene conc. (mg/ml)	
					Experimental	Predicted
1	70.00	4.00	30.00	Ethanol	0.110	0.090
2	70.00	4.00	50.00	Ethanol	0.106	0.100
3	60.00	3.00	40.00	Ethylacetate	0.092	0.071
4	60.00	3.00	40.00	Ethylacetate	0.052	0.071
5	50.00	4.00	30.00	Ethylacetate	0.075	0.091
6	60.00	3.00	40.00	Ethanol	0.113	0.100
7	50.00	2.00	30.00	Ethanol	0.094	0.098
8	60.00	5.00	40.00	Ethylacetate	0.079	0.096
9	60.00	3.00	40.00	Ethylacetate	0.076	0.071
10	60.00	3.00	40.00	Ethylacetate	0.046	0.071
11	40.00	3.00	40.00	Ethylacetate	0.061	0.065
12	50.00	2.00	30.00	Ethylacetate	0.084	0.066
13	60.00	3.00	40.00	Ethylacetate	0.099	0.071
14	60.00	3.00	40.00	Ethanol	0.083	0.100
15	60.00	3.00	40.00	Ethanol	0.110	0.100
16	50.00	4.00	50.00	Ethanol	0.121	0.130
17	60.00	5.00	40.00	Ethanol	0.100	0.130
18	70.00	2.00	50.00	Ethylacetate	0.007	0.045
19	60.00	3.00	40.00	Ethanol	0.112	0.100
20	70.00	2.00	30.00	Ethylacetate	0.010	0.033
21	60.00	1.00	40.00	Ethanol	0.092	0.079
22	60.00	3.00	40.00	Ethylacetate	0.055	0.071
23	50.00	2.00	50.00	Ethanol	0.102	0.110
24	70.00	4.00	30.00	Ethylacetate	0.079	0.058
25	80.00	3.00	40.00	Ethanol	0.0001	0.031
26	60.00	3.00	60.00	Ethanol	0.114	0.140
27	70.00	2.00	30.00	Ethanol	0.071	0.065
28	60.00	1.00	40.00	Ethylacetate	0.044	0.047
29	50.00	4.00	50.00	Ethylacetate	0.120	0.100
30	60.00	3.00	40.00	Ethanol	0.113	0.100
31	70.00	2.00	50.00	Ethanol	0.098	0.077
32	50.00	2.00	50.00	Ethylacetate	0.053	0.078
33	40.00	3.00	40.00	Ethanol	0.128	0.097
34	60.00	3.00	20.00	Ethylacetate	0.091	0.085
35	70.00	4.00	50.00	Ethylacetate	0.114	0.069
36	80.00	3.00	40.00	Ethylacetate	0.004	-0.001
37	60.00	3.00	60.00	Ethylacetate	0.134	0.110
38	50.00	4.00	30.00	Ethanol	0.104	0.120
39	60.00	3.00	20.00	Ethanol	0.112	0.120
40	60.00	3.00	40.00	Ethanol	0.129	0.100

The "Pred R-Squared" of 0.4962 is in rational agreement with the "Adj R-Squared" of 0.6212. While the "Adeq Precision" measures the signal to error ratio with the ratio greater than 4. Thus, ratio of 15.439 indicates an adequate signal which can be used to navigate the design space (Nnanwube *et al*., 2020(a,b,c); Nnanwube and Onukwuli, 2020(a,b); Nnanwube *et al*., 2022; Onukwuli and Nnanwube, 2022).

Table 3. Results of ANOVA for beta carotene production

Source Value	Sum of Squares	Df	Mean Square	F-value	P-Value
Model	0.033	6	5.582E-003	11.66	<0.0001
A-Temp(ºC)	8.752E-003	1	8.752E-003	18.28	0.0002
B-Dosage(g)	4.812E-003	1	4.812E-003	10.05	0.0033
C-Time(min)	1.063E-003	1	1.063E-003	2.22	< 0.0001
D-Type of solvent	0.010	1	0.010	21.41	0.1457
A^2	4.973E-003	1	4.973E-003	10.39	0.0029
C^2	2.201E-003	1	2.201E-003	4.60	0.0395
Residual	0.016	33			
Lack of Fit	0.012	23	5.311E-004	1.48	0.2641
Pure Error	3.585E-003	10	3.585E-004		
Cor Total	0.049	39			

Table 4. Other regresion values

R-Squared	ADJ R-Squared	Pred R-Squared	Adeq Precision
0.6794	0.6212	0.4962	15.439

3.4 Quadratic model equations

3.4.1 Final equation in terms of coded and actual factors

The final equations in terms coded factors for beta carotene production using ethanol and ethyl acetate are shown in Equations (2) and (3). The final equations in terms of actual factors are shown in Equations (4) and (5).

Type of solvent = Ethanol

$$\text{Betacarotene} = -0.087+0.0102*A+0.002263*B-4.99299E\text{-}003*C-7.71338E\text{-}005*A^2 + 8.46163E\text{-}005*C^2 \quad (2)$$

Type of solvent = Ethylacetate

$$\text{Betacarotene} = -0.03573+0.0002*A+0.0063*B-6.59299E\text{-}003*C-8.71338E\text{-}005*A^2+9.46163E\text{-}005*C^2 \quad (3)$$

3.4.2 Final equation in terms of actual factors

Type of solvent = Ethanol

$$Betacarotene = -0.10371+0.010002*Temp + 0.012263*Dosage-4.59299E-003*Time -9.71338E-005*Temp^2 + 6.46163E-005*Time^2 \quad (4)$$

Type of solvent = Ethylacetate

$$Betacarotene = -0.13573+0.010002*Temp + 0.012263*Dosage – 4.59299E-003*Time -9.71338E-005*Temp^2 + 6.46163E-005*Time^2 \quad (5)$$

The summary of the optimum conditions is shown in Table 5.

Table 5. Summary of the optimum conditions

Dosage (g)	Temperature (°C)	Time (min)	Type of Solvent	Beta carotene (mg/ml)		Error (%)
				Predicted	Experimental	
3.96	64.59	49.79	Ethanol	0.133877	0.133864	0.01
4.0	61.49	50	Ethylacetate	0.102702	0.102681	0.002

3.5 3D plots

The distribution of normal plot shows whether the residuals follow a normal dispersal in which case the points will follow a striaght line and perphaps some moderate scatter. Definite patterns like an "S-shaped" curve also indicate that a change in the response did provide a better analysis for the extraction process. From Figure 1, the residuals did follow a straight line with even scatter indicating that the experimental error that occurrs will not reduce the yield of the product (Joglekar and May, 1987). Figure 2 shows an increased beta carotene concentration as temperature decreases and dosage increases until the attainment of equilibrium at temperature above 55 °C due to the boiling range of the solvent used and limited surface area of dosage of 5g in the solution (Nambiar, 2006).

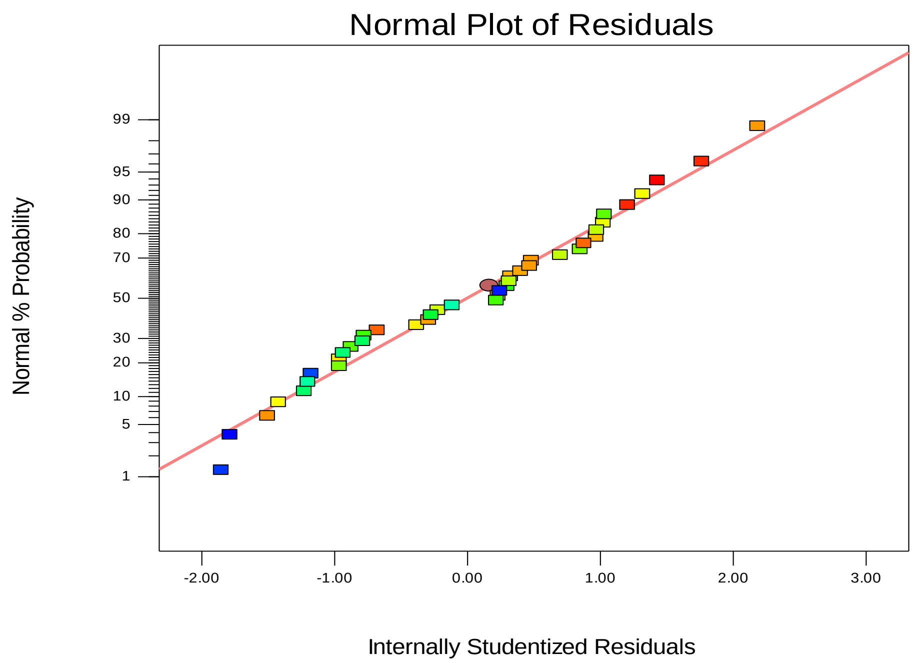


Figure 1. Normal plot of residuals

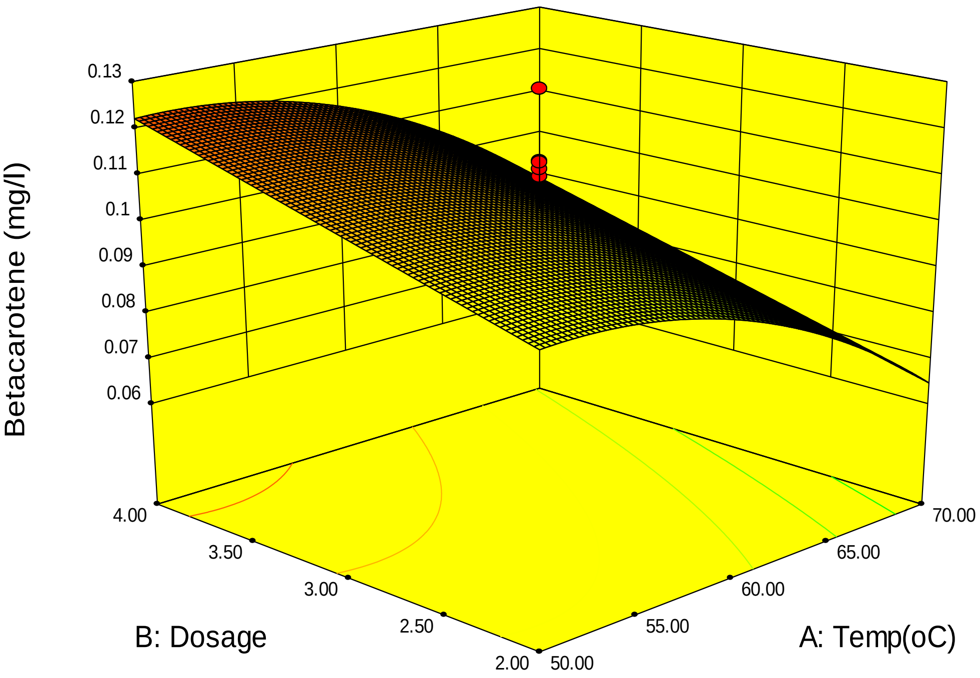


Figure 2. A plot of interaction between Temperature and Dosage of the extraction process

Figure 3 shows a graph of predicted values versus the actual response values. This helps to detect a value or group of values that are not easily predicted by the model. With the data points split evenly by the 45 degree line and an almost equal run on each side, it indicates that the expected value was not far reached.

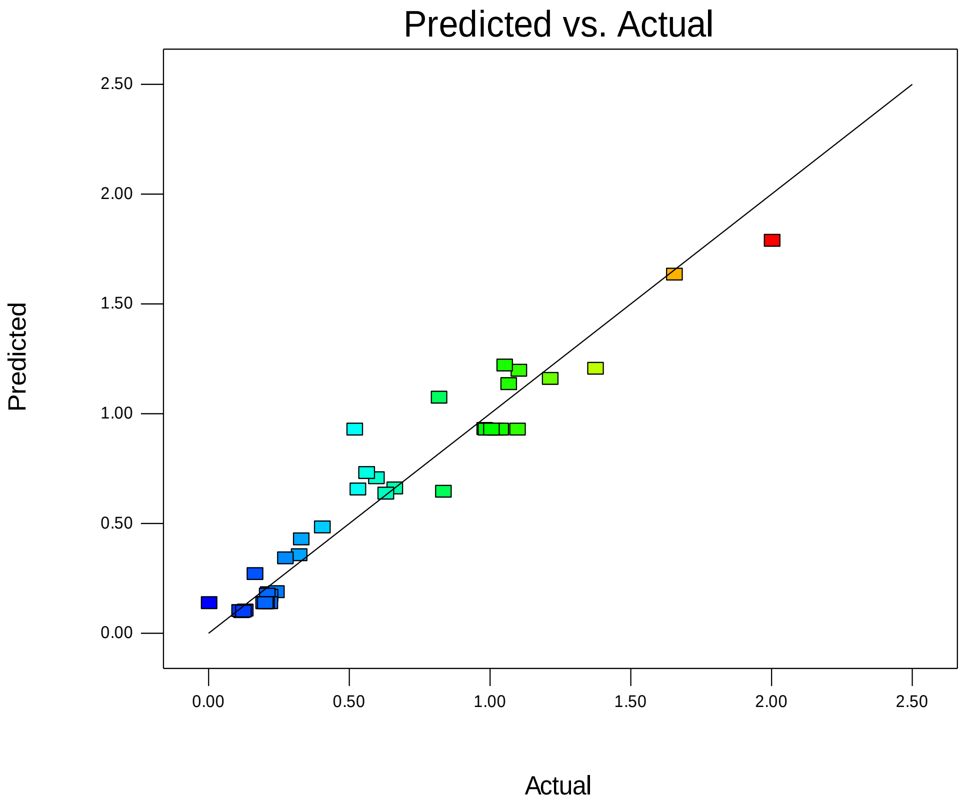


Figure 3. A plot of Predicted versus Actual values

3.6 Fourier transform infrared results

Table 6 shows the FTIR frequency and functional groups of Maize and Moringa leaves extract containing beta carotene. From the table of moringa extract as illustrated below, the frequencies of 754.4972 and 849.3967 shows the presence of Cis and Trans rings of the alkene's family respectively. The frequency of 1607.718 indicates the presence of aromatic double bond while 2218.048 indicates triple bond alkynes of aromatic family. 3071.664 and 3293.809 proves the presence of the alkynes of the unsaturated aromatic group. Also, according to Trivedi *et al.* (2017) from the African journal of biotechnology, the highest peak area of 205.1612 corresponding to the wavelength of 2218.048 demonstrates that there is a beta carotene derivative. Hence, the moringa extract contain beta carotene compound among other substances. From the maize extract, the frequency of 770.7714 represents the presence of the Cis-Alkenes of the aromatic bend, 1607.808 and 2047.514 shows the presence of alkene double bond while the 2180.357 indicates triple bond and 3137.944 frequencies demonstrate the unsaturated general family. Also the highest peak area of 811.744 corresponding to the wavelength of 2047.514 demonstrates that there is a beta carotene derivative. In all, the cycle of these frequencies proves the presence of beta carotene as the carotene is an aromatic unsaturated compound (Nyambaka and Ryley, 2001). The FTIR spectra is shown in Figure 4.

Table 6. FTIR frequency and functional groups of Maize and Moringa leaves extract

Maize Extract			Moringa Leaves Extract		
Frequency (cm^{-1})	Peak Area	Functional groups	Frequency (cm^{-1})	Peak Area	Functional groups
770.7714	38.75271	C-H aromatic bend	754.4972	61.20626	C-H aromatic
900.3966	32.17158	C-O stretch	849.3967	44.26033	C-H aromatic
1065.196	12.56015	C-O stretch	1020.326	6.66305	C-O
1284.632	79.2639	C-O stretch	1296.326	6.2749	C-O
1397.412	102.259	C-O stretch	1395.267	71.4454	C-O
1607.808	85.45763	C=C Alkene	1470.918	4.14560	C=C aromatic
1924.431	60.65326	C-O	1607.718	24.2702	C=C aromatic
2047.514	811.7441	C=C	1819.06	17.1375	C-O
2180.358	126.0147	C≡C stretch Alkynes	1955.693	138.4456	C-O
2448.362	175.7304	C=N stretch	2218.048	205.1612	C≡C stretch alkynes
2522.613	282.8152	C-H	2454.82	52.0333	C≡N stretch
2812.295	311.0367	C-H	2669.96	62.3612	C-H
2714.951	220.5888	C-H Aliphatic	2805.091	46.877	C-H stretch
2843.308	465.9971	C-H Aliphatic	2871.751	40.072	C-H stretch
3137.944	377.7536	C-H Unsaturated	2985.23	61.3784	C-H stretch
3346.092	173.7795	N-H stretch	3071.664	82.5625	C-H alkyne
3513.21	142.4362	O-H Water stretch	3293.809	60.8943	C-H alkyne
3706.789	16.52966	O-H Water stretch	3436.456	30.08455	N-H stretch
3793.453	59.34163	O-H Water stretch	3572.556	45.3659	O-H water stretch

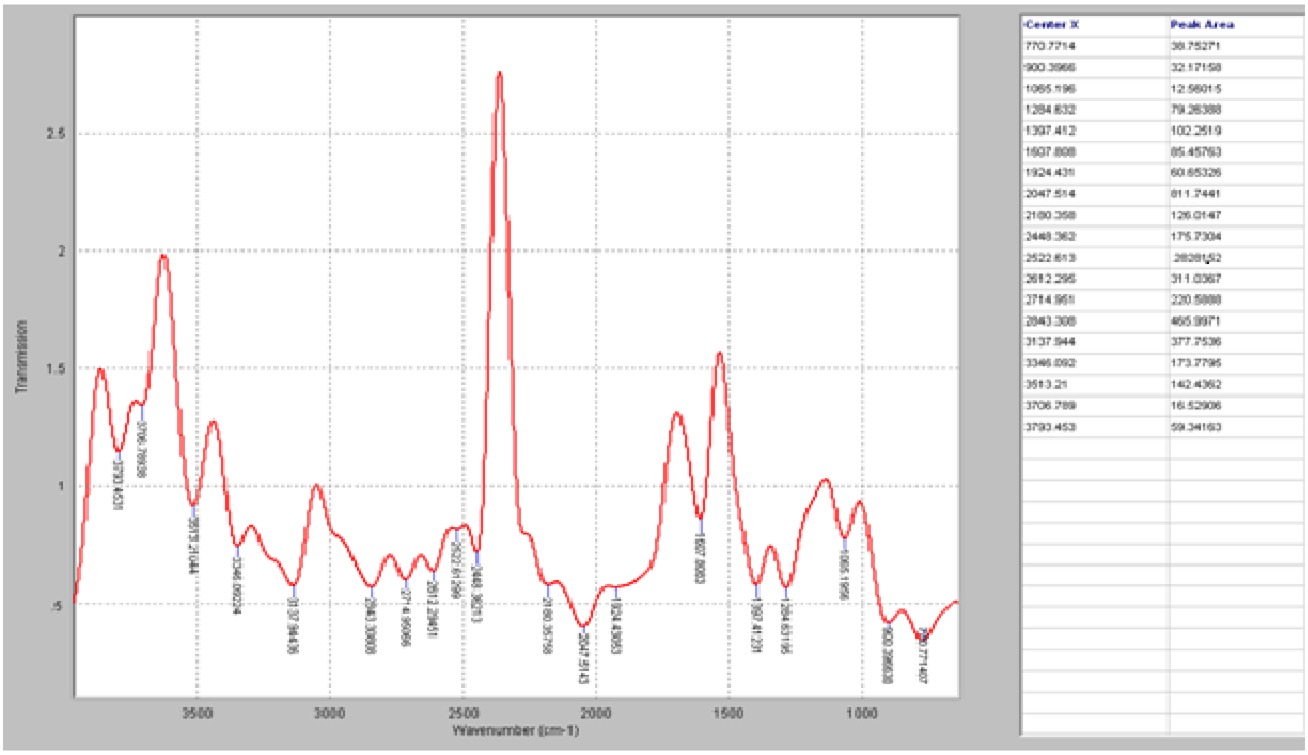


Figure 4. The Chart of FTIR of the produced Beta Carotene

Conclusion

It can be concluded that the maize and moringa leaves are two abundant and under tapped plants in Nigeria that are vital sources of carotenoids. The response surface methodology was useful in optimizing the extraction process.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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