Optimization on Rubber Seed Oil Epoxidation Process Parameters Using Response Surface Methodology

Nwosu-Obieogu, Kenechi**+; Aguele, Felix Osarumehnsen; Chiemenem, Linus
Chemical Engineering Department, Michael Okpara University of Agriculture, Umudike, Abia State, NIGERIA

ABSTRACT: *Pure rubber seed oil was epoxidized via in situ conventional method using hydrogen peroxide and acetic acid in the presence of Sulfuric acid as catalyst. Optimization of the effect of process parameters such as time, temperature, and catalyst concentration was studied using Response Surface Methodology (RSM). The optimal condition for the predicted oxirane value, 1.5333%, was obtained at a reaction time of 6.49 hours, stirring speed of 667.26, and catalyst concentration of 1.82 mol. The resultant epoxide product was confirmed using Fourier transform infrared spectroscopy (FTIR) (at 1636.3 cm^{-1}). These findings demonstrated the effects of process parameters on the rate of epoxide formation and the possibility to synthesize bio-based resin from rubber seed oil.*

KEYWORDS: *Rubber seed oil; Optimization; Catalyst; Response surface methodology; Oxirane value.*

INTRODUCTION

Plant (vegetable) and animal oils are natural renewable materials that differ in structure and unsaturation. Vegetable oil is a class of plant oil that is abundant, inexpensive, biodegradable, environmentally benign, and offers varying degrees of unsaturation depending on the nature of the plant [1]. Vegetable oil, such as groundnut, sunflower, melon, Karanja, soybean, castor, linseed, okra seed oil, etc. are considered unreactive chemical materials that can be made reactive through chemical modification [2]. They are triglycerides, which contain saturated fatty acids such as palmitic or stearic acids, and unsaturated fatty acids such as palmitoleic, oleic, linoleic, or linolenic acids, containing one, two, or more double bonds between two carbon atoms. The unsaturated fatty acids are the reactive sites for chemical modification in vegetable oils, hence the more unsaturated the fatty acids, the more reactive the oil.[3]

Rubber tree (*Hevea brasiliensis*) is the source of latex which is a feedstock for the production of rubber tires for automotive and aerospace applications. The rubber the tree also produces seeds with an average oil content of 35 - 40% [4]. The seeds are not suitable for human consumption and so its industrial applications do not deplete the food supply. The oil is unsaturated and exhibits a semi-drying property, hence it can be used in the manufacture of paint, soap, alkyd resin, and wood polish, same as other plant oils. [5,6]

Various chemical modifications on the double bonds (reactive sites) of these vegetable oils have been done. In the chemical industry, the most common value-adding chemical modification of vegetable oils is epoxidation and hydroxylation. [7]. Epoxidation of fatty acids is a reaction of a C=C double bond with active oxygen to form a three-membered oxirane ring or epoxide group [8,9].

* *To whom correspondence should be addressed.*
\+ *E-mail*: *kenenwosuobie@gmail.com*
1021-9986/2021/5/1575-1583 *9/$/5.09*

Industrial methods usually involve the reaction of these unsaturated C=C double bonds with a peracid (that is, an acid with additional oxygen). This acid is formed by the reaction of an ordinary carboxylic acid (e.g. acetic acid) with hydrogen peroxide. The epoxides formed can be used as raw materials to synthesize cross-linkable bio-resins [10].

The epoxides obtained from higher alkenes, esters, and triglycerides of unsaturated fatty acids are intermediates for the preparation of oligoestrols, glycols, hydroxy ethers, alcanolamines. Without further modifications, they are used as plasticizers and stabilizers for plastics, inks, coatings, and cutting fluid for metalworking processes or coatings obtained by UV initiates cross-linking [3, 7, 11-15]. Recent studies have attempted to improve the efficiency of epoxidation under milder conditions that minimize by-product formation, reaction time, increase the rate of peracid formation using a catalyst and optimize the process parameters [16]. Tables 1 and 2 below show the physicochemical characteristics and fatty acid profile of rubber seed oil as reported by *Okiemen et al*. [5] which is a pointer to the fact that it can be suitably epoxidized. Also, kinetic and thermodynamics studies on rubber seed oil epoxidation have been carried out by *Okiemen et al* [5] where the rate constant, activation energy, enthalpy, entropy, and free energy activation were determined as 5.01 X 10^{-6} l $mol^{-1}s^{-1}$, 15.7 kcal/mol, 15.2,-31.94, and 25.44 respectively, this indicated that an increase in temperature increased the rate of epoxide formation, it was validated by *Obanla et al* [17]. Optimization of the process parameter of some vegetable oils like canola, soya bean, rapeseed, and sesame seed oil has been investigated by [18-21] respectively. Comparative analysis of different epoxidation procedures of Cynara cardunculus seed oil has been carried out by *Turco et al* [22]. Hence this work investigates the optimum epoxidation condition for rubber seed oil varying the process parameters such as time, stirring speed, and catalyst concentration.

EXPERIMENTAL SECTION

Materials

Pure rubber seed oil used in this study was soxhlet extracted with hexane from ground seed material; acetic acid (85%) obtained from Sigma Aldrich, Poole, England, hydrogen peroxide (30wt %) from MERCK. sodium carbonate obtained from GFS Chemicals, Inc. USA.

Table 1: Physico-chemical characteristics of rubber seed oil.

Iodine value (gI_2/100g)	155.56
Specific gravity (30ºC)	0.926
Acid value (MgKOH/g)	23.00
Free fatty acid (% oleic acid)	11.29
Peroxide value (meq/kg)	0.40
Saponification value (MgKOH/g)	192.93

Source: Okieimen et al,(5)

Table 2: Fatty acid profile of rubber seed oil.

Myristic C_{14}:0	2.2
Palmitic C_{16}:0	7.6
Stearic C_{18}:1	10.7
Oleic C_{18}:1	20.0
Linoleic C_{18}:2	36.0
Linolenic C_{18}:3	23.5

Source: Okieimen, et al (5)

Equipment Used

Magnetic heater, three-necked round bottom flask, thermometer, condenser, feed funnel, stirring bulb, measuring cylinder, weighing balance, separation funnel, rotary evaporator.

Design of Experiment

The experiment was designed with Box Behnken considering 3 factors (temperature, time, stirring speed, and catalyst concentration) and 2 responses (iodine value and oxirane value) comprising of 17 experimental runs using Design Expert Software.

Epoxidation procedure

The epoxidation method reported by *Goud et al*. [23] and *Nwosu-Obieogu et al*. [24] was used with little variation in the procedure and was repeated for all the experimental runs with the same concentration while varying reaction time, stirring speed, and catalyst concentration. 30g of rubber seed oil was placed in a three-necked bottom flask, 4 g of acetic acid was added to the flask after about 5 minutes, the mixture was stirred continuously for 30 minutes. Then 16.15 g of 30 wt% aqueous hydrogen peroxide was added dropwise to the reaction mixture, as oxygen donor, at a rate such that

Table 3: Independence factors and their coded value levels.

				level		
Factors	Name	Unit	Type	-1	0	1
A	Catalyst conc.	Mol	Numeric	0.9	1.8	2.7
B	Time	Hours	Numeric	5.0	6.0	7.0
C	Stirring speed	Rpm	numeric	500	1000	1500

the hydrogen peroxide addition was completed within half an hour. The mole ratio of the components used is 1:1.5:0.5; H_2O_2: HCOOH. After the complete addition of hydrogen peroxide, the mixture was heated under reflux at the same desired temperature (65 ºC) with rapid stirring. The collected samples of the Epoxidised Rubber seed oil (ERSO) were washed with sodium carbonate (Na_2CO_3) which was dissolved in distilled water to remove the free acids and other unreacted components. 10g of Na_2C0_3 was first dissolved in 100mL of distilled water. Then, another 100mL of distilled water was further added to the mixture. The total mixture was added to the sample and separated by a separating funnel. Subsequent extraction was used to recover the remaining samples after washing.

Synthesis of Acrylated Rubber Seed Oil

30g of the epoxidized rubber seed oil was heated at room temperature, 9.79g of acrylic acid-containing hydroquinone (0.02g, 0.25wt %) was added to the oil at 30 minutes. The reaction mixture was heated under reflux for 6hours at 90°C with constant stirring. The mixture was then cooled to room temperature. The obtained product, Acrylated Epoxidized rubber Oil (AESO) was washed with distilled water and isolated.

Analytical techniques

Iodine value

The iodine value of the test oil sample was determined by the Wijs method for iodine value [The American Oil Chemist's Society Official Method]. 0.5 g of the sample was poured into a conical flask. 10 ml of carbon tetrachloride was added to the oil and was shaken to allow the oil to dissolve. Also, 20 mL of the Wijs iodine solution was later added to the mixture. It was stirred vigorously, stoppered, and kept in the dark for 30 minutes. Subsequently, 15 ml of potassium iodide solution followed by 100 ml of distilled water was added. The mixture was titrated against 0.01N sodium thiosulphate solution. A reagent black was titrated as well.

The iodine value of epoxidized samples was calculated after analysis using the formula:

$$IV = \frac{(B-S)\times M\times 12.69}{W} \quad (1)$$

Where:

IV = Iodine value of samples

S = Volume of $Na_2S_2O_3$ used for sample (ml),

B = Volume of $Na_2S_2O_3$ used for blank (ml),

W = Weight of sample used (g),

M = Molarity of the $Na_2S_2O_3$ used.

Oxirane Oxygen content

The percentage of the oxirane oxygen was determined by a direct method established by using a hydrobromic acid solution in glacial acetic acid. The Oxirane Oxygen (OO) content was calculated according to the consumed amount of the halogen atom.

The Oxirane Oxygen Content of the analyzed samples was calculated using the formula:

$$OV = \frac{(B-S)\times M\times A_o\times 100}{1000W} \quad (2)$$

Where:

S = Volume of NaOH used for sample (ml)

B = Volume of NaOH used for blank (ml)

M = Molarity of the NaOH used

W = Weight of sample used (g)

A_o = Atomic weight of oxygen

FT-IR analysis

The pure and epoxidized rubber seed oil was characterized using Fourier Transform InfraRed (FT-IR) Spectroscopy Technique to determine surface functional groups present. The FT-IR analyses were carried out on the

Table 4: Experimental design layout for iodine and oxirane value of rubber seed oil

Std	Run	Factor 1 A: Catalyst conc. mol	Factor 2 B: time Hours	Factor 3 C: Stirring speed rpm	Response 1 Iodine value g/100g of oil	Response 2 Oxirane value %
11	1	1.8	5.0	1500	6.22	0.1
13	2	1.8	6.0	1000	3	0.87
17	3	1.8	6.0	1000	3	0.87
7	4	0.9	6.0	1500	2.7	1.39
1	5	0.9	5.0	1000	5.97	1.6
2	6	2.7	5.0	1000	8.33	1.13
8	7	2.7	6.0	1500	5.3	0.34
15	8	1.8	6.0	1000	2.1	0.6
10	9	1.8	7.0	500	3.4	0.8
12	10	1.8	7.0	1500	6.02	0.5
16	11	1.8	6.0	1000	2.5	0.21
3	12	0.9	7.0	1000	0.6	1.55
6	13	2.7	6.0	500	7.87	1.3
5	14	0.9	6.0	500	9.53	0.36
14	15	1.8	6.0	1000	4.6	0.83
9	16	1.8	5.0	500	2.24	0.42
4	17	2.7	7.0	1000	9.14	1.04

samples using Shimadzu FT-IR-8400S Spectrophotometer with a resolution of 4 cm-1 in the range of 4000 - 500 cm.

RESULTS AND DISCUSSION

Statistical analysis of data for epoxidation of rubber seed oil

The results of the iodine and oxirane value presented in Table 4 were determined using Equations 1 and 2, respectively. The varying responses are indications that the process parameters considerably affected the iodine and oxirane value. The minimum iodine value of 0.6g /100g oil was obtained at a catalyst concentration of 0.9 mol, after 7 h, and at a stirring speed of 1000 rpm. The maximum oxirane value of 1.6% was obtained at a catalyst concentration of 0.9 mol, time of 5 hours, and stirring speed of 1000 rpm, inline with what was reported by *Paul et al*. [25], hence this is an indication that the iodine and oxirane values from the epoxidation of rubber seed oil were affected by process conditions. The statistical analysis for epoxidation of rubber seed oil was done using analysis of variance (ANOVA). Table 5 shows the ANOVA results for rubber seed oil epoxidation for oxirane value. The multiple regression analysis of the experimental data gives a second-order polynomial equation. The quadratic model developed depicts the interaction between the oxirane value respectively (Y) and the coded values of the independent variables A, B, and C (catalyst concentration, time, and stirring speed).

$$Y = 0.75 - 0.28A - 4.167X10 - 3B - 0.29C + 0.54A^2 + 0.038B^2 - 0.16C^2 - 1.000X10 - 2AB - 0.22AC + 0.17C \quad (3)$$

Where Y represents response variable oxirane value measured in %

Adequacy of the model

The significance and adequacy of the model were tested using ANOVA. It was observed from Table 5 that the model and all the coded factors are significant except the interaction effect between time and stirring speed (BC),

Table 5: Analysis of variance (ANOVA) results for response surface quadratic model of oxirane value

Source	Sum of squares	DF	Mean square	F value	Prob> F	
Model	2.57	9	0.29	16.67	0.0078	significant
A	0.37	1	0.37	21.29	0.00999	
B	8.333E-005	1	8.333E-005	4.85E-005	0.0078	
C	0.23	1	0.23	13.30	0.0218	
A^2	0.88	1	0.88	51.31	0.0020	
B^2	4.408E-003	1	4.408E-003	0.26	0.6389	
C^2	0.054	1	0.054	3.17	0.1494	
AB	4.000E-004	1	4.000E-004	0.023	0.8860	
AC	0.082	1	0.082	4.76	0.0945	
BC	0.052	1	0.052	3.00	0.1582	
Residual	0.069	4	0.017			
Lack of fit	0.023	1	0.023	1.49	0.3088	
Pure Error	0.046	3	0.015			
Cor Total	2.64	13				
R-Squared	0.9740					
Adj R-Squared	0.9156					

catalyst concentration and time (AB) and catalyst concentration and stirring speed (AC) that is not significant, quadratic models, B^2, C^2 also is not significant. The greater the F-value, the more certain it is that the model explains adequately the variation in the data, it was obtained as 16.67 which is adequate for the model. [26,27] The fitness of the polynomial model was expressed by the coefficient of determination (R^2) and the coefficient of adjusted R^2, which were obtained as 0.9740 and 0.9156 respectively, it is an indication that the regression model is acceptable. The lack of fit value of 0.3088 for oxirane value depicts non-significance, this implies pure error and low for the model, it shows an adequate representation of the interaction by the model. The non-significant lack of fit of the model is good as the model could be used for the theoretical prediction of the oxirane value.

Table 6 shows the assessment of experimental errors and the confidence interval of the experimental variables indicating that the overall model for the response is significant.

The 3D response surface plots are the graphical representation of the regression equations used to visualize the relationship between the responses and experimental levels of each factor. The variation in oxirane value is displayed on the z-axis showing the three-dimensional relationship with factor variables on y and x-axis respectively. The interactions of the two factors are reflected in the contour of the plots. The rounded contour line indicates a weak interaction of two factors and a distorted contour indicates a significant interaction of two factors [28], all the interaction between the factors (stirring speed, time, and catalyst concentration) on the oxirane value displayed a distorted contour, which indicates that the interaction between the factors is significant.

Normalization plots in Fig. 4 helped in ascertaining if the models are satisfactory. The data was plotted against a theoretical normal distribution in such a way that the points should form an approximately straight line and a departure from this line indicates a falling out from a normal distribution. Some data fell out of line, the ones distributed along the 45-degree line was enough to validate the model [29].

Table 6: ANOVA analysis of experimental errors and confidence intervals for oxirane value.

Factor	Coefficient estimate	Standard error	95% Cl low	95% Cl high
Intercept	0.75	0.065	0.57	0.93
A. Catalyst conc	-0.28	0.060	-0.44	-0.11
B. Time	-4.167E-003	0.060	-0.17	0.16
C. stirring speed	-0.29	0.080	-0.52	-0.070
A^2	0.54	0.076	-0.33	0.75
B^2	0.038	0.076	-0.17	0.25
C^2	-0.16	0.093	-0.42	0.092
AB	-1.000E-002	0.065	-0.19	0.17
AC	-0.22	0.10	-0.50	0.059
BC	0.17	0.10	-0.10	0.45

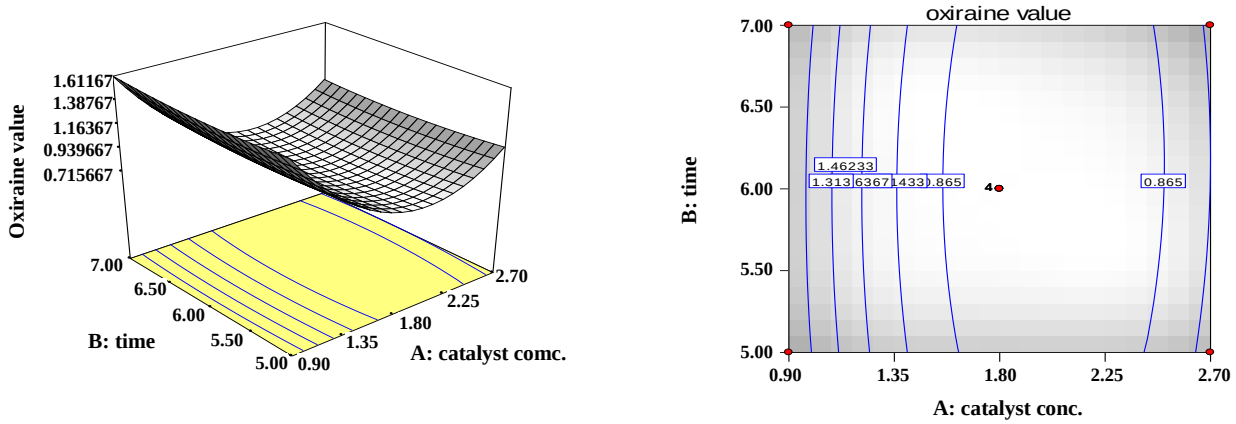


Fig 1: The 3D and contour plots of the effect of catalyst concentration and time on the oxirane value of ERSO.

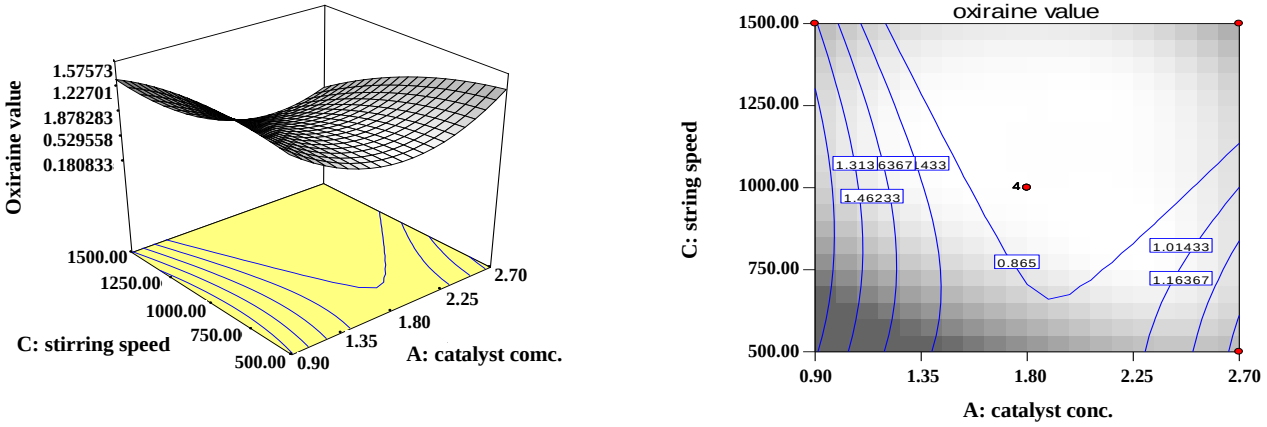


Fig 2: The 3D and contour plots of the effect of catalyst concentration and time on the oxirane value of ERSO.

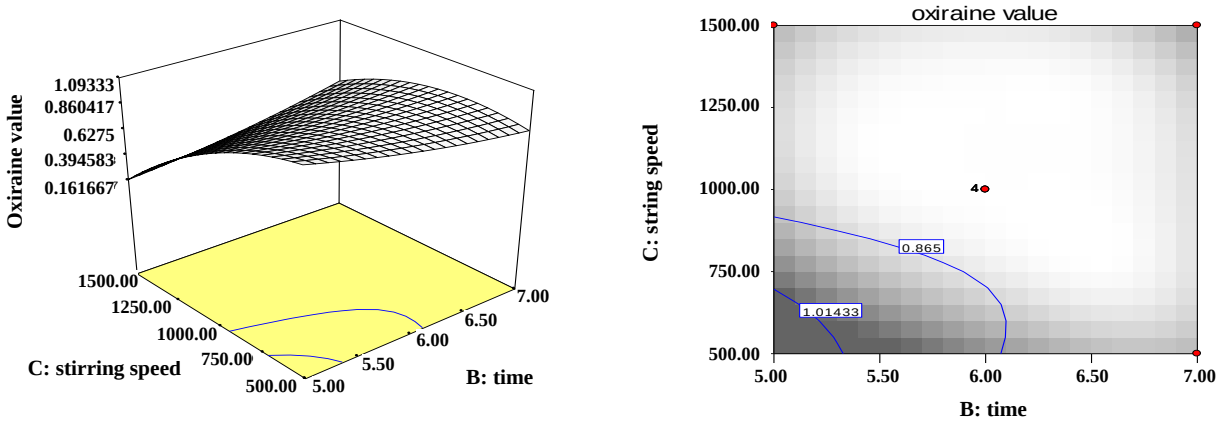


Fig 3: The 3D and contour plots of the effect of stirring speed and time on the oxirane value of ERSO.

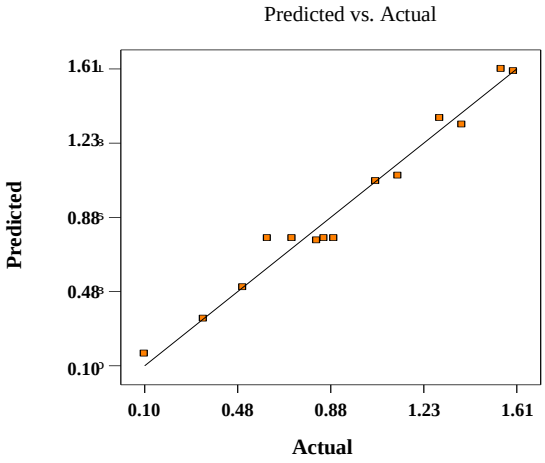


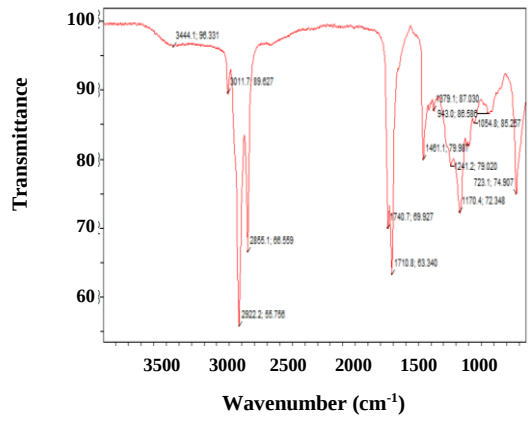
Fig 4: Normalization plot for oxirane value

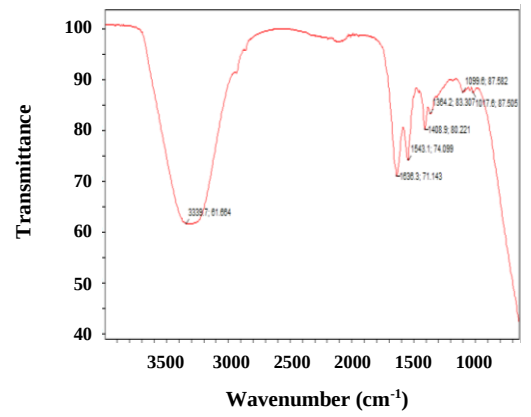
FT-IR graphs

In the FT-IR spectra, the presence of carbon-carbon double bonds (C=C) in the untreated rubber seed oil was indicated by the appearance of a peak at 1710.8cm^{-1}. The absorption band for the epoxy group in the RSO was indicated by the single peak at 1636.3cm^{-1}, this peak was missing in the untreated oil. Hence, a pointer to the fact that the oil has been suitably epoxidized. The acrylate epoxy resins of rubber seed oil were obtained at the wavenumber of 2120cm^{-1} to form the acrylic group, which indicates that it has been modified and can be applied in biobased thermoset development.

CONCLUSIONS

The development of epoxidized rubber seed oil was demonstrated and the formation of epoxy groups was confirmed by FT-IR spectroscopy analysis. The result of the investigation shows that rubber seed oil can be successfully utilized for epoxidation using peroxyacid generated in situ. The optimal condition for the predicted oxirane value of 1.5333%, was obtained at a reaction time of 6.49 hours, stirring speed of 667.26, and catalyst concentration of 1.82 mol. Results of the statistical analysis showed that the process parameters (catalyst concentration, time, and stirring speed) have significant effects on the response, its application in biobased resin was validated through acrylation of the epoxidized rubber seed oil. Hence these findings are for possible utilization of rubber seed oils in the production of thermosets and composite materials.





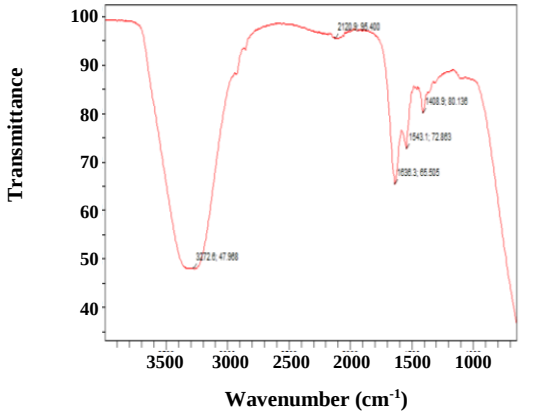


Fig 5: The Fourier transform infrared spectroscopy of the pure sample of, a) pure rubber seed oil, b) epoxidized rubber seed oil, c) acrylate epoxidized rubber seed oil.

Received : Nov. 15, 2019 ; Accepted : May 11, 2020

REFERENCES

[1] Petrovic Z.S., Polymers from Biological Oils, *Contemporary Materials*, **1**: 39-50 (2010).

[2] Saurabh T., Patnaik M., Bhagat S. L, Renge V.C., Studies on the Synthesis of Biobased Epoxideusing Cottonseed Oil, *Int. J. Adv. Eng. Res. Stud*, **1(2)**: 279-284 (2012).

[3] Jabar J.M., Olagboye S.A., Kinetics Studies on Epoxidation of Jatropha Curcas and Thevetia Peruviana Oil, *Journal of Sustainable Technology*, **8(1)**: 117-127 (2017).

[4] Nwankwo B.A., Aigbekaen S.G.A., "Estimates of Rubber (ev) Seed Production in Nigeria". in: "Industrial Utilization of Natural Rubber, Seed Latex, and Wood", *Proceedings of Natural Conference (Ed: Ephraim E. Enabor).* Rubb. *Res. Inst. of Nigeria.;*78-87 (1985)

[5] Okieimen F. E., Bakare O. I., Okieimen C.O., Studies on the Epoxidation of Rubber Seed Oil, *Industrial Crops, and Products*, **15**: 139-144 (2002)

[6] Ramadhas A. S., Jayaraj S., Muraleedharan C., Biodiesel Production from High FFA Rubber Oil, *Fuel*, **84(4)**: 335-340 (2009).

[7] Goud V. V., Patwardhan A. V., Pradhan N. C., Studies on the Epoxidation of Mahua Oil (*Madhumica indica*) by Hydrogen Peroxide, *Bioresource Technol*., **97**: 1364-1371 (2006).

[8] Dinda S., Patwardhan A. V., Goud V.V., Pradhan N. C., Epoxidation of Cottonseed Oil by Aqueoushydrogen Peroxide Catalyzed by Liquid Inorganic Acids, *Bioresource*. **99 (9)**: 3737-3744 (2008).

[9] Nwosu-Obieogu K., Kalu U.C., *In Situ* epoxidation of Sesame Seed Oil for the Synthesis of a Bio-Based Resin, *European J. Sustainab*le *Dev*., **4(3)**: em0121 (2020).

[10] Latif F.E.A, Abidin Z.Z., Cardona F., Awang Biak K.A., Tahir P.M., Ern L.K., Bio-Resin Production Through Ethylene Unsaturated Carbon Using Vegetable Oils, *Processes*., **8(48)**: 1-15 (2020).

[11] Rios L.A., Weckes P.P., Schuster H., Hoelderich W.F., Resin Catalyzed Alcoholysis of Epoxidized Fatty Esters: Effect of the Alcohol and the Resin Structures, *Applied Catalysis A*: *General*, **284**: 155-161 (2005).

[12] Metzger J.O., Bornscheuer U., Lipids as Renewable Resources. Current State of Chemical and Biotechnological Conversion and Diversification, *Appl. Microbiol. Biotechnol.*, **71**: 13–22 (2006)

[13] Quinchia L.A., Delgado M.A., Reddyhoff T., Gallegos C., Spikes H.A., Tribological Studies of Potential Vegetable Oil-Based Lubricants, *Tribol. Int.*, **69**: 110–117 (2014).

[14] Dinda S., Ravisankar D., Puri P., Development of Bio-Epoxide from Nahor (*Mesua ferrea Linn*) Oil, *Journal of the Taiwan Institute of Chemical Engineers*, **65**: 399-404 (2016).

[15] Silviana S., Anggoro D.D., Kumoro A.C., Kinetic Study of Waste Cooking Oil Epoxidation with Peroxyacetic Acid Using Acid Catalysts, *Rasayan J.Chem*., **12(3)**: 1369-1374 (2019).

[16] Thames S.F., Yu H., Biopolymer from Renewable Resources, *Surf. Coat. Technol.*, **115**: 208-214 (2009)

[17] Obanla O.R., Udonne J.D., Ajani O.O., Ojewumi M.E., Omodara O.J., Oni B.A., Studies of the in-Situ Epoxidation of Rubber (Hevea Brasiliensis) Seed Oil by Performic Acid, *J. Phys. Conf. Ser. 1378*, 1-8. (2019).

[18] Matusiak M., Milcher, E., Optimization of Selective Epoxidation of Canola Oil with in Situ Generated Peracetic Acid, *Journal of Advanced Oxidation Technologies* **21(1)**: - (2018).

[19] Sinadovic-Fiser S., Milovan J., Zoran S., Kinetics of in Situ Epoxidation of Soyabean Oil in Bulk Catalyzed by Ion Exchange Resin, *Kansas Polymer Research*, **78(7)**: 725-731 (2001).

[20] Arumugam S., Sriram, G., Rajmohan, T., Multi-Response Optimization of Epoxidation Processparameters of Rapeseed Oil Using Response Surface Methodology(RSM)-Based Desirability Analysis, *Arab. J. Sci. Eng*., **39**: 2277-2287 (2014).

[21] Musik M., Milchert E., Selective Epoxidation of Sesame Oil with Peracetic Acid, *Molecular catalysis*, **433**: 170-174 (2017).

[22] Turco R., Tesser R., Russo V., Vitiello R., Fagnano M., Di Serio M., Comparison of Different Possible Technologies for Epoxidation of *Cynara cardunculus* Seed Oil, *Eur. J. Lipid Sci. Technol*., 1900100, pp 1-8 (2019).

[23] Goud V.V., Patwardhan A.V., Dinda S., Pradhan N.C., Kinetics of Epoxidation of Jatropha Oil with Peroxyacetic and Peroxyformic Acid Catalyzed by Acidic Ion Exchange Resin, *Chem Eng Sci*; **62(15)**: 4065-4076 (2007)

[24] Nwosu-Obieogu K., Hamed J.O., Anike E.N., Aguele F.O., Ukandu O., Obasi H.C., Uduma C.K., Chiemenem L.I., Studies on the Epoxidation of Groundnut Seed Oil with Carboxylic Acid, *FUTOJNLS*, **5(2)**: 94-101 (2019)

[25] Paul K.A., Borugadda B.U., Bhalerao S.M., Goud V.V., In Situ Epoxidation of Waste Soybean Cooking Oil for Synthesis of Biolubricant Basestock: A Process Parameter Optimization and Comparison with RSM, ANN and GA, *Can. J. Chem. Eng.*, **9999**:1451-1461 (2017)

[26] Montgomery D.C., "Design and Analysis of Experiments". 6th ed., John Wiley & Sons. Inc, Hoboken NJ (2005).

[27] Trinh T.K., Kaug L.S., Response Surface Methodological Approach to Optimize the Coagulation/Flocculation Process in Drinking Water Treatment, *Chem. Eng. Res. Design.*, **89**: 1126-1130 (2011).

[28] Holetz F. B., Ueda-Nakamura T., Filho B. P. D, Cortez D.A.G., Morgado-Diaz J. A., Nakamura C.V., Effect of Essential Oil of Ocimum Gratissimum on the Trypanosomatid Herpetomonas Samuelpessoai. *Acta Protozoologica*, **42(4)**: 269–276 (2003).

[29] Chen Y., Nie J., Chen M., Zhang D., "Biosorptional Removal of Lead and Cadmium from Water by Waste Materials", Jiangxi University of Science and Technology, School of Resource and Environmental Engineering. Ganzhou Jiangxi: IEEE. (2011).