Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2015, 6(4):50-60

ISSN: 0976-8610
CODEN (USA): AASRFC

A novel method of using refractive index as a tool for finding the quality of aqueous enzymatic extracted algae oil

Anwesa Sarkar[1], J. P. Pandey[1], Anupama Singh[1], Lakshmi Tiwari[2] and Anil Kumar[3]

[1]*Department of Post Harvest Process and Food Engineering, College of Technology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*
[2]*Department of Microbiology, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*
[3]*Department of Food Science and Technology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*

ABSTRACT

Any physical parameter should find applications in our day-to-day life. In this paper, it has been shown that that how the refractive index can be used as a tool for finding the quality of oil. The refractive index of algae oil extracted by different processing condition has been determined and presented here.

Keywords: Algae oil, Refractive index, oil quality.

INTRODUCTION

Optics is a branch of physics which deals with the study of light. In optics the refractive index or index of refraction n of an optical medium is a dimensionless number that describes how light, or any other radiation, propagates through that medium. But in chemistry of oil it indicates the possible chances of rancidity development in oil. Higher the refractive index higher is the chances of spoilage due to oxidation. Refractive index is an important optical parameter to analyze the light rays traversing through materials medium. In laboratory, the refractive index of liquids can be found out by spectrometer using hollow prism. The Abbe's refractometer can also used for finding the refractive index with very good accuracy. Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oil industry since it offers many advantages compared to conventional extraction. For instance, it eliminates solvent consumption which lowers investment costs and energy requirements. Also, it enables simultaneous recovery of oil and protein and the process yields good quality oil. The need for further degumming operations is eliminated and the process removes some toxins or anti nutritional compounds from oils. In this sense, it is an emerging and innovative technology in the oil extraction sector which has benefits such as cost savings and nutritional issues. The use of enzyme allows higher extraction efficiencies can potentially influence the physical and chemical properties of oil. Over the last four decades, several studies have been carried out on aqueous processing in the sector of oilseeds. But very little work has been reported to apply this innovative and efficient technique for extraction of algal oil. There is a lot of scope for research to optimise a process which can be successfully scaled up and used for commercial application as an alternative method for algae oil extraction. Present study deals with the refractive index and quality of oils which was extracted from algae biomass with the help of enzymes.

MATERIALS AND METHODS

Procurement of algae strain

Algae strain was provided by the Department of Microbiology, Gobindh Ballav Pant University of Agriculture & Technology, Pantnagar.

Preparation of growth media

Algae were cultivated in specific media which provide nutrients for its growth and help to produce oil. The composition of the media was given by Buriew, 1976 and described in Table 1 and 2. All the ingredients were added in their specific amount in 1000 ml of distilled water and dissolved properly. The conical was then cotton plucked and autoclaved. After sterilisation the media was cooled to optimum temperature before inoculation.

Table 1 Composition of the media and its specification (Buriew, 1976)

Sr no.	Chemical	Specific wt/vol
1	$NaNO_3$	1.5 g
2	K_2HPO_4	0.04 g
3	$MgSO_4$,7 H_2O	0.075 g
4	$CaCl_2$,2H_2O	0.036 g
5	Citric Acid	0.0006 g
6	Ferric ammonium Citrate	0.0006 g
7	EDTA Disodium Salt	0.0001 g
8	Trace metal solution	1 ml
9	Distilled water	1000 ml

Cultivation of algae

Mass culture of algae was done in open condition in trays under sunlight. Initially 500 ml of algae culture in broth was added to 5 l of media and then media was added time to time according to the growth rate of algae. Biomass was collected after 15 to 20 days followed by immediate experimentation.

Table 2: Composition for trace metal solution (Buriew, 1976)

Sr no.	Chemical	Specific wt/vol
1	Boric acid H_3BO_3	2.86 g
2	$MnCl_2$,7 H_2O	1.181 g
3	$CuSO_4$, 7 H_2O	0.222 g
4	NaMO O_4, 2 H_2O	0.39 g
5	$CuSO_4$, 5 H_2O	0.079 g
6	$CO(NO_3)_2$, 6 H_2O	49.4 µg
7	Distilled Water	1000 ml

Collection of Biomass

Biomass was collected by filtering the algae with muslin cloth and repeated washing with distilled water to remove the impurities. After washing it was again filtered to remove any traces of media in it. The solid to water ratio used for the entire experiment was 10:3 Enzymatic treatment

1. Algae biomass was collected by filtration and washed several times with distilled water.
2. pH of the sample were adjusted (3, 4, 5, 6, 7) as per the design levels with the help of HCl/ NaOH solutions. Solutions were added accordingly drop by drop with vigorous shaking and pH was measured after each drop.
3. Cellulase and Lipase enzyme used in this experiment were purchased from Hi-Media. The cellulase was in powdered form so its solution was prepared as per the instruction for desired activity. Lipase was already in liquid form.
4. Both the enzyme solution (5 ml) of different concentration were added to the conical as per the design (0, 2, 4, 6, 8 ml/100 g) and properly shaked.
5. Then conical were cotton plucked and kept inside the incubator at different temperature (45, 50, 55, 60, 65 ◦C) according to the design.
6. Agitation speed of the incubator shaker was kept constant at rpm of 100 to provide proper mixing.
7. Samples were withdrawn at different time intervals (0, 6, 12, 18, 24 h) and immediately centrifuged.

Separation of oil

1. Withdrawn samples were kept in open condition to gain the optimum temperature.
2. 50 ml were taken in centrifuge tubes.
3. Centrifugation was done at a constant rpm of 5000 for 10 minutes.
4. The supernatant phase were pipetted out and collected.
5. The extracted oil yield was measured in measuring cylinder.
6. The separated oil was stored for further use.

Experimental Design

Selection of oil extraction parameters and there ranges were carried out on the basis of review of literature, the variables: cellulase and lipase enzyme concentration, temperature time and pH were selected as independent

parameters to see the effect on aqueous enzymatic extraction of oil from algae biomass. The variables and their coded and uncoded levels used in the experimental plan are given in Table 3.

Table 3: Independent Variables coded and actual value for experiment

Independent variables		Coded Levels		
Name	Code	-2	0	2
		Actual Levels		
Enzyme concentration (ml/100 g sample)	X_1	0	4	8
Time (h)	X_2	0	12	24
Temperature	X_3	45	55	65
pH	X_4	3	5	7

Response surface methodology (RSM) was used for the design and analysis of all experiments for four independent variables at five levels. It's also helped to reduce the number of experiments without affecting the accuracy of results and to decide the interactive effects of independent variables on the response. Central Composite Rotatable Design (CCRD) which is efficient design tool for fitting second order model was selected for the study.

The experimental plan and design of experiment has been shown in table 4. The design includes six repeated experiments at the central point of the codded variables. This was necessary for finding out the "error sum of square" and the" lack of fit" of regression equations developed between the dependent and independent variables. Total numbers of experiments designed by software were found to be 30. refractive index were determined as dependent variable for aqueous enzymatic extraction.

Table 4: Experimental Design for Final experiment

Expt no.	X_1	X_2	X_3	X_4	enzyme conc.	time	Temp.	pH
1	-1	-1	-1	-1	2	6	50	4
2	1	-1	-1	-1	6	6	50	4
3	-1	1	-1	-1	2	18	50	4
4	1	1	-1	-1	6	18	50	4
5	-1	-1	1	-1	2	6	60	4
6	1	-1	1	-1	6	6	60	4
7	-1	1	1	-1	2	18	60	4
8	1	1	1	-1	6	18	60	4
9	-1	-1	-1	1	2	6	50	6
10	1	-1	-1	1	6	6	50	6
11	-1	1	-1	1	2	18	50	6
12	1	1	-1	1	6	18	50	6
13	-1	-1	1	1	2	6	60	6
14	1	-1	1	1	6	6	60	6
15	-1	1	1	1	2	18	60	6
16	1	1	1	1	6	18	60	6
17	-2	0	0	0	0	12	55	5
18	2	0	0	0	8	12	55	5
19	0	-2	0	0	4	0	55	5
20	0	2	0	0	4	24	55	5
21	0	0	-2	0	4	12	45	5
22	0	0	2	0	4	12	65	5
23	0	0	0	-2	4	12	55	3
24	0	0	0	2	4	12	55	7
25	0	0	0	0	4	12	55	5
26	0	0	0	0	4	12	55	5
27	0	0	0	0	4	12	55	5
28	0	0	0	0	4	12	55	5
29	0	0	0	0	4	12	55	5
30	0	0	0	0	4	12	55	5

Coding of the variables was done as per the following:
The independent variables were coded as X_1, X_2, X_3 and X_4 for enzyme concentration, Time, Temperature and pH with help of equations 1 – 4, respectively.

$$X_1 = \frac{\text{enzyme conc.} - 4}{2} \quad \text{....(1)}$$

$$X_2 = \frac{\text{Time} - 12}{6} \quad \text{.... (2)}$$

$$X_3 = \frac{\text{Temp.} - 55}{5} \quad \text{....(3)}$$

$$X_4 = \frac{\text{pH} - 5}{1} \quad \text{....(4)}$$

Determination of Refractive Index

Temperature of the refractometer was adjusted and the oil sample was smear on the cleaned prism and readings were taken. After the measuring was complete the prism was cleaned with hot water. Readings were corrected using equation 5 (Ranganna, 2005)

$$R=R'+K(T'-T) \quad \text{.....(5)}$$

Where,
R= Adjusted reading
R′= Reading at T ◦C
T′= temp at which readings taken
T= specified temp 40 ◦C
K= 0.00385 for oil

RESULTS AND DISCUSSION

Designed experiments were conducted to produce oil from algae biomass. Effect of aqueous enzymatic extraction on refractive index were studied. The experiments were planned using the central composite rotatable design (CCRD) design in four independent variables namely enzyme concentration, incubation temperature, incubation time and pH. The levels of parameters considered were cellulase and lipase enzyme concentration (0, 2, 4, 6 and 8 v/v %), incubation temperature (45, 50, 55, 60 and 65°C), incubation time (0, 6, 12, 18 and 24 h) and pH (3, 4, 5, 6, 7). The results are presented in Tables (4.1, 4.7, 4.13, 4.19 and 4.25).

A complete second order model (Eq. 6) was fitted to the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination), Fisher's F-test and lack of fit. The predicted models were used to interpret the effect of various parameters on the response. Optimization of process parameters was carried out and contours were developed for selected parameters.

A second order response function for four independent variables had the following general form:

$$Y = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{4}\beta_{ij}X_i X_j + \sum_{i=1}^{4}\beta_{ii}X_i^2 \quad \text{...(6)}$$

where,
β_0 is constant
β_i, β_{ii}, β_{ij} are coefficients
X_i, X_j are variables (coded)

The experimental data were analyzed employing multiple regression techniques to develop response functions and variable parameters optimized for best outputs. The regression coefficients of complete second order model and their significance were compared.

Regression analysis of Eqn. 6 gives the results in terms of ANOVA, regression coefficients and associated statistics, standard deviation, coefficient of determination (R^2), Lack of fit, etc. These are used to determine adequacy of the predictive model and effect of independent variables on the response. The models were compared based on the coefficient of determination (R^2), adjusted coefficient of determination (R^2-adj) and predicted coefficient of

determination (R^2-pred). The coefficient of determination (R^2) is defined as the regression of sum of squares proportion to the total sum of squares which illustrates the adequacy of a model. R^2 ranges from 0 to 1. R^2 values closer to 1(in decimal), means the model is more accurate. The high adjusted and predicted coefficient of determination also illustrate whether the model adequately fits the data (Badwaik *et al.*, 2012). After selecting the most accurate model, the analysis of variance (ANOVA) was used to investigate the statistical significance of the regression coefficients by conducting the Fisher's F-test at 95% confidence level. The interactive effects of the factors were observed using surface plots, derived from the chosen model. Finally, the entire process was optimised. The aim of the optimisation was to maximise the responses with the desirable weight and the credibility of the optimum conditions was diagnosed through the desirability values of the responses which range from 0 to 1. The closer values of desirability to 1 showed the more desirable and credible optimal conditions (Yolmeh *et al.*, 2014).

The probability of significance of predictor's coefficient indicates the extent of effect of predictor on the response. The sign and magnitude of the coefficient explain the nature of the effect. Negative sign at linear level means decrease in response when the level of the predictor is increased while positive sign indicates increase in the response. Significant negative interaction suggests that the level of one of the predictors can be increased while that of other decreased for constant value of the response. Positive interaction means the response is minimum at center point and it increases with increase or decrease of both the variables from center point. Positive coefficient of a quadratic term indicated the minimum response at center value of the parameter and it increases with increase or decrease in parameter level. Negative coefficient of the quadratic term shows the maximum response at the centre value and it decreases with increase/decrease in parameter level. The result of experimentation and mathematical analysis are given below.

It was revealed from Table 5 that refractive index of oil was in the range of 8.003 to 10.23 throughout the experimental conditions. Maximum and minimum refractive index of oil was observed at Experiment No. 10 and 27 respectively. Enzyme concentration of 4 % (X_1= 0), incubation temperature of 55°C (X_2 = 0), time 18 h (X_3 = 0) and pH 5(X_4=0) gives oil of maximum refractive index while enzyme concentration of 2% (X_1 = -1), incubation temperature of 60°C (X_2 = 1) time 18 h (X_3 =1) and pH 6 (X_4= 1) gives oil of minimum refractive index.

Table 5 Design matrix of CCRD and data of responses for aqueous enzymatic extraction of algae biomass

Expt. no.	Enzyme concentration (v/v)	Time (h)	Temperature (°C)	pH	Refractive Index
1	0	0	0	0	1.395
2	1	1	-1	1	1.325
3	0	0	0	0	1.445
4	0	0	-2	0	1.239*
5	-1	1	-1	1	1.351
6	1	1	1	1	1.412
7	0	0	0	0	1.431
8	0	0	0	0	1.425
9	1	-1	-1	-1	1.284
10	0	0	0	0	1.436
11	0	-2	0	0	1.258
12	-1	-1	1	-1	1.308
13	0	0	2	0	1.456
14	-1	1	-1	-1	1.295
15	1	1	-1	-1	1.311
16	1	-1	1	1	1.369
17	0	0	0	0	1.463**
18	2	0	0	0	1.314
19	1	-1	-1	1	1.387
20	1	1	1	-1	1.375
21	0	2	0	0	1.368
22	1	-1	1	-1	1.328
23	-1	-1	-1	-1	1.253
24	-1	-1	-1	1	1.401
25	0	0	0	-2	1.326
26	-2	0	0	0	1.245
27	-1	1	1	1	1.386
28	-1	-1	1	1	1.335
29	-1	1	1	-1	1.348
30	0	0	0	2	1.352

***, * indicates maximum and minimum values*

Full second order model, Eq. 6 was fitted into refractive index data and experimental conditions using multiple regression analysis and the results are given in Table 6. The coefficient of determination (R^2) for the regression

model for oil yield was 82.41 %, which implies that the model could account for 82.41 % data. The values of R^2- adj and R^2-pred for the refractive index of oil were 65.99 and 7.93 respectively. The F_{cal} value (5.0192) was greater than table F_{tab} value (3.65) suggesting model was significant at 1% level of significance. Positive linear coefficients of the variables (enzyme concentration, incubation temperature incubation time and pH) indicated that the refractive index of oil had a directly proportional relation with the variables. That means if the level of the variables will increase refractive index will also increase. Lack of fit was insignificant. Therefore, the equation which is a regression model adequate in describing oil yield is given below:

$$RI = 1.433 + 0.011X_1 + 0.015\ X_2 + 0.029X_3 + 0.022\ X_4 - 0.002\ X_1X_2 + 0.006X_1\ X_3 - 0.005X_1X_4 + 0.014X_2X_3 - 0.011X_2X_4 - 0.011\ X_3X_4 - 0.035X_1^2 - 0.026X_2^2 - 0.018X_3^2 - 0.020X_4^2 \quad ...7$$

where, RI = Refractive index
X_1 = enzyme concentration (v/v)
X_2 = incubation temperature (°C)
X_3 = incubation time (h) and
X_4 = pH.

Table 6 Estimated regression coefficients of refractive index for aqueous enzymatic extraction of algae biomass

Source	Refractive index	
	Coefficient	P value %
Models	1.433	0.002***
X_1	0.011	0.190
X_2	0.015	0.070*
X_3	0.029	0.002***
X_4	0.022	0.013
$X_1\ X_2$	-0.001	0.854
$X_1\ X_3$	0.006	0.515
$X_1\ X_4$	-0.005	0.629
$X_2\ X_3$	0.014	0.156
$X_2\ X_4$	-0.011	0.264
$X_3\ X_4$	-0.011	0.253
X_1^2	-0.035	0.0002***
X_2^2	-0.027	0.002***
X_3^2	-0.018	0.027**
X_4^2	-0.020	0.015**
R^2	82.41	
R-adj	65.99	
R-pre	7.93	
F_{cal} value	5.0192	
LOF	**NS**	

Analysis of variance for response surface quadratic model and variables for refractive index can be seen from Table 7. It was clearly indicated that independent variables had very high significance (1%) on refractive index of oil at linear and quadratic level. But at interactive level the variables had only 5 % level of significance.

Total effect of individual parameter on refractive index of oil was calculated using the sequential sum of squares, and shown in Table 8. It was from Table 8 observed that all of the variables namely enzyme concentration (X_1), incubation time (X_2), Incubation temperature (X_3) and pH (X_4) had high significant effect at 1 % level of significance on the refractive index of oil.

On the basis of individual effect of independent variables on refractive index of oil reported in Table 4.30. the model can be simplified by omitting the non-significant terms and rewritten as:

$$RI = 1.433 + 0.015\ X_2 + 0.029X_3 - 0.035X_1^2 - 0.026X_2^2 - 0.018X_3^2 - 0.020X_4^2 \quad ...8$$

Table 7 Analysis of variance for response surface quadratic model and variables for refractive index

SOURCE	DF	SS	MS	F-Value
Model	14	0.098618	0.007044	5.019267***
Linear	4	0.038803	0.009701	18.90976***
Quadratic	4	0.070779	0.017695	34.49257***
Interactive	6	0.008025	0.001337	2.607131**
Error	15	0.002564	0.000513	
Total	29	0.218789		

****, **,* Significant at 1, 5 and 10 % level of significance respectively*

$F_{tab}(4, 15) = 14.1981$; $F_{tab}(6, 15) = 7.5591$; $F_{tab}(14,15) = 3.6557$ (1%)
$F_{tab}(4, 15) = 5.8578$; $F_{tab}(6, 15) = 3.9381$; $F_{tab}(14,15) = 2.463$(5%)
$F_{tab}(4, 15) = 3.8704$; $F_{tab}(6, 15) = 2.8712$; $F_{tab}(14,15) = 2.0095$ (10%)

These observations are in close agreement with the earlier findings of Dickey *et al.*, 2008; Sineiro *et al.*, 1997.

Table 8. Overall effect of individual parameters on refractive index

SOURCE	DF	SS	MS	F-Value
Model	14	0.098618	0.007044	5.019267***
Enzyme concentration(X_1)	5	0.036467	0.007293	14.21716***
Incubation time(X_2)	5	0.029257	0.005851	11.40642***
Incubation temperature(X_3)	5	0.033944	0.006789	13.23357***
pH (X_4)	5	0.025963	0.005193	10.12184***
Error	15	0.002564	0.000513	
Total	29	0.226813		

****, **,* Significant at 1, 5 and 10 % level of significance respectively*

$F_{tab}(5, 15) = 9.7223$; $F_{tab}(14,15) = 3.6557$ (1%)
$F_{tab}(5, 15) = 4.6187$; $F_{tab}(14,15) = 2.463$(5%)
$F_{tab}(5, 15) = 3.2380$; $F_{tab}(14,15) = 2.0095$ (10%)

The objective of the study was to get the optimized conditions for maximum quality of oil can be obtained using the optimized parameters among the experiments performed. The optimized condition could be a single point or a range of points in which all the possible combinations would yield good results. While using any optimization technique some constraints have to be decided, keeping in view the optimized conditions are obtained. These constraints set the guidelines to get the desired results. One of the techniques used to visualize the response surface is to plot the 3D graphs of the response surface equation (Eqn. 6). In a 3D plot, lines or curves of constant response values create a plane or graph whose coordinate axes represent the levels of independent variables and the response is visualized perpendicular to the plane of paper. Series of contour lines of equal response value were generated which provided useful information for understanding the effect of two independent parameters on the dependent variable. Optimizatiom is a process of making compromises between responses, to achieve a common target. Numerical optimization was carried out using Design-Expert 9.0.3 statistical software. The goal seeking begins at a random starting point and proceeds up and down the steepest slope on the response surface for a maximum or minimum value of the response respectively. All the responses and independent variables were given similar (+++) importance. The goal setup for optimization of oil extraction from algae biomass is given in the Table 9.

Table 9 Constraints for optimization for aqueous enzymatic extraction of algae biomass

Name	Goal	Limit	Limit
enzyme concentration(X_1)	minimize	-2	2
incubation time(X_2)	minimize	-2	2
incubation temperature (X_3)	is in range	-2	2
pH (X_4)	is in range	-2	2
Refractive index	minimum	1.239	1.463

Optimum result of aqueous enzymatic oil extraction of algae biomass was obtained when enzyme concentration is 2.5 %, temperature of incubation is 60°C, time is 7 h and pH 4.

A response-surface generated with the Design Expert 9.0.3 program is constructed for refractive index of oil. By using the experimental effect of any two independent variable response curve is constructed for each response alone. The 3D graphs are shown in Fig. 1 to 6 for various combinations of interactive terms at optimum value i.e. at various combinations of enzyme concentration, incubation time, incubation temperature and pH of algae biomass. Surface plots were drawn between X_1, X_2, X_3 and X_4.

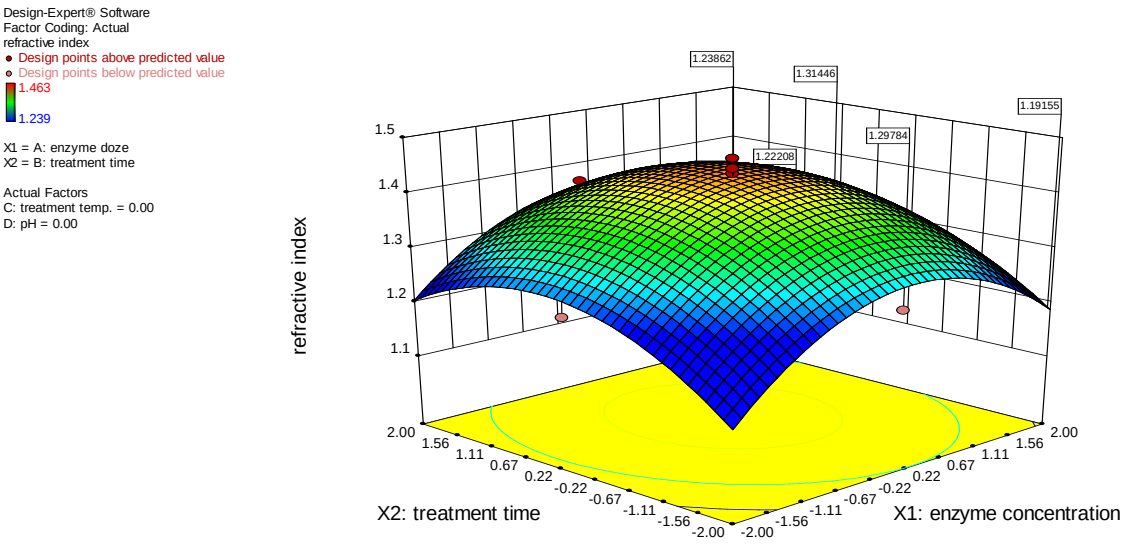

Fig. 1 surface plot of enzyme concentration(X_1) and incubation time (X_2) on refractive index

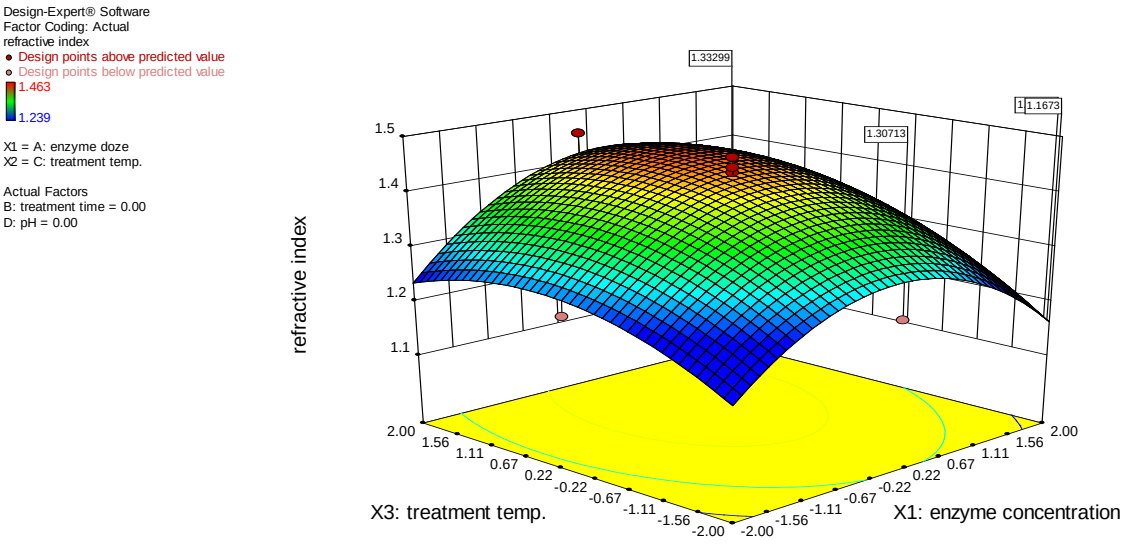

Fig.2 surface plot of enzyme concentration(X_1) and incubation temperature (X_3) on refractive index

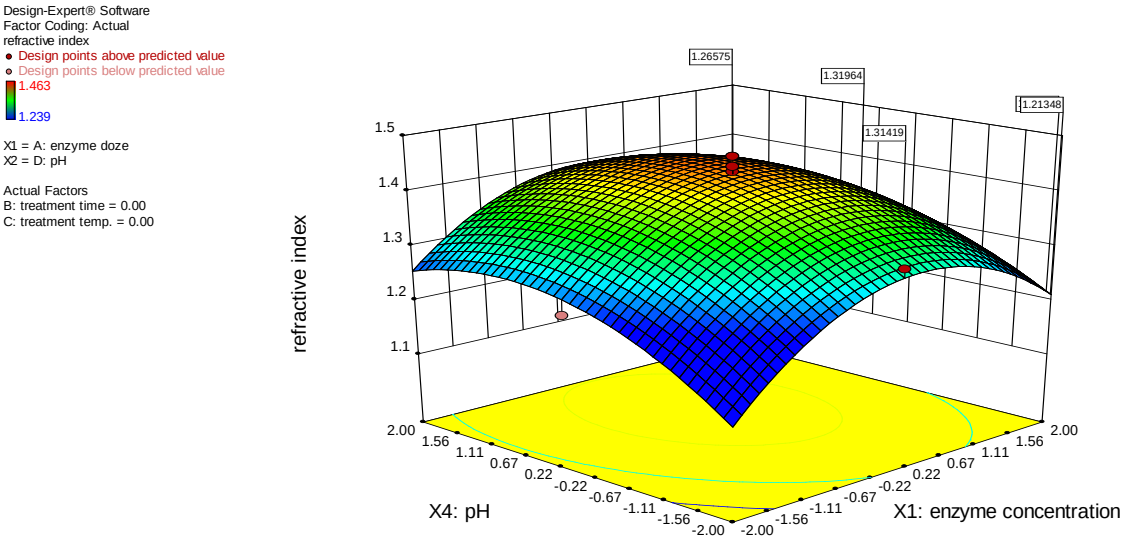

Fig. 3 surface plot of enzyme concentration(X_1) and pH (X_4) on refractive index

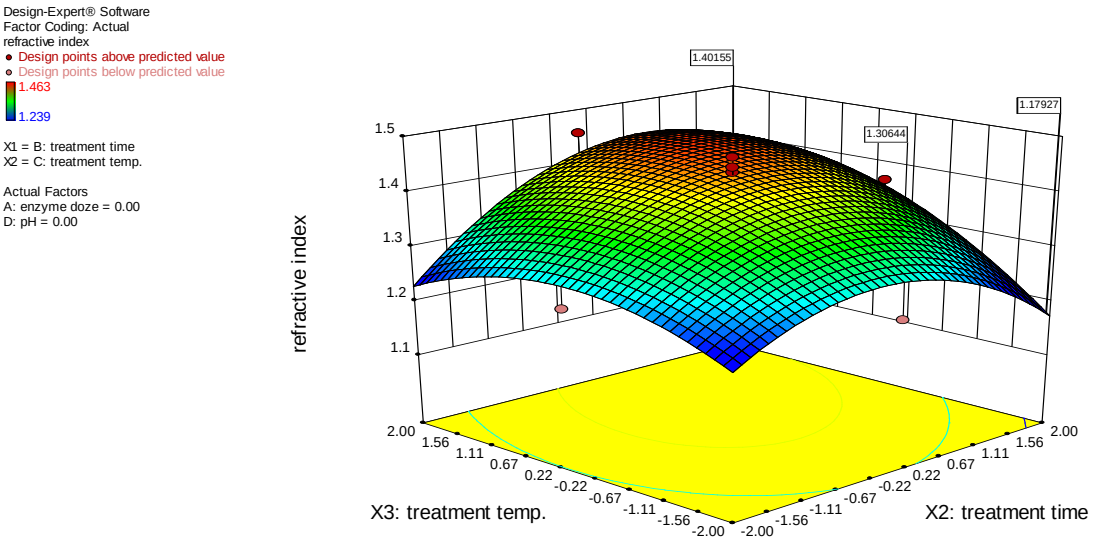

Fig. 4 surface plot incubation time (X_2) and incubation temperature (X_3) on refractive index

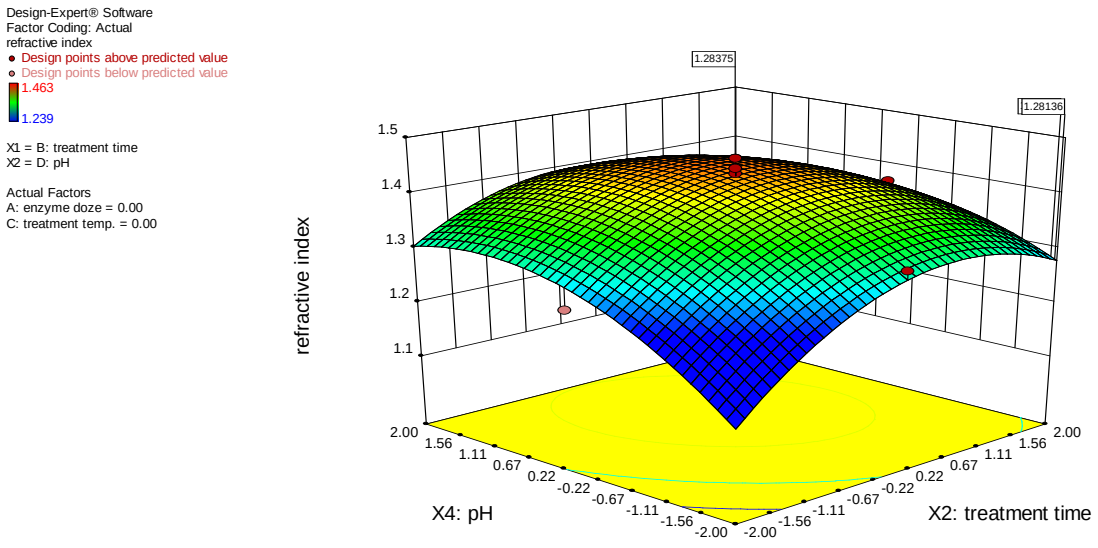

Fig. 5 surface plot of incubation time (X_2) and pH (X_4) on refractive index

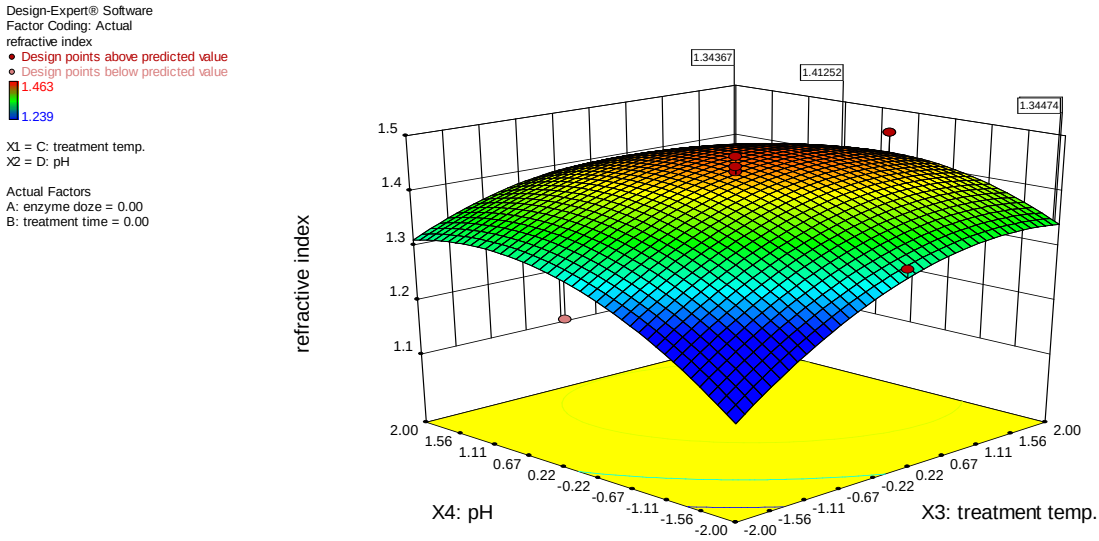

Fig. 6 surface plot of incubation temperature (X_3) and pH (X_4) on refractive index

CONCLUSION

The refractive indices of thirty oil samples have been determined. The quality of these oils has been deduced by using refractive index as a tool. This reveals that the simple laboratory measurement of refractive index can also be used as a quality control technique.

REFERENCES

[1]Ariponnammal S. *Research Journal of Recent Sciences*. Vol. 1(7), 77-79, July (**2012**)
[2]Subramaniyam N. and Brijlal, A Text book of optics, 18th ed. S. Chand and Company Pvt Ltd, **(1987)**
[3]Eugene Hecht., Optics, Addison-Wesley **(2002)**
[4]Lawrence E. Kinsler, Austin R. Frey, Alan B. Coppens and James V. Sanders, Fundamentals of Acoustics, John Wiley and Sons, Inc., 136 **(2000)**
[5]Jee M. and Jee M., ed., Oils and Fats Authentication, Blackwell Publishing (CRC Press), Boca Raton, Florida,1–24 **(2002)**
[6]Gordon M.H. and Jee M., ed., Oils and Fats Authentication, Blackwell Publishing (CRC Press), BocaRaton, Florida, 143–155**(2002)**

[7]Edmiston M. D., *J. Chem. Educ.*, **78,** 1479–80 **(2001)**
[8]Neder A., Garcia E., and Viana L.N., *J. Chem. Educ.*, **78,** 1481–3 **(2001)**
[9]Farkas N., Henriksen P. N. and Ramsier R.D., *Phys. Educ*, **41,** 69–72 **(2006)**
[10]Singh S., *Phys. Educ*., **39,** 235 **(2004)**
[11]Khanna D. R. and Gulati H. R., Fundamentals of optics, R. Chand and Co Publishers, 12th ed. **(1985)**