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Optimization of the Colour Index of the Neutral Glanded Cottonseed

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Abstract: The main factors controlling the colour index of the neutral glanded cottonseed oil during the neutralization step which is the most critical of the refining process of glanded cottonseed oil were determined as storage conditions of crude glanded cottonseed oil, mass ratio of water to crude oil (X_1); mass ratio of sodium hydroxide to crude oil (X_2), temperature of the neutralization (X_3). After determining the optimal storage conditions, a 2^3 factorial central composite experimental design was used to study the combined effect of these factors and to optimize their levels to reduce the colour index of the neutral glanded cottonseed oil. A polynomial model of the colour index of the neutral glanded cottonseed oil was deducted from statistical analysis of experiments. Paréto analysis indicated that the influences of the main factors (X_1, X_2 and X_3) on the colour index of the neutral oil were 9.17%, while the interaction effects (X_1X_2, X_1X_3, $X_1X_2X_3$) and quadratic effects (X_1*X_1, X_2*X_2, X_3*X_3) were 37.47 and 53.32%, respectively. Under the optimized conditions, the colour index of the neutral glanded cottonseed oil was ranged from 9.58 to 12.53, value that is more than 50% lower than the colour index (23.7) of the previous industrial neutral glanded cottonseed oil. The optimal combination of the neutralization parameters for the minimum colour index of the neutral glanded cottonseed oil from contour plot were: mass ratio of water to crude glanded cottonseed oil 10.75%, mass ratio of sodium hydroxide to crude glanded cottonseed oil 2.8%, temperature of the neutralization 70°C. Isoresponse curves and contour plot were drawn from the mathematical model enable one to demonstrate the robustness of this optimization method.

Key words: Colour index, optimization, neutralization, glanded cottonseed oil

INTRODUCTION

Glanded cottonseed oil widely produced in tropical african countries is one of the major sources of food lipids. Food lipids are an important source of energy, essential fatty acids and vitamins A and E in the diet. Nowadays the cotton variety widely cultivated in many african countries mainly in Cameroon is the *Gossypium hirsutum* L. that contains gossypol (Fig. 1). This gossypol is the main cause of the fixing of the colour of the neutral and refined glanded cottonseed oil (Frampton and Kuck, 1974). Gossypol is also a substance known to be toxic to monogastric animals including man (Yildirim-Aksoy *et al*., 2004; Blackstaffe *et al*., 1997; Yuan and Shi, 2000).

This fixed colour oil is therefore not only of poor commercial quality but also is a public health problem due to the toxicity of gossypol (Yildirim-Aksoy *et al*., 2004; Blackstaffe *et al*., 1997; Yuan and Shi, 2000). Deacidification or neutralization is considered as the most critical step of vegetable oil refining process (Christianne *et al*., 2007). Chemical deacidification using sodium hydroxide causes high losses of neutral oil due to the

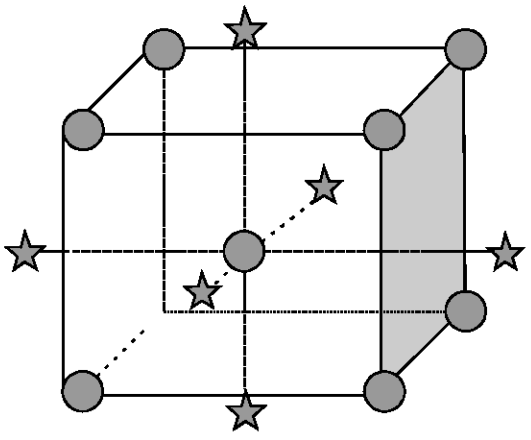

Fig. 1: Central composite design

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saponification and emulsification. In addition, in the presence of sodium hydroxide not only saponification of free fatty acids and colouring substances take place, but part of the phenolic compounds such as gossypol, phenolic acids, flavonoids are likely to be transformed into quinones and polyquinones capable of either being soluble in oil and/or form reactions with it, thus giving rise to a fixed colour oil (Frampton and Kuck, 1974). Several methods alternative to saponification technique are described in the literature, such as supercritical extraction of free fatty acids (Brunetti *et al*., 1989; Bhosle and Subramanian, 2005), membrane technology (Raman *et al*., 1994; Subramanian *et al*., 1998; Zwijnenberg *et al*., 1999; Kale *et al*., 1999), biological deacidification (Cho *et al*., 1990), chemical reesterification (Bhattacharyya and Bhattacharyya, 1987; Anderson, 1962) and many selective solvent extractions (Sreenivasan, Viswanath, 1973; Tomopoulos, 1971; Pina and Meirelles, 2000; Bhattacharyya *et al*., 1987; Shah and Venkatesan , 1989). In physical deacidification such as distillation, much energy is required and the refined oil is subject to undesirable alterations in colour and also to a reduction of stability to oxidation (Antoniassi *et al*., 1998). In order to improve the value of glanded cottonseed oil and meal, planting of a glandless variety was being considered in some areas (Lusas and Jividen, 1987). This glandless variety is not yet widely cultivated in tropical african regions. In spite of the advantages of all these methods cited, limitation of their application in african developing countries is the high initial investment cost of equipments (Gingas, 2000). To improve the value of glanded cottonseed oil, optimization of the colour index of the neutral oil during the neutralization process that is the critical step of the refining process is usually performed by classical method such as systematic alteration of one variable while keeping the others constant. This conventional practice of single factor optimization does not depict the combined effect of all the factors involved (Sunitha *et al*., 1998).

Taking the survey of optimization of the colour index of the neutral glanded cottonseed oil, in the first step we study the storage conditions of crude glanded cottonseed oil before neutralization and secondly a 2^3 factorial central composite experimental design (Mathieu *et al*., 1997; Deming and Morgan, 1993) that was not yet used to study the colour index of the neutral glanded cottonseed oil is employed. This technique generates optimal mathematical function in which all important factors are changed simultaneously, thereby facilitating the identification of process relations as well as the location of the real process optimum.

MATERIALS AND METHODS

A spectronic 20D+ (Milton Roy and Co, New York, USA), a A200s Sartorius analytic balance, accurate to 0,0001g and a water bath (prolabo) accurate to 2°C were used. Glanded cottonseeds, crude glanded cottonseed oils, neutral glanded cottonseed oils were kindly supplied by the Cotton Development Society "Sodecoton" of Garoua and Maroua (Cameroon). All chemicals from Prolabo (France) Phosphoric acid, sodium hydroxide were of analytical reagent-grade and were used as received. Doubly distilled water was used throughout.

Crude glanded cottonseed oil extraction and storage conditions: Maroua industrial crude glanded cottonseed oil was obtained by pre-pressing with the use of continuous screw press or expeller. Garoua industrial crude glanded cottonseed oil was obtained by pre-pressing and solvent extraction of the meal with hexane. The laboratory crude glanded cottonseed oil used as a reference sample was obtained by solvent extraction with hexane. The crude oil obtained were homogenized and distributed in portions of 100 g corresponding to the size of an experimental sample. Six sets of samples were stored at following conditions -18 and -18°C under nitrogen, -12 and -12°C under nitrogen, 26 and 26°C under nitrogen. The storage time of a sample ranged from 2-30 days before the neutralization.

Degumming the crude oil with phosphoric acid: Crude cottonseed oil contains significant quantity of phospholipids. Degumming exploits the affinity of phosphatides for water by converting them to hydrated gums, which is insoluble in oil. The use of low concentration of phosphoric acid is due to the fact that in addition of the formation of hydrate gums, phosphoric acid removes the traces of non-hydratable phosphatide. Crude oil (100 g) was introduced into a 500 mL 3 necks angled round-bottom flask, heated to 80°C and agitated. 3% v/m of phosphoric acid (d = 1.85) was added and agitated for 15 min. Degummed oil was separated from gums by decantation and washed twice with deionised water as follows: Degummed oil was introduced into the flask, heated to a temperature of 80°C and agitated. Five percent (v/m) of deionised water was added under agitation, which is pursued for 10 min before stopping. After decantation, the water phase was drained off.

Neutralization of degummed cottonseed oil: Degummed oil (100 g) was introduced into a 500 mL three necks angled round-bottom flask and heated to a temperature of

50-60°C under agitation. The stoechiometric amount of 20°Be sodium hydroxide with an increase of by 7-10 percent according to the amount of free acid content of the crude oil was added and temperature raised to 90-100°C. The soapstock was well flocculated after 15-20 min and agitation stopped. After settling, the soapstock was discharged and the neutral oil washed.

Washing the neutral oil: Neutral oil was introduced into a 500 mL three necks angled round-bottom flask and heated to a temperature of 70°C under agitation. Deionised water containing 2% sodium chloride at the same temperature was added. Agitation was carried on for about 5 minutes before stopping. The aqueous phase was drained off after decantation. Washing operation was repeated twice.

Neutral oil colour index determination: Spectrophotometric method Cc 13c-50 (AOCS, 1978) was adopted and Eq. (1) used to determine the colour index of the neutral oil.

$$\text{Colour index} = 1.29*A460 + 69.7*A550 + 41.7*A620 - 56.4*A670 \quad (1)$$

Experimental design: A 2^3 central composite experimental design (Mathieu *et al*., 1997; Deming and Morgan, 1993) was used to investigate the influence of three most predominant variables on the colour index of the neutral glanded cottonseed oil: the mass ratio of total water to crude glanded cottonseed oil (X_1), the mass ratio of sodium hydroxide to crude glanded cottonseed oil (X_2), the neutralization temperature (X_3).

With the aim of comparing the effects of the different factors on the response, these factors U_i were coded in reduced variables X_i. The lower and the upper limits of the variable (Table 1) gave the variation domain of each of these variables with the variation step that enables the regular increase or decrease from one variable level to another using the following relationship.

$$U_i = U_0 + \Delta U*X_i \quad (2)$$

U_i = Real variable value.
X_i = Coded variable value.
U_o = Real value of variable at the centre of the domain.
ΔU = Variation step.

Generally, in case of the central composite experimental design with n variables, $(2^n + 2n + C_0)$ experiments are necessary. Total 2^n experiments are in the corners of the cube representing the experimental domain, 2^n points are the star points and C_0 points are the replicates at the centre of the cube (Fig. 2). C_0 points are necessary to estimate the variability of the experimental measurements. Each variable will assume 5 levels in coded variable ($-\alpha$, -1, 0, 1, α). In this study, with 3 factors and α = 1.682 with 4 replicates at the centre point, 18 experiments are performed in random order to minimise the effect of uncontrolled factors (Table 2).

Table 1: Coded and experimental values of neutralization parameters in the studied domain

Xi (coded variable)	-α	-1	0	+1	+α	ΔU
U1 (% water)	7	8,5	11	13,5	15	2,5
U2 (% NaOH)	2	2.3	2.7	3.1	3.4	0.4
U3 (temperature °C)	50	60	70	80	90	10

Table 2: Experimental and calculated colour index of the neutral glanded cottonseed oil

Coded values			Real values			Laboratory sample		
X_1	X_2	X_3	U_1	U_2	U_3	EXP	PRE	RES
-1	-1	-1	8.5	4.6	60	11	11	0
1	-1	-1	13.5	4.6	60	12	11.9	0.1
-1	1	-1	8.5	6.2	60	10	9.8	0.2
1	1	-1	13.5	6.2	60	11.8	11.8	0
-1	-1	1	8.5	4.6	80	12	12	0
1	-1	1	13.5	4.6	80	9.8	10	-0.2
-1	1	1	8.5	6.2	80	10.5	10.6	-0.1
1	1	1	13.5	6.2	80	10	9.9	0.1
-α	0	0	7	5.4	70	11.2	10.7	0.5
α	0	0	15	5.4	70	11.6	11.9	-0.3
0	-α	0	11	4	70	12	12	0
0	α	0	11	6.8	70	11.2	11	0.2
0	0	-α	11	5.4	50	10	10.4	-0.4
0	0	α	11	5.4	90	9.5	9.9	-0.4
0	0	0	11	5.4	70	9.6	9.4	0.2
0	0	0	11	5.4	70	9.7	9.4	+0.3
0	0	0	11	5.4	70	9.7	9.4	+0.3
0	0	0	11	5.4	70	9.6	9.4	+0.2

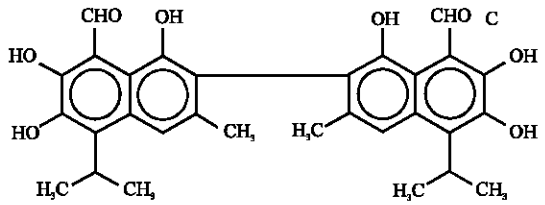

Fig. 2: Structure of gossypol

The postulated equation of the model for the response Yi including the interaction and quadratic effects of each controlled factors is:

$$Y = b_0 + \Sigma b_i X_i + \Sigma b_{ii} X_i^2 + \Sigma b_{ij} X_i X_j + \Sigma b_{ijk} X_i X_j X_k \quad (3)$$

Y = Neutral oil colour index.
b_0 = Estimation of average responses.
b_i = Estimation of main effects of the factor I.
b_{ii} = Estimation of quadratic effects of the factor I.

b_{ij} = Estimation of interactions between the factors I. j,b_{ijk} = Estimation of interactions between the i, j and k factors.

The coefficients b_i, b_{ij}, b_{ii}, b_{ijk}, which are proportional to the influence of the corresponding factor are determined by means of regression analysis using Eq. (4).

$$b_i = (X' X)^{-1}.X' .Y \quad (4)$$

X = Matrix of model.
X' = Transposed matrix of X.
(X' X) = Matrix of information.
$(X' X)^{-1}$ = Matrix of scattering.
Y = Vector of experiment responses.

Estimation of the weight of different response parameters: To estimate the weight of the different parameters on the response, Paréto analysis method (Haaland, 1989) that expresses the effect of X_i parameter by relation Eq. (5) was used.

$$Fi = 100(bi^2 / \Sigma b_j^2) \quad (5)$$

Fi is the percentage of the effect of parameter i on the response, bi^2 is the quadratic estimation of the Xi coefficient, Σb_j^2 is the sum of quadratic estimations of all coefficients.

Software: Response surfaces were drawn and coefficients for the regression model were calculated using Modde 4 software package (Modde 4.0).

RESULTS AND DISCUSSION

Effect of storage conditions: Before examining the storage conditions of the crude glanded cottonseed oil on the colour index of the neutral oil, we must have in mind that extraction conditions are the first step of fixing of the colour of oil. In many steps of the cottonseed processing the temperature ranged from 100-110°C (cooking process of meat), 130-150°C (pre-pressing or full pressing of meat). In fact Vix *et al.* (1946) reported that colour fixation of the oil became objectionable between 65.5-82.2°C and beyond 82.2°C it increased rapidly. Lionet *et al.* (1955) also reported that when heating the meat from prime seed at 75.5-95°C, the colour of refined oil ranged from 5.51-12.89 AOCS colour. According to Liu (1951) allowing the cottonseed oil miscella to stand 6 or ten weeks before desolventizing or neutralization the crude oil caused the oil to be darkened considerably. Meanwhile, the optimal storage time and temperature were not mentioned in these studies. Considering that the extraction process is an industrial constraint, the study of the storage time and temperature of the crude glanded cottonseed oil in inert atmosphere or in the presence of oxygen was investigated. Figure 3 presents the results and as can be seen, the colour index of the neutral glanded cottonseed oil increases with the storage time and temperature.

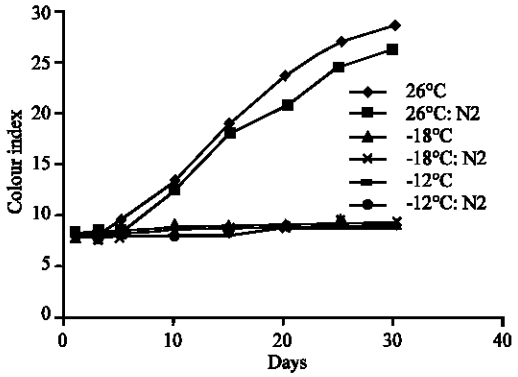

Fig. 3: Crude glanded cottonseed oil storage conditions

Storage time up to 10 days at 26°C seems to be a limit conservation time to have acceptable colour index of the neutral glanded cottonseed oil. With this maximum storage time, the value of the colour index of the resulting neutral glanded cottonseed oil ranged from 9-13 giving the colour index slightly higher than the color index of the soy bean oil 8.8. The inert storage atmosphere of the crude oil plays minor influence on the colour of the neutral glanded cottonseed oil. When the crude glanded cottonseed oil was stored at -12°C the neutralization could be performed after more than one month without significant influence of the storage time on the colour index of the neutral oil. Possibility of such conservation at a low cost is available by solar energy technology in tropical african countries.

Analysis of the process factors: Three factors were selected as potentially affecting the colour index of neutral glanded cottonseed oil, namely: the mass ratio of water to crude glanded cottonseed oil (X_1), the mass ratio of sodium hydroxide to crude glanded cottonseed oil (X_2), the neutralization temperature (X_3). Using the 2^3 factorial central composite experimental design, the model of the neutralization process was obtained. It was postulated a second-order response model. The coefficients of the model presented in Table 3 were estimated by least square multiple regression method. The sign of the effect indicates its direction. The positive or negative sign indicates the positive or negative effect of the factor on the response. The importance of these effects was graphically represented on Fig. 4. The higher the effect of a factor (absolute value), the more significant it was on the neutralization process of gland cottonseed oil. The

Table 3: Regression coefficients for main factors and their interactions

	Factor/interaction		Regression coefficient
	Mean/interaction b0		9.621
X1	Water/oil ratio (L1) b1		0.057
	Water/oil ratio (Q) b11		0.607
X2	NaOH/Oil ratio (L2) b2		-0.282
	NaOH/Oil ratio (Q2) b22		0.678
X3	Temperature (L3) b3		-0.245
	Temperature (Q) b33		0.024
	X1X2	b12	0.313
	X1X3	b13	-0.687
	X2X3	b23	-0.013
	X1X2X3	b123	0.112

L= linear, Q= quadratic

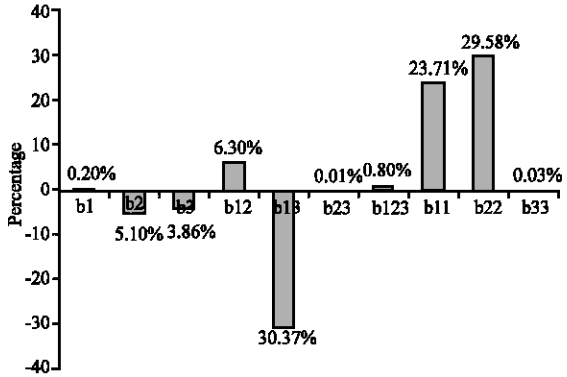

Fig. 4: Pareto analysis of the process factors of the neutral oil colour index

colour index of the resulting neutral oil will depend therefore, on the relative sum of the weight of each factor on the response.

It was observed according to Paréto diagram (Fig. 4) that the mass ratio of water to crude glanded cottonseed oil (X^1) had no effect on the colour index of the neutral oil, while this factor associated to the temperature of the neutralization (X^3), showed a strong influence (30.37%). The quadratic effects of the mass ratio of water to crude glanded cottonseed oil (X_1^2, 23.71%) as soon as the mass ratio of sodium hydroxide to crude glanded cottonseed oil (X_2^2, 29.58%) greatly influence the colour index of the neutral glanded cottonseed oil. Thus attention must be paid not only on main effects but also on interaction and quadratic factors.

The equation of the model for the response (laboratory crude oil extraction), including the interaction and quadratic terms of each controlled factor were:

$$F(X_1, X_2, X_3) = 9.621 + 0.057X_1 - 0.282X_2 - 0.245X_3 + 0.313X_1X_2 - 0.687X_1X_3 - .013X_2X_3 + 0.112X_1X_2X_3 + 0.607X_1^2 + 0.678X_2^2 + 0.024X_3^2 \quad (6)$$

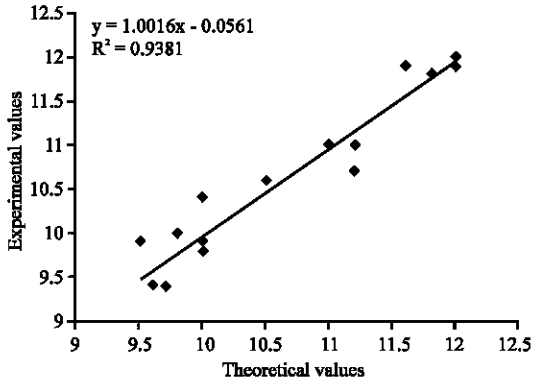

Fig. 5: Validation of the model equation

Verification of the model equation: It was a postulated second-order polynomial model, which must be validated. If the model represents the phenomenon in the studied domain, the difference between the experimental results and the theoretical results calculated by the model (Table 2) was due solely to experimental error.

The coefficient of determination obtained ($r^2 = 0.9381$) when plotting the experimental colour index versus the theoretical colour index (Fig. 5) indicated that the variation of 93.8% for colour index of neutral glanded cottonseed oil was attributed to the independent variables, mass ratio of water to crude oil (X_1); mass ratio of sodium hydroxide to crude oil (X_2), temperature of the neutralization (X_3). We admitted in using this method of validation that the experimental results do not contain errors since they were the average of results of the three replications and that even if there were an error, it was constant and did not depend on the variation of parameters involved. Admitting this, it can be ascertained that in the overall, the model used represents the studied phenomenon in the variation domain.

Optimal neutralization conditions: From Eq. (6) the colour index of the neutral glanded cottonseed oil at any point in the interval of our experimental domain can be predicted. The 3D response surface and the 2D contour plot are the graphical representations of the regression equation. The response surface curves allow visualizing the simultaneous evolution of the colour index of the neutral glanded cottonseed oil with the change of the process factors. The 3D response surface (Fig. 6) of colour index of the neutral oil as a function of water/crude oil ratio and alkali/crude oil ratio at a fixed neutralization temperature level of 70°C indicates that the colour index of the neutral oil decreases (12.3-9) as the water/oil ratio and alkali/crude oil ratio decrease down to 11 and 2.7%, respectively. The main goal of response surface is to

Table 4: Optimal conditions and results of the oil crude oil neutralization

Optimal neutralization conditions		
Factor	Coded value	Real value
X1(%water)	-0.1	10.75
X2(%NaOH)	0.25	2.80
X3(°C)	0.00	70.00
Neutral glanded cottonseed oil colour index		
	Experimental	Predicted
Lab. Sample	9.86	9.585
Garoua sample	12.53	12.74
Maroua sample	11.76	12.02

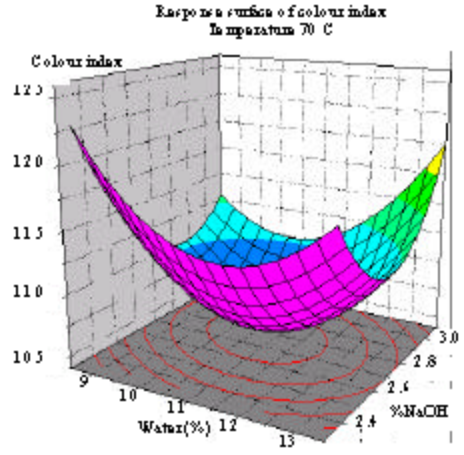

Fig. 6: Response surface of colour index of neutral glanded cottonseed oil at 70°C

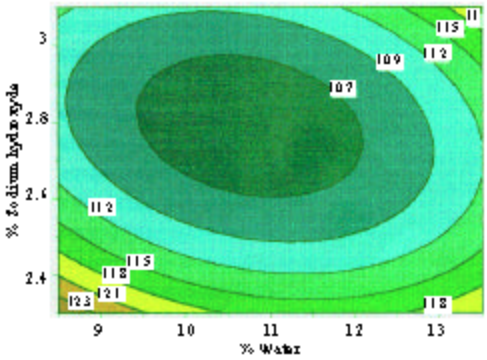

Fig. 7: Contour plot of the colour index of neutral glanded cottonseed oil at 70°C

efficiently hunt for the optimum values of the variables such that the response is optimized. The use of contour plot (Fig. 7) is both to best indicate technically and economically the values of process factors for obtaining a minimum colour index of the neutral glanded cottonseed oil.

The optimal neutralization conditions determined graphically according to the contour plot (Fig.7) with a neutralization temperature of 70°C were presented in Table 4 with experimental and predicted values of the colour index of the neutral glanded cottonseed oil of different sources. At a more elevated temperature 82°C, the colour fixation of the oil increases rapidly (Vix *et al*., 1946) and hydrolysis of cottonseed oil was accelerated giving poor neutralization yield. As can be seen in Table 4, the predicted results and experimental results obtained demonstrate that the mathematical model represents very well the studied phenomenon in the experimental domain indicating the robustness of the method used.

CONCLUSION

Storage conditions of crude glanded cottonseed oil before neutralization had been studied and optimization of the neutralization process has been done. Central composite experimental design was used to study the influence of neutralization parameters on the colour index of the neutral glanded cottonseed oil. Polynomial regression equation has been derived, showing the greatest influence of interaction and quadratic factors on the colour index of the neutral oil. The validity of the model has been proved by plotting the experimental colour index versus the theoretical colour index. The coefficient of determination of 0.938% indicated that the variation of 93.8% for colour index of neutral glanded cottonseed oil was attributed to the independent variables, mass ratio of water to crude oil (X_1); mass ratio of sodium hydroxide to crude oil (X_2), temperature of the neutralization (X_3). Using the contour plot, the optimal neutralization conditions were determined graphically and experimental results obtained using these optimal conditions agreed squarely with the predicted results showing the robustness of the method and indicating that the model developed was a predictable mathematical model for the neutralization of glanded cottonseed oil.

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