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Conjugated Linoleic Acid Production by Alkali Isomerization of Linoleic Acid from *Idesia polycarpa* Maxim. *var. vestita* Diels Oil

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Increasing attention to conjugated linoleic acid for its significant biological effects attracted growing demand for conjugated linoleic acid related products. The isomerization of linoleic acid was regarded as a more promising option for large-scale production of conjugated linoleic acid. However, the availability of linoleic acid is a prerequisite for the technology. High purity linoleic acid from *Idesia polycarpa* Maxim. *var. vestita* Diels oil was used to synthesize conjugated linoleic acid in this work and response surface methodology was successfully employed to optimize the process. The optimal conditions for production of conjugated linoleic acid by alkaline isomerization were: a ratio of 1.0:0.4:5.2 (w/w/w) of linoleic acid/KOH/ethylene glycol, 188.3 ºC and 4.4 h. After the optimization process, the experimental purity of conjugated linoleic acid (89.72 %) corresponded to the predicted value. The high quality conjugated linoleic acid with a total yield of 30.84 % (w/w) from *Idesia polycarpa* Maxim. *var. vestita* Diels oil possesses a bright prospect as potential linoleic acid source for the production of conjugated linoleic acid.

Key Words: *Idesia polycarpa* Maxim. *var. vestita* Diels, Conjugated linoleic acid, Response surface methodology, Isomerization.

INTRODUCTION

Conjugated linoleic acid refers to a group of positional and geometrical isomers of linoleic acid containing conjugated double bonds[1]. Conjugated linoleic acid has attracted growing interest due to its wide-range of biological effects such as immune modulation[2], anticarcinogenic activity[3], fat partitioning and metabolism[4] and lowering atherosclerosis[5]. However, endogenous production of conjugated linoleic acid isomers by humans from vaccenic acid is limited[6]. Increasing interest in its effects has resulted in development and wide availability of commercial conjugated linoleic acid nutritional supplements. Up to now, various methods have been developed to produce synthetic conjugated linoleic acid from linoleic acid, such as bacterial biosynthesis[7], lipases[8] and photoisomerization[9]. However, biosynthesis from bacteria or lipase is expensive, requiring sensitive conditions and complex follow-up processes to recover the product[10]. Likewise, photoisomerization of linoleic acid is time-consuming and requires complicated equipment[11]. A more promising option for conjugated linoleic acid production is alkali isomerization, whereby linoleic acid is isomerized to conjugated linoleic acid under alkaline conditions[12] to produce mixtures consisting mostly of approximately equal amounts of c-9, t-11 conjugated linoleic acid and t-10, c-12 conjugated linoleic acid isomers[13]. Employing this technique, the availability of free linoleic acid is a prerequisite for the isomerization reaction[14]. Linoleic acid-rich oils are always regarded as promising candidates of linoleic acid for the production of conjugated linoleic acid. In previous studies, safflower, corn, soybean and cottonseed oils have been used as linoleic acid sources[15,16]. With increasing demand for conjugated linoleic acid related products, increasing research efforts have been directed toward development of new linoleic acid raw materials for conjugated linoleic acid production.

Idesia polycarpa Maxim. *var. vestita* Diels (IPMVVD) is native to and widely distributed throughout China (Fig. 1). As seen in the distribution map, wild IPMVVD is present in 12 of 34 provinces of China. Furthermore, successful cultivation of the plant is readily achievable due to its strong resistance to cold, drought and pests and its low selectivity for soil type. The oil content, oil yields and fatty acid profiles of IPMVVD and other feedstocks are tabulated in Table-1. It is notable that, the concentration of linoleic acid in IPMVVD oil is similar to safflower oil and higher than the other oils. Furthermore, because the oil yield of IPMVVD is higher than other feedstocks, it is considered to be a promising new raw material source for the production of conjugated linoleic acid.

In this study, preparation for conjugated linoleic acid by alkaline isomerization of linoleic acid from IPMVVD oil was

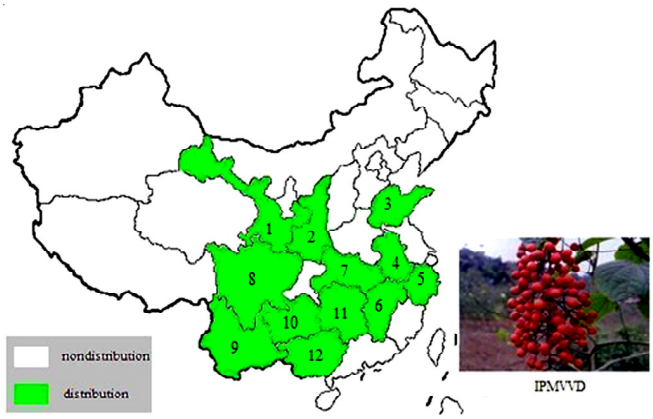

Fig. 1. Distribution of IPMVVD in China

described. The whole treatment process is described in Fig. 2. Response surface methodology coupled with central composite rotatable design (CCRD) was applied to optimize the procedure for the production of conjugated linoleic acid.

EXPERIMENTAL

Idesia polycarpa Maxim. *var. vestita* Diels oil was obtained from a local oil company (Sichuan Guo Zheng Biomass Energy Technology Development Co., Ltd.). A conjugated linoleic acid standard was purchased from Sigma Chemical Co., Ltd. The chemicals and solvents used were analytical reagent grade. A UV-TU1810 (Beijing Puxi General Instrument Co., Ltd., Beijing, China) was used to detect conjugated linoleic acid. A GC-MS-QP2010E (Shimadzu Corp. Kyoto, Japan) was employed to determine the fatty acid composition of IPMVVD oil.

Physical and chemical properties of IPMVVD oil: As a new raw material source for conjugated linoleic acid, the physical and chemical properties of IPMVVD oil were measured. The fatty acid composition of IPMVVD oil was determined by GC-MS[22] and listed in Table-1. Properties of the feedstocks were analyzed in our laboratory following American Oil Chemists' Society (AOCS) standard methods[23]. Acid value was determined by (AOCS Cd-3a-63); iodine value was obtained by (AOCS Cd-1-25); saponification value was measured by (AOCS T1-1a-64); Density was estimated by the AOCS Cc-10a-25 method using a pycnometer and moisture content was determined by Karl Fisher titration. Results were as follows: acid value of 15.56 mg/g; iodine value of 120.46 g/100g; saponification number of 204.3 mg/g; density (20 ºC) of 0.91 g/mL and moisture content of 0.21 %.

High purity linoleic acid from IPMVVD oil: High purity linoleic acid was obtained by the process described in our previous paper[24]. Briefly as follows, IPMVVD oil was

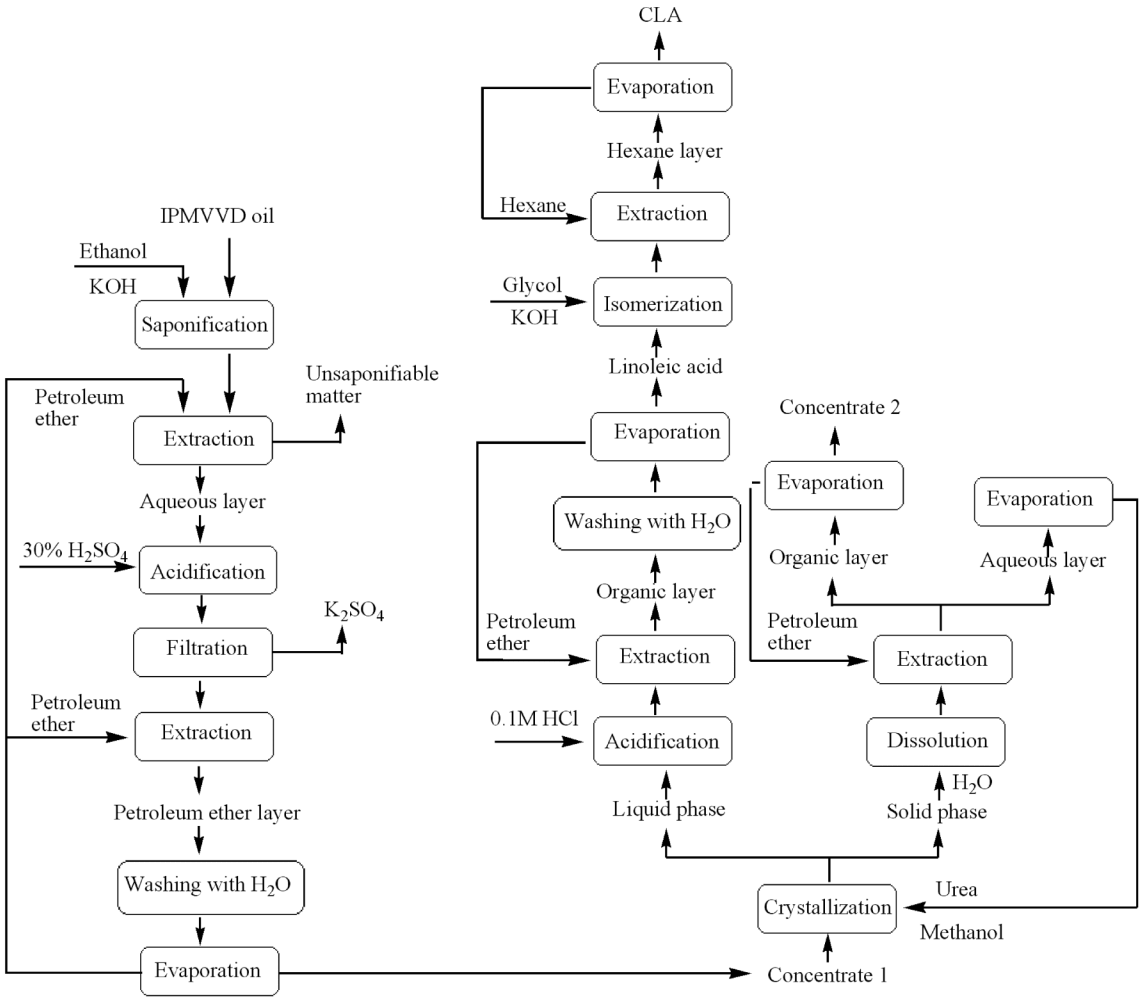

Fig. 2. Flowchart for the production of conjugated linoleic acid from IPMVVD oil

TABLE-1
FATTY ACID COMPOSITION (%) AND YIELDS OF THE FEEDSTOCK OILS FOR CONJUGATED LINOLEIC ACID

Oil	Fatty acid						Oil content (%)	Oil yield (L/ha)
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3		
IPMVVD*	14.6*	5.6*	1.6*	7.2*	68.6*	0.9*	36-38	4895
Soyabean	14.0[a]	–	2.4[a]	23.5[a]	51.2[a]	8.5[a]	15-20[d]	446[d]
Corn	12.31[b]	0.12[b]	1.91[b]	25.39[b]	58.65[b]	0.93[b]	48[d]	172[d]
Cottonseed	22.0[b]	1.0[b]	3.0[b]	19.0[b]	54.0[b]	1.0[b]	18-25[d]	325[d]
Safflower	6-7.5[c]	–	1-3[c]	13-15[c]	61-79[c]	–	≥ 28[e]	685.9[e]

Very low or non-existent; *: Measured by us with the methods described in the following section. a: Ref 17; b: Ref 18; c: Ref 19; d: Ref 20; e: calculated from Ref 21.

saponified and acidized, then mixed fatty acids (concentrate 1) with a yield of 80.73 % were removed by filtration and extraction. Subsequently, linoleic acid was extracted and separated from mixed fatty acids by urea adduction fractionation under the following condition: a ratio of 4.4:1.0:1.0 (w/w/w) of methanol/urea/mixed fatty acids, temperature of -5.9 ºC and time of 24.6 h. The yield and purity of the obtained linoleic acid were 48.65 and 98.74 %, respectively.

Isomerization of linoleic acid: Ethylene glycol and KOH were added to a round-bottom flask equipped with a branched-hollow stopper and heated to the designated temperature with high-speed stirring in a nitrogen environment. As soon as the KOH was dissolved and evenly mixed, purified linoleic acid was introduced. The reaction conditions are shown in Table-2. After the reaction and acidification with HCl (6 M), methanol was added to the mixture. Conjugated linoleic acid was then extracted with hexane and washed with 30 % aqueous methanol. The hexane was removed and the conjugated linoleic acid detected using the UV spectrophotometer.

Experimental designs for response surface methodology analysis: At present, as a useful statistical technique, response surface methodology has been universally employed to optimize experimental conditions in the fields of synthesis[25] and natural product extraction[26], *etc*. In this work, central composite rotatable design coupled with response surface methodology was applied to optimize the isomerization of linoleic acid. The independent variables (X_i) were coded at five levels (-2, -1, 0, 1 and 2) and response values (Y) of the procedure are shown in Table-2. To avoid bias, 30 runs, including 6 replications of the center points, were performed in random order.

TABLE-2
CENTRAL COMPOSITE ROTATABLE DESIGN AND RESPONSE VALUES FOR ISOMERIZATION OF LINOLEIC ACID (LA) TO CONJUGATED LINOLEIC ACID (CLA)

Experiments	Factors				Purity of CLA (%)	
	KOH/LA (w/w)	Ethylene glycol/LA (w/w)	Time (h)	Temperature (ºC)	Y	
	X_1	X_2	X_3	X_4	Actual value	Predicted value
1	0.3 (-1)	4.5(-1)	4.5(1)	190(1)	80.03	80.19
2	0.4(0)	5(0)	4(0)	180(0)	86.41	85.81
3	0.4(0)	4(-2)	4(0)	180(0)	75.89	74.01
4	0.4(0)	5(0)	4(0)	160(-2)	52.03	53.53
5	0.4(0)	5(0)	4(0)	180(0)	85.05	85.81
6	0.3(-1)	5.5(1)	4.5(1)	170(-1)	73.25	71.14
7	0.4(0)	5(0)	4(0)	180(0)	86.53	85.81
8	0.4(0)	5(0)	4(0)	200(2)	85.95	85.43
9	0.5(1)	4.5(-1)	3.5(-1)	190(1)	79.64	81.07
10	0.3(-1)	5.5(1)	3.5(-1)	190(1)	80.79	80.26
11	0.3(-1)	5.5(1)	4.5(1)	190(1)	84.43	83.95
12	0.4(0)	5(0)	4(0)	180(0)	85.18	85.81
13	0.3(-1)	4.5(-1)	3.5(-1)	190(1)	77.68	78.41
14	0.5(1)	5.5(1)	4.5(1)	190(1)	87.93	87.30
15	0.5(1)	4.5(-1)	4.5(1)	190(1)	84.14	84.22
16	0.4(0)	5(0)	4(0)	180(0)	85.13	85.81
17	0.5(1)	5.5(1)	3.5(-1)	170(-1)	68.03	67.20
18	0.3(-1)	5.5(1)	3.5(-1)	170(-1)	64.23	63.84
19	0.5(1)	4.5(-1)	4.5(1)	170(-1)	68.89	68.75
20	0.6(2)	5(0)	4(0)	180(0)	72.35	72.68
21	0.3(-1)	4.5(-1)	3.5(-1)	170(-1)	57.98	57.94
22	0.4(0)	5(0)	4(0)	180(0)	86.58	85.81
23	0.4(0)	5(0)	5(2)	180(0)	83.79	85.19
24	0.4(0)	5(0)	3(-2)	180(0)	75.16	74.74
25	0.5(1)	5.5(1)	3.5(-1)	190(1)	82.92	82.24
26	0.5(1)	4.5(-1)	3.5(-1)	170(-1)	61.80	61.98
27	0.3(-1)	4.5(-1)	4.5(1)	170(-1)	62.95	63.33
28	0.4(0)	6(2)	4(0)	180(0)	80.13	82.99
29	0.2(-2)	5(0)	4(0)	180(0)	64.63	65.28
30	0.5(1)	5.5(1)	4.5(1)	170(-1)	76.91	75.88

The experimental data were analyzed by response surface regression (RSREG) (Design expert 7.0) and fitted to the following second-order polynomial eqn. 1:

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j X_j + \sum_{j=1}^{k}\beta_{jj} X_j^2 + \sum_{i<j}^{k}\beta_{ij} X_i X_j \quad (1)$$

where X_i, X_j were independent coded variables which affected the response of Y; β_0, β_j, β_{jj} and β_{ij} were the regression coefficients for intercept, linearity, quadratic and interaction, respectively. Then analysis of variance (ANOVA) was used to test the models and the optimal conditions for maximized response values were pre-established by ridge analysis.

RESULTS AND DISCUSSION

Model fitting and analysis of variance: Experimental designs for isomerization of linoleic acid were randomized and the details were tabulated in Table-2. The complete second-degree regression models in terms of coded factors were presented in eqn. 2 in the following:

$$\text{Purity of CLA} = 85.81 + 1.85X_1 + 2.24X_2 + 2.61X_3 + 7.97X_4 - 0.17X_1X_2 + 0.34X_1X_3 - 0.34X_1X_4 + 0.48X_2X_3 - 1.01X_2X_4 - 0.90X_3X_4 - 4.21X_1^2 - 1.83X_2^2 - 1.46X_3^2 - 4.08X_4^2 \quad (2)$$

The predicted responses were calculated by using the model and compared with actual values (Table-2); value for the coefficient of determination (R^2) was 0.9892, indicating that the predicted responses matched well with the actual ones. Consequently, the model could be used to navigate the design space. Furthmore, significance of the model and non-significance of the lacks of fit from the analysis of variance (Table-3) validate the model.

TABLE-3
ANALYSIS OF VARIANCE (ANOVA) FOR THE MODEL FOR PURITY OF CONJUGATED LINOLEIC ACID

Source	Sum of square	DF[a]	Mean square	F-Value
Model	2776.46	14	198.32	97.98[b]
Residual	30.36	15	2.02	–
Lack of fit	27.45	10	2.75	4.72[c]
Pure error	2.91	5	0.58	–
Total	2806.81	29	–	–

[a]Degrees of freedom. [b]Significant. [c]Insignificant.

The individual effects of the various variables, their interactions and the quadratic effects could be evaluated by the models. The value and sign of the regression coefficients in the models denote influences of the variables on the objective function[27]. Given that, the importance of the factors and their interactions on the purity of conjugated linoleic acid were discussed relative to the eqn. 2. It can be seen that X_4 (reaction temperature) was the significant factor impacting the purity of conjugated linoleic acid among the four single parameters with positive effects to the response value. Quadratic terms of X_1^2, X_2^2, X_4^2 and X_3^2 contributed significant negative effects on the purity of conjugated linoleic acid. Likewise, significant interactions were also found between X_2X_4 and X_3X_4.

Analysis of response surfaces for the isomerization of linoleic acid: The relationship between the processing parameters and the purity of conjugated linoleic acid could be well understood from the 3D-plots (Fig. 3) derived from the eqn. 2. Fig. 3a shows that it was possible to obtain high purity conjugated linoleic acid with a longer reaction time, but at intermediate levels of the KOH/linoleic acid ratio. An increase in the purity of conjugated linoleic acid was noted with prolonged reaction time, before the isomerization reached equilibrium. A quadratic effect of the KOH/linoleic acid ratio might be found in Fig. 3a. That was because the low levels of KOH resulted in less desirable catalytic efficiency and was not conducive to the isomerization reaction. However, more catalyst would tend to result in added saponification, causing more foam during the acidification process and increasing the difficulty of separation, resulting in a loss of conjugated linoleic acid.

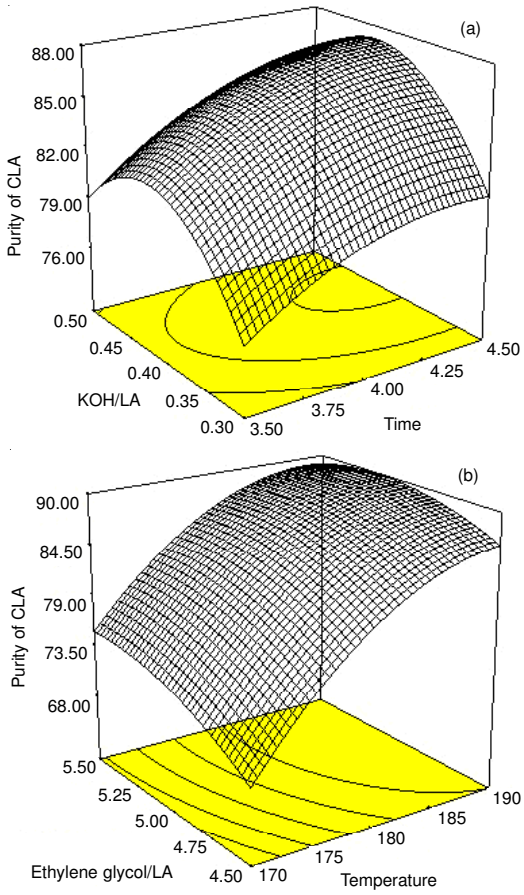

Fig. 3. 3D-plot between two parameters for the purity of conjugated linoleic acid (Conditions: (a) Ethylene glycol/linoleic acid = 5.00, temperature = 180 ºC; (b) KOH/linoleic acid = 0.40, t = 4 h)

The reaction temperature has a significant effect on the purity of conjugated linoleic acid as observed in Fig. 3b. Elevating the reaction temperature could increase the rate of isomerization, which is advantageous to the purity of conjugated linoleic acid. Fig. 3b indicates that the ethylene glycol/linoleic acid ratio also has a positive effect on the purity of conjugated linoleic acid. The sparing solvent would raise the viscosity of reaction solution, leading to a greater resistance in mass transfer and reducing isomerization.

Optimization: The optimum conditions of isomerization of linoleic acid were determined by the ridge analysis maximum. Ridge analysis generated the estimated ridge of maximum response for increasing radii from the center of original design. The conditions predicted by the ridge analysis maximum are given in Table-4. Experiments were performed according to predicted conditions to validate the predicted results; the purity and yield and conjugated linoleic acid are shown in Table-4. The experimental value was found to be reasonably close to the predicted one, which demonstrated the validity and adequacy of the predicted model.

TABLE-4
OPTIMUM CONDITIONS FOUND BY THE MODEL AND VERIFICATION OF THE MODEL

Source	Value
KOH/linoleic acid (w/w)	0.4
Ethylene glycol/linoleic acid (w/w)	5.2
Reaction time (h)	4.4
Reaction temperature (ºC)	188.3
Predicted value of conjugated linoleic acid purity (%)	90.80
Experimental value of conjugated linoleic acid purity (%)	89.72
Conjugated linoleic acid yield (%)	78.53

Conclusion

The preparation of conjugated linoleic acid from a new non-edible vegetable oil (IPMVVD oil) was studied. Response surface methodology was successfully applied to estimate the effect of independent variables on the process and to determine the operating conditions required to optimize conjugated linoleic acid purity. Under the optimized condition, the yield and purity of conjugated linoleic acid were 78.53 and 89.72 %, respectively. Additionally, a total conjugated linoleic acid yield of 30.84 % (w/w) was obtained, which is superior to other reported synthesis methods[11,28]. This newly developed method identified a new raw material source for the production of conjugated linoleic acid and serves to provide meaningful guidance to researchers in this area.

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REFERENCES

1. A.S. Demir and F.N. Talpur, *J. Agric. Food Chem*., **58**, 1646 (2010).
2. J. Bassaganya-Riera, R. Hontecillas-Magarzo, K. Bregendahl, M.J. Wannemuehler and D.R. Zimmerman, *J. Anim. Sci*., **79**, 714 (2001).
3. N.E. Hubbard, D. Lim, L. Summers and K.L. Erickson, *Cancer Lett*., **150**, 93 (2000).
4. T. Yang, X. Xu and L. Li, *J. Food Lipids*, **8**, 149 (2001).
5. T.A. Wilson, R.J. Nicolosi, M. Chrysam and D. Kritchevsky, *Nutr. Res*., **20**, 1795 (2000).
6. I. Salminen, M. Mutanen, M. Jauhiainen and A. Aro, *J. Nutr. Biochem*., **9**, 93 (1998).
7. C.R. Kelper, W.P. Tucker and S.B. Tove, *J. Biol. Chem*., **245**, 3612 (1970).
8. T. Nagao, Y. Shimada, Y. Yamauchi-Sato, T. Yamamoto, M. Kasai, K. Tsutsumi, A. Sugihara and Y. Tominaga, *J. Am. Oil Chem. Soc*., **79**, 303 (2002).
9. R.R. Gangidi and A. Proctor, *Lipids*, **39**, 577 (2004).
10. J. Ogawa, S. Kishino, A. Ando, S. Sugimoto, K. Mihara and S. Shimizu, *J. Biosci. Bioeng*., **100**, 355 (2005).
11. P.J. Vishal and A. Proctor, *J. Agric. Food Chem*., **54**, 5590 (2006).
12. J.P.L. Nichols, S.F. Herb and R.W. Riemenschneider, *J. Am. Oil Chem. Soc*., **73**, 247 (1951).
13. S.J. Kim, G.B. Park, C.B. Kang, S.D. Park, M.Y. Jung, J.O. Kim and Y.L. Ha, *J. Agric. Food Chem*., **48**, 3922 (2000).
14. M. Vahvaselkä and S. Laakso, *J. Agric. Food Chem*., **58**, 2479 (2010).
15. R.W. Riemenschneider, S.F. Herb and P.L. Nichols, *J. Am. Oil Chem. Soc*., **26**, 371 (1949).
16. W. Gammill, A. Proctor and V. Jain, *J. Agric. Food Chem*., **58**, 2952 (2010).
17. S.M.P. Meneghetti, M.R. Meneghetti, T.M. Serra, D.C. Barbosa and C.R. Wolf, *Energy Fuels*, **21**, 3746 (2007).
18. J.D. Radcliffe, D.M. Czajka-narins and V. Imrhan, *Plant Food Hum. Nutr*., **59**, 73 (2004).
19. A. Isigigür, F. Karaosmanoglu and H.A. Aksoy, *J. Am. Oil Chem. Soc*., **72**, 1223 (1995).
20. A. Karmakar, S. Karmakar and S. Mukherjee, *Bioresour. Technol*., **101**, 7201 (2010).
21. R.L. Ravikumar, V.K. Roopa, C.D. Soregaon and D. Satish, 7th International Safflower Conference, Proc. 7th Int. Safflower Conf., Australian Oilseeds Federation, Wagga (2008).
22. F.E. Luddy, R.A. Barford and R.W. Riemenschneider, *J. Am. Oil Chem. Soc*., **37**, 447 (1960).
23. American Oil Chemist's Society, Official and Tentative Methods, Chicago (1980).
24. Q. Wang, K. Du, C. Jia, X. Li, S. Yao and H. Song, *Adv. Mater. Res*., **396**, 1222 (2012).
25. A.L. Ahmad, P.C. Oh and S.R. AbdShukor, *Chem. Eng. J*., **171**, 640 (2011).
26. Z. Hou, Y. Zheng, Y. Gao, X. Liu, F. Yuan and G. Liu, *Food Bioprod. Proces*., **88**, 298 (2010).
27. P.D. Haaland, Experimental Design in Biotechnology, Marcel Decker, New York (1989).
28. S. Kishino, J. Ogawa, Y. Omura, K. Matsumura and S. Shimizu, *J. Am. Oil Chem. Soc*., **79**, 159 (2002).