Biodiesel Production from Limed Fleshing Waste of Leather Industry: Alkaline Versus Bio-Catalysis

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Abstract

Limed fleshing is generated as waste during the processing of skin into leather, which has rich source of fat. Here we report the recovery of fat from limed fleshing waste and subsequent conversion into fatty-acid methyl ester (FAME) or biodiesel. The acid value of the fleshing oil after rendering the fleshing fat was 8.13 mg KOH/g. Hence, esterification followed by transesterification was carried out to produce FAME. For esterification, methanol was used as alcohol and sulfuric acid was used as a catalyst, while transesterification was performed with alkaline catalyst (KOH). Response surface methodology was applied to optimize the transesterification of fleshing oil. A significant reduction in the acid value implies the conversion of fatty oil into FAME. When the optimal factors were employed experimentally, an average acid value of 0.351±0.059 mg KOH/g was achieved, which is comparable to the value predicted by the model. In another attempt towards green chemistry, transesterification was performed using lipase as catalyst (from *Candida rugosa*) using the above optimal conditions yielding an average acid value of 0.287±0.068 mg KOH/g. The use of activators (Na^+ and K^+) enhanced the biocatalytic biodiesel production by reducing the acid value up to 0.224±0.115 and 0.256±0.089 mg KOH/g for KCl and NaCl salts, respectively. The results confirmed the formation of biodiesel with best set of properties for fuel usage thereby demonstrating the successful utilization of limed fleshing waste through both alkaline and enzymatic catalysis.

Key Words: Flesh, Acid value, Esterification, Biodiesel, Catalysis

Introduction

Leather industry plays significant role in Indian economy in the light of its scope for employment, growth and exports. Indian leather industry possesses a major place in the world leather trade. Tamil Nadu, Uttar Pradesh, West Bengal and Punjab produce leather in India (Indian Leather & Tanning Industry Profile 2010). The leather industry, one of the polluting industries, produces large quantity of solid wastes (Ravindran and Sekaran 2010). Pre-tanning stages produce most of the solid waste in the leather production process. Pre-fleshing is performed to remove flesh and to improve the penetration of the chemicals for better leather products. Solid waste disposal technologies have received less attention, perhaps due to a perception that solid wastes are less damaging to the environment. Handling and disposal of such quantities of sludge can represent 40% of the operating cost of wastewater treatment

system. Moreover, the fat content of the leather industry wastes is remarkable. Hence, solid wastes must be utilized effectively as feedstock in biodiesel production due to their rich fat content (Isler et al. 2010; Ozgunay et al. 2007). Thus, the pollution from the leather industry wastes can be reduced and more valuable products can be obtained.

Biodiesel is an alternative to conventional diesel because it improves energy security and reduces air pollution. Over the past two decades, compressed natural gas (CNG), liquefied petroleum gas (LPG) and electricity power have emerged as fuels. These fuels posses a number of drawbacks like engine modification and separate fuel distribution. Biodiesel has the potential to overcome those disadvantages. Biodiesel can be produced from a wide variety of feedstocks. The choice of feedstocks depends largely upon geography (Jeong et al. 2009). Chemically, biodiesel is alkyl esters of fatty acids derived from animal fats (Yang et al. 2007), vegetable oils (Freedman et al. 1984) and waste cooking oil (Dorado et al. 2002). Biodiesel is produced in the presence of alkali catalyst in which acid catalyzed esterification step is required in order to reduce the free fatty acids content of the initial feedstock. The two major factors that affect the biodiesel production are the raw material cost and the processing cost, though the commercialization of glycerol can share the cost of production with biodiesel, improving the profitability of overall process (Melero et al. 2009).

Most research studies have depicted no appreciable difference between biodiesel and diesel in engine durability or in carbon deposits (Kwon et al. 2012). In spite of the advantages of biodiesel such as low emissions, biodegradable, non-toxic, and better lubricity, it is not yet commercialized all over the world (Mangesh et al. 2006). It has fuel properties comparable to conventional diesel and hence they can be mixed in proportions from B10 to B25 for use in standard diesel engines with minor or no modifications. Biodiesel works well with new technologies such as catalysts (which can reduce the soluble fraction of diesel particulates but not the solid carbon fraction), particulate traps and exhaust gas re-circulation (Ma and Hanna 1999). Animal fat obtained from leather industry fleshing wastes was used to produce biodiesel. The fuel properties of FOME were comparable to EN 14214 and ASTM D6751 biodiesel standards (Alptekin et al. 2012; Colak at al. 2005; Isler et al. 2010; Ozgunay et al. 2007; Ramos et al. 2008).

Multivariant techniques are used widely since the past few years for the process optimization (Ferreira et al. 2007; Joa et al. 2007; Imandi et al. 2007). The traditional method of process optimization is laborious, time consuming and mostly erroneous. Design of experiments can be performed with less number of trials and minimum or no error than traditional method of experimentation. Design of experiments based on response surface methodology can be applied for process optimization through the development of mathematical model. Central composite and Box-Behnken designs are among the principal response surface methodologies used in experimental design. Central composite design is five-level design which consumes more time and less efficient than Box-Behnken design. Box-Behnken design is three level design and slightly more efficient than other methods (Colak et al. 2005). Using Box-Behnken design of experiments, a mathematical correlation between alcohol to oil molar ratio, catalyst concentration and temperature were determined to obtain optimal biodiesel yield.

In this paper, oil from limed fleshing waste with about 8% FFA level was used as feedstock in order to transform to its methyl ester by transesterification using alkaline and enzyme

catalysts. The objectives were to (i) recover fat from limed fleshing waste produced from leather industries and to render the fleshing fat and characterize the fleshing oil for the quantification of FFA, dynamic viscosity, kinematic viscosity and density; (ii) optimise biodiesel production from fleshing oil through two-step esterification-transesetrification reaction by adopting three-level three-factor Box-Behnken design (BBD) of response surface methodology (RSM) and to study the effects of alcohol to oil molar ratio, catalyst concentration and reaction temperature on FFA of methyl ester; (iii) produce biodiesel from fleshing oil through two-step esterification-transesetrification reaction by using *candida rugosa* lipase as catalyst; (iv) study the effect of metal activators (Na^+ and K^+) on biodiesel production; (v) characterise biodiesel produced from fleshing oil for profiling of fatty acids and density, dynamic viscosity, kinematic viscosity, flash point, FFA and cetane number determination.

Materials and Methods

In this study, the limed fleshing waste was kindly provided by the tannery unit of Central Leather Research Institute, Chennai, India. The fleshing oil was separated from limed fleshing waste as follows: The fleshing waste was mixed with water (1:1) and heated to 60°C and then the fat rich top phase is treated with n-hexane to extract the fat and remove the protein residue. The characteristics of limed fleshing are tabulated in Table 1. The fleshing fat was in solid state at room temperature and subjected to heating at 110°C for 1 h to remove excess water and then filtered to remove the insoluble residue. Some properties of the fleshing oil are shown in Table 2. The fatty acid composition of the fleshing oil is given in Table 3.

Table 1. Characteristics of limed fleshing

Property	Unit	Limed fleshing
Fat	%	9.81±0.29
Protein	%	8.19±0.03
Insolubles	%	1.51±0.06
Water	%	80.49±0.32

Table 2. Properties of fleshing oil and esterified fleshing oil

Property	Unit	Fleshing oil	Esterified fleshing oil
Density	g/mL	1.053±0.042	1.048±0.058
Dynamic viscosity	cP	5.51±0.75	2.89±0.62
Kinematic viscosity	cSt	5.25±0.92	2.61±0.81
Free fatty acid	%	8.13±0.42	2. 38±0.26

The fleshing oil contains FFA value of 8.13% and therefore the direct transesterfication was not possible. The feedstock with FFA more than 4% can cause foaming during transesterification. The FFA level of the feedstock should be reduced to less than 4% before using an alkali catalyst (Liu, 1994). Therefore, it was needed to perform esterification reaction with the feedstock using sulphuric acid as catalyst and methanol as alcohol. The esterification process of fleshing oil was performed with alcohol to oil molar ratio of 30:1 and sulphuric acid concentration of 3% (based on FFA) at 60°C for 1 h. The molecular weight of the fleshing oil was calculated based on molecular weight of the fatty acids in the fleshing oil. The fleshing oil was added in the reaction flask fitted with reflux condenser, magnetic stirrer

and thermometer, and heated. When the temperature reached 60°C, the alcohol-acid catalyst mixture was added into the oil and the final mixture was stirred for 1 h. The mixture was settled overnight and the two phases were obtained. The upper phase consists of the alcohol-water-acid mixture and the lower phase consists of esterified and unreacted fleshing oil. The lower phase was subjected to heating at 110°C for one hour to remove excess alcohol-water and then filtered. The FFA of the esterified oil mixture was measured (Cox and Pearson 1962). Some properties of the esterified fleshing oil are also shown in Table 2.

Transesterification was performed using methanol and KOH as catalyst to investigate the effect of alcohol to oil molar ratio, catalyst concentration and temperature on FFA level of FOME. The catalyst concentration was selected as 0.5%, 1% and 1.5% w/w (based on FFA). Alcohol to oil molar ratio was selected as 3:1, 6:1 and 9:1 for the transesterification reaction. Mean molecular weight of the fleshing oil was found to be 850 g/mol according to the fatty acid composition for transesterification reaction. The transesterification process and laboratory apparatus were the same as those of pretreatment experiments except for catalyst. The reaction temperature was selected as 60°C, and reaction time was selected as 1 h. After the transesterification reaction, the glycerine layer was separated in a separating funnel and the ester layer was washed with warm water. After washing process, the methyl ester was subjected to a heating at 110°C to remove excess alcohol and water, and then filtered. The produced methyl esters were characterized for its properties. The fatty acid composition of the fleshing oil methyl ester is given in Table 3.

A three-level three-factor Box-Behnken design of response surface methodology is employed for optimization of biodiesel production with 15 experiments (Cochran and Cox 1957). Alcohol to oil molar ratio (M), catalyst concentration (C) and reaction temperature (T) are the independent variables and FFA is the dependent variable used to optimize the biodiesel production from limed fleshing. The coded and uncoded levels of the independent variables are given in Table 4. Two replications are carried out for all design points except the centre point (0, 0, 0) and the experiments are carried out in randomized order. The experimental data obtained by following the above procedure are analyzed by the response surface regression procedure using the following second-order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ij} {X_i}^2 + \sum_{i<j}^{k} \sum_{j}^{k} b_{ij} X_i X_j \qquad (1)$$

where Y is predicted response, and i, j are linear, quadratic coefficients, respectively. b and k are regression coefficients and the number of factors studied in the experiment respectively.

A trial version of Design Expert 8 is used for regression analysis of variance (ANOVA). Response surfaces and contour plots are developed using the fitted quadratic polynomial equation obtained from regression analysis, holding one of the independent variables at a constant value corresponding to the stationary point and changing the other two variables. Confirmatory experiments are carried out to validate the equation, using combinations of independent variables which were not part of the original experimental design but within the experimental region.

Transesterification reaction was conducted using *Candida rugosa* lipase following a suggested method (Devanesan et al. 2007): Experiment was carried out at the optimum temperature of 40°C, inoculum size of 10% v/v, 1:4.16 molar ratio of oil to methanol. After a period of reaction time of 1 h, the reaction was stopped by boiling the mixture for 10 min and the biomass was removed from the reaction mixture. The glycerine layer was separated in a

separating funnel and the ester layer was washed with warm water. After washing process, the methyl ester was subjected to a heating at 110°C to remove excess alcohol and water, and then filtered. The produced methyl esters were characterized for its properties. The fatty acid composition of the fleshing oil methyl ester is given in Table 3. The effect of two metal activators (Na^+ and K^+) on FFA level of fleshing oil was investigated by adding 2% w/v each of NaCl and KCl solutions at the optimum temperature of 40°C, inoculum size of 10% v/v, 1:4.16 molar ratio of oil to methanol and for reaction time of 1 h.

Results and Discussion

In this study, the potential of limed fleshing waste from leather industries as biodiesel feedstock was investigated and the process parameters of biodiesel production were optimized. Sulphuric acid was used s catalyst for the esterification of fleshing oil. The esterification process was performed with 30:1 alcohol to oil molar ratio and 3% w/w (based on FFA) sulphuric acid for 1 h reaction at 60°C. Some properties of the fleshing oil and esterified fleshing oil were characterized to understand the effect of esterification on properties of fleshing oil. The FFA level was reduced from 8.13% to 2.38% as a result of esterification. After reducing the FFA level of the fleshing oil to below 4%, transesterification reaction was performed with varying alcohol to oil molar ratio, alkali catalyst concentration and temperature.

Table 3. Fatty acid profile of fleshing oil and fleshing oil methyl ester

Product	Fatty acid distribution								Ref
	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	
Fleshing oil	4.2	6.3	22.9	2.1	10.4	47.9	ND	ND	Present study
FOME[a]	4.34	2.02	28.56	7.94	10.89	43.61	1.92	0.72	Present study
FOME[b]	6.3	ND	20.8	ND	10.4	54.2	ND	ND	Present study
Fleshing oil	4.2	2.16	28.4	8.1	10.67	43.83	1.8	0.84	Isler *et al.*, 2010
Fleshing oil	3.05	ND	20.59	4.6	8.36	41.08	2.97	ND	Colak *et al.*, 2005
Fleshing oil	3.18	1.27	27.25	5.1	18.23	42.06	2.04	0.21	Alptekin *et al.*, 2012
FOME	3.29	1.23	27.76	4.95	13.40	41.16	2.19	0.22	Alptekin *et al.*, 2012

a : alkali catalyst; b : enzyme catalyst; ND – Not detected

Table 4. Factors and their levels for Box-Behnken design

Variable	Symbol	Coded factor levels		
		-1	0	+1
Alcohol to oil molar ratio	M	3	6	9
Catalyst concentration (%)	C	0.5	1.0	1.5
Temperature (°C)	T	55	65	75

In order to optimize the reaction conditions of FOME production, the Box-Behnken design, which is generally the best design for response surface optimization, was selected with three-level three-factors: i.e., alcohol to oil molar ratio, catalyst amount, and reaction temperature. Table 4 lists the experimental factor settings and results on the basis of the experimental design. All 15 of the designed experiments were conducted, and the results were analyzed via multiple regression. The coefficients of a full model were evaluated via regression analysis and tested for significance. Finally, the best fitting model was determined via regression. This showed that three linear coefficients (M, C and T), three quadratic coefficients (M^2, C^2 and T^2) and three cross-product coefficients (M C, M T, C T) were significant (Tables 4 and 5).

Table 5. Box-Behnken design, experimental and estimated data for three-level three-factor response surface analysis

Run	M	C (%)	T (°C)	FFA (%)	
				Experimental	Estimated
1	6	1	65	0.554	0.558
2	3	1	75	1.435	1.370
3	6	0.5	75	1.27	1.196
4	6	1	65	0.562	0.558
5	9	1	55	1.325	1.389
6	3	1	55	1.215	1.083
7	3	1.5	65	0.61	0.668
8	9	1	75	1.159	1.291
9	9	1.5	65	0.994	0.856
10	6	1.5	55	0.527	0.601
11	9	0.5	65	1.341	1.283
12	6	0.5	55	1.025	1.019
13	6	1.5	75	0.607	0.613
14	3	0.5	65	1.104	1.242
15	6	1	65	0.558	0.558

The ANOVA for the response surface quadratic model is provided in Table 6. The coefficients of the response surface model as provided by Eq. (1) were also evaluated. A p-value showed that all of the linear coefficients were more highly significant than their quadratic and cross-product terms. However, in order to minimize error, all of the coefficients were considered in the design. According to the ANOVA analysis of factors, low lack of fit was observed. This indicates that the model does indeed represent the actual relationships of reaction parameters, which are well within the selected ranges (Table 6). The final estimative response model equation (based on the actual value) by which the production of FOME was estimated as follows:

$$Y = 12.861875 - 0.383458\,M - 0.22325\,C - 0.338538\,T + 0.048917\,M^2 + 0.056\,C^2 + 0.002853\,T^2 + 0.00245\,M\,C - 0.003217\,M\,T - 0.00825\,C\,T \quad (2)$$

where Y is the FFA (%) M, C and T are the values of the independent factors, alcohol to oil molar ratio, catalyst amount (% w/w) and reaction temperature (°C) respectively. The model coefficients and probability values (coded value) are shown in Table 7. The model proved suitable (R^2 = 93.97%) for the adequate representation of the real relationship among the selected factors.

Table 6. ANOVA for response surface quadratic model analysis of variance table

Source	Sum of Squares	df	Mean Square	F value	Prob > F
Model	1.549996	9	0.172222	8.660172	0.0143
M	0.025878	1	0.025878	1.301281	0.3057
C	0.501001	1	0.501001	25.19281	0.0040
T	0.017955	1	0.017955	0.902873	0.3856
M C	0.005402	1	0.005402	0.271652	0.6245
M T	0.037249	1	0.037249	1.873066	0.2294
C T	0.006806	1	0.006806	0.342252	0.5839
M^2	0.715643	1	0.715643	35.98612	0.0018
C^2	0.000724	1	0.000724	0.036391	0.8562
T^2	0.300434	1	0.300434	15.10732	0.0116
Residual	0.099433	5	0.019887		
Lack of Fit	0.099401	3	0.033134	2070.859	0.0005
Pure Error	0.000032	2	0.000016		
Cor Total	1.64943	14			

df – degree of freedom

Table 7. Regression coefficients and significance of response surface quadratic model

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.558	1	0.081418	0.348708	0.767292	
M	0.056875	1	0.049858	-0.07129	0.185039	1
C	-0.25025	1	0.049858	-0.37841	-0.12209	1
T	0.047375	1	0.049858	-0.08079	0.175539	1
M C	0.03675	1	0.07051	-0.1445	0.218002	1
M T	-0.0965	1	0.07051	-0.27775	0.084752	1
C T	-0.04125	1	0.07051	-0.2225	0.140002	1
M^2	0.44025	1	0.073389	0.251597	0.628903	1.011111
C^2	0.014	1	0.073389	-0.17465	0.202653	1.011111
T^2	0.28525	1	0.073389	0.096597	0.473903	1.011111

df – degree of freedom; CI – Confidence interval; VIF – Variation inflation factor

Figure 1 represents the effects of catalyst concentration and alcohol to oil molar ratio on FOME production at a constant temperature of 65°C. At any designed quantity of catalyst from 0.5% to 1.5%, the decrease of FFA resulted in increase in alcohol to oil molar ratio. An increase of introduced quantities of catalyst decreased FFA level at 65°C. The appropriate minimal content was determined at a catalyst amount of 1.44% w/w (based on FFA) at high reaction temperature and alcohol to oil molar ratio.

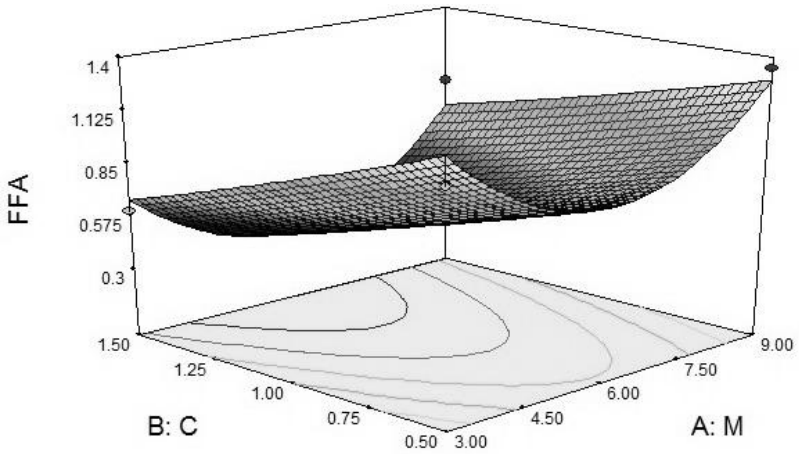

Figure 1. Response surface plots representing the effect of alcohol to oil molar ratio and catalyst concentration and their reciprocal interaction on FFA

Figure 2 shows the effects of reaction temperature and alcohol to oil molar ratio and their reciprocal interactions on FOME production at a catalyst quantity of 1% and a reaction time of 1 h. At the lowest reaction temperature (55°C), an increase in the alcohol to oil molar ratio diminished the FFA level. At a low alcohol to oil molar ratio, high reaction temperatures caused low FFA level. Biodiesel production was affected significantly by reaction temperature and alcohol to oil molar ratio.

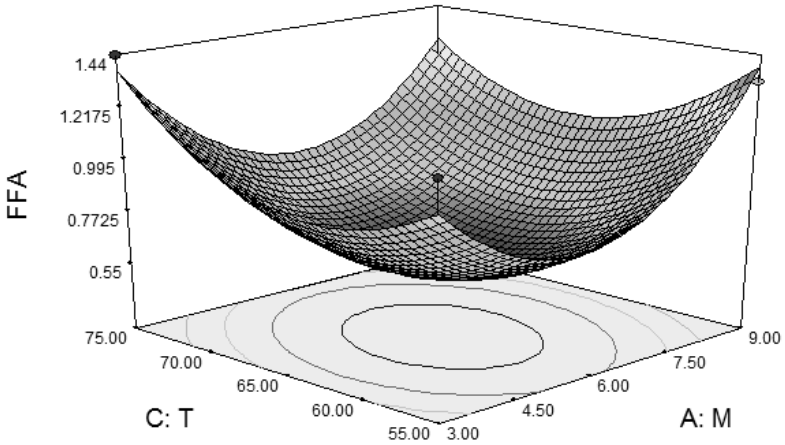

Figure 2. Response surface plots representing the effect of alcohol to oil molar ratio and temperature and their reciprocal interaction on FFA

The effect of catalyst concentration and temperature on FFA level at alcohol to oil molar ratio of 6 is provided in Figure 3. At low concentration of introduced catalyst, FFA level was slightly affected by the reaction temperature; whereas, at high catalyst amounts, the reaction temperature was extremely relevant to the decrease of FFA.

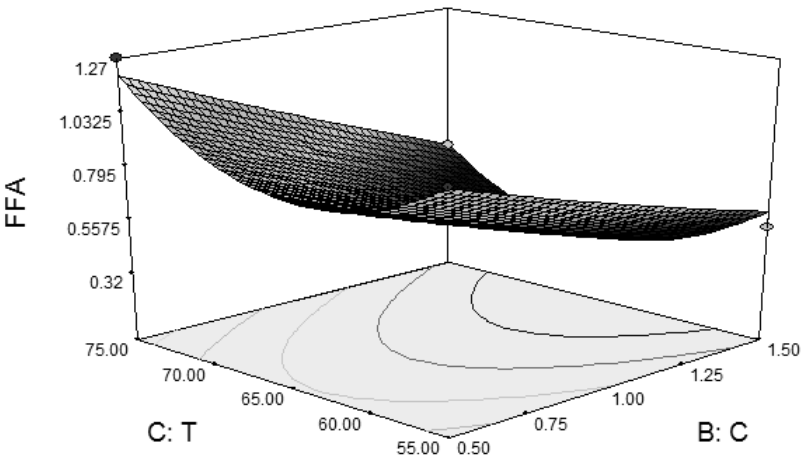

Figure 3. Response surface plots representing the effect of temperature and catalyst concentration and their reciprocal interaction on FFA

The reaction temperature and alcohol to oil molar ratio exerted significant influence on FFA level. High reaction temperature (above 65°C) tended to induce methanol evaporation. At any of the designated reaction temperatures, an increase in the concentration of catalyst diminishes FFA level in a linear fashion. As compared to the results shown in Figures 1–3, the optimal condition for the minimal acquisition of FFA was achieved with a high reaction temperature, alcohol to oil molar ratio and catalyst concentration. The optimal values of the selected variables were obtained by solving the regression equation (Eq. (2)) using Design Expert 8 software. The optimal conditions for FOME production estimated by the model equation were as follows: M = 4.16, C = 1.44% w/w (based on FFA) and T = 61°C. The theoretical FFA level predicted under the above conditions was 0.478%. In order to verify the prediction of the model, the optimal reaction conditions were applied to three independent replicates for FOME production. The average FFA was 0.351±0.058%, a figure well within the estimated value of the model equation. This demonstrated that response surface methodology with appropriate experimental design can be effectively applied to the optimization of the process of factors in a chemical reaction. This study focused on the application of response surface methodology to the optimization of FOME production conditions using alkali catalyst. This may provide useful information regarding the development of economic and efficient process using alkali catalyzed reaction system.

In an attempt towards green chemistry, transesterification was performed using *Candida rugosa* lipase as catalyst using the above optimal conditions yielding an average FFA level of 0.287±0.068%. It was also found that the use of activators (Na^+ and K^+) enhanced the biocatalytic biodiesel production by reducing the free fatty acid level upto 0.224±0.115% and 0.256±0.089% for KCl and NaCl salts, respectively.

In order to evaluate the quality of biodiesel generated from limed fleshing, the content and composition of FOME, FFA, density, dynamic viscosity, kinematic viscosity, flash point and cetane number were analysed. The FOME content was 97.1% at the predicted conditions of the response surface methodology experiments. The composition of FOME was as follows: 43.61% oleic acid methyl ester, 36.5% palmitic and palmitoleic acid methyl esters, 10.89% stearic acid methyl ester, and 6.1% others. The composition of FOME evidenced a pattern similar to that of limed fleshing. The FFA and dynamic viscosity were 0.351% and 3.683 cP

respectively. These results satisfied the Indian standard for biodiesel quality (Table 8). The problems inherent to using pure biodiesel can be resolved by blending with conventional diesel.

Table 8. Fuel properties of FOME and its comparison with Indian, European and American standards

Property	Unit	EN 14214 / BIS	ASTM D6751-10	FOME
Ester content	%	>96.5	>96.5	97.1
Density	g/mL	0.86 – 0.90	NA	0.874
Dynamic viscosity	cP	3.01 – 4.5	NA	3.683
Kinematic viscosity	cSt	3.5 – 5	1.9 – 6	4.214
FFA	%	<0.5	<0.5	0.351
Flash point	°C	>101	>130	120
Cetane number	-	>51	>47	53

NA – Not available

Conclusion

In this study, the experiments using response surface methodology was performed to determine the optimal reaction conditions for the production of biodiesel from fleshing waste. According to response surface methodology experiments, the optimal values of the variables were as follows: reaction temperature of 61°C, catalyst amount of 1.44% w/w (based on FFA), and alcohol to oil molar ratio of 4.16, with a 1 h reaction time. At this predicted optimum condition, the predicted FFA level was reached at 0.315%. The experimental value was well within the estimated value of the model. The demonstration of response surface methodology can be applied effectively to the optimization of the process parameters in a biodiesel production. Transesterification was also performed using *Candida rugosa* lipase as catalyst using the above optimal conditions yielding an average FFA level of 0.287±0.068%. The use of activators (Na^+ and K^+) enhanced the biocatalytic biodiesel production by reducing the free fatty acid levels.

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