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Optimization of degummed *Linum usitatissimum* methyl ester from methanolysis as a potential resource for diesel engines

Sangeeta Kanakraj[1]*, Savita Dixit[1], A. Rehman[2]

[1] Department of chemistry, Maulana Azad National Institute of Technology, Bhopal M.P, India.

[2] Department of Mechanical Engineering, Maulana Azad National Institute of Technology, Bhopal M.P, India.

* Corresponding author: Sangeeta Kanakraj, skskanakraj5@gmail.com, skanakraj@yahoo.com, +91 9827212930 ,Fax 0755-267056.

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ABSTRACT Studies were carried out on the methanolysis of crude and degummed *Linum usitatissimum* seed oil with methanol for methyl ester synthesis. In this study, response surface methodology was applied in order to optimize the reaction factors for methyl ester synthesis. The present work also studies the experimental conditions such as catalyst concentration, molar ratio (methanol:oil) and reaction time on the conversion of *Linum usitatissimum* seed oil to methyl esters. The conversion was above 96.2% under the condition of 60 °C, methanol-oil molar ratio of 8:1, reaction time of 60 minutes and catalyst concentration (catalyst/oil) of 0.6 wt%. The resulting methyl esters (biodiesel) and its diesel blends were characterized for basic fuel properties including density, viscosity, cloud and pour point, flash and fire point, calorific value and acid value. The results show that the enzymatic degummed *Linum usitatissimum* seed oil used with a phosphorus (phospholipids) level of 9.8 ppm provides an excellent methyl ester yield of over 96.2% and basic fuel properties of these blends are comparable to those of conventional diesel fuel at 20% of methyl esters concentrations.

KEYWORDS Enzymatic degumming, *Linum usitatissimum* seed oil, methyl ester, methanolysis

Introduction

Linseed, or *Linum usitatissimum L.*, is a member of the family Linaceae and the genus *Linum* [Herchi et al., 2012; Minor vegetal oil, 2011]. It is an annual species of the *Linaceae* family, growing to a height of 0.3-1.0 m, and is cultivated for the production of textile fibre, seed, linseed oil; most commonly used as a carrier in oil-based paint and varnish [Flaxseed Oil, 2011]. Linseed is best suited for fertile, fine textured and loamy soils and an important factor is the amount of rainfall during the growing period. Adequate moisture and relatively cool temperatures, particularly during the period from flowering to maturity, seem to favour both oil content and oil quality [Minor vegetal oil, 2011]. The seed is located in the extremities of the branches in round capsules, each of which contains from one to ten seeds. It is highly oleaginous; the seeds of this plant contain 33-47% which is non-edible in nature. It originated from

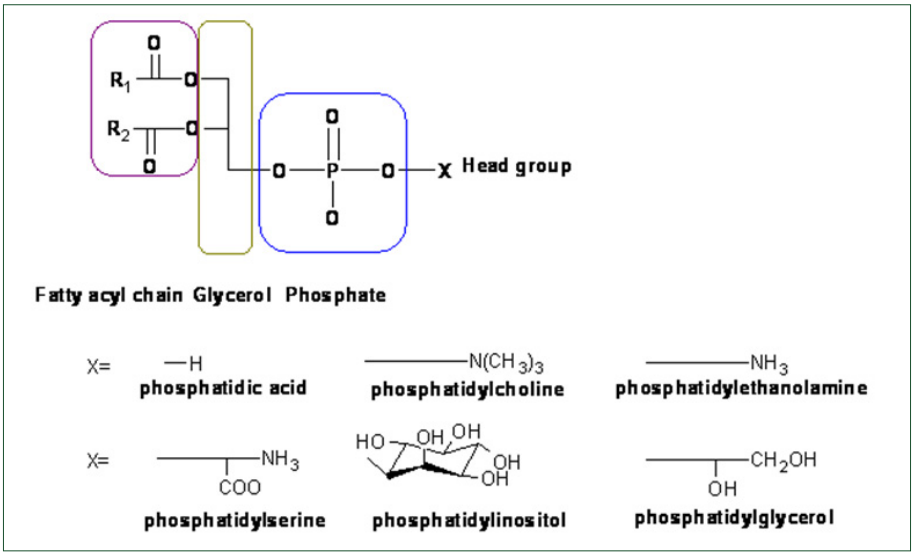

Figure 1 Structure and major classes of phospholipids [Dijkstra, 2011].

Mediterranean coastal countries and is cultivated in Canada, Argentina, India and the USA [Lopes et al., 2011; Flaxseed Oil, 2011; Nabi and Najmul, 2008]. In India, various states have sufficient forest area for the cultivation of linseed. Madhya Pradesh is the leading producer of this crop. Madhya Pradesh and Uttar Pradesh together contribute to the national oilseed production to the extent of about 70% [Galvao et al., 2012]. India is considered the third largest producer of linseed in the world among the linseed-producing countries Australia and Canada. In India, Linseed is mainly cultivated as a Rabi crop in the months of October-November [Linseed, 2011]. The climatic and soil conditions of India are convenient for the production of Linseed crops [Dixit et al., 2012; Lingaraju et al., 2012; Linseed, 2011].

Energy crisis contributes to the development of biofuel production. Fuel crisis and environmental concerns have renewed interests in the scientific community to look for alternative fuels of bio-origin such as vegetable oils and biodiesel [Dixit et al., 2012]. The concept for producing fuel from vegetable oil for diesel engines is not new. In the year 1900, Rudolph Diesel first successfully tested a compression ignition engine on peanut oil as a fuel in place of petroleum diesel [Adebayo et al., 2011; Fangui and Milford, 1999]. India and other developing countries can secure energy production with the use of renewable energy from non-edible vegetable oil such as linseed oil, karanj oil, Neem oil, Mahua oil and Jatropha oil [Tiwari et al., 2006]. The linseed plant contains high amount of oil in its seeds which can be converted to biofuel methyl esters [Dixit et al., 2012]. This article highlights the efforts made to develop biodiesel from linseed oil, which is readily available in India.

Fatty acid profile of *Linum usitatissimum* seed oil

The triglycerides of linseed oil contain five major fatty acids, [Juita et al., 2011] depicted in Table 1.

Gum in vegetable oil

Vegetable oils contain phospholipid, which is a class of esters of phosphoric acid containing one or two molecules of fatty acid, an alcohol, and a nitrogenous base also known as phosphatide [Phospholipid]. The phospholipid molecule is made up a head and 2 tails; the head is made up of phosphate and glycerol and is hydrophilic, given its polarity. In contrast, the tail is comprised of fatty acid and is hydrophobic [Phospholipid, 2012]. The basic structure of the phosphatides (phospholipids) is *sn*-glycerol-3-phosphate. Two fatty acids are esterified to the hydroxyl groups at positions *sn*-1 and *sn*-2, while a phosphorus-containing acid is esterified at the hydroxyl group at position *sn*-3 shown

in Fig.1. The amount of phospholipids was determined as the total phosphors of oil. Phosphorus content in the oil was analyzed and expressed in parts per million (ppm). This value is multiplied by a factor of 25 to give the phosphatide content of the oil. This factor is derived from the ratio of the specific weight of the phosphorus and the phosphatides [Hamm et al., 2002; Kelly and Jacobs, 2011]. All vegetable oils contain two types of gums: (phospholipids), hydratable (HPL) and non-hydratable (NHPL), and are removed from oil by a degumming process. The level of hydratable and non-hydratable gums varies depending on a number of factors such as seed quality, seed type and oil milling conditions [Zufarov et al., 2008]. It is reported that *Linum usitatissimum* seed oil contains a high amount of phospholipids. The most important members of this class of lipids found in *Linum usitatissimum* seed oil are phosphatidylethanolamine (PE) phosphatidylinositol (PI) and phosphatidylcholine PC) [Herchi et al., 2012]. PC and PI phospholipids are completely hydratable and PE is partially hydratable.

Influence of phospholipids (phosphorus) on methanolysis (transesterification)

The phosphorus content in feedstocks (vegetable oils) indicates the presence of phospholipids (gums). This parameter is very important for biodiesel production from vegetable oils. Phospholipids are suggested as a source of catalyst destruction and create problems during oil processing and transesterification process. Due to the presence of phospholipids in feedstocks which are used for biodiesel production, the saponification of glyceride tends to be accelerated before the completion of alcoholysis. It is reported that the produced soap consumes the catalyst and reduces the catalytic efficiency. The soap formation can cause problems with separation of glycerol from the esters after the reaction is complete. These impurities not only contaminate biodiesel production but reduce biodiesel yield as well [Gerpen, 2005; Houfang et al., 2009; Freedman et al., 1984; Mullenix, 2011]. Thus, vegetable oils to be used for biodiesel production must be degummed.

Degumming process for vegetable oil

Degumming is a process of removing the impurities such as phospholipids (gum), free fatty acids, water, wax and trace of metals from vegetable oils. There are various degumming techniques reported for removal of phospholipids from vegetable oils. The conventional degumming processes are water and acid degumming. These process are limited to oils with low phospholipids contents [Dixit and Kanakraj, 2010]. Acid degumming methods have several disadvantages including corrosion of processing equipment and environmental hazards [Prabhaharan and Rakshit, 2009]. Enzymatic and soft degumming are the most current degumming processes. Soft degumming involves EDTA (Ethylene Diamine Tetra Acetic acid) as a degumming agent, and it is able to completely eliminate phospholipids, but due to the high cost of EDTA it is not found in industrial applications [Choukri et al., 2001]. However, based on the literature available, the enzymatic degumming technique provides significant benefits in the pretreatment processes utilized during preparation of seed oil for biodiesel. These benefits include increased yield, reduced chemical usage, improved operating efficiency, cost effectiveness and reduced waste product [Zufarov et al., 2008; Prabhaharan and Rakshit, 2009; Bo et al., 2006]. The enzymatic degumming technique is reported for rapeseed, soybean, sunflower and rice bran oils [Roy et al., 2002]. To date, work has been done for *Linum usitatissimum* seed oil. In the current study, fuel properties of enzymatic degummed seed oil and its methyl esters are discussed, and an attempt is made to confirm enzymatic degummed oil as a potential resource for diesel engines.

Materials and methods

Mechanically pressed *Linum usitatissimum* seed oil used in this experiment was obtained from a local market and commercially available diesel fuel was purchased from a nearby petrol pump. Lecitase ultra (phospholipase enzyme) was used as a degumming agent, purchased from Zytex Biotech Private Limited,

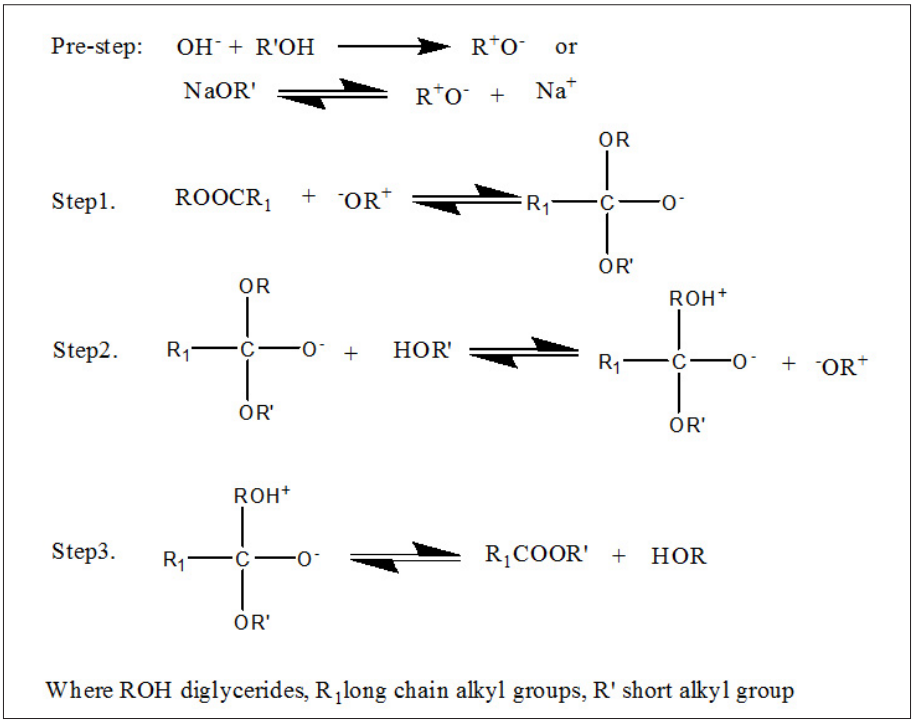

Figure 2 The mechanism of the alkali catalyzed transesterification[Schuchardtet al.,1998].

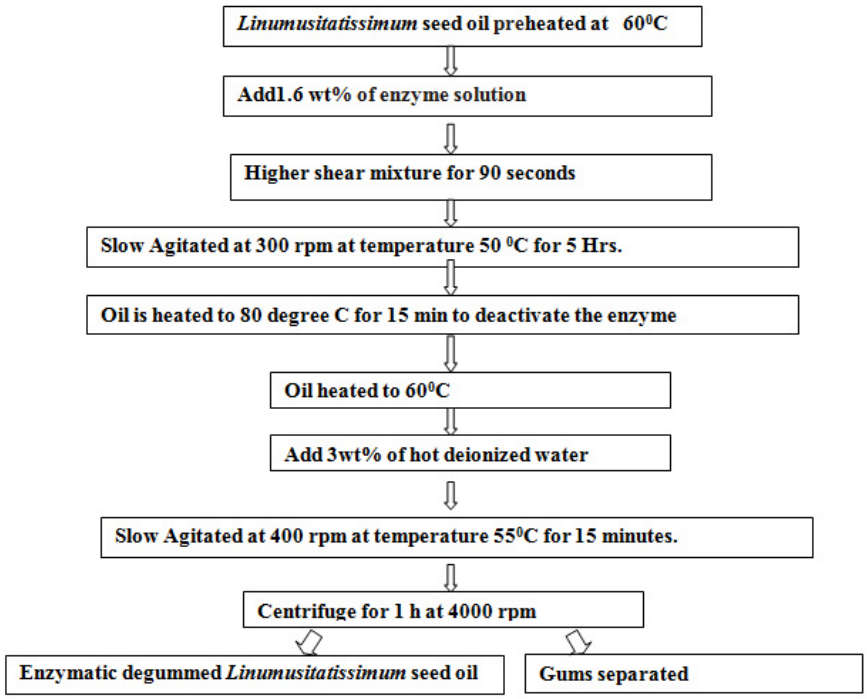

Figure 3 Process flow schematic for production of enzymatic degummed Linumusitatissimum seed oil.

Table 1 Fatty acid profile of *Linum usitatissimum* seed oil [Galvao et al., 2012; Singh et al., 2010; Myint et al., 2007; Lazzari and Chintore, 1999]

Fatty acids	Chemical name	Structure	Formula	Percentage % by weight of total fatty acids
Saturated acid				
Palmitic	Haxadecanoic	C16:0	$C_{16}H_{32}O_2$	6-7
Stearic	Octadacanoic	C18:0	$C_{18}H_{36}O_2$	3-6
Monounsaturated acid				
Oleic	Octadecenoic	C18:1	$C_{18}H_{34}O_2$	14-24
Polyunsaturated				
Linoleic cis-9, cis-12	Octadecadienoic	C18:2	$C_{18}H_{32}O_2$	14-19
Alpha Linolenic		C18:3	$C_{18}H_{30}O_2$	48-60

Table 2 Phosphatides content of EDLO

Oil type	Phosphorus (ppm)	Phosphatides* (%)
Crude *Linum usitatissimum* seed oil (CLO)	150±1	0.375±0.001
Enzymatic degummed *Linum usitatissimum* seed oil (EDLO)	9.8±0.1	0.245±0.001
Enzymatic degummed *Linum usitatissimum* methyl ester (EDLOME)	0.2±0.1	0.0005±0.0001
*Phosphorus (ppm) = phosphatides (%) x10^4/25 [Hamm, 2002]		

Mumbai, India. Other reagents used were of analytical grade. In the experimental set up, a reactor used for experiments was a 500 mL three-necked glass flask. The flask was placed in a water bath. The center neck was fitted with a stirrer. One of the two side necks was equipped with a thermometer and another is closed.

Transesterification is also called alcoholysis, which uses alcohols in the presence of a catalyst (KOH, NaOH) that chemically breaks the molecule of triglyceride into alkyl esters as biodiesel fuel and yields glycerine as a byproduct. When methanol is used, the process is known as methanolysis (Fig. 2).

For this study, crude *Linum usitatissimum* seed oil was degummed and further transesterified with methanol.

Enzymatic degumming process of *Linum usitatissimum* seed oil

Crude *Linum usitatissimum* seed oil was placed into a 500 mL conical flask, and an enzyme (100 mL) buffer solution was (prepared by dissolving 4 g of citric acid and 1.4 g of caustic (NaOH), pH was adjusted to 4.8 to 5 and 350 to 400 mg of enzyme (*Lecitase ultra*) was added to the buffer solution) added to oil in different wt% (0.6%, 1% and 1.6 wt%) by using a high shear mixer for 90 seconds reaction after that the mixture was transferred to the three-necked glass flask. The flask was put in a water bath with slow agitation for incubation at temperature 48 to 50 °C for 5 h. After 5 h the oil was heated to 80 °C for 15 min to deactivate the enzyme. After deactivation of enzyme, the oil was heated to 60 °C, and then 5 wt% of hot deio-

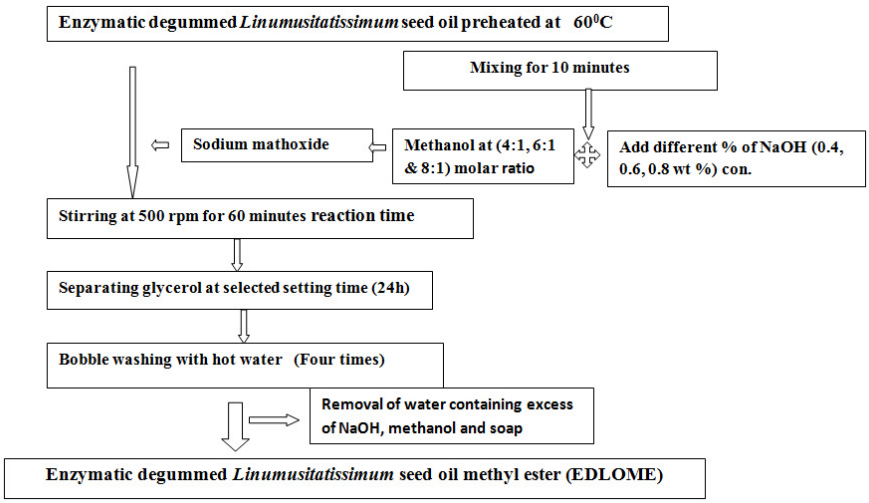

Figure 4 Schematic diagram of simple procedure developed for the production of enzymatic degummed Linumusitatissimum methyl ester (EDLOME) at different condition.

nized water was added into the reaction flask. The temperature of the system was maintained at 55 °C for 15 minutes. The stirrer operating at 400 rpm and the mixture was transferred to a centrifuge. Finally, the degummed oil was obtained after centrifuging for 1 h at 4000 rpm. Samples were drawn for phosphorus analysis Fig. 3. The optimal condition identified based on the minimum residual phosphorus content in the degummed oil is given in Table 2.

Methanolysis of enzymatic degummed *Linum usitatissimum* seed oil (EDLO)

For this synthesis, a defined amount of EDLO oil was preheated at 60 °C and taken in a biodiesel reactor vessel. The methanol oil ratio 4:1 to 8:1 and the alkali catalyst (NaOH) 0.4% - 0.8 wt% were premixed for 10 minutes in a flask and added rapidly into the oil. The free fatty acids were neutrilized by using sodium methoxide creating a methyl ester under 60 °C reaction temperature with suitable speed, stir for agitating the mixture, within reaction time of 30-90 minutes biodiesel is formed along with the glycerin with clear phase separation.The methyl ester was purified by washing gently with boiled water to remove residual catalyst, glycerol and methanol. Finally the ester was dried by heating at 120 °C for an hour and pure methyl ester was obtained. In order to standardize the process parameters, three levels of molar ratios (4:1, 6:1 and 8:1), three levels of reaction times (30, 60 and 90 minutes) were set with a constant reaction temperature of (60 °C.

The specific experimental factors are described in Table 3. The methanolysis of the enzymatic degummed *Linum usitatissimum* seed oil was carried out using the procedure described in Fig. 4.

Experimental design and central composite design (CCD)

In order to optimize the reaction factors, a three-level, three-factor central composite rotatable design (CCD) was utilized in this study. The CCD applied three design factors: catalyst amount (A), methanol to oil (B) and reaction time (C). The total number of experiments was 20 determined by the expression: 2^n (2^3=8: factor points) + 2n (2×3 = 6: axial points) + 6 (center points: six replications). Table 3 describes the coded and uncoded levels of the independent variables, and the test ranges for the parameter. The Design Expert 8.0 software was used for regression and graphical analysis of the data obtained. The maximum values of the yield were taken as the responses of the design experiment. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA).

Table 3 Factor and their level for response surface design

Factors	Symbol	Range and levels		
		-1	0	+1
Catalyst amount Wt%	A	0.4	0.6	0.8
Methanol/oil (Molar ratio)	B	4:1	6:1	8:1
Reaction time minutes	C	30	60	90

Once the experiments were performed, the response variable (conversion to biodiesel) was fitted to a second-order model in to correct the response variable to the independent variable. The general form of the second-degree polynomial equation is expressed in the equation: [Yuan et al., 2008]

$$Y = \beta_0 \sum_{}^{k} \beta_i x_i + \sum_{}^{k-1} \sum_{}^{k} \beta_{ij} x_i x_j + \sum_{}^{k} \beta_{ii} x_i^2 + e \qquad \text{Eq. (1)}$$

Where Y is the predicted response β_0 and the offset term, β_i the linear effect, β_{ij} is the squared effect and β_{ii} is the interaction effect. n is the number of significant independent variables A, B and C [Jeong et al., 2009].

Characterization of enzymatic degummed *Linum usitatissimum* seed oil methyl ester (EDLOME) obtained from EDLO

Three blends (10, 20 and 30 %) of EDLOME by volume with diesel fuel were prepared and used to characterize basic fuel properties, in order to determine the fuel properties of the diesel fuel, EDLOME and its diesel blends. The characterization covered the physical and chemical properties of the oil and its methyl ester. The following test methods were used: density at 15 °C (IS: 1448: [P: 16]:1990), viscosity at 40 °C (ASTM D445), pour point (ASTM D97), flash point (ASTM D93), calorific value measured by bomb calorimeter (ASTM- D240), acid number (AOCS Cd 3a-64), phosphorus (AOCS official method Ca 12-55), cloud point (ASTM d2500) and fire point (ASTM D92-93). The analysis was performed in duplicate. The FTIR spectra were obtained in a spectrophotometer Shimadzu FTIR affinity-1, an interval of range 4000-400 cm^{-1}.

Results and discussion

Effect of enzymatic degumming method on phosphorus (phospholipids) content in *Linum usitatissimum* seed oil and its methyl ester

The amount of phospholipids was determined as the total phosphors of oil according to AOCS official method ca 12-55 using a UV- visible spectrophotometer. ASTM D 6751-09 requires the maximum amount of 10 ppm phosphorus content in the final biodiesel fuel. Degumming removed phospholipids (phosphatides) and lowered the phosphorus content in *Linum usitatissimum* seed oil. During enzymatic degumming, the conventional degumming processes was improved with the use of an enzymatic technology involving a phospholipase-A1 enzyme. In this process the Lecitase - Ultra (phospholipase A1) hydrolyzed the Carboxylic group at the C1 Position and non-hydratable or partially hydratable phospholipids in oil were initially converted to fully hydratable phospholipid (lyso-phospholipids), which were easy to eliminate with the water phase by centrifugation as shown in Fig.5. The enzymatically hydrolyzed lysophospholipids are hydrophilic and mostly soluble in water. The residual phosphorus content in degummed oil and its methyl ester are about 9.8 ppm and

Table 4 Full-factorial central composite design matrix of three variables in coded and natural units along with the observed responses

Std	Run	Variable in coded levels			Catalyst amount Wt%	Methanol/ oil Molar ratio	Reaction time Minutes	NDLOME Conversion % Y_1	EDLOME Conversion % Y_2
		A	B	C					
20	1	0	0	0	0.6	6:1	60	86.2	89.39
18	2	0	0	0	0.6	6:1	60	86.54	90
11	3	0	-1	0	0.6	4:1	60	85	87
5	4	-1	-1	1	0.4	4:1	90	84	87
13	5	0	0	-1	0.6	6:1	30	81	84
17	6	0	0	0	0.6	6:1	60	86.29	89.29
15	7	0	0	0	0.6	6:1	60	87	89.55
3	8	-1	1	-1	0.4	8:1	30	88	89
8	9	1	1	1	0.8	8:1	90	85	89
12	10	0	1	0	0.6	8:1	60	95	96.2
7	11	-1	1	1	0.4	8:1	90	91	93
10	12	1	0	0	0.8	6:1	60	85	87
6	13	1	-1	1	0.8	4:1	90	74	76
14	14	0	0	1	0..6	6:1	90	84	86
1	15	-1	-1	-1	0.4	4:1	30	83	85
2	16	1	-1	-1	0.8	4:1	30	72	74
16	17	0	0	0	0.6	6:1	60	87	90
4	18	1	1	-1	0.8	8:1	30	84	85
19	19	0	0	0	0.6	6:1	60	86.87	89.44
9	20	-1	0	0	0.4	6:1	60	88	90

0.2 ppm respectively after enzymatic degumming to meet the phosphorus level requirement as proposed by the ASTM standard shown in Table 2.some degummed samples are given in Fig. 6.

Optimization of reaction condition by response surface methodology for EDLOME synthesis

Table 4 describes the experimental parameters and their results, on the basis of the CCD experimental design. Regression analysis yielded three linear coeffi-

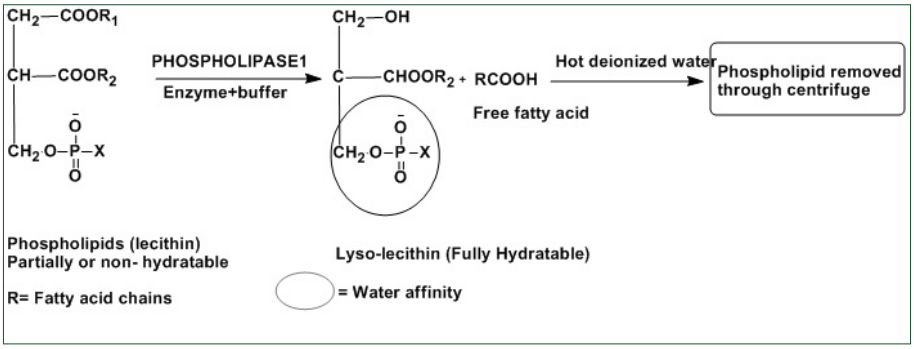

Figure 5 Structure of enzymatic degumming of phospholipid.

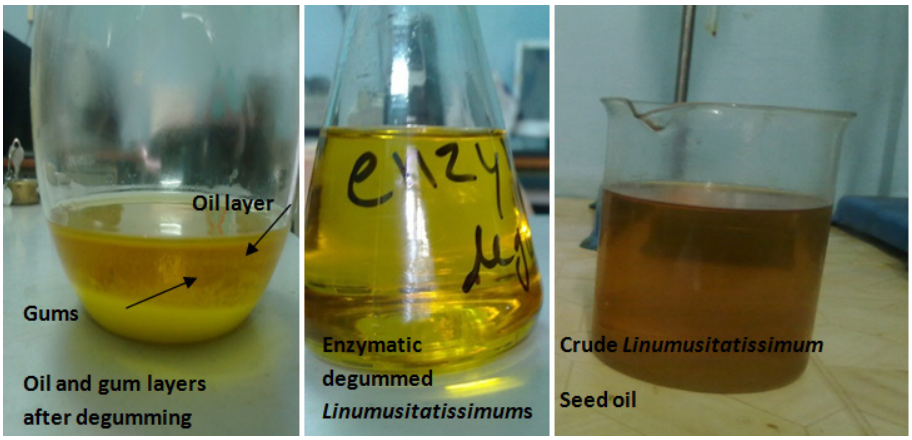

Figure 6 Water degummed *Linumusitatissimum* seed oil samples.

$$Y_2 = 89.78 - 3.30A + 4.32B + 1.40C + 1.75AB + OAC + 0.50BC - 1.52A^2 + 1.58B^2 - 5.02C^2 \quad \text{Eq. (2)}$$

cients (A, B, C), three quadratic coefficients (A^2, B^2, C^2), and three cross product coefficients (AB, AC, BC) for the full model (Table 5). Table 5 also describes the ANOVA for the response-surface quadratic model.

The final value estimate response model equation (based on the final empirical value), by which we analyzed EDLOME synthesis, was as follows:

In which Y_2 is the response factor, methyl ester (EDLOME) content wt%, A, B, C are the values of the three independent variables, catalyst amount, molar ratio methanol to oil and reaction time. The model coefficient values are provided in Table 6. The 3D response surface plots are generally represent the graphical representations of the regression equation are presented in Fig.7 (A, B, C). The 3D response surface in Fig. 7a shows that there is a response surface plots representing the effects of molar ratio (methanol to oil), catalyst amount and their reciprocal interaction on EDLOME synthesis. Other factors are constant. Fig. 7b indicates the effects of reaction time, catalyst amount and their reciprocal interaction on EDLOME synthesis. In Fig.7c it is shown that there is a significant mutual interaction between reaction time, molar ratio (methanol to oil) on EDLOME synthesis. The optimal conditions for methyl ester synthesis from enzymatic degummed *Linum usitatissimum* seed oil as estimated by the model equation, were as follows: A (catalyst amount) = 0.6 wt%, B (molar ratio methanol to oil) = 8:1 and C (reaction time)= 60 °C. the model predicts that the maximum conversion to methyl ester that can be obtained under the above optimum conditions of the variables is 96.2 %.

Table 5 Analysis of variance (ANOVA) for the quadratic model for EDLOME Conversion %

Source of variations	Sum of squares	Degree of freedom	Mean square	F- value	Prob >F
Model	475.59	9	52.84	54.27	<0.0001
A	108.90	1	108.90	111.84	<0.0001
B	186.62	1	186.62	191.66	<0.0001
C	19.60	1	19.60	20.13	0.0012
AB	24.50	1	24.50	25.16	0.0005
AC	0.000	1	0.000	0.000	1.0000
A^2	6.37	1	6.37	6.54	0.0285
B^2	6.85	1	6.85	7.04	0.0242
C^2	69.34	1	69.34	71.21	<0.0001
Residual	9.74	10	0.97		
Lack of fit	9.25	5	1.85	18.97	0.0029
Pure error	0.49	5	0.097		
Total	485.33				

Table 6 Regression coefficient and significance of response surface quadratic model

Factor	Coefficient estimate	df	Standard error	95% CI low	95% CI high	VIF
Interceopt	89.78	1	0.34	89.02	90.53	1.00
A	-3.30	1	0.31	-4.00	-2.60	1.00
B	4.32	1	0.31	3.62	5.02	1.00
C	1.40	1	0.31	0.70	2.10	1.00
AB	1.74	1	0.35	0.97	2.53	1.00
AC	0.000	1	0.35	-0.78	0.78	1.00
BC	0.50	1	0.35	-0.28	1.28	1.00
A^2	-1.52	1	0.60	-2.85	-0.20	1.82
B^2	1.58	1	0.60	0.25	2.90	1.82
C^2	-5.02	1	0.60	-6.35	-3.70	1.82

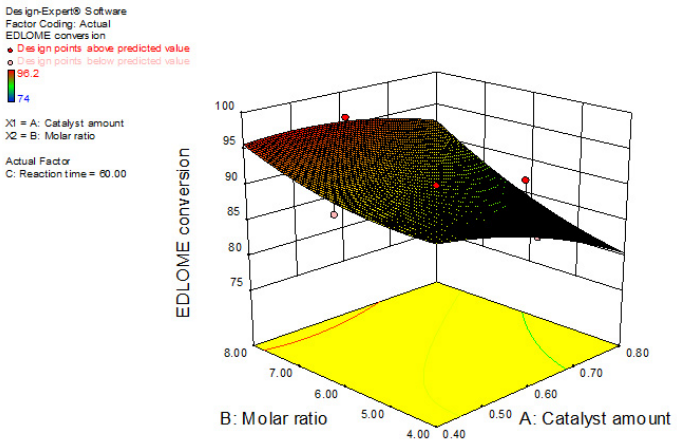

Figure 7a Response surface plots representing the effects of molar ratio (methanol to oil), catalyst amount and their reciprocal intraction on EDLOME synthesis other factor are constant.

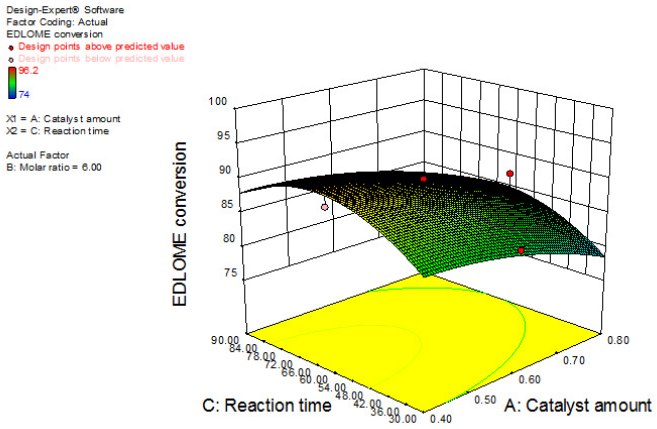

Figure 7b Response surface plots representing the effects of reaction time, catalyst amount and their reciprocal intraction on EDLOME synthesis . other factor are constant.

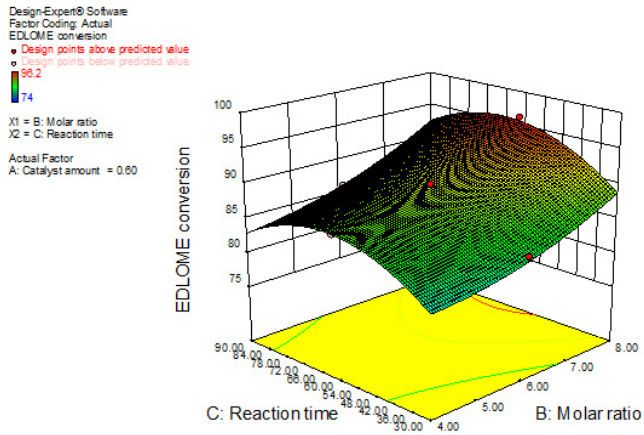

Figure 7c Response surface plots representing the effects of reaction time, molar ratio (methanol to oil) and their reciprocal intraction on EDLOME synthesis . other factor are constant.

Effect of mass ratio of catalyst to oil on esters conversion

The effect of catalyst (NaOH) content was studied in the range of 0.4% to 0.8 (wt/wt of the oil taken). It was found that the highest conversions of methyl ester from EDLO were obtained at 0.6 wt% (optimum catalyst concentration) of catalyst, and the conversion of ester increased as the amount of catalyst decreased from 0.8 to 0.6 wt%. Every reaction obtained its optimum catalyst concentration value. Beyond this optimum value, excessive catalyst will participate in saponification which reacts with triglyceride to form soap and water. Soap formation reduces the ester conversion [Hossain and Mazen, 2010].

Effect of molar ratio of methanol to oil on esters conversion

One of the most important parameters affecting the yield of ester is the molar ratio of alcohol to vegetable oil. It was found that the ester yield increased with increases in molar ratio of 4:1 to 8:1. The maximum conversion efficiency was achieved at a molar ratio of 8:1, and it was selected as the optimization ratio. Increasing alcohol amount beyond the optimal ratio does not increase the yield [Encinar et al., 2010]. However, the high molar ratio of alcohol to oil interferes with the separation of glycerol because there is an increase in solubility. When glycerol remains in the solution, it was lowering the yield of esters [Taufiq et al., 2011].

Effect of reaction time on ester conversion

It was found that the maximum conversion efficiency was achieved at 60 minutes reaction time, showing that this amount of time is sufficient to complete the methanolysis. It was noticed that for reaction times less than 60 minutes, the conversions were lower, due to incomplete methanolysis. If the reaction time was increased beyond 60 minutes, the conversion was decreased slightly. This fact can be explained by a possible higher degree of the reverse reaction [Ivanoiu, 2011].

The FTIR spectrum indicates the conversion of triacylglycerols to methyl esters, and was used as a fast and precise method for methyl ester (biodiesel) analysis. The methoxycarbonyl group in biodiesel showed a different band position. The peaks of this band changed from 1748.4 in degummed *Linum usitatissimum* oil to 1741 in biodiesel. Most prominent was the peak at 1741 cm^{-1} (C=O stretch), showing that this was an ester. The band observed at 1159 cm^{-1} in oil is observed in biodiesel at 1169 cm^{-1} and is attributed to methyl groups near carbonyl groups. The major change was also observed at 2922 cm^{-1} which is characteristic of fatty acid methyl esters. The FTIR spectrum of EDLOME is presented in Fig. 8a and 8b.

Characterization and fuel properties of methyl ester obtained from enzymatic degummed Linum usitatissimum seed oil

Density

The density of EDLOME and its diesel blends are very close, and in range of 0.853 to 0.871 (g mL^{-1}) at 15 °C. The density of the blends increases with the increase of the amount of EDLOME in the blend. Hence, the density of 10% and 20% blends was comparable to those of diesel fuel as seen in Table 7.

Viscosity

The Indian standard (IS: 1448[p: 25] 1976) was used for the range of the viscosity of diesel from 2 to 4 cSt for the use in high speed diesel engines. In this study, the viscosity comparison of the EDLOME and its blends suggest that EDLOME can be blended with diesel in proportions of 10% to 30% to meet the viscosity requirement as proposed by bureau of Indian standards, and is presented in Table 7.

Calorific value

The calorific value of diesel, Linseed oil, NDLOME and EDLOME were found as 45.71, 39.63, 41.2 and 43.5 MJ kg^{-1}, respectively. The calorific value of NDLOME increased by 3.9% more than that of Linseed

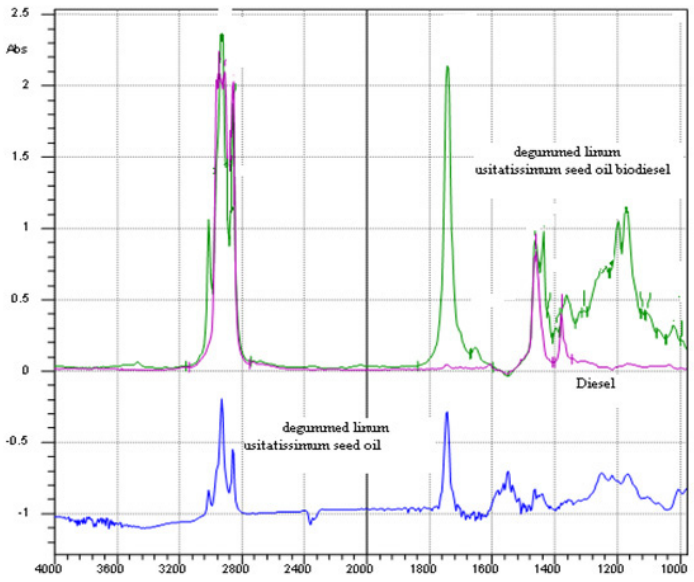

Figure 8a FTIR spectrum of Linumusitatissimum methyl ester

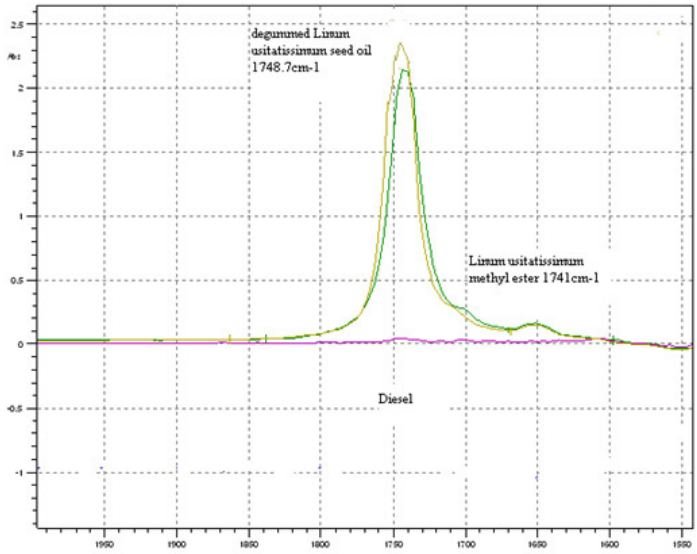

Figure 8b FTIR spectrum of *Linumusitatissimum* methyl ester

oil, whereas the calorific value of EDLOME increased by 9.8% more than that of Linseed oil. The calorific value of EDLOME was found to very close to that of diesel fuel (Table 7).

Flash and fire point

The Flash and Fire point of EDLOME was higher than those of diesel fuels, but it can be improved by blending it with diesel fuel. The flash point increased significantly for the blends of 10% and 30% is shown in Table 7.

Cloud and pour point

The results show that the cloud and pour point of EDLOME was higher than those of diesel fuel. However, it can be improved by blending it with diesel fuel, as shown in Table 7.

Table 7 Basic fuel properties of enzymatic degummed Linum usitatissimum oil methyl ester and its diesel

Fuel Parameters	CLO	EDLO	NDLOME	EDLOME	DLOME10	DLOME20	DLOME30	Diesel
Density at 15^{0}C(g/ml)	0.922	0.906	0.882	0.871	0.820	0.829	0.853	0.816
Kinematic viscosity (cSt) at 40 ^{0}C	25.7	19.7	3.5	2.9	2.5	2.6	2.8	2.4-4
Calorific value (MJ/kg)	39.63	40.00	41.2	43.5	45	44.5	44.0	45.71
Flash point ^{0}C	230	210	170	164	64	70	90	56
Fire point ^{0}C	241	220	176	168	70	84	100	68
Cloud point ^{0}C	1.7	-1	-3	-5	-6	-6	-7	-8
Pour point ^{0}C	-14	-15	-16	-18	-19	-19	-18	-20
Acid value mg KOH/g	1.9	1.4	0.36	0.37	0.3	0.3	0.3	0.22
Saponification value mg KOH/g	188	185	-	-	-	-	-	-

Conclusions

In this study the production of biodiesel from alkali-catalyzed methanolysis of crude and enzymatic degummed *Linum usitatissimum* seed oil was systematically investigated. The process parameters, such as catalyst concentration, reaction time and molar ratio of methanol to oil, were optimized on the conversion of Fatty Acid Methyl Ester (FAME).The fuel properties were determined as per the ASTM standards and procedures. As a result of the analysis carried out in this study, the following conclusions can be made:

1. It was found that the highest recovery of 96.2% of methyl ester was obtained at 8:1 molar ratio when enzymatic degummed oil was reacted with 0.6% NaOH at 60 °C for 60 minutes, and it yielded better conversion of FAME than crude Linseed oil, the optimal condition identified based on the higher % yield of methyl ester. The result of crude Linseed oil methanolysis shows that the presence of gums or phospholipid compounds decreases catalytic activity and results in a reduction in FAME conversion. Hence the degumming of crude oil is essential in improving the conversion efficiency. Degumming is not only effective to increase the FAME conversion, but also improve the basic fuel properties of biodiesel.

2. The synthesis of methyl ester (biodiesel) was confirmed by the FTIR analysis.

3. The fuel properties of enzymatic degummed *Linum usitatissimum* methyl ester (EDLOME) including density, viscosity, and calorific value of methyl esters were comparable to those of conventional diesel fuel at 20% methyl esters concentrations.

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