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Biofuel derived from enzymatic degummed *linum usitatissimum* seed oil : as a potential resource for diesel engine

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Abstract

A bio-fuel derived from enzymatic degummed *Linum usitatissimum* seed oil (EDLO). We applied response surface methodology in order to optimize the reaction conditions of degumming process using microbial enzyme (Licitase ultra) in *Linum usitatissimum* seed oil, Enzyme dosage, temperature and reaction time were important determining factors affecting oil degumming. Optimum operating conditions were found to be reaction time of 5 h, enzyme dosage of 1.6 wt% and temperature of 50°C at pH 5. At this optimum condition point the phosphorus content of degummed oil were found to be 7.8 mg/kg. This study also discusses the EDLO-diesel fuel blends as a potential source for blend production. Furthermore, the blends of various proportions of EDLO:diesel,25:75, 35:65, 45:55 and 55: 45 were prepared, characterized and compared with diesel fuel. The fuel properties of EDLO25 diesel fuel blend was found comparable to those of diesel on the basis of experimental analysis..

Keywords: Enzymatic degumming; *Linum usitatisimum* seed oil; Diesel blend; Biofuel; fuel properties

Introduction

The energy crisis contributed the development of biofuel production. Fuel crisis and environmental concerns have renewed interest of the scientific community to look for alternative fuel of bio-origin such as vegetable oils. Biofuel is a less polluting and sustainable fuel derived from renewable sources such as vegetable oils, lipids, sugar and polysaccharides (Adebayo *et al.,* 2011).The concept for producing fuel from vegetable oils for diesel engine is not radically new concept. In 1900 Mr. Rudolph diesel first tested his compression ignition engine on peanut oil as a fuel without any problem (Fangui *et al.*,1999, Parawira, 2010, Andrade *et al.,* 2011). A number of problems were encountered in using straight vegetable oil (SVO) directly as a diesel engine fuel. The high viscosity of SVOs is the main cause reported in the literature for the problems encountered in engines. High SVO viscosity results in poor fuel atomization, incomplete combustion, and deposits in the combustion chamber (Pramanik, 2003). There are many appropriate methods for utilizing vegetable oils as fuels for diesel engines, such as dilution, blending, heating, transesterification and emulsification (Rathore *et al.,* 2007) and degummed vegetable oil. Among these appropriate methods, degummed *Linum usitatissimum* seed oil is considered as an alternative fuel.

Linum usitatissimum seed oil in India

Linseed oil, its scientific name is *Linum usitatissimum,* belongs to the family Linaceae and the genus *Linum*. It originated and is cultivated in Canada, Argentina and India (Minor vegetal, 2011, Linseed basic, 2011). India is considered as the third largest producer of linseed in the world. In India linseed is mainly cultivated as rabi crop in with October-November being the main sowing season (Puhan *et al.,* 2010). The various Indian states have sufficient forest area for the plantation of Linseed such as Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Karnataka and West Bengal. Madhya Pradesh the center part of India is the leading producer of this crop. Madhya Pradesh and Uttar Pradesh together contribute to the national linseed production to the extent of about 70 per cent. (Linseed basic, 2011, Dixit *et al.*, 2012). Fig. 1 shows the production of Linseed in India (IOPEPC,2013)

Influence of phospholipids (phosphorus) on vegetable oil based fuels

The phosphorus content in oil indicates the presence of phospholipids (gums). This parameter is very important for fuel use Indeed, phospholipids are responsible for the fouling of

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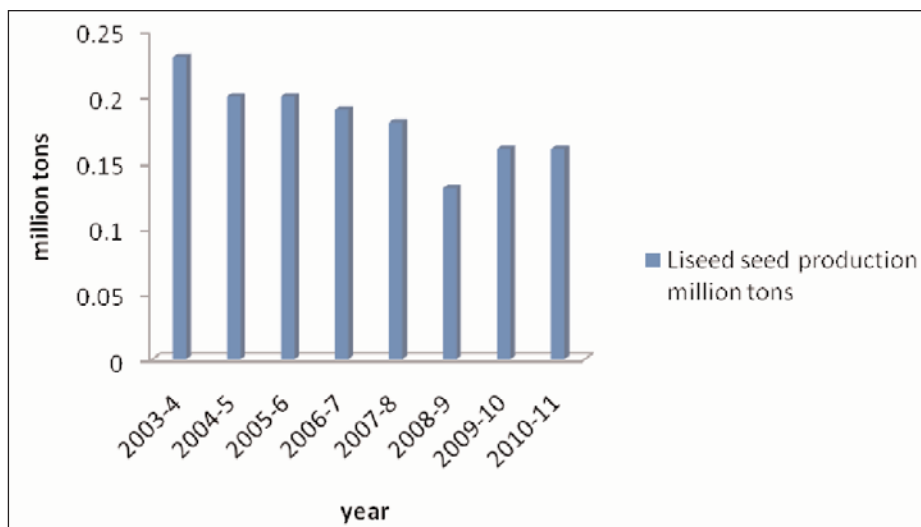*Corresponding author. e-mail: skskanakraj5@gmail.com

**Fig. 1. Linseed seed production in India from 2003-2011(IOPEPC,2013)**

valves, combustion chamber and cylinders when straight vegetable oils are used (gumming phenomenon) (Zufarov *et al.*,2008,Sidibe *et al.*, 2010). Gums can cause problems during the transportation of fuels by settling and forming deposit on tanks walls or clogging pipelines (Fugue *et al.,*1999, Srivastava *et al.*, 2000, Mullanix,2011). In engine where vegetable oil is used, excess phosphorus (gums) in the fuel can cause damage to catalytic converters since it can coat the inside of the converters or coking within the engine (Mullanix, 2011, Xiaohu *et al.,* 2010) several researchers have also reported that formation of deposit in diesel engines (Ali *et al.,* 1997, Zufarov *et al.*, 2008). Therefore, it must be removed from the oil through an appropriate degumming process.There are several traditional degumming concepts have been introduced in the recent years to refining of vegetable oil including water degumming. Total degumming, ultra filtration process, acid degumming etc. cannot always achievement of phosphorus contents required for physical refining and are not always optimally suited for all oil qualities (Yang *et al.,* 2006) Unfortunaty these methods have several disadvantages including corrosion of processing equipment; the yield loss, the equipment required and the energy expenditure of the process are also high (Prabharan *et al.,* 2009). Enzymatic degumming is a suitable process for refining of oil. It is cost effective and very simple in opration as compared to traditional degumming process, this process does not alter the fatty acid composition of the oil (Yang and Yang *et al.,* 2006) simple water degumming will not remove non-hydratable gums hence this degumming can be removed all type of

gums from oil. This degumming has only been reported for rapseed, soyabean, sunflower and rice bran oils (Roy *et al.,* 2002). But no work has been done for *Linum usitatissimum* seed oil. In this research, an attempt has been made to consider enzymatic degummed *Linum usitatissimum* seed oil as a fuel for compression ignition engines.

Chemistry of gum and enzymatic degumming:

Gums

Oil & Fats contain complex organo-phosphorus compound referred to as phospholipids (phosphatides). All Vegetable Oils contains two types of gums (phospholipids), hydratable phospholipids (HPL) and non-hydratable phospholipids (NHPL), and they are removed from oil by degumming process (Zufarov *et al.,* 2008). However, the amount of hydratable and non-hydratable gums vary significantly depending on a number of factors such as seed quality, seed type and oil milling conditions, but crude vegetable oil contains roughly 90 % hydratable gums while the remaining 10 % consists of non- hydratables. The basic structure of the phosphatides (phospholipids) given in Fig. 2. Phosphorus content in vegetable oils is analyzed and expressed in parts per million (ppm). This value is multiplied by the factor 25 to give the phosphatide content of the oil. This factor is derived from the ratio of the specific weight of phosphorus and the phosphatides (Hmm *et al.,* 2002). *Linum usitatissimum* seed oil contains a high amount of phospholipids. The most important members of the lipids class found in this oil are phosphatidylethanolamine (PE) phosphatidyli
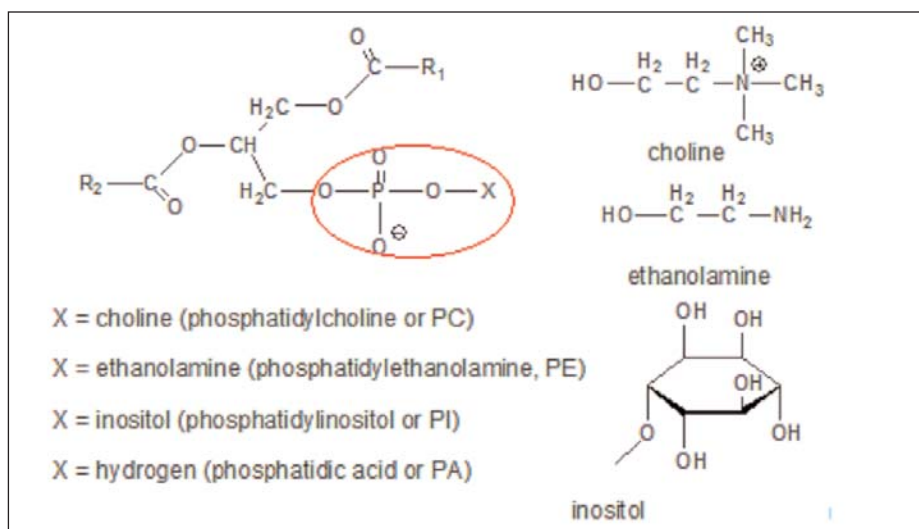
Fig . 2. Chemical structure of phospholipids

nositol (PI) and phosphotidylcholine (PC) (Heechi *et al.,* 2012). Most of these (PC and PI phospholipids) are completely hydratable phospholipids and PE is partially hydratable. The objectives of the present work to analyze the effect of enzymatic degumming on reducing phospholipids from crude *Linum usitatissimum* seed oil and also discusses the EDLO- diesel fuel blends as a potential source for blend production.

Enzymatic degumming

In this process the Lecitase - Ultra (phospholipase A) hydrolyzed the Carboxylic group at C1 Position and non-hydratable or partially hydratable phospholipids in oil can initially be converted to fully hydratable phospholipid (lyso-phopholipids). The enzymatically hydrolyzed lysophospholipids are hydrophilic and mostly soluble in water.

Materials and methods

Materials

Mechanically expelled *Linum usitassimum* seed oil used in this experiment obtained from local market, commercially available diesel fuel was purchased from nearby petrol pump. Lecitase ultra (phospholipase enzyme) used as a degumming agent was purchased from Zytex biotech private limited Mumbai India. Other reagents were of analytical grade.

2.2 Experimental setup

The reactor used for experiments was a 500 ml three-necked glass flask. The flask is placed on a water bath. The center neck is fitted with a stirrer. One of the two side necks is equipped with a thermometer and the other is closed by a glass stopper. The set up is shown in Fig. 3.
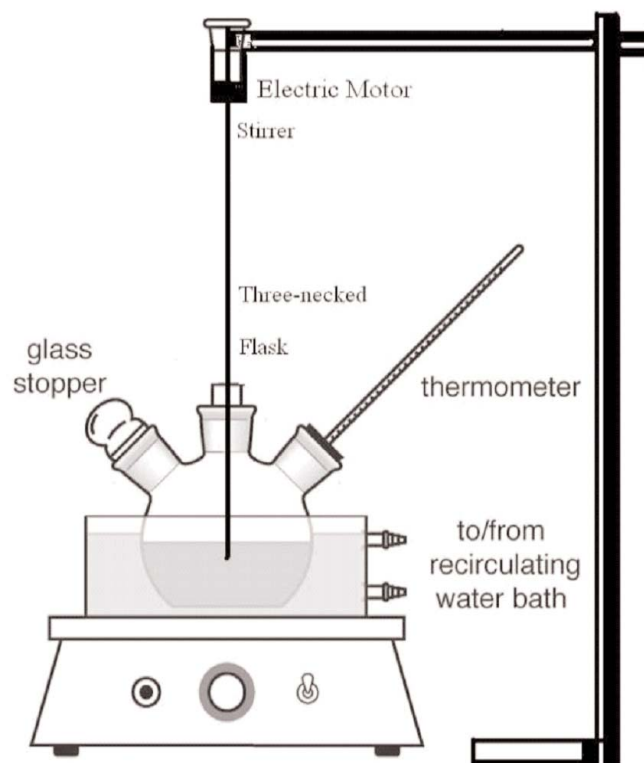
Fig. 3. Experimental set-up for preparation of degummed *Linum usitatissimum* seed oil

Methodology

Enzymatic degumming of Linum usitatissimum seed oil

Measured quantity of *Linum usitatissimum* seed oil is taken in 500 ml conical flask, and enzyme solution (100 ml buffer solution is prepared by dissolving 4 gm of citric acid and 1.4 gm of caustic (NaOH), the PH is adjusted to 4.8 to 5 and 350 to 400 mg of enzyme is added to the buffer solution) is taken by pipette and added to the oil in different wt% (0.6-2.2 wt%) by using high shear mixer for 90 sec. the reaction mixture is transfer into the three-necked glass flask .The flask is kept in the water bath with slow agitation for incubation at temperature 40 to 50 °C with slow agitation for 3-5 hours. After incubation the oil is heated to 75 to 80°C for 15 min to deactivate the enzyme. After deactivation of the enzyme, the oil was heated to 60°C, and 1wt% of hot deionized water is added into the reaction flask. The temperature of the system was maintained at 55°C for 15 minutes while the stirrer operating at 400 rpm. The mixture was then transferred to a centrifuge. Degummed oil was obtained after centrifuging for 1 h at 4500 rpm. Samples were drawn for phosphorus analysis Fig. 4. The optimal condition was identified based on the minimum residual phosphorus content in the degummed oil.

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. A CCD was applied three design factors, namely, Enzyme amount (A), Reaction temperature (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 X 3=6: axial points) + 6 (center points: six replications). Table 1 describes the coded and uncoded levels of the independent variables, the test ranges for the parameter. The Design expert 8.0 software was used for regression and graphical analysis of the data obtained. The residual phosphorus content in the degummed oil was taken as the responses of the design experiment. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). Once the experiments are performed, the response variable was fitted a second- order model in to correct the response variable to the independent variable. The general form of the second-degree polynomial equation (Youn *et al.,* 2008).

$$Y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k-1}\sum_{j=2}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + e$$
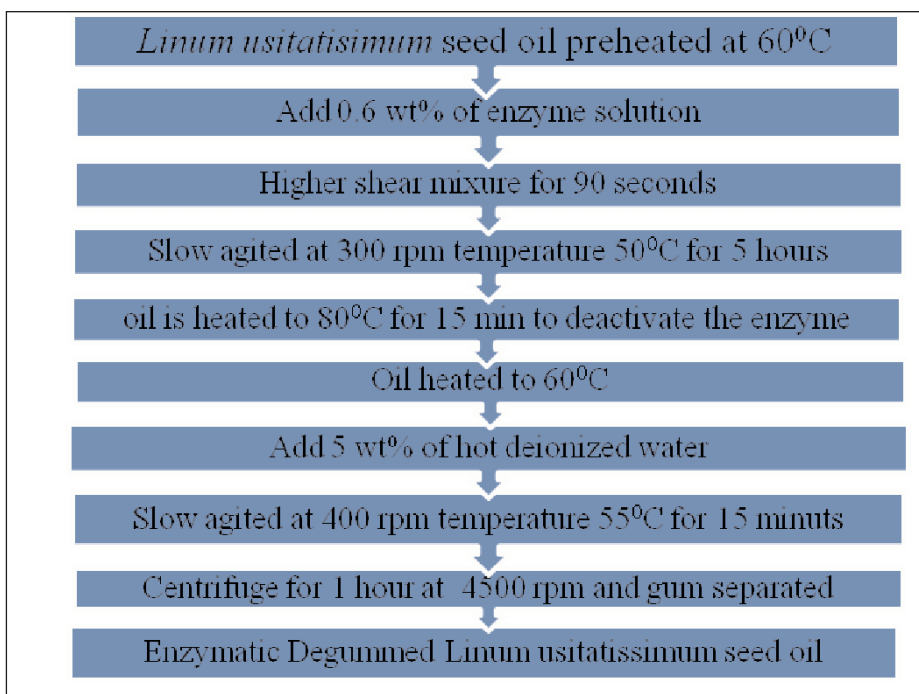
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Fig. 4. Schematic diagram of simple procedure developed for the synthesis of enzymatic degummed *Linum usitatissimum* seed oil

Table I. Factor and their level for response surface design

Factors	Symbol Range	and levels		
		-1	0	+1
Enzyme amount Wt%	A	0.6	1.6	2.2
Reaction Temperature	B	40	45	50
Reaction time Hour	C	3	4	5

Where Y is the predicted response β_0 and the offset term, β_i the linear effect, β_{ij} the squared effect and β_{jj} is the interaction effect , n is the number of significant independent variable A, B and C(Jeong *et al.,* 2009).

Enzymatic degummed Linum usitatissimum seed oil and diesel fuel blending

Different blends of EDLO with conventional diesel fuel were prepared. The fuel properties of the EDLO and diesel fuel are presented in Table 6. The EDLO was blended with diesel fuels at 25%, 35%, 45% and 55% on a volume basis. In order to determine the properties of the diesel fuels, the EDLO and its diesel blends, the following test methods were used, Density at 15°C (IS: 1448:[P:16]:1990), Viscosity at 40°C (IS:1448:[P:25]:1976), 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 2500).Some EDLO-diesel blends given below in Table II. For proper mixing of EDLO with diesel fuel, it important to first pour the diesel fuel in the container and then the EDLO is added. Stirring should be done for few seconds. Blending improves fuel properties to suit the CI engine operation.

Table II. EDLO -diesel blends

Sample	Blending with diesel
EDLO25	25% EDLO and 75% diesel
EDLO35	35% EDLO and 65% diesel
EDLO45	45% EDLO and 55% diesel
EDLO55	55% EDLO and 45% diesel

Results and discussion

3.1Effect of Enzymatic degumming method on phosphorus (phospholipids) content in Linum usitatissimum seed oil

The amount of phospholipids was determined as the total phosphorus in oil according to AOCS official method ca 12-55. ASTM D 6751-09 requires the maximum amount of 10 ppm phosphorus content in the final biodiesel fuel. Degumming was removed phospholipids (phosphatides) and lowered down phosphorus content in the *Linum usitatissimum* seed oil.The residual phosphorus content in enzymatic degummed oil is about 7.8 ppm after enzymatic degumming which meet the phosphorus level requirement as proposed by the ASTM standard. Straight LO presents the highest phosphorus concentration (100 ppm) Phosphatide content in EDLO.

Optimization of Reaction condition of Response surface Methodology for EDLO synthesis.

Table III describes the experimental parameters and their results, on the basis of the CCD experimental design. Regression analysis yield three linear coefficients (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 IV). Table IV also describes the ANOVA for the response surface quadratic model.

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

Run	Variable in coded levels			Enzyme amount Wt%	Reaction temperature °C	Reaction time hour	Phosphorus (mg/kg) Y
	A	B	C				
1				1.6	45	4	14.2
2				1.6	40	4	16.5
3				1.6	50	4	13.2
4				1.6	45	4	14
5				0.6	50	5	11.9
6				2.2	40	3	19.8
7				2.2	50	5	7.8
8				0.6	40	5	16
9				1.6	45	4	14.2
10				0.6	50	3	17
11				1.6	45	3	17
12				1.6	45	4	14.1
13				1.6	45	5	9.6
14				2.2	50	3	15
15				2.2	45	4	14.1
16				1.6	45	4	14
17				2.2	40	5	12.1
18				0.6	40	3	25
19				0.6	45	4	18
20				1.6	45	4	14.2

Table IV. Analysis of variance (ANOVA) for the quadratic model

Source of variations	Sum of squares	Degree of freedom	Mean square	F- value	Prob >F
Model	247.96	9	27.55	96.13	<0.0001
A	36.48	1	36.48	127.29	<0.0001
B	60.87	1	60.87	121.38	<0.0001
C	131.72	1	131.72	459.57	0.0001
AB	1.43	1	1.43	4.98	0.0497
AC	0.085	1	0.085	0.30	0.5979
BC	2.42	1	2.42	8.44	0.0157
A^2	4.66	1	4.66	16.27	0.0024
B^2	0.96	1	0.96	3.35	0.0971
C^2	2.53	1	2.53	8.83	<0.0140
Residual	2.87	10	0.29		
Lack of fit	2.82	5	0.56	58.30	0.0002
Pure error	0.048	5	9.66E-003		
Total	250.83	19			

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

$$Y=14.56-1.91A-2.47B-3.63C+0.42- 0.10AC+0.55BC$$
$$1.40A^2+0.59B^2-0.96C^2 \quad\quad ……………..\ (2)$$

In which Y is the response factor, (EDLO) content wt%, A, B, C are the value of the three independent variable, enzyme amount (wt%), Reaction temperature and reaction time (minutes). The model coefficient values are provided in Table V. The 3D response surface plots are generally represent the graphical representations of the regression equation are presented in Fig. 5 (a,b,c,). The 3D response surface in Fig. 5(a) show that there is a response surface plots represent

ing the effects of reaction temperature, enzyme amount and their reciprocal interaction on EDLO synthesis. Other factor is constant. Fig. 5b indicated that the effects of reaction time, enzyme amount and their reciprocal interaction on EDLO synthesis and Fig. 5c shows that there is a significant mutual interaction between reaction time, reaction temperature on EDLO synthesis. The optimal conditions for EDLO synthesis from crude *Linum usitatissimum* seed oil as estimated by the model equation, were as follows: A (enzyme amount) = 1.6 wt% ,B (Reaction temperature)= 50 and C (reaction time)= 5h. The model predicts that the minimum phosphorus content in degummed oil that can be obtained under the above optimum conditions of the variables is 7.8 mg/kg.

Fuel related properties of EDLO- diesel blends

Density

In this study the density of EDLO- diesel blends are very close to each other and range from 0.849 to 0.890 (g/ ml) at 15 ºC and they are slightly higher than those of the diesel fuel as seen in Table III Fig.6a, presents the density values of EDLO diesel blends. There is a linear increase in the density of the blends as the amount of EDLO increases. Hence, the density of 25 % EDLO is comparable to that of diesel fuel.

Kinematic viscosity

The viscosity of the blend increases as the EDLO amount increases in the fuel mixture as observed from Fig. 6b the
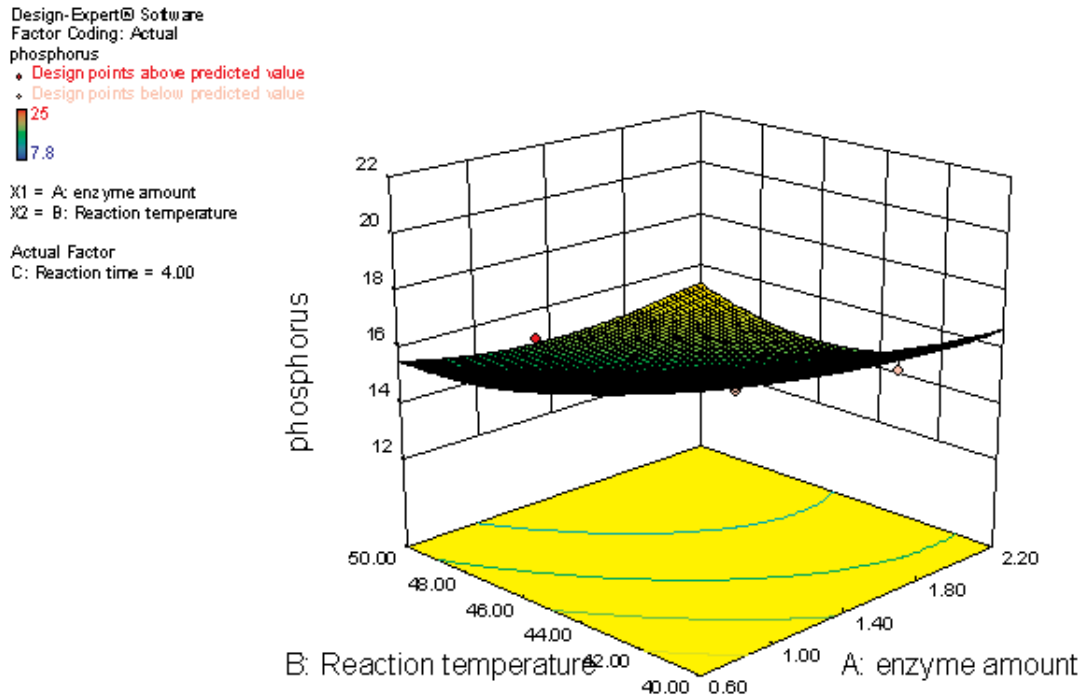
Table V. Regression coefficient and significance of response surface quadratic model

Factor	Coefficient estimate	df	Standard error	95% CI low	95%CI high	VIF
Intercept	14.56	1	0.20	14.13	15.0	1.03
A	-1.91	1	0.17	-2.29	-1.53	1.00
B	-2.47	1	0.17	-2.85	-2.09	1.00
C	-3.63	1	0.17	-4.01	-3.26	1.00
AB	0.42	1	0.19	6.290E-004	0.84	1.00
AC	-0.10	1	0.19	-0.52	0.32	1.00
BC	0.55	1	0.19	0.13	0.97	1.00
A^2	1.40	1	0.35	0.63	2.17	1.85
B^2	-0.59	1	0.32	-0.13	1.31	1.82
C^2	-0.96	1	0.32	-1.68	-0.24	1.82

Fig. 5(a). Response surface plots representing the effects of reaction temperature, enzyme amount, and their reciprocal interaction on EDLO synthesis. Other factor is constant
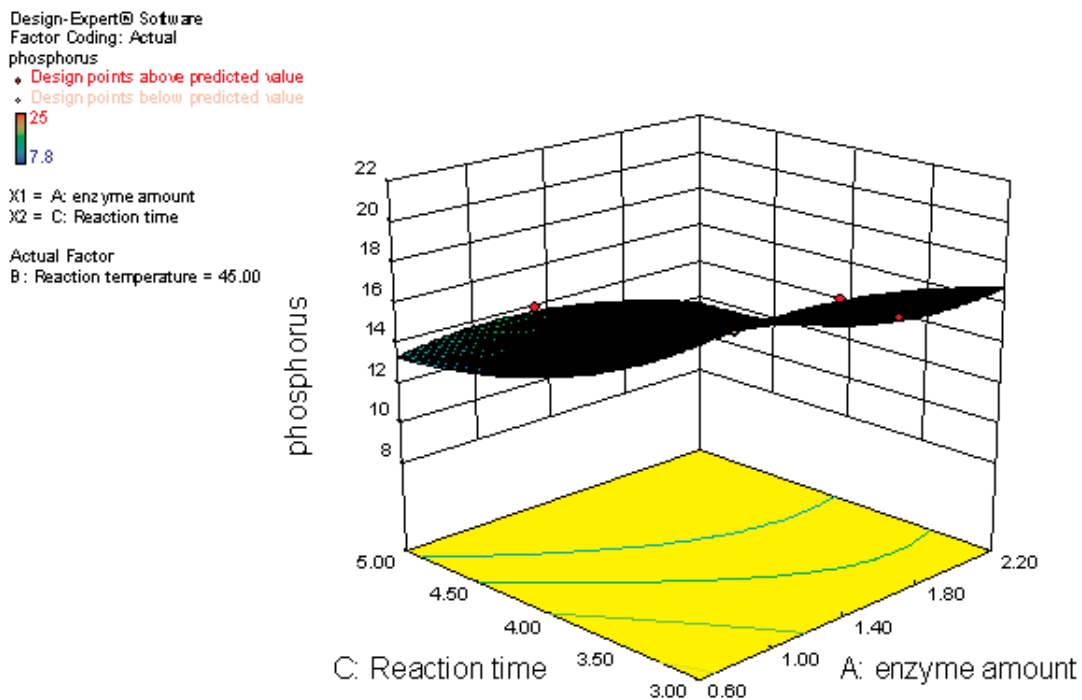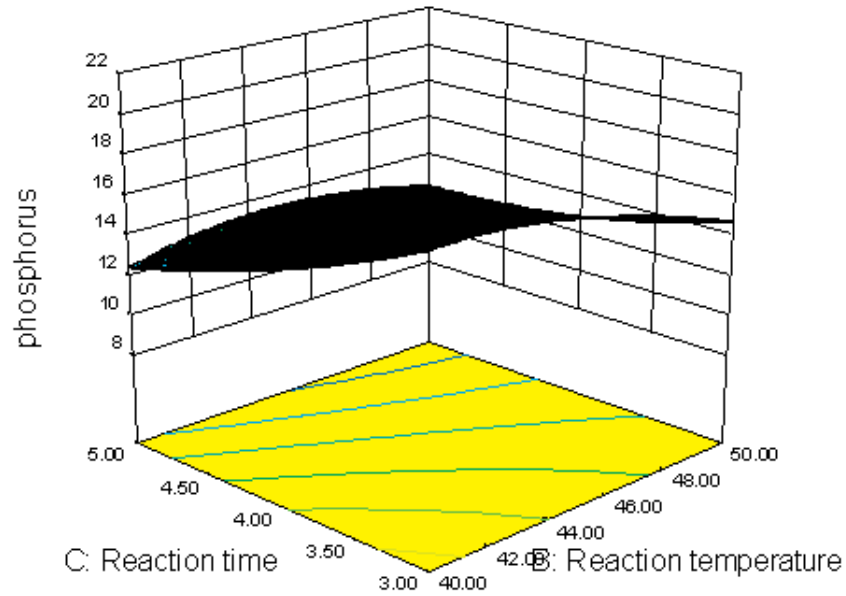

Fig. 5(b). Response surface plots representing the effects of reaction time, enzyme amount and their reciprocal interaction on EDLO synthesis. Other factor is constant

Fig. 5(c). Response surface plots representing the effects of reaction temperature, reaction time and their reciprocal interaction on EDLO synthesis. Other factor is constant

viscosity of EDLO was observed to be higher than that of diesel fuels but the viscosity of 25% EDLO is very close to that of diesel fuel as given in Table III. The Indian standard (IS: 1448 [p: 25] 1976) recommends the range of the viscosity of diesel from 2 to 4 cSt for the use in high speed diesel engines. Thus the comparison of the viscosity of EDLO and its blends suggests that EDLO can be blended with diesel in proportion of 25 % to meet the viscosity requirement as proposed by Bureau of Indian Standards (IS: 1448 [p: 25]).
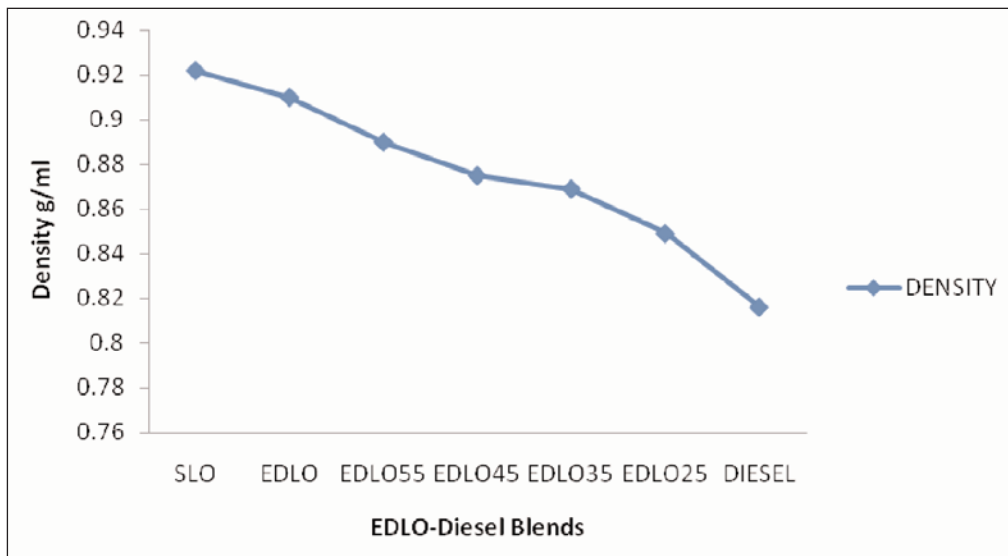
Fig. 6a. Density of EDLO -diesel fuel blends
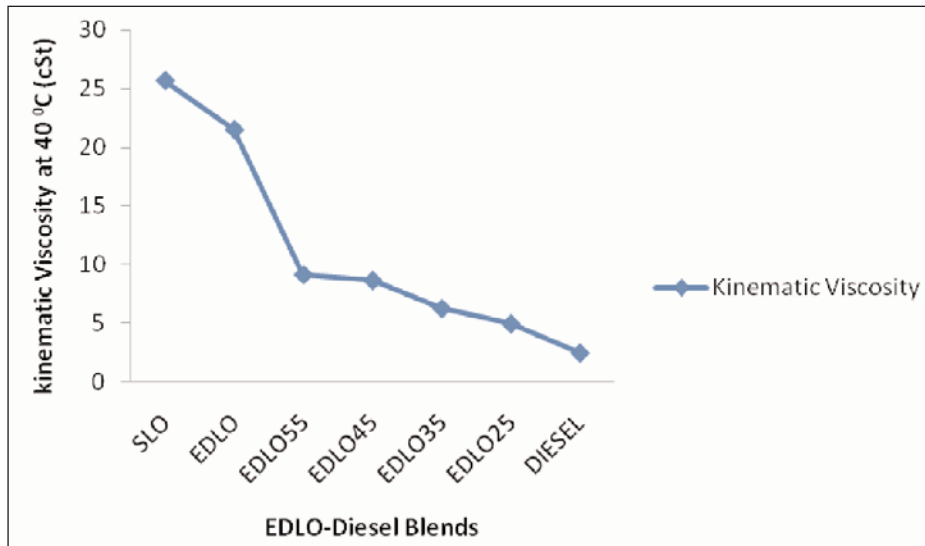
**Fig. 6b. KinematicViscosity of EDLO-Diesel**

Cloud and Pour point

The cloud and pour point are of great importance to engine operating in cold climate. Both properties may also indicate the tendency towards filter plugging and flow problems in the fuel line. The cloud and pour points of the blends demonstrated an increase as the concentration of EDLO increases in relation to the diesel fuels, as given in Fig.7a. According to these results, the cloud and pour point of EDLO can be reduced significantly by blending it with diesel fuel, which means that the fuel flow will be improved in the fuel injection system at low temperature.

Flash and fire points

These are the properties, which shall be considered in assessing the flammability and hazard of fuel. The flash and fire point of EDLO are much higher than those of diesel fuels but it's both flash and fire point of diesel can be improved by blending it with EDLO as shown in Table III and Fig.7b.
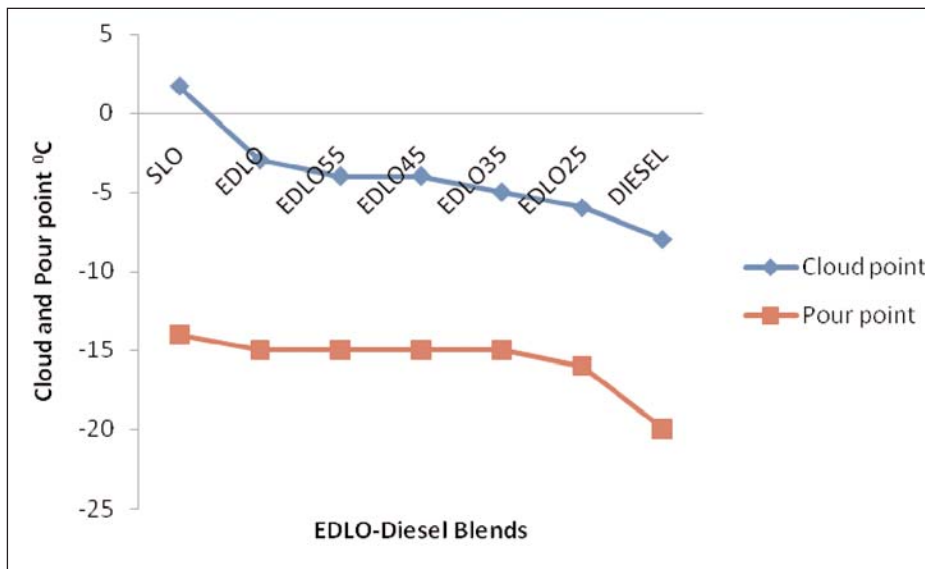
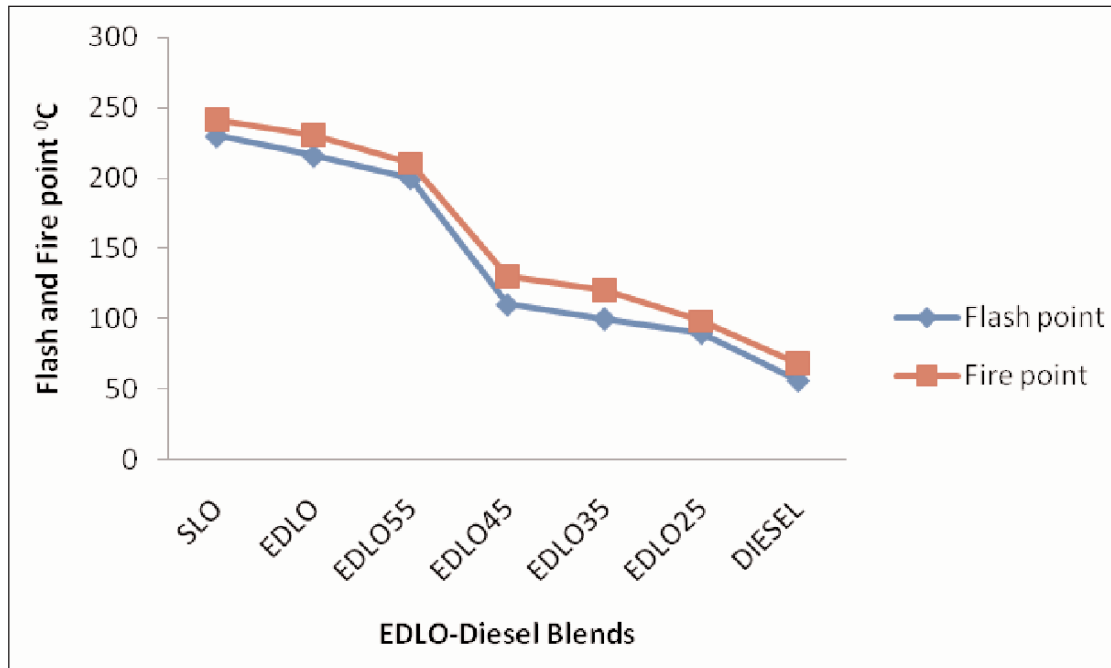**Fig.7a. Cloud and Pour point of EDLO-diesel blends**

Fig.7b. Flash and Fire point of EDLO-diesel blends

Table VI. Fuel properties of Diesel,Straight *Linum usitatissimum* seed oil and EDLO -diesel blends

| Fuel parameter	Straight *Linum usitatissimum* seed oil (SLO)	Enzymatic degummed *Linum usitatissimum* seed oil (EDLO)	EDLO- Diesel blends				Diesel
			EDLO55	EDLO45	EDLO35	EDLO25	
Density at 15°C(g/ml)	0.922	0.910	0.890	0.875	0.869	0.849	0.816
Kinematic viscosity (cSt) at 40 °C	25.7	21.5	9.1	8.6	6.2	4.9	2.4-4
Calorific value (MJ/kg)	39.63	40.1	40.9	41.45	41.65	41.95	45.71
Flash point °C	230	215	200	110	100	90	55
Fire point °C	241	225	210	130	115	95	68
Cloud point °C	1.7	-3	-4	-4	-5	-6	-8
Pour point °C	-14	-15	-15	-15	-15	-16	-20
Acid value mg KOH/g	1.0	0.85	0.84	0.84	0.84	0.84	0.22
Saponification value mg KOH/g	188	182					
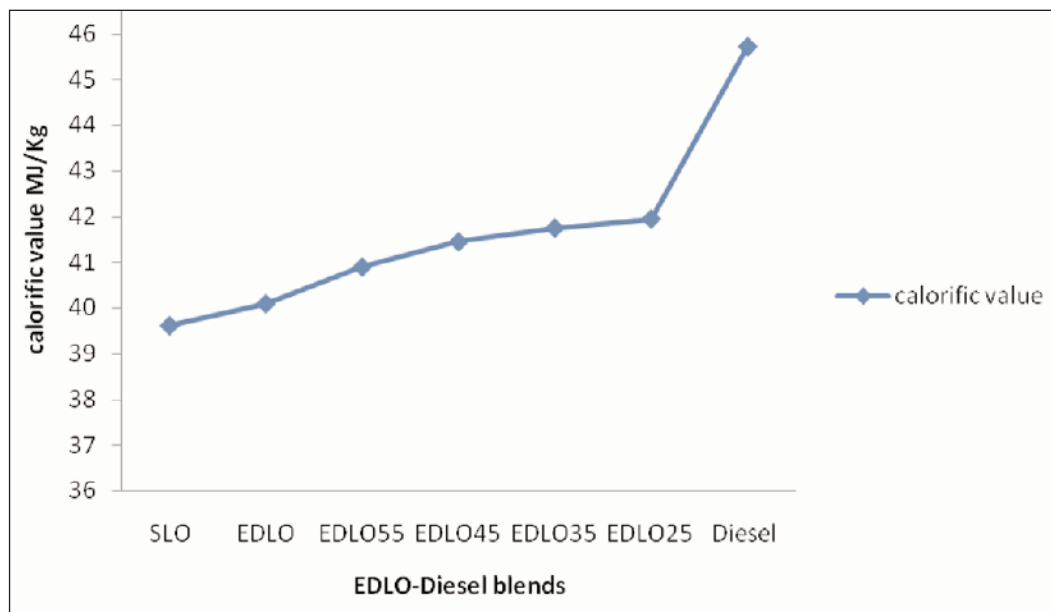
Fig. 8. Calorific value of EDLO -diesel fuel blends

Calorific value

The calorific value is an important property defining the energy content and thereby the efficiency of fuels. Calorific value for the EDLO- diesel blends was observed. It was found that EDLO25 blends calorific value is very close to that of diesel fuel. As observed in Fig.8

Conclusion

The enzymatic degummed *Linum usitatissimum* seed oil is chosen as a potential non-edible vegetable oil for the production of biofuel. This process not only reduced the phosphorus (gm) content of the *Linum usitatissimum* seed oil from 100 ppm to 7.8 ppm but also it can prove to be an effective method of enhanced its fuel properties. As a general conclusion, the enzymatic degumming is an environment friendly method for the removal of gums from vegetable oil and it is suggested as a way to use non-esterified *Linum usitatisimum* seed oil in diesel engine. India is considered as the third largest producer of Linseed (*Linum usitatissimum* seed) in the world. Madhya Pradesh the central part of India is the leading producer of this crop. In the near future, Enzymatic degummed *Linum usitatissimum* seed oil can be expected as a potential energy resource for diesel engine in India.

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