

Article

Enzymatic Production of DMC-BioD from Different Plant Seed Oil Using Dimethyl Carbonate

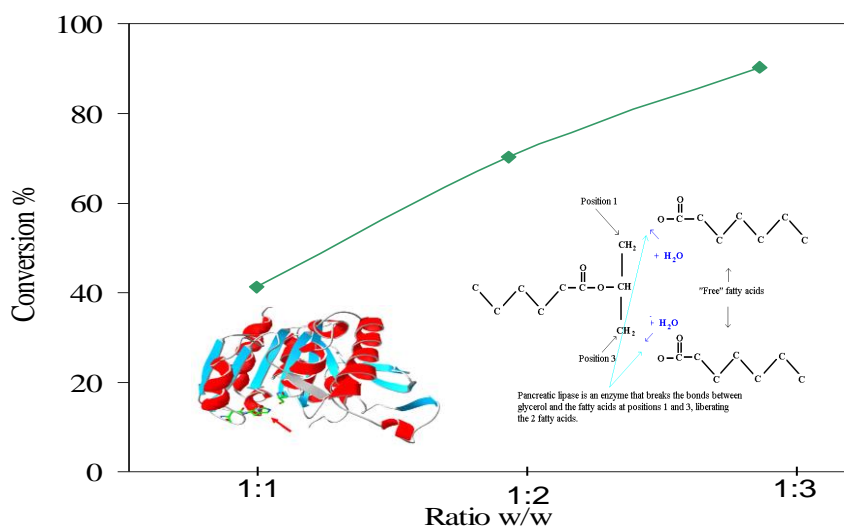
Balaji Panchal *, Sanjay Deshmukh and Munish Sharma

Nurture Earth R and D Pvt. Ltd. MIT campus, Beed Bypass Road, Aurangabad-431028 M.S. India

* Author to whom correspondence should be addressed; E-Mail: panchalbalaji@yahoo.co.in

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Abstract: Enzymatic transesterification by using lipase as catalyst has become more attractive for the production of biodiesel due to easy separation of products such as glycerol and methyl-esters. Biocatalytic synthesis is a promising environmentally friendly process for biodiesel production, from renewable plant resources. The maximum yield of different plant seed oil biodiesel could reached up to $93 \pm 0.02\%$ with the optimal reaction conditions: oil to dimethyl carbonate ratio of 1:3w/w (100g oil: 300g DMC), 200mg of lipase mass ratio based on oil basis, reaction time 6h and agitation speed 300rpm. Properties of biodiesel as measured according to accepted methods were found to satisfy nearly all prescribed ASTM D 6751-02 specification, where applicable. Aim of the present research study was to produce biodiesel by environmentally safer method and low cost.



Keywords: DMC-BioD; porcine pancreatic lipase; seed oil, dimethyl carbonate.

1. Introduction

Biodiesel is the name given to any diesel equivalent biofuel which can be used in an unmodified diesel engine vehicle. Biodiesel is mixture of alkyl esters of fatty acids derived from the transesterification of vegetable or animal fats with alcohol (Parichart et al., 2011). It contains mono-alkyl esters of long-chain fatty acids, preferentially methyl and ethyl esters, derived from renewable feedstocks, such as vegetable oils, palm fatty acid distillate (PFAD), animal fats, rendered animal fats, grease, waste cooking oil, recycled vegetable oil, soya acid oil, palm acid oil etc. The environmental problems caused by using organic solvents and the high operating costs related to the severe reaction conditions (Raghunath et al., 2008; Bobade and Khyade, 2012). It has been reported that 60-90% of biodiesel cost arises from the cost of the feedstock (Raghunath et al., 2008).

Among these alternative sources, vegetable oils have gained considerable attention, since they are derived from renewable resources, can be domestically produced and not as harmful as petroleum to the environment (Demirbas, 2009). Biodiesel has a superior lubricates to petrodiesel and hence its addition allows the overall reduction of sulfur in the fuel to almost nil (Drown et al., 2001)

Enzymatic transesterification of triglycerides has a good alternative to chemical processes due to its eco-friendly, selective nature and low temperature requirements (Du et al., 2005; Gnanaprakasam et al., 2013). Thus, many investigators have intensively studied enzymatic processes for biodiesel production to solve those problems (Bobade and Khyade, 2012). In the last few years the study of the enzymatic production of biodiesel, encouraged by pollution and by product separation problems of the chemical catalyzed process has shown significant progress (Zhang et al., 2003). The interests for using lipases as biocatalyst for oil transesterification are due to the high efficiency, selectivity, facilitation of glycerol removal and biodiesel purification, toleration of water into the oil, low energy consumption and low waste amounts (Akoh et al., 2007).

Over 23000ha of land in India is devoted to production of the fruits, a 10 fold increase from just 20 years ago (Kamala Jayanthi and Verghese, 2010). The oil content of the *Citrus maxima* seeds was 34% (Okoye et al., 2011). Oil having high percentages of peroxide is unstable and grows rancid easily (Nzikou et al., 2007). Free fatty acid (FFA) concentration of the *Citrus maxima* seed oil reaches 25.70% (Okoye et al., 2011). *Citrus limetta* and *Citrus limon* belong to the Rutaceae family. The *Citrus limetta* seed oils mainly consisted of linoleic acid (36.1-39.8%), palmitic acid (25.8-32.2%), oleic acid (21.9-24.1%), linolenic acid (3.4-4.4%) and stearic acid (2.8-4.4%) (Anwar et al., 2008). *Citrus limon* is

important fruit of genus *Citrus* (Kamal et al., 2011). Maximum percentage oil recovered was higher than 36% and 36.5% reported for unfrosted *Citrus Sinesis* seed (Nwobi et al., 2015). Wastes from industrial processing are composed of peels, seeds and pulps representing 45 to 58% of the raw processed fruit (Reazai et al., 2014). Researchers on the chemical composition of the *Citrus limon* seed oils of various citrus species like *Citrus yuko*, *Citrus sudachi*, and *Citrus junos* have been carried out (Amran et al., 2009). *Cucurbita maxima* D. oil content ranged from 10.9 to 30.9% (David et al., 2007). The *Cantaloupe melon* and *Citrullus colocynthis* L. belong to the Cucurbitaceae family. *Citrullus colocynthis* L. the predominant fatty acid was linoleic acid (18:2) in 62.2%. The presence of other fatty acids ranged in 10-14% for oleic acid (18:1), stearic acid (18:0) and palmitic acid (16:0), respectively (Mirjana and Ksenija, 2005). *Annona squamosa* belongs to the family Rubiaceae. *A. squamosa* fruits containing hard, brown or black bean-like glossy seeds (Amod et al., 2008). *A. squamosa* seeds potent source of biodiesel (Sharma et al., 2012). It has been reported that *A. squamosa* seeds contain 23% oil of which 9.8% is a hydroxyl acid, the oil contains 38.6% saturated fatty acids and 61.4% unsaturated fatty acids (29.0% oleic and 32.0% linoleic) (Mariod et al., 2011).

Dimethyl carbonate (DMC) is a nontoxic and biodegradable molecule with versatile reactivity (Tundo and Selva, 2002). Glycerol is not synthesized during the conversion of oil and DMC for the preparation of biodiesel (Liping et al., 2010). DMC is promising alternative solvent for methanol for biodiesel production.

The present research aims to investigate the DMC is used in biodiesel production. Also investigate the research was to production of biodiesel by environmentally safer method. Characterizations of DMC-BioD and compare it with existing standards.

2. Experiments

2.1. Materials and Reagents

Citrullus colocynthis L, *Cantaloupe melon*, *Citrus maxima*, *Citrus limetta*, *Citrus Limon*, *Manikara zapota*, *Cucurbita maxima* D and *Annona squamosa* fruits were purchased from a local market in Aurangabad (MS), India. *Porcine pancreatic* lipase enzyme purchased from SRL chemicals, Mumbai, India. All chemicals used in the experiments such as dimethyl carbonate (DMC), hexane, toluene and iodine crystal were of analytical reagent (AR) grade.

2.2. Oil Extraction from Different Plant Seeds

The outer dried surrounding pith of the fruit was removed and the seeds were dried in an oven at 40 °C for 24h (Malacrida et al., 2011). The dried seeds were cracked manually, the shells carefully

removed and the kernels thus obtained were crushed and ground into fine powder with a mixer grinder. The ground kernels with particle size of 0.5mm were used for oil extraction. These seeds powder were extracted using hexane solvent for 6h with ratio of different plant seed powder weight 100g (in equal weight 14.32g of each seed) to solvent volume (600ml) of 1:6w/v. The mixture was then filtered twice using glass wool. The clear filtrate was concentrated using a rotary evaporator to remove the solvent from the oil. Then the oil was weighed to calculate the concentration of oil in the solution. Extractions were conducted at five temperature level (30, 35, 40, 45 and 50 °C).

2.3. Enzyme Catalyzed Transesterification

Extracted oil of different plant seeds to DMC ratio have been taken in a glass reactor (1:3w/w) to this mixture, 10ml of hexane and 200mg of enzyme (*Porcine pancreatic lipase*) as a catalyst have been added and incubated with constant stirring at 300rpm. The reaction condition has been optimized by carrying out different sets of experiments with varying of oil to dimethyl carbonate ratio, enzyme concentration, reaction time and agitation speed. The product formation has been monitored by Gas chromatography (GC). At the end of reaction, the content has been dissolved with hexane (30ml). Hexane was added to the reaction mixture to increase the solubility of the reactants. The reaction mixture was washed with distilled water to remove the glycerol and excess DMC. The methyl-ester phase was then dried by using anhydrous sodium sulphate. Hexane was removed by keeping the dried sample in the open air for one to two days. The yield of DMC- BioD was calculated using the following formula;
$$\text{Yields of DMC-BioD (\%)} = (\text{Grams of DMC-BioD produced}) / (\text{Grams of oil used in reaction}) \times 100$$

2.4. Analysis of Different Plant Seed Oil into DMC-BioD

The transesterification of different plant seed oil into its DMC-BioD was evaluated using Gas Chromatograph (Make-Netel India Ltd.) using EN14103 test method (Nakpong and Wootthikanokkhan, 2010). The injection port, column and detector were heated to a pre-specified temperature of 140 to 240 °C in 20minutes and then they remained at 240 °C for 10min. Since the mobile phase was a carrier gas, the components present in the analytical mixture should be vaporized, so that it can be effectively carried through the column. The biodiesel sample was prepared by diluting 250mg DMC-BioD with 5ml methyl hepta deconate standard solution (500mg per 50ml hexane) in a small vial. 1.0µl of the diluted sample was injected into Flame Ionization Detector (FID) for methyl-ester analysed.

2.5. Properties of Oil and DMC-BioD

The properties of the oil and different plant seed oil DMC-BioD produced was determined following, ASTM (ASTM, 2003). The determination of density, kinematic viscosity, FP, CP, PP, copper strip corrosion, acid value, water content, ash content and carbone residue were made in accordance

with ASTM D5002, ASTM D445, ASTM D93, ASTM D2500, ASTM D97, ASTM D130, ASTM D664, ASTM D6304, ASTM D874 and ASTM D4530.

3. Results and Discussion

3.1. Characterization of Different Plant Seed Extracted Oil

The different plant seed extracted oil was clear and viscous. Important properties of extracted oil feedstock were shown in Table 1. The fatty acid compositions of oil have an important role in the performance of biodiesel in diesel engines. Saturation fatty acid biodiesel increase the cloud point, cetane number and improve stability whereas more polyunsaturation reduce the cloud point, cetane number and stability (Kumar and Bhattacharya, 2008). The high kinematic viscosity of oils leads to many problems after they are directly used in diesel engines (Meher et al., 2006a). The reduction in kinematic viscosity leads to improve atomization, fuel vaporization and combustion (Ramadhas et al., 2005b).

Table 1. Properties of different plant seed extracted oil

Sr. no.	Properties	Unit	Results
1	Kinematic viscosity, 40 °C	mm ² /sec.	10.44±0.01
2	Density at 25 °C	°C	0.99±0.01
3	Cloud point at °C	°C	8±0.02
4	Pour point at °C	°C	2±0.01
5	Acid value	mgKOH/g	5.72±0.01
6	Iodine value	g/100g	78±0.01
7	Saponification value	mg KOH/g	202±0.03
8	pH		6±0.01

3.2. Effect of Reaction Conditions on DMC-BioD Production

3.2.1. Effect of oil to DMC ratio on the yield of DMC-BioD

The optimum level of DMC concentration for the maximum production of biodiesel was investigated. The oil to DMC ratio (1:1, 1:2, 1:3 and 1:4w/w) was varied in four experiments to determine the effect of amount of DMC on DMC-BioD production. In all experiments, 200mg concentration of *Porcine pancreatic* lipase (on oil bases), 300rpm agitation speed and room temperature were employed. Optimum yield 93±0.02% of DMC-BioD was achieved with ratio 1:3w/w as shown in Figure 1. However, when the ratio of oil to DMC was increased to 1:4w/w, significant reduction in conversion was observed. It might be explained that the enzyme was inactivated by contact with high concentration of insoluble DMC. The DMC-BioD content for oil to DMC ratio of 1:1, 1:2, and 1:4w/w

were, respectively, 41 ± 0.05 , 70 ± 0.1 and $82 \pm 0.03\%$. The DMC-BioD content for *Pongamia pinnata* oil to DMC ratio of 1:3w/w was 96% after 6h (Panchal et al., 2013). The maximum biodiesel production rates from rapeseed oil and cotton seed oil were reported at 1:3.6 and 1:4 oil to methanol ratio, respectively (Li et al., 2006; Royon et al., 2007). Therefore, the ratio of the oil to DMC at 1:3w/w was used in this study.

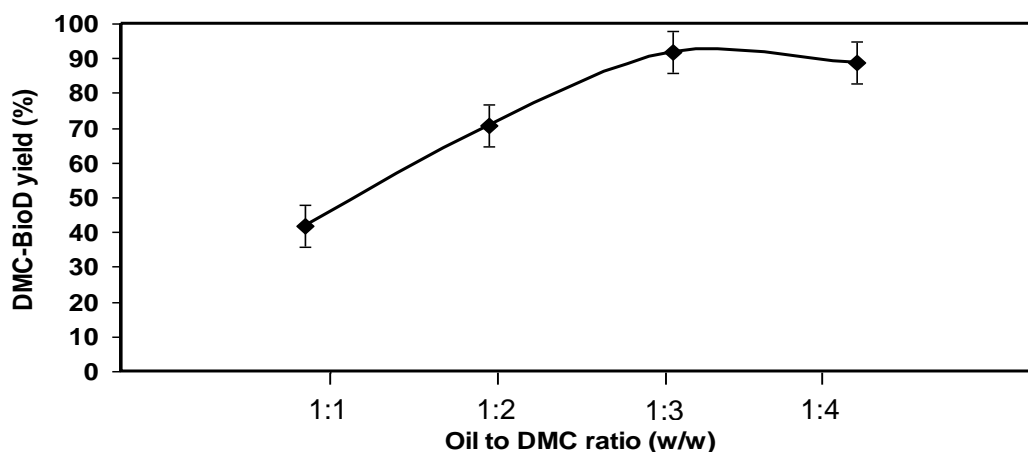


Figure 1. Effect of oil to DMC ratio on DMC-BioD production. Reaction conditions: 200mg of *Porcine pancreatic* lipase, agitation speed 200rpm, reaction time 6h and various ratio of oil to DMC. Data are represented as mean \pm standard deviation of triplicate observation.

3.2.2. Effect of enzyme dosages on the yield of DMC-BioD

The amount of enzyme added to reaction is also an important factor for DMC-BioD production, because it affects reaction rate (typically, the higher the enzyme dosage, the faster the reaction), but there has a limit in which the addition of enzyme does not alter any more the rate of product formation or that the amount turns the process more economically prohibitive. In this context, enzyme-catalyzed DMC-BioD production was investigated using dosages of *Porcine pancreatic* lipase from 100 to 300mg. Observed the highest conversion of oil to DMC-BioD ($93 \pm 0.02\%$) when 200mg of the *Porcine pancreatic* (on oil bases) was used as shown in Figure 2. The methyl-ester content increased with increased enzyme dosage. However, with the enzyme dosage increased to 300mg provided $92 \pm 0.1\%$ (DMC-BioD), there was no significant increase in the yield. Although, the enzyme dosage 100mg provided a little $25 \pm 0.09\%$ (DMC-BioD), such as 100mg and 300mg enzyme dosage should be avoided. Regarding the effect of lipase specificity, Pandey, (2009) reported the use of some specific and non-specific lipases (from *C. rugosa*, *P. cepacia* and *P. fluorescens*) in biodiesel production. Specific lipases need gradual addition of DMC to achieve high yields (between 80 and $93 \pm 0.02\%$) and this was probably due to acyl migration of sn-2 to sn-1, which occurs spontaneously in glycerides (Wang et al., 2010). Thus, the optimal enzyme dosgae for DMC-BioD production was chosen 200mg.

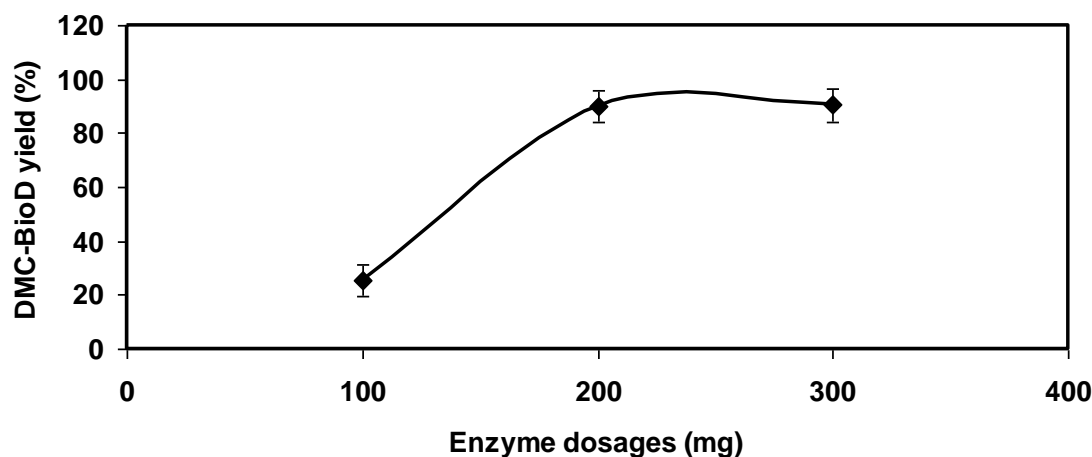


Figure 2. Effect of enzyme dosages on DMC-BioD production. Reaction conditions: 1:3w/w oil to DMC ratio, agitation speed 200rpm, reaction time 6h and various enzyme dosages. Data are represented as mean \pm standard deviation of triplicate observation.

3.2.3. Effect of reaction time on the yield of DMC-BioD

Effect of reaction time on DMC-BioD production from different plant seed oil using *Porcine pancreatic* lipase was studied by conducting experiments with different periods of 2, 4, 6 and 8h. Experiments were carried out at the room temperature, *Porcine pancreatic* lipase concentration 200mg (on oil bases), ratio of oil to DMC 1:3w/w and agitation speed 300rpm. Observed the highest conversion of DMC-BioD ($93 \pm 0.02\%$) in 6h. Figure 3, shows the yield percentage of DMC-BioD at 2, 4, 6 and 8h of reaction times. In 2, 4 and 8h time DMC-BioD produced were 30 ± 0.01 , 70 ± 0.05 and $90 \pm 0.07\%$, respectively. Based on the transesterification of fatty acids with a dialkyl carbonates (especially dimethyl or diethyl carbonate) which firstly reported (Tjahjono et al., 2014). From the results, 6h of reaction time gave better yield when compared with 2, 4 and 8h, in reaction time.

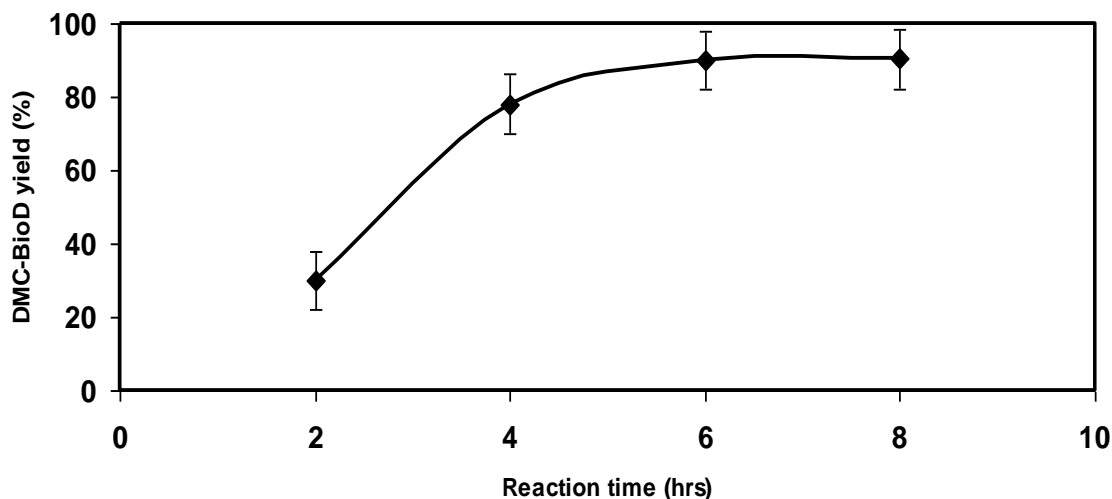


Figure 3. Effect of reaction time on DMC-BioD production. Reaction conditions: 1:3w/w oil to DMC ratio, 200mg of *Porcine pancreatic* lipase, agitation speed 200rpm and various reaction time. Data are represented as mean \pm standard deviation of triplicate observation.

3.2.4. Effect of agitation speed on the yield of DMC- BioD

Lifka and Ondruschka, (2004), reported that the agitation speed was important factor for biodiesel production. The effect of speed of agitation on conversion was studied in the range of 100 to 400rpm Figure 4. It was found that the percentage yield increased with speeds from 100 to 300rpm. However, no further increase in percentage yield was observed at 300rpm. The yield of DMC-BioD was $93 \pm 0.02\%$ at 300rpm. This may explain the results obtained in this study, in which the lowest agitation was more efficient in terms of biodiesel yield. A similar effect was observed by Babicz et al. (2010). External mass transfer limitations can be minimized by carrying out the reaction at an optimum speed of agitation (Yadav and Trivedi, 2003). Thus, the optimal agitation speed for DMC-BioD production was chosen at 300rpm.

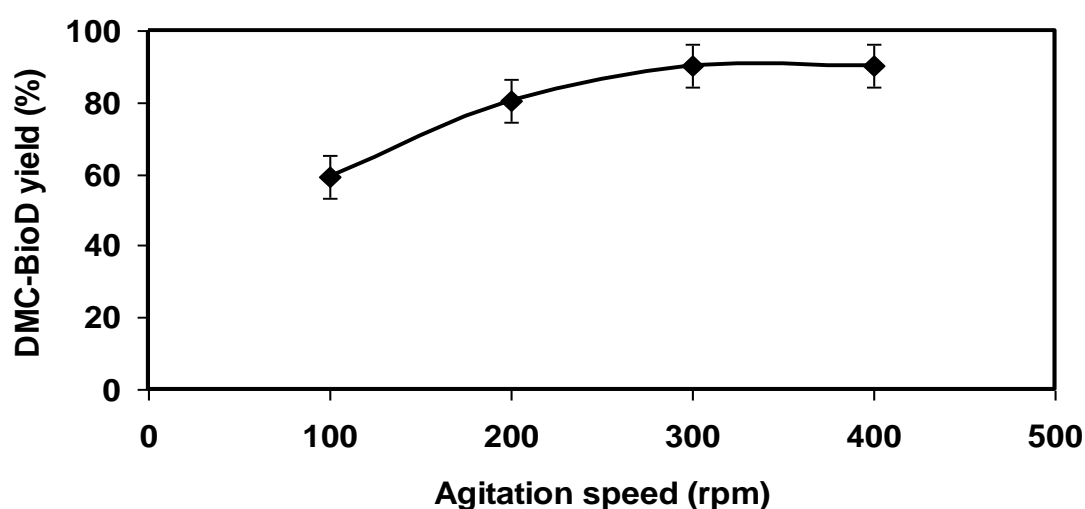


Figure 4. Effect of agitation speed on DMC-BioD production. Reaction conditions: 1:3w/w oil to DMC ratio, 200mg of *Porcine pancreatic* lipase, reaction time 6h and various agitation speeds. Data are represented as mean \pm standard deviation of triplicate observation.

3.2.5. DMC-BioD content

A higher conversion of triglycerides into biodiesel leads to a better engine performance. Yield of biodiesel can vary greatly depending on the different technologies used and the raw materials available. The DMC-BioD yield obtained by enzymatic transesterification of different plant seed oil was determined by gas chromatography; with methyl heptadecanoate as internal standard. The resulted data were processed, yielding of $93 \pm 0.02\%$ DMC-BioD, indicating an almost complete conversion of triglycerides into DMC- BioD. Fatty acid and its weight percent analysis of different plant seed extracted oil DMC-BioD were shown in Table 2.

Table 2. Fatty acid analysis of different plant seed extracted oil DMC-BioD

Sr. No.	Fatty acids	Wt %
1	Methyl palmitate (C16:0)	9.38±0.3
2	Methyl Stearate (C18:0)	7.34±0.1
3	Methyl oleate (C18:1)	31.3±0.4
4	Methyl linoleate (C18:2)	42.21±0.6
5	Methyl linolenate (C18:3)	4.09±0.1
6	Methyl Arachidic (C20:0)	2.31±0.2
7	Other	1.2±0.1

3.2.6. Fuel properties of DMC-BioD compared with ASTM D 6751-02 standard

The quality of biodiesel is very important for the performance and emission characteristics of a diesel engine. The fuel properties of the DMC-BioD were shown in Table 3. The present results showed that the acid value of the DMC-BioD was closer to the ASTM D 6751-02 standard. The kinematic viscosity of DMC-BioD was relatively closer to the ASTM D6751-02 standard values. The reported technical implication of higher viscosity of biodiesel was that it decreases the leakages of fuel in a plunger pair and in turn it changes the parameters of a fuel supply process (Lebedevas and Vaicekauskas, 2006). The flash point of the DMC-BioD was 125 °C, which was nearer to the flash point of ASTM D 6751-02 standard values. Pour point (PP) is one of the important parameters associated with engine performance in cold weather conditions (Dorado et al., 2004). The tested properties of DMC-BioD were found to be in reasonable agreement with ASTM D 6751-02.

Table 3. The fuel properties of different plant seed oil biodiesel in comparison with that of biodiesel standards.

Sr. no	Properties	Unit	Method	Limits	Results
1	Kinematic viscosity, 40°C	mm ² /sec.	D445	1.9 - 6.0	4.45±0.01
2	Density at 25°C	gm/cm ³	D4052	Reports	0.84±0.01
3	Flash point at °C	°C	D93	Reports	125±0.02
4	Cloud point at °C	°C	D2500	Reports	3±0.01
5	Pour point at °C	°C	D5950	Reports	-2±0.0
6	Copper corrosion strip	1	D130	3max	1a±0.0
7	Acid value	mgKOH/g	D664	0.50max	0.28±0.01
8	Water content	wt%	D6304	0.03max	0.02±0.0
9	Ash content	wt%	D874	0.02max	0.01±0.0
10	Carbone residue	wt%	D4530	0	0
11	Methyl -Ester content	%			93±0.02

4. Conclusion

This study revealed that DMC-BioD could be produced successfully from the different plant seeds (*Citrus limetta*, *Citrus Limon*, *Manikara zapota*, *Cucurbita maxima D* and *Annona squamosa* seeds) extracted oil with *Porcine pancreatic* lipase catalyzed using DMC. The DMC-BioD obtained has fuel properties that completely met and exceeded ASTM D 6751-02 standard. Therefore, as feedstock, can be potentially used as raw materials for DMC-BioD production on a commercial scale. Results of present study demonstrated that the optimum conditions were 1:3w/w ratio of oil to DMC, room temperature, 200mg *Porcine pancreatic* lipase (on oil bases), reaction time 6h and agitation speed 300rpm. The results indicate that all of the reaction variables in this study had effects on the reaction. The optimized conditions provided DMC-BioD in high yield ($93 \pm 0.02\%$). These optimum conditions can be used in large-scale production to reduce the cost of production.

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