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Production of biodiesel from sea mango (*Cerbera odollam*) seed using in situ subcritical methanol–water under a non-catalytic process

Jenni Lie¹ · Maria Bangun Rizkiana¹ · Felycia Edi Soetaredjo¹ · Yi-Hsu Ju² · Suryadi Ismadji¹

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Abstract

A catalyst-free and environmentally friendly process was employed for the production of biodiesel from sea mango seed oil. This oil is non-edible and contains several fatty acids such as palmitic acid (C16), *trans*-9-elaidic acid (C18:1t), oleic acid (C18:1), linoleic acid (C18:2), and linolelaidic acid (C18:2n6t). The in situ extraction and transesterification of the oil were carried at the subcritical methanol—water condition. The effect of reaction temperature and time on the yield of biodiesel was studied. The maximum yield of biodiesel was 98.58% and achieved at 200 °C, 40 bar and reaction time of 6 h. The ratio among sea mango seed, methanol, and water was 2:10:1.

Keywords Sea mango seed · Biodiesel · Subcritical methanol–water

Introduction

A large number of studies on biodiesel production have been conducted due to its potential to reduce the dependence of fossil-based liquid fuel [1] and to lessen the CO_2 emission from the transportation sector [2]. Aside from reducing the net CO_2 emission, biodiesel is also known for its biodegradability [3], non-toxicity [4], and low emission profile of SO_x [5] and other pollutants, such as CO_x , hydrocarbon, NO_x [6], and low particulate matter [7]. Biodiesel is a renewable biofuel. Thus, it is environmentally friendly [8]. Rashid et al. [9] defined biodiesel as mono-alkyl esters derived from vegetable oils/animal fats by a chemical process called the transesterification process [9]. However, vegetable oils/animal fats may contain high free fatty acid (FFA) and water [10] that may cause the formation of soap during the reaction [11].

- Felycia Edi Soetaredjo felyciae@yahoo.com
- Suryadi Ismadji suryadiismadji@yahoo.com
- Department of Chemical Engineering, Widya Mandala Surabaya Catholic University, Kalijudan 37, Surabaya 60114, Indonesia
- Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Sec 4, Taipei 10607, Taiwan

Due to the lack of non-consumable resources of vegetable oils and animal fats as raw materials for biodiesel production, in the early studies of biodiesel production high-quality food-grade oils were used as the starting materials. In the USA, biodiesel is produced in industrial scale using soybean oil as the raw material, while palm oil is used to produce biodiesel in Malaysia [12]. However, the use of edible feedstock creates several economic and environmental issues, the competition between crop land used for food production and biodiesel can become intense especially in developing and less develop countries with food shorage problems [12–14]. Non-edible and non-conventional feedstocks have been selected to substitute edible feedstocks for biodiesel production, and in this study we utilized sea mango (Cerbera odollam), which is known as a poisonous fruit as the raw material for biodiesel preparation.

Sea mango is a tree of the *Apocynaceae* family. The seed of this poisonous fibrous-kernelled fruit contains a significant amount of oil [15]. The presence of non-edible oil in sea mango makes it a potential candidate for use as non-edible feedstock for biodiesel production [15]. Kansedo et al. [16] studied the production of biodiesel from sea mango seed using sulfated zirconia catalyst. They found that the yield of FAME from sea mango seed could reach up to 83% via an ex situ method. Unfortunately, the use of the conventional method of biodiesel production has some drawbacks, such as the high price of the catalyst, the high cost of the pretreatment process, and slow reaction time [17].



Water is environmentally friendly and the cheapest solvent for many kinds of processes [18]; however at ambient condition, water is not a suitable solvent or reactant for organic compounds due to its high polarity, and it also cannot be used as extracting agent for non-polar compounds. Studies have found out that by increasing its temperature and pressure, water can be utilized as an extracting agent. Water in the liquid state condition at temperature above the atmospheric boiling point and below the critical temperature (374 °C) is called as subcritical water (SubCW) [19], and at this condition water can be used as extracting and reaction media for many kinds of reaction systems with or without the presence of catalyst [20].

In the present, there is no study on the utilization of in situ subcritical methanol—water for the production of biodiesel from sea mango seed oil without the presence of a catalyst. One of the main drawbacks of using catalyst, especially homogeneous catalyst, is the difficulty in the purification step, which often requires a complicated procedure and at the end will affect the cost of production itself [21]. In this study, we utilized the non-catalytic process for the production of biodiesel from sea mango seed.

This study aimed to find the best condition for the manufacture of biodiesel from sea mango seed with the non-catalytic process using subcritical water and methanol. The effects of temperature and reaction time on the yield of biodiesel were studied at constant pressure (40 bar) and a constant mass ratio among methanol, water, and sea mango seed (10:1:2).

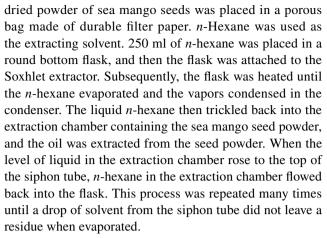
Materials and methods

Materials

Sea mango seeds were collected from several streets in Surabaya, East Java, Indonesia. The sea mango seeds were repeatedly washed using tap water to remove dirt and other impurities and subsequently dried at 100 °C for 24 h. The dry sea mango seed was pulverized in Junke and Kunkel hammer mill. The chemicals used in this study such as n-hexane (90%) and anhydrous methanol (99.9%) were purchased from Merck, Germany. Ultrapure N_2 gas (99.9%) was obtained from Aneka Gas Pty. LTD. The reference standard mix of (Supelco) fatty acid methyl esters (FAMEs) consists of C_{14} – C_{22} fatty acids and was purchased from Sigma-Aldrich Co. USA. All the chemicals were used without any further purification.

Analysis of sea mango seed oil mass fraction

The oil mass fraction in the sea mango seed was determined by Soxhlet extraction method. Forty-five grams of



The liquid from the Soxhlet extractor was evaporated using IKA RV10 B rotary evaporator to remove *n*-hexane, and the mass of oil obtained was weighed using Mettler Toledo analytical balance.

Yield % =
$$\frac{\text{Total weight of oil}}{\text{Total weight of the sample}} \times 100\%$$
. (1)

The chemical characteristics of sea mango seed oil used in this study are given in Table 1.

In situ subcritical methanol transesterification

In situ subcritical water methanol transesterification of sea mango seed oil was carried out in a laboratory-scale high-pressure batch reactor (150 cm³, temperature limit: 100–300 °C, pressure range: 0–80 bar). The stainless steel reactor was equipped with an external heater, a pressure gauge, and a thermos controller to maintain the system at the desired temperature. The ratio of methanol, sea mango seed powder, and distilled water was 10:2:1, this ratio was based on the stoichiometry of transesterification reaction between triglycerides and methanol. The reactor was closed with its cap using M8 screws, and it was heated

Table 1 Crude sea mango seed oil characteristics

Parameters	Sea mango oil	Methods
Fatty acid composition (%)		GC
C16:0 (palmitic acid)	31.52	
C18:0 (stearic acid)	3.64	
C18:1 (oleic acid)	47.85	
C18:2 (linoleic acid)	16.99	
Water content (%)	0.94	Karl-Fisher
Free fatty acid (%)	6.53	MPOB P2.5
Acid value (mg KOH g ⁻¹)	12.84	Standard titration
Density (kg m ⁻³)	921.85	ASTM
Viscosity at 40 °C (mm ² s ⁻¹)	28.97	ASTM





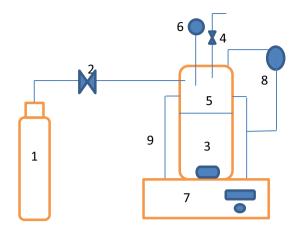


Fig. 1 Diagram of subcritical reactor— N_2 gas cylinder (1); relief valve (2); magnetic bar (3); release valve (4); reactor (5); pressure gauge (6); RPM controller (7); thermocouple (8); heating element (9)

at a rate of 20 °C min⁻¹ to the desired temperatures (140, 160, and 180 °C). The temperatures were kept constant for specific times (4, 5, and 6 h). To keep the temperature and pressure constant, it was controlled by a PID-type controller with an uncertainty of ± 1 °C and pressure gauge to monitor the pressure at 40 bar. The schematic diagram of the subcritical reactor is given in Fig. 1. N₂ gas was injected into the reactor to maintain the pressure of the system and to ensure that water and methanol existed in the liquid form. During the reaction, the system was kept under constant stirring at 400 rpm using a magnetic stirrer. After the reaction was complete, the reactor was suddenly cooled to room temperature and the vent valve was opened to release nitrogen gas. The liquid was separated from the mixture using Whatman filter paper no. 41. The biodiesel was extracted from the filtrate using *n*-hexane in a separatory funnel, and then glycerol (bottom phase) was separated from the organic phase. The organic phase was evaporated using IKA RV 10 B rotary evaporator to remove *n*-hexane. The yield of biodiesel (FAMEs) was weighed and analyzed using gas chromatography.

Yield
$$\% = \frac{\text{Total weight of methyl esters}}{\text{Total weight of oil in the sample}} \times 100\%.$$

Analysis of FAME in biodiesel

The content of fatty acid methyl ester in biodiesel was analyzed using Shimadzu gas chromatograph 2014. Gas chromatography was equipped with FID and 30 m \times 0.25 mm DB-WAX capillary column; the film thickness was 0.25 μm . The analysis was conducted using helium as carrier gas at a linear velocity of 34 cm s $^{-1}$ and the injector temperature was kept constant at 250 °C, while the FID temperature was kept at 300 °C. The column temperature

was programmed at 120 °C for 3 min, and then heated to 300 °C with a heating rate of 20 °C min⁻¹ and kept at 300 °C for 2 min. The amount of the sample injected into the column was 1 μ l with 1:100 split ratio. Other physical and chemical properties of the biodiesel were determined by the ASTM D6751 method.

Results and discussion

Oil content of sea mango seed

To determine the oil content of the sea mango seeds, the extraction of oil from sea mango seeds was conducted using the Soxhlet extraction procedure. The extraction experiments were conducted three times, and the results are given in Table 2. High oil content in the sea mango seed (average 48.72 wt. %) indicates that this unedible seed is a suitable precursor for biodiesel production.

Effect of reaction temperature on FAME yields

The yield of biodiesel obtained from the transesterification of sea mango oil using subcritical water-methanol at various temperatures and reaction time is summarized in Table 3. From this table, it can be seen that the maximum yield of biodiesel was 98.58%. The highest yield of the biodiesel was obtained at a temperature of 200 °C and reaction time of 6 h. At 200 °C, the increase in the reaction time gave no significant effect on the yield of biodiesel. In the in situ production of biodiesel, temperature played a significant role in the yield of biodiesel as indicated in Table 3. At temperature 140 °C and reaction time of 4 h, the yield of biodiesel was 14.08% and increased almost two times when the temperature was increased to 160 °C. At subcritical condition, the increase in temperature decreased the polarity of methanol, leading to increase in the miscibility of sea mango oil in methanol. With the increase of the miscibility of oil in methanol, the amount of oil extracted from the solid interior of mango seed also increases. The oil and methanol become one homogeneous phase [22]. The transesterification reaction of sea mango oil with methanol is reversible and endothermic; therefore, with the increase of temperature

Table 2 Oil content in the sea mango seed

(2)

Run	Weight of sea mango seed (g)	Weight of oil (g)	Yield (% w/w)
1	45.0005	21.6088	48.02
2	44.9997	21.8357	48.52
3	45.0003	22.3241	49.61
Average			48.72





Table 3 The yield of biodiesel from sea mango seed

Temperature (°C)	Pressure (bar)	Reaction time (h)			
		4	5	6	
140	40	14.08% ± 1.55	$19.04\% \pm 0.78$	$25.24\% \pm 1.02$	
160	40	$28.27\% \pm 0.98$	$32.62\% \pm 1.19$	$48.36\% \pm 0.01$	
180	40	$88.88\% \pm 1.25$	$90.12\% \pm 0.43$	$90.20\% \pm 1.71$	
200	40	$98.12\% \pm 1.18$	$98.41\% \pm 0.96$	$98.58\% \pm 0.21$	

and miscibility of oil in methanol (become one phase), the rate of transesterification reaction to form fatty acid methyl ester also increases. The esterification reaction also occurred simultaneously in this process due to the 6.4% free fatty acid contained in sea mango seed oil as shown in Table 1. The high content of free fatty acid in the feedstock was not a problem for biodiesel production using the subcritical water methanol process, because no alkaline catalyst was used in this process that may cause a saponification reaction and give a low yield of biodiesel.

Effect of water on FAME yields

The addition of water into the subcritical system also plays an important role. When water was added to the system, the biodiesel yield rose to 98.58%. This value is ~ 26% higher compared to biodiesel yield from water-free process under similar conditions (200 °C, 40 bar for 6 h). Silva et al. [23] investigated the effect of water addition on biodiesel production from Jatropha curcas L. oil under supercritical ethanol. In the water-free system, the maximum fatty acid ethyl ester obtained was about 80%, and when 10% water was introduced to the process the yield became 87%. It was reported that the presence of water would stimulate the parallel reactions of transesterification and decrease the decomposition degree of fatty acid [23–25]. At subcritical condition, the dielectric constant of water is lower than at standard pressure and ambient temperature. Therefore, the water is easily dissociated into H₃O⁺ and OH⁻. With the presence of excess H₃O⁺, the system becomes acidic. The presence of H₃O⁺ (hydroxonium) in the system represents the nature of the proton in aqueous solution, and this proton subsequently acts as a catalyst for the transesterification process at the temperature below the critical point of the mixture [26] and reduce the reaction time to achieve the same biodiesel yield.

Effect of reaction time on FAME yields

In general, the reaction time gives a positive effect on the yield of biodiesel as seen in Table 3. The longer the reaction time, the higher is the yield of biodiesel. As mentioned before, the transesterification reaction to produce biodiesel is an endothermic and reversible process; it requires energy

to shift the reaction toward the biodiesel product (fatty acid methyl ester). With the increase of reaction time, the energy added to the system also increases and more energy is available to shift the reaction toward the formation of fatty acid methyl ester. Since the reaction was maintained at subcritical condition under nitrogen environment without the presence of any catalyst, the oxidation or secondary reaction of the biodiesel product did not occur. Therefore, the yield increased with the increase of reaction time.

FAME content in biodiesel

It has been reported that sea mango seed mainly contains oleic acid (48.1%), followed by palmitic acid (30.3%), linoleic acid (17.8%), and stearic acid (3.8%) [27]. GC analysis showed that sea mango seed oil contains several fatty acids such as palmitic acid (C16), *trans*-9-elaidic acid (C18:1t), oleic acid (C18:1), linoleic acid (C18:2), and linolelaidic acid (C18:2n6t). During the transesterification reaction with methanol, these fatty acids were converted to their esters [C16 (methyl palmitate), C18:1t (*trans*-9-elaidic methyl ester), C18:1c (*cis*-9-oleic methyl ester), C18:2 (methyl linoleate), C18:2n6t (methyl ester)]. The fatty acid methyl ester (FAME) compositions in the biodiesel obtained from sea mango seed oil are given in Table 4.

Fuel properties of biodiesel

The physical and chemical characteristics of the biodiesel such as density, kinematic viscosity, flash point, cetane number, and acid number were determined according to the ASTM standard. The density was determined by ASTM D1298, and the kinematic viscosity was determined using ASTM D445-10. ASTM D93 was employed for the determination of the flash point of the biodiesel. The cetane number was determined by ASTM D613, which is a standard method for the determination of the cetane number of diesel fuel oil. The values of chemical and physical characteristics of the biodiesel are given in Table 5.

In a combustion chamber, the density of the biodiesel plays an essential role since it determines the mass of the biodiesel injected into the chamber [28]. The standard density of biodiesel at 15 °C is in the range of 860–900 kg m⁻³. The density of the biodiesel obtained in this study is in the





Table 4 Fatty acid methyl ester content in biodiesel from sea mango seed oil

Sample	Parameters			Composition, %				
	T(°C)	P (bar)	t (h)	C16	C18:1t	C18:1c	C18:2	C18:2n6t
1	140	40	4	31.80 ± 1.04	4.66 ± 0.18	53.35 ± 2.31	3.99 ± 1.42	6.20 ± 0.45
2	160	40	4	48.75 ± 2.56	9.06 ± 1.07	24.12 ± 2.98	11.67 ± 0.39	6.38 ± 1.22
3	180	40	4	36.07 ± 1.82	5.41 ± 0.53	50.95 ± 1.75	6.17 ± 0.01	1.39 ± 0.03
4	200	40	4	32.64 ± 3.91	6.31 ± 1.42	48.51 ± 2.43	9.81 ± 1.11	2.73 ± 0.05
5	140	40	5	45.24 ± 2.78	8.33 ± 1.90	32.97 ± 0.59	9.87 ± 1.72	3.59 ± 2.43
6	160	40	5	49.26 ± 1.13	0.66 ± 0.09	41.39 ± 2.73	7.94 ± 0.90	0.76 ± 0.01
7	180	40	5	38.41 ± 2.09	3.90 ± 1.07	48.92 ± 1.19	4.04 ± 0.02	4.73 ± 0.87
8	200	40	5	43.39 ± 1.27	5.17 ± 0.81	43.10 ± 1.19	3.11 ± 0.18	5.23 ± 0.07
9	140	40	6	38.90 ± 2.31	3.69 ± 0.09	20.48 ± 2.26	14.22 ± 1.76	22.72 ± 1.36
10	160	40	6	34.72 ± 1.53	1.71 ± 0.02	54.40 ± 2.31	8.46 ± 0.58	0.70 ± 0.08
11	180	40	6	23.62 ± 0.94	1.76 ± 0.05	43.29 ± 1.09	2.57 ± 0.03	28.76 ± 0.77
12	200	40	6	30.91 ± 1.42	2.85 ± 0.54	58.01 ± 2.41	4.76 ± 1.62	3.47 ± 0.90

Fatty acid methyl esters: C16 (methyl palmitate), C18:1t (*trans*-9-elaidic methyl ester), C18:1c (*cis*-9-oleic methyl ester), C18:2 (methyl linoleate), C18:2n6t (linolelaidic acid methyl ester)

Table 5 Fuel properties of biodiesel from sea mango seed oil

Sample $(T/t)^a$	D @ 15 °C (kg m ⁻³)	KV @ 40 °C (mm ² s ⁻¹)	FP (°C)	CN	AN (mg KOH g ⁻¹)
140/4	885.1	2.89	133	55.1	0.3761
140/5	883.4	2.91	132	55.6	0.3302
140/6	881.7	2.93	133	55.4	0.3549
160/4	882.9	2.96	129	53.8	0.3618
160/5	887.5	2.94	125	52.9	0.3446
160/6	885.4	2.92	127	53.4	0.4014
180/4	882.9	2.99	124	53.2	0.3785
180/5	881.6	2.94	125	54.6	0.4151
180/6	884.2	2.97	122	53.4	0.3493
200/4	886.4	2.91	119	54.7	0.3526
200/5	887.3	2.95	120	53.9	0.3857
200/6	885.9	3.01	119	53.7	0.4067
ASTM D6751	Na	1.9–6.0	93 °C min	47 min	0.5 max

D, density; KV, kinematic viscosity; FP, flash point; CN, cetane number; AN, acid number

range of 881.6–887.5 kg m⁻³. These values are in the range of the standard density of biodiesel. The kinematic viscosity strongly influences the volatilization and atomization of the biodiesel upon injection in the combustion chamber. This physical property also influences the engine life. The higher viscosity enhances the fuel spray penetration, resulting in the improvement of air–fuel mixing. However, fuel with high viscosity tends to form a deposit in the engine and reduce the combustion efficiency [20, 28, 29]. Nevertheless, fuel with low viscosity may not provide sufficient lubrication in the combustion system [28]. The ASTM kinematic viscosity standard for biodiesel is 1.9–6.0 cSt (mm² s⁻¹), and the kinematic viscosity of the sea mango biodiesel is in the range of 2.89–3.01 cSt.

Flash point is one of the essential characteristics of biodiesel. This physical property relates to the ignition of biodiesel. The moderate value of a flash point is required for the safety of handling and storage. Since the flash point of the biodiesel produced in this study is above the minimum value of the ASTM standard, this biodiesel is safe for handling and storage for a period of time. Cetane number is an index that measures the combustion quality of diesel fuel during compression ignition [28]. This index strongly influences the performance of the engine during fuel combustion and exhaust emission. The low value of cetane number causes difficulties in the startup of the engine and, during combustion, produces noise in the engine and smokes of exhaust gas. The cetane number of biodiesel



^aT/t: temperature (°C)/reaction time (h)

produced in this study is higher than the minimum value of the ASTM standard D6751. The compositions of fatty acids in the feedstock or raw material also have significant effect on the cetane number of the biodiesel product. If the oil contains a high percentage of saturated fatty acid, the value of cetane number of the resulting biodiesel is high. On the contrary, a low value of cetane number will be obtained if the oil contains a significant amount of unsaturated fatty acids [20, 30].

One of the crucial properties of biodiesel is the acid number. The acid number indicates the quantity of free fatty acids and mineral acids present in the biodiesel. High acid number causes corrosion problem in the engine and fuel injectors [28]. The degradation of biodiesel during storage will increase the acid number; therefore, this property can be used as a measure of biodiesel degradation. The acid number of biodiesel obtained in this experiment is in the range of 330–415 mg kg⁻¹. The values are still in the inadmissible range, since the maximum acid number allowed in the ASTM D6751 is 500 mg kg⁻¹.

Conclusion

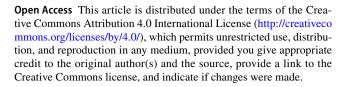
In this study, the biodiesel was produced from non-edible seed oil using the subcritical methanol-water process. The sea mango seed, which contains highly toxic compounds, was used as the source of the non-edible oil. The in situ extraction and transesterification of the oil were carried out without the presence of acid or base catalyst. This process drastically reduces the production cost, since it does not require extraction and separation of the catalyst. However, the main disadvantage of this process is that the transesterification reaction must be conducted at high temperature and pressure. The maximum FAME yield of 98.58% was obtained at 200 °C, at a pressure of 40 bar and reaction time of 6 h.

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Authors contribution JL and MBR conducted the experiments, FES performed statistical analysis, YHJ drafted the manuscript, and SI performed the experiment design and corrected the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.



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