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EVALUATING PHYSICOCHEMICAL PROPERTIES OF MUTATED JATROPHA CURCAS SEED OILS AND THEIR FEASIBILITY AS NEW FEEDSTOCKS FOR BIODIESEL PRODUCTION

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KEYWORDS

biodiesel, fatty acid, mutated JC, physicochemical properties, triacylglycerol.

ABSTRACT

The physicochemical properties of *Jatropha curcas* (JC) seed oils are related to the plant varieties and affect the biodiesel quality when it is used as feedstock. This work investigates the physicochemical properties and feasibility of mutated JC seed oil for biodiesel feedstock. Three mutated JC seed oils, from JC-150, JC-226 and JC-300, were successfully evaluated. The oil contents were determined by using gravimetry methods. The AV, FFA, IV and PV were determined by using titrimetric methods. Types of fatty acids were analyzed by using a GC-FID. The triacylglycerol (TAG) and PE compositions were determined by using a HPLC-ELSD. The results show that the oil contents of JC-150, JC-226 and JC-300 seeds were 48.3%, 45.8%, and 51.7%, respectively. The PE contents in JC-150, JC-226 and JC-300 were lower (approximately 33.4%, 46.9% and 96.4%) compared to the control. The oleic and linoleic acids were two main components of all samples, with compositions in the range 41.82-42.45% and 36.68-37.45%, respectively. The compositions of polyunsaturated and monounsaturated TAG were obtained in the range 71.60–76.22% and 19.62–24.53%, respectively. These results show that the properties of mutated JC seed oils meet with the requirements for biodiesel production.

INTRODUCTION

The *jatropha curcas* (JC) plant is a species in the *Euphorbeaceae* family, which has short, vascular, woody plants and is categorized in the same family as rubber and cassava. The JC plant can easily be found in Central and South America, Africa, India and Southeast Asia. JC has many functions, such as traditional soap, organic pesticide, and medicinal herbs. In addition, JC seeds can be applied as a raw material in organic fertilizer because it contains nitrogen, phosphor and potassium. Also, the solid waste of JC seeds can be used as raw material for biogas production. The most interesting JC seeds are those with high oil content, around 70-75% of the seeds contain oil (Das et al., 2021).

As reported in the literature, JC is considered to be a promising candidate plant to produce bio-lubricant and biodiesel feedstocks. It is well known that JC seed oil can be processed to produce various products, such as bio-lubricant (Ho et al., 2019, Hoong et al., 2019) and biodiesel (Koh & Ghazi, 2011, Maftuchah et al., 2020, Silitonga et al., 2011). A lubricant is defined as a substance (generally in liquid form) which is used to reduce the friction between two or more moving surfaces, to improve material efficiencies and reduce wear (Chowdary et al., 2021). Biodiesel is a biofuel that is produced from vegetable oils (i.e., palm, soy, canola or jatropha) through the transesterification reaction with a catalyst (Gutierrez-Lopez et al., 2021). Based on these facts, JC has become an interesting topic to be explored and developed in future research.

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The characteristics of JC seed oil are strongly associated with genetic factors such as the age of the plants, environmental conditions, geography, soil fertility, and applied agronomic practices (Kumar & Singh, 2014, Lama et al., 2018). Several researchers have reported that different genotypes of JC produce different yields of oil seed (Lama et al., 2018, Maftuchah et al., 2020). In China, researchers found that the seed oil content in mutated jatropha was around 6% higher than the original jatropha (Yang et al., 2010). In addition to the seed yields, the toxicity (based on the phorbol esters content) of JC seed oil is affected by the type of jatropha genotype used (Abou-Arab et al., 2019). Non-toxic jatropha genotypes have also been found in Mexico (Sanghamitra et al., 2014 , Valdes-Rodriguez et al., 2013) but these genotypes are less abundant. The non-toxic jatropha are those that contain phorbol esters (PE) at a concentration less than 0.05 mg/g, while toxic jatropha contain PE more than 0.05 mg/g (Kumar & Singh, 2014). Currently, the researchers have tried approaches such as agrobiological or breeding programs (i.e., crossbreeding) and physical methods (gamma irradiation) (Ha et al., 2019, Maftuchah et al., 2020) to improve the quantity and quality of JC seed oil contents and/or reduce their toxicity.

It is well known that the JC seed oil is one of the popular non-edible feedstocks of low price. The JC can be easily grown in semi-arable and uneconomic lands (Tan et al., 2019). This has encouraged researchers to explore and investigate the characteristics of seed oil from several JC genotypes. As mentioned above, the agrobiological and physical methods can be used to mutate the genotype of JC. Hence, the different genotypes produce different characteristics of seed oil. To the best of our knowledge, there are no reports on the feasibility of the mutated jatropha seed oil for producing biodiesel, especially the mutated JCs with high intensities of gamma radiation (150-300 kGy). Therefore, this work investigates the characteristics of seed oil from some of the mutated JCs, particularly the feasibility of the mutated JC seed oils for biodiesel feedstocks. The current study presents valuable information related to the physicochemical properties of the seed oil of mutated JC as feedstock for biodiesel production. The mutated JCs were produced by the gamma irradiation process on original jatropha seeds (article unpublished). All of the samples were obtained from the experimental plots at the Faculty of Science and Technology (FST), Universiti Kebangsaan, Malaysia (UKM).

MATERIAL AND METHODS

Chemicals and JC seeds

The chemicals used were: hydrochloric acid (HCl), petroleum ether (C_6H_{14}), anhydrous sodium sulfate (Na_2SO_4), potassium hydroxide (KOH) pellets, potassium iodide (KI), sodium thiosulfate ($Na_2S_2O_3$), ethanol (C_2H_6O), methanol (CH_4O), diethyl ether ($C_4H_{10}O$), acetone (C_3H_6O), acetonitrile (C_2H_3N), glacial acetic acid ($C_2H_4O_2$), chloroform ($CHCl_3$), hydranal, Wijs solution, starch, and phenolphthalein; all were purchased from Fischer Chemical.

The fresh seeds from JC-150, JC-226 and JC-300 were collected from the experimental plots (Biobased Lubes) at FST, UKM. The JC-150, JC-226 and JC-300 were labelled based on the 150 kilo Gray (kGy), 226 kGy and 300 kGy of gamma ray intensities which were used in the irradiation process to mutate the JC genotypes (article unpublished). All seed samples were dried separately in the open air for one week and then peeled, finely ground, weighed and placed in different sample bags at room temperature for further analysis.

Oil extraction

A total of 500 g of dried seed samples were weighed using an analytical balance (KERN ABS 220-4). Dried seed of JC–150, JC–226, JC–300 and JC-0 (control) were extracted by the Soxhlet apparatus method using petroleum ether as a solvent at a ratio of 6:1 (v/w). Extraction processes were conducted at 70°C for 12 hours (Satar et al., 2015, Sayyar et al., 2009). After the extraction process was finished, the solvent was removed from the extract by using a rotary evaporator (BUCHI Rotavapor R-124) at 65–70°C. The seed oil yield (expressed as a percentage, %) was then determined by using [eq. (1)].

$$Oil\ yields\ (\%) = \frac{W_0}{W_3} x\ 100\ \% \tag{1}$$

Where:

W_0 and Ws are are the weight of oil (g) and seed sample (g), respectively. The collected oils were kept in a refrigerator for further analysis.

Oil characterizations

Some physicochemical properties, such as acid value (AV, mg KOH/g sample), free fatty acid (FFA, %), peroxide value (PV), and saponification value (SV), were determined by using the titrimetric methods described by Ewunie et al. (2021). As described by Asmare & Gabbiye (2014), the AV of the mutated JC seed oil can be determined by titration using potassium hydroxide. The FFA (%) was calculated based on the AV multiplied by 0.501, as described by Ewunie et al. (2021). The iodine value (IV) was determined, based on ASTM D5554, as described by Yusuff (2021). In brief, the mathematical expressions for the AV, IV, SV and PV are shown in [eq. (2)], [eq. (3)], [eq. (4)], and [eq. (5)], respectively.

For determining the AV, a total of 5 g of oil was dissolved in a 25 ml diethyl ether and ethanol (50: 50) mixture. Then, a few droplets of phenolphthalein indicator was added to the oil-solvent mixture and carefully titrated with 0.1 N alcoholic KOH solution, until a light pink color appeared. The AV was calculated by using [eq. (2)], as follows:

$$AV = \frac{56.1\ x\ N\ x\ V}{W_{oil}} \tag{2}$$

N and V are the concentration and volume (ml) of alcoholic KOH solution used in the titration, respectively, and

W_{oil} is the weight (g) of the oil sample.

For determining the IV: a total 0.3 g oil sample was dissolved in 15 ml chloroform. Then, 25 ml of Wijs solution was added to the oil sample. The oil sample-Wijs solution mixture was kept in a dark place for 60 mins. After that, 20 ml KI saturated solution and 100 ml deionized water were added to the mixture. The mixture was then titrated by using 0.1 N $Na_2S_2O_3$ solution, with some droplets of starch solution as an indicator. The endpoint of titration was indicated by the disappearance of the blue color. The blank solution was tested using the same procedure. The IV (mg I_2/100 g oil) was calculated based on [eq. (3)]:

$$IV = \frac{(V_b - V_s)}{W_{oil}} \; x \; N \; x \; 12.69 \quad (3)$$

Where:

N (0.1 N) is the concentration of $Na_2S_2O_3$, and

Vb and Vs are the volume (ml) of $Na_2S_2O_3$ utilized for titrating the blank and the sample, respectively. The numeral 12.69 is a constant associated with the equivalent weight of iodine.

For determining the SV: a total 2.0 g of oil sample and 50 ml of excess ethanolic KOH solution (0.1 N) were mixed in a boiling flask. Then, the mixture was refluxed and stirred with a magnetic bar for 45 mins. Subsequently, an excess of KOH was titrated with the 0.1 N HCl. Three droplets of phenolphthalein were used as indicators. The endpoint of titration was indicated by the disappearance of the pink color. The SV was calculated using [eq. (4)], as follows:

$$SV = \frac{V_b - V_s}{W_{oil}} x \; N \; KOH \; x \; 56.1 \quad (4)$$

Where:

V_b and V_s are the volumes (ml) of HCl used for the blank and the sample, respectively.

To determine the PV: a total 2.0 g of oil sample and 10 ml of $CHCl_3$ were mixed in a 250 ml conical flask. The mixed solution was then added to 15 ml of glacial $C_2H_4O_2$ and 1.0 ml of saturated KI solution, while being gently shaken for 2 mins. The mixture was subsequently placed in a dark room for 15 mins. Finally, a total of 15 ml deionized water was added to the mixture and titrated with 0.002 M of $Na_2S_2O_3$ using starch as an indicator. The PV was calculated by using [eq. (5)], as follows:

$$PV = \frac{(V_b - V_s)}{W_{oil}} \; x \; M \; x \; 1000 \quad (5)$$

Where:

V_b and V_s are the volumes (ml) of $Na_2S_2O_3$ used for the blank and the sample and M is the molarity of $Na_2S_2O_3$.

The physical properties, such as density and kinematic viscosity, were measured by using a Pycnometer and Rheometer (Anton Parr, Physica MCR 301), respectively. However, the energy contents of seed oil were measured using a Bomb Calorimeter (Anton Parr, 6100 Calorimeter).

The composition of fatty acids

Fatty acid profiles and compositions were analyzed using gas chromatography equipped with a flame ionization detector (GC–FID, Shimadzu). A total 0.1 ml of oil sample was put into a 10 ml vial and 2 ml of n-hexane and 1 ml of $NaOCH_3$ (1M) were added, while shaking for 10 mins. The upper layers from two phases of mixtures were taken and injected into the GC-FID. The stationary phase of the GC-FID is a capillary column with dimensions of 30 m x 0.25 mm x 0.25 mm. Nitrogen gas was used as a mobile phase, with a flow rate set at 1 ml/minute and total running time of 60 mins. The detector temperature was adjusted at 280^{o}C during GC-FID operation. Lastly, the identified peaks of fatty acids in oil seed samples were characterized based on the standard fatty acids.

The composition of triacylglycerols (TAGs)

A total 0.25 ml of oil sample was diluted into 2 ml of an acetone and acetonitrile mixture (63.5:36.5) and homogeneously mixed together. To remove the suspended solids, the mixture was filtered using a filter membrane with a pore size of 0.45 μm. Subsequently, the filtered mixture was analyzed using high-performance liquid chromatography equipped with an evaporator light scattering detector (HPLC–ELSD, Ultimate DIONEX). The stationary phase in the HPLC-ELSD was column C18, with the dimensions 5 μm x 120 Å (4.6 x 250 nm), while the mobile phase was an acetone and acetonitrile mixture (63.5:36.5). The flow rate was set at 1 ml/min and total running time was 30 min. To characterize the type of TAG, the identified peaks were compared with the standard TAG peaks.

Biodiesel production

Biodiesel, or fatty acid methyl ester (FAME), was produced from the mutated JCs through the transesterification process with KOH as a catalyst. The mutated JC seed oil and methanol-KOH were mixed at a ratio of 1:6 and placed inside a three-necked 500 ml flask. The KOH catalyst was prepared by diluting 1% (w/w oil) of KOH pellets in methanol (called methanol-KOH). The transesterification was conducted at 70^{o}C for 2 hours, while being stirred using a magnetic bar during the process. At the end of the transesterification process, the product was separated into two phases: biodiesel (top layer) and glycerol (bottom layer). The biodiesel yield was calculated by using [eq. (6)], as follows:

$$Yield \; of \; biodiesel \; (\%) = \frac{W_B}{W_O} x \; 100 \; \% \quad (6)$$

Where:

W_B is the weight of biodiesel, and

W_O is the weight of the oil sample.

RESULTS AND DISCUSSION

Oil contents

This work investigated and characterized the seed oil from mutated JC-150, JC-226 and JC-300 and the control JC (JC-0). Overall, the oil contents of the mutated JCs were higher than the control sample, JC-0. As reported by Lama et al. (2018), the genotype can affect the seed yield. Similarly, this work also shows that JC genotypes contribute to the oil seed content. Although the gamma irradiation may change the JC genotypes that have high oil seed content and low PE, extended work must be performed to ensure the most suitable intensity for the JC genetic mutations. As presented in Table 1, JC-300 shows the highest oil content, 51.7 ± 0.1%, then JC-150 (48.3 ± 0.1%), JC-226 (45.8 ± 0.1%) and the control JC-0 (45.5 ± 0.1%), respectively. There was no clear correlation between the intensity of gamma irradiation on the physicochemical properties of seed oil (Figure 1).

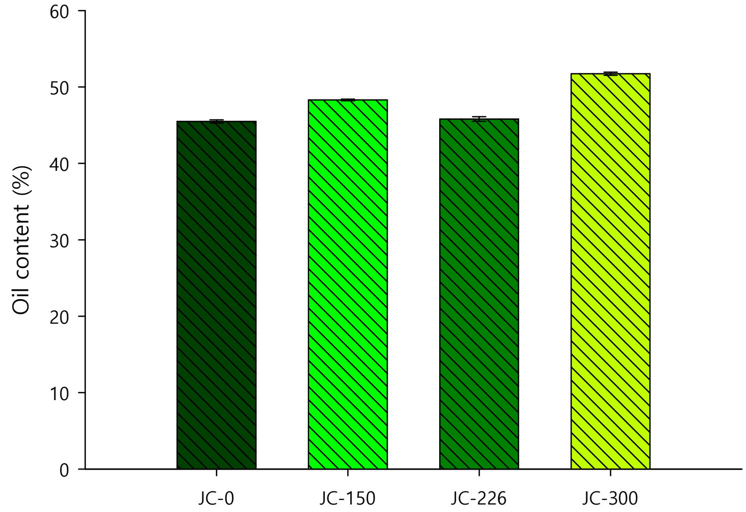

FIGURE 1. Trends of oil content from the mutated JCs and control sample.

The AV and FFA

The AV is one of the most important parameters in identifying oil quality, particularly for biodiesel feedstock. In general, high AV is caused by the presence of free fatty acids (FFAs) at high concentrations. During biodiesel production, the FFAs can reduce the quantity of biodiesel because the FFAs generate soap when base catalysts are used (Bouaid et al., 2016, Mićić et al., 2019). In addition to the quantity of biodiesel, the quality (i.e., purity) of biodiesel is also affected by high FFA. According to Bouaid et al., (2016), the purity of biodiesel decreases from 97.2% to 95.0% when FFA increases from 0% to 4%. In general, the maximum FFA content in good quality feedstock for biodiesel production is less than 1.0% (Litinas et al., 2020). Unfortunately, the FFA content in the jatropha seed oil is generally higher than 0.5%, hence the acid pre-treatment processes are needed to reduce the FFA (Andrade-Tacca et al., 2014, Chai et al., 2014). As shown in this work, the FFA contents in all samples were obtained in the range 1.2-3.5%. The typical moisture effects on the seed oil FFA are described in Figure 2, where high seed moisture results in high FFA. The high FFA content is associated with the water content or the moisture of the seed oil as a result of hydrolyzing TAG to release the organic acids. If the FFA content in seed oil is higher than another one, then the transesterification reactions for biodiesel production will need more base catalyst to neutralize the acid. In addition, the high FFA increases the saponification reactions, consequently reducing biodiesel production (Zulqarnain et al., 2021).

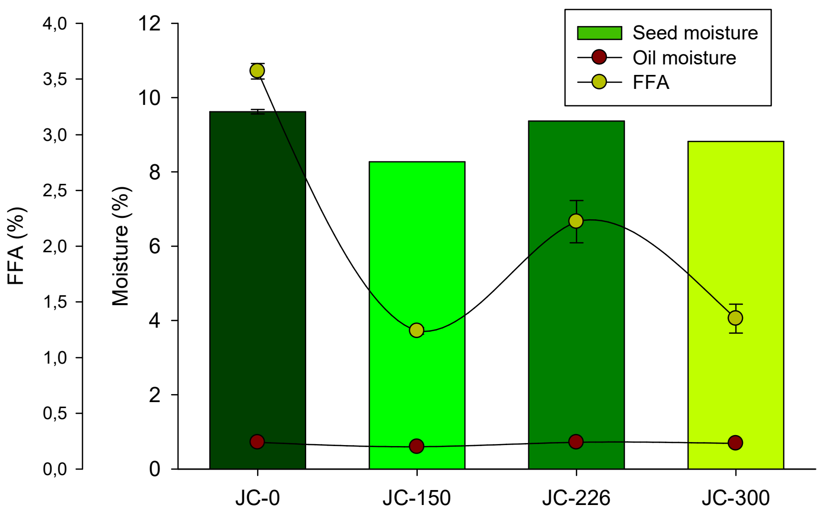

FIGURE 2. Trends of seed moisture, oil moisture and FFA compositions of the mutated JC seed oils and control.

Density

Density, or specific gravity, is one of the most important parameters that must be investigated in the fatty acid industries. Biodiesel fuels are produced from vegetable oils or animal fats and so their characteristics are determined by their feedstock properties. If the seed oil has a high density, so the biodiesel produced also has a high density. The high density indicates large diameter oil droplets. The atomization formation can easily be attained if the oil has a low density. In addition, lower density causes the penetration of oil in the combustion chamber to be fast. Therefore, oil with a lower density is more favorable than that with a higher density (Canakci & Sanli, 2008). In this work, density for all seed oils was observed in the range 0.89-0.90 g/l. These results meet with the EN ISO 3675/EN ISO 12185 standards; the density limit must in the range 0.86-0.90 g/l.

Viscosity

The viscosity is associated with the chemical structure of oil and/or TAG compositions. The viscosity increases with the increasing length of the carbon chain and decreases with an increasing number of double bound of carbon chains. Like density, the viscosity also contributes to the quality of biodiesel as an engine fuel (Canakci & Sanli, 2008). The viscosity is one of the paramount features of an engine fuel because it plays a key role in the fuel spray, mixture formation and combustion process. A high viscosity leads to reduced fuel atomization and interferes with the injection process (Woo & Kim, 2020). In general, the viscosity of oil seed will be reduced after the transesterification process because the big TAG structures are cracked to the simpler structure of fatty acid methyl ester. In this work, the viscosity for all seed oil samples was obtained in the range 23.5-24.6 cSt. These values were similar to vegetable oil viscosity (31.6-51.2 cSt) and much higher than biodiesel viscosity (4.08-5.16 cSt) (Canakci & Sanli, 2008).

The IV

The IV describes the degree of unsaturated oils, animal fats, and fatty acid methyl ester. The IV increases with an increase in the degree of unsaturated oil. The unsaturated oil is determined by the amount of double bond of carbon chains in the fatty acid or TAG structures (Alviso et al., 2021, Giakoumis, 2018). Based on DIN 51605 and DIN 51623, the maximum IV for vegetable oil is around 125 mg I_2/g oil. Meanwhile, the EN 14214 and ASTM 6584 standards assign a maximum IV for vegetable oils of 120 mg I_2/g and 115 mg I_2/g, respectively. From Table 1, the IV for oil samples was lower than that of the required standards. These facts suggest that the JC–150, JC–226 JC–300 and JC-O oils have good feasibility for biodiesel feedstocks.

The SV

The higher SV describes the shorter chain of fatty acids or smaller fatty acid molecules (Jumat et al., 2006, Kuntom et al., 2005, Siew et al., 1995). The SV for all oil samples were quite similar to each other and were obtained in the range 206.0-213.7 mg KOH/g. The highest SV (213.7 mg KOH/g) was obtained for JC-0. However, the SV for these samples were quite similar. This might be due to the fact that the fatty acid composition in TAG is similar. The percentage of un-saponification values were obtained in the range 0.57-0.79%. The type and number of materials in the oils are closely related to the percentage of un-saponification. The percentage of un-saponification values describes the number of substances frequently dissolved in oils or fats which cannot be saponified by the usual caustic processes (Vidal et al., 2018).

The PV and energy content

The PV is an indicator of deterioration or rancidity of oil due to the oxidation at the double bond of an unsaturated fatty acid (Azeez et al., 2019). The PV of oil samples were observed in the range 0.3-1.3 meq/kg. The highest PV was obtained for JC-226, followed by JC-150 (0.3 meq/kg), JC-300 (0.5 meq/kg) and JC-0 (0.5 meq/kg). These results indicate that JC-226 was more easily damaged than JC-150, JC-300 and JC-0. In addition to the PV, the unsaturation level and the presence of oxygen bonds contributed to the energy content of the oil (Van Gerpen, 2005). The energy content of all oil samples was quite similar and in the range 38.1-40.5 kJ/kg. This indicates that the levels of unsaturated fatty acid in the oil samples were also similar. In general, the high energy content is determined by the low unsaturation level and fewer oxygen bonds.

The PE compositions

Phorbol esters (PE) are the main toxic constituents generally present in the jatropha seeds and oils, hence, jatropha seed oils can not be used for nutritional purposes or in the food industry. Furthermore, the presence of PE in the jatropha seeds makes them toxic to some vertebrates, snails and insects (Subhalaxmi et al., 2010). In general, the level of PE composition in the toxic jatropha is around 0.05 mg/g (0.005%) or more (Jonas et al., 2021). Based on Table 1, the PE contents in all seed oils were much higher than 0.005%. The JC-0 oil shows the highest PE composition obtained (3.84 ± 0.58% or 0.38 mg/g). Meanwhile, the PE contents in JC-150, JC-226 and JC-300 were observed to be 2.56 ± 0.67% (0.26 mg/g), 2.04 ± 0.76% (0.24 mg/g) and 0.14 ± 0.05% (0.14 mg/g), respectively. Figure 3 shows that the reduction of PE contents in JC-150, JC-226 and JC-300 were found to be approximately 33.4%, 46.9% and 96.4%, respectively, compared to JC-0. These results may indicate that the gamma irradiation successfully changed the genetics of jatropha, hence the PE contents were decreased. This agrees with Lama et al. (2018), who reported that different genetics produce different seed yield, oil content and toxicity.

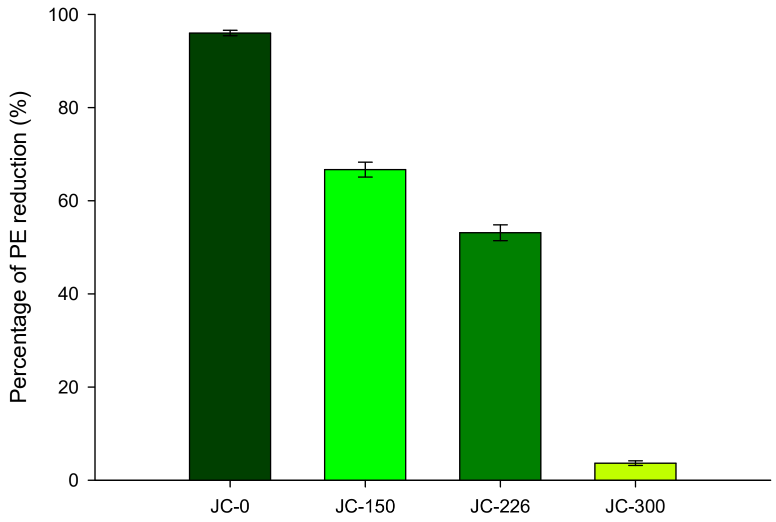

FIGURE 3. The reduction of PE composition in the mutated jatropha oil and control.

TABLE 1. Summary of physicochemical properties of the mutated JC seed oils and control.

Parameter	JC – 0	JC–150	JC–226	JC–300
Seed moisture (%)	9.63 ± 0.06	8.30 ± 0.01	9.40 ± 0.01	8.80 ± 0.01
Oil content (%)	45.5 ± 0.1	48.3 ± 0.1	45.8 ± 0.1	51.7 ± 0.1
Oil moisture (%)	0.24 ± 0.01	0.20 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Acid value (mg KOH/g)	7.0 ± 0.2	2.5 ± 0.1	4.4 ± 0.2	2.7 ± 0.3
% FFA as oleic acid	3.5 ± 0.1	1.2 ± 0.1	2.2 ± 0.1	1.4 ± 0.1
Density (g/ml)	0.90 ± 0.01	0.89 ± 0.01	0.90 ± 0.01	0.89 ± 0.01
Viscosity (cSt)	23.9 ± 0.1	23.6 ± 0.1	24.9 ± 0.1	23.5 ± 0.1
Iodine (mg I_2/g)	95.7 ± 0.1	94.2 ± 0.1	95.4 ± 0.1	94.2 ± 0.8
Saponification value (mg KOH/g)	213.7 ± 0.8	207.1 ± 3.2	208.5 ± 0.8	206.0 ±2.2
Un-saponification material (%)	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
Peroxide (meq/kg)	0.5 ± 0.1	0.3 ± 0.1	1.3 ± 0.1	0.5 ± 0.1
Energy content (kJ/g)	40.5 ± 0.1	39.2 ± 0.1	39.1 ± 0.1	38.1 ± 0.1
Phorbol ester content (%) *	3.84 ± 0.58	2.56 ± 0.67	2.04 ± 0.76	0.14 ± 0.05

Note: All parameters were tested in triplicate. *Amount of PE compositions in seed and oil.

Fatty acid compositions

The types and compositions of fatty acid in the mutated JC seed oils are presented in Table 2. The mutated JC seed oils and *Taxus baccata* can be categorized into the second-generation of biodiesel feedstock, while *Scenedesmus obliquus* and *Ulothrix* are third-generation biodiesel feedstock. *Mentha pulegium* is the fourth-generation feedstock. The types of fatty acid in the mutated JC seed oils were observed as palmitic (P), palmitoleic (Pl), stearic (S), oleic (O), and linoleic (L). The palmitic, oleic and linoleic acids were the three main types of fatty acid in all samples. In the mutated JC seed oils, the relative compositions of oleic, linoleic, palmitic acids were obtained in the ranges 41.82-44.39%, 35.09-37.45% and 12.8-13.4%, respectively. Meanwhile, two main components of fatty acids in the selected references (e.g., *Scenedesmus obliquus* (Mendonça et al., 2022), *Taxus baccata*, *Ulothrix* and *Mentha pulegium* (Jafarihaghighi et al. 2022) were palmitic-linoleic and palmitic-oleic. Based on these results, the mutated JC seed oils can be categorized as oleic-linoleic groups because the oleic and linoleic acids were the main types of fatty acid in the samples (Akintayo, 2004).

Furthermore, the compositions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the mutated JC seed oils were higher than the saturated fatty acids (SFA). Meanwhile, the SFA compositions in *Taxus baccata, Scenedesmus obliquus* and *Ulothrix* were higher than MUFA and PUFA. In the *Mentha pulegium* sample, the composition of MUFA was much higher than SFA and PUFA. The types of fatty acids and their compositions are important parameters for evaluating the quality of feedstocks for biodiesel production (Folayan et al., 2019, Khethiwe et al., 2020). The types of fatty acids and MUFA and PUFA compositions affect the density, viscosity, flashpoint, cloud point and cetane number (CN). The high unsaturated fatty acid content results in a high density, low viscosity, low flashpoint, low CN and high cold point of biodiesel (Folayan et al., 2019), while the oils that have a higher SFA level are easily crystallized. In addition, the carbon chain lengths (CCLs) are associated with the CN, and increased CCLs lead to an enhanced quality of biodiesel burning in the engine (Jafarihaghighi et al., 2020). The

CCLs of fatty acid in the mutated JCs and *Scenedesmus obliquus* were observed to be lineic ($C_{18:2}$) while they are Gondoic ($C_{20:1}$), Erucic ($C_{22:1}$) and Nervonic ($C_{24:1}$) in *Ulothrix*, *Taxus baccata* and *Mentha pulegium*, respectively. This suggests that the JC seed oils and *Scenedesmus obliquus* can produce biodiesel with about the same amount of CN but, when JC seed oils are compared to the selected references, they might produce biodiesel with lower CN.

TABLE 2. Relative fatty acids compositions of the second generation of mutated *JCs* and selected references of the second, third and fourth generations of biodiesel feedstock.

Fatty acids (%)	JC-T (2nd gen.)	JC–150 (2nd gen.)	JC–226 (2nd gen.)	JC–226 (2nd gen.)	*Taxus baccata* (2nd gen.)	*Scenedesmus obliquus* (3rd gen.)	*Ulothrix* (3rd gen.)	*Mentha pulegium* (4th gen.)
Palmitic ($C_{16:0}$)	12.76 ± 0.14	13.28 ± 0.38	12.99 ± 0.62	13.39 ± 0.26	30.59	42	38.65	19.30
Palmitolic ($C_{16:1}$)	0.15 ± 0.25	0.51 ± 0.04	0.56 ± 0.02	0.52 ± 0.07	0.16	-	-	1.00
Stearic ($C_{18:0}$)	7.61 ± 0.18	7.16 ± 1.40	6.61 ± 0.03	6.82 ± 0.66	4.03	7	5.30	1.49
Oleic ($C_{18:1}$)	44.39 ± 0.41	41.82 ± 2.90	42.39 ± 0.01	42.45 ± 1.41	20.02	36	26.75	54.04
Linoleic ($C_{18:2}$)	35.09 ± 0.17	36.68 ± 4.32	37.45 ± 0.08	36.81 ± 2.25	30.37	20	19.38	10.52
Linolenic ($C_{18:3}$)	-	-	-	-	1.69	13.8	3.20	1.74
Others	-	-	-	-	13.14[a]	-	6.72[b]	11.91[c]
SFA	20.37	20.44	19.60	20.21	43.96*	49*	44.02*	22.49*
MUFA	44.54	42.33	42.95	42.97	22.53*	36*	33.45*	65.25*
PUFA	35.09 ± 0.17	36.68 ± 4.32	37.45 ± 0.08	36.81 ± 2.25	33.51*	33.8*	22.53*	12.26*
References	This work	This work	This work	This work	(Jafarihaghighi et al. 2022)	(Mendonça et al. 2022)	(Jafarihaghighi et al. 2022)	(Jafarihaghighi et al. 2022)

Note: 2nd gen, 3rd gen and 4th gen. = the second, third and fourth generation feedstock; [a] = total composition of Capric ($C_{10:0}$) = 2.11%; Myristoleic ($C_{14:1}$) = 2.35, Pentadecanoic ($C_{15:0}$) = 1.01%, Arachidic ($C_{20:0}$) = 6.01%, and Behenic ($C_{22:0}$) = 0.21%; [b] = total composition of Arachidic ($C_{20:0}$) = 0.02% and Gondoic ($C_{20:1}$) = 6.70%; [c] = total composition of Arachidic ($C_{20:0}$) = 1.70%, Erucic ($C_{22:1}$) = 8.10% and Nervonic ($C_{24:1}$) = 2.11%; * = calculated

TAG composition of the mutated JC seed oil

Eleven TAGs were detected in all samples, which consisted of seven polyunsaturated TAGs, three monounsaturated TAGs, and one saturated TAG (Table 3). Polyunsaturated TAGs were categorized as LLL, OLL, POL+SLL, PLL, OOL, OOO, and SOO. Monounsaturated TAGs consisted of PPL, POP, and POS, while the saturated TAG detected was PPP. The total amount of polyunsaturated TAGs in JC-0, JC–150, JC–226, and JC–300 oils were found to be around 75.5%, 71.6%, 71.6%, and 76.2%, respectively. The total compositions of the monounsaturated TAGs in JC-0, JC–150, JC–226, and JC–300 oils were found to be around 19.6%, 24.5%, 23.9%, and 20.6%, respectively. However, the total compostion of saturated TAGs in JC-0, JC–150, JC–226, and JC–300 oils were observed to be around 3.1%, 2.8%, 3.2%, and 2.1%, respectively. Like the fatty acids, the composition and types of TAGs also affected the quality of biodiesel because the fatty acids are the main components in TAGs. In general, the typical TAG distributions in the jatropha oil are presented clearly in Figure 4.

TABLE 3. Relative compositions of TAG in JC-O, JC–150, JC–226 and JC–300.

TAGs	ECN	Relative compositions (%)			
		JC – 0	JC–150	JC–226	JC–300
Polyunsaturated					
LLL	42	4.8 ± 0.1	2.7 ± 1.2	3.9 ± 0.9	3.8 ± 1.0
OLL	44	17.1 ± 1.1	13.3 ± 1.5	14.8 ± 2.3	17.5 ± 0.4
POL+SLL	44	6.7 ± 0.3	5.3 ± 0.4	7.2 ± 0.6	6.4 ± 0.8
PLL	44	19.1 ± 1.2	17.3 ± 0.3	17.3 ± 0.3	21.4 ± 1.3
OOL	46	17.6 ± 1.1	23.5 ± 2.9	17.9 ± 0.3	18.5 ± 0.6
OOO	48	9.3 ± 0.6	8.7 ± 0.8	9.5 ± 0.2	9.9 ± 0.6
SOO	48	0.2 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.1 ± 0.1
Monounsaturated					
PPL	46	1.3 ± 0.5	1.7 ± 0.4	0.8 ± 0.5	1.5 ± 0.2
POP	48	18.2 ± 1.5	22.7 ± 0.2	22.9 ± 0.2	17.1 ± 1.1
POS	50	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0
Saturated					
PPP	48	3.1 ± 1.0	2.8 ± 0.9	3.2 ± 0.4	2.6 ± 0.6
Unknown	-	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.2

Note; ECN = Equivalent carbon number; L = linoleic; O = oleic; P = palmitic; S = stearic

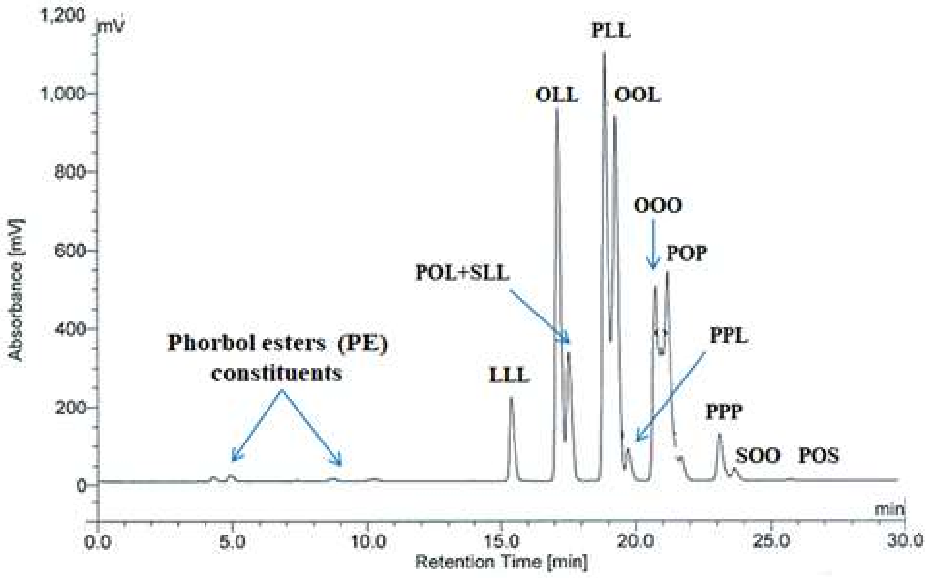

FIGURE 4. Typical TAG distributions and PE constituents in the jatropha seed oil.

Characterization of biodiesel derived from the mutated JC seed oils

Biodiesel feedstocks can be divided into four groups: first, second, third and fourth generation biodiesel. The first and second generations are derived from edible and non-edible oils. The third and fourth generation are derived from algae and genetically modified (GM) algae biomass (Abdullah et al., 2019). Based on these classifications, the samples of the mutated JCs and JC-0 can be categorized as second generation feedstock. In general, the biodiesel derived from the second generation feedstock is of lower quality than the third and fourth generations. This is due to the higher concentration of unsaturated fatty acid in the second generation than in the third and fourth generations. According to Jafarihaghighi et al. (2022), the CCLs and high concentrations of unsaturated fatty acids are strongly associated with the CN of biodiesel and emissions of nitrogen oxide (NOx). To date, the first and second generations of biodiesel are commercially produced (Jafarihaghighi et al., 2022). Meanwhile, the third and fourth generations are not commercially available, as yet. In addition to the quality, the quantity of biodiesel produced must be considered. Either the first, second, third or fourth generation feedstock can be converted to 81–99%, depending on the catalyst used. This suggests that one kilogram of feedstock can produce approximately 800-900 g of biodiesel. Based on these facts, the mutated JCs could be assumed to be a feedstock for producing biodiesel of an appropriate quality and quality. As presented in Table 4, the quality of mutated jatropha oil methyl ester (JOME) meet the EN 14214 European standard.

TABLE 4. Biodiesel quality and European standard EN 14214.

Parameter	Biodiesel				EN 14214
	JOME – 0*	JOME–150	JOME-226	JOME–300	
Acid value (mg KOH/g)	0.32	0.26	0.30	0.26	max. 0.5
FFA (%)	0.16	0.13	0.15	0.13	-
Water content (ppm)	150	100	140	130	500
Iodine value (mg I_2/g)	111.5	121.3	113.4	111.5	120
Density (24 ^{0}C, g/ml)	0.87	0.87	0.87	0.87	0.86 - 0.90
Viscosity (40, cSt)	4.9	4.8	4.8	4.9	3.5 – 5.0
Caloric value (kJ/g)	38.9	37.9	38.4	38.9	-
Cetane number	52.2	52.5	52.0	52.2	min. 47
Cloud point (^{0}C)	7	7	7	8	-
Flash point (^{0}C)	157	158	158	157	-
Ash content (%)	0.001	0.001	0.002	0.010	-
Methyl ester (%)	99.1	98.6	98.8	99.1	>96.5
Yield (%) using NaOH as catalyst	89.3	91.1	89.4	91.2	-

Note: JOME-0, JOME-150, JOME-226 and JOME 300 = *Jatropha* oil methyl ester of JC-0, JC-150, JC-226 and JC-300; * = data were collected from a published paper by Satar et al. (2015).

CONCLUSIONS

Physicochemical properties of mutated JC seed oils (JC-150, JC-226 and JC-300) have been successfully evaluated in this work. The type of TAGs and fatty acid compositions of the mutated JC seed oils were similar to the type of TAGs and fatty acid compositions of JC-0. Meanwhile, the PE content in all mutated JC seed oils was much lower than the JC-0 control. The quality of biodiesel derived from the mutated JC oils was comparable to the second generation of biodiesel. These results show that mutated JC seed oils could be used as one of the second generation feedstocks for biodiesel production. More research is needed to find a suitable physical method of gamma ray intensity to produce mutated jatropha with much higher oil content and zero PE. Thus, the next generation of JCs can probably be used in the energy field and food product sectors.

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REFERENCES

Abdullah B, Muhammad SAFS, Shokravi Z, Ismail S, Kassim KA, Mahmood AN, Aziz MMA (2019) Fourth generation biofuel: A review on risks and mitigation strategies. Renew Sustainable Energy Reviews 107:37-50.

Abou-Arab AA, Mahmoud MH, Ahmed DMM, Abu-Salem FM (2019) Comparative study between chemical, physical and enzymatic methods for *Jatropha curcas* kernel meal phorbol ester detoxification. Heliyon 5:e01689.

Akintayo ET (2004) Characteristics and compositions of Parkiabiglobbossa and Jatropha curcas oils and cakes. Bioresource and Technology 92:307-310.

Alviso D, Zárate C, Duriez T (2021) Modeling of vegetable oils cloud point, pour point, cetane number and iodine number from their composition using genetic programming. Fuel 284:119026.

Andrade-Tacca CA, Chang C-C, Chen Y-H, Ji D-R, Wang Y-Y, Yen Y-Q, Chang C-Y (2014) Reduction of FFA in jatropha curcas oil via sequential direct-ultrasonic irradiation and dosage of methanol/sulfuric acid catalyst mixture on esterification process. Energy Conversion Management 88:1078-1085.

Asmare M, Gabbiye N (2014) Synthesis and characterization of biodiesel from castor bean as alternative fuel for diesel engine American Journal Energy of Engineering 1:1-15.

Azeez AM, Fasakin AO, Orege JI (2019) Production, characterisation and fatty acid composition of jatropha curcas biodiesel as a viable alternative to conventional diesel fuel in nigeria. Green Sustainable Chemistry 9:1-10.

Bouaid A, Vázquez R, Martinez M, Aracil J (2016) Effect of free fatty acids contents on biodiesel quality. Pilot plant studies. Fuel 174:54-62.

Canakci M, Sanli H (2008) Biodiesel production from various feedstocks and their effects on the fuel properties. Journal of Industrial Microbiology & Biotechnology 35:431–441.

Chai M, Tu Q, Lu M, Yang AYJ (2014) Esterification pretreatment of free fatty acid in biodiesel production, from laboratory to industry. Fuel Processing Technology 125:106-113.

Chowdary K, Kotia A, Lakshmanan V, Elsheikh AH, Ali MKA (2021) A review of the tribological and thermophysical mechanisms of bio-lubricants based nanomaterials in automotive applications. Journal of Molecular Liquids 339:116717.

Das AK, Chavan AS, Ch. Shill D, Chatterjee S (2021) Jatropha Curcas oil for distribution transformer – A comparative review. Sustainable Energy Technologies and Assessments 46:101259.

Ewunie GA, Lekang OI, Morken J, Yigezu ZD (2021) Characterizing the potential and suitability of Ethiopian variety Jatropha curcas for biodiesel production: Variation in yield and physicochemical properties of oil across different growing areas. Energy Reports 7:439-452.

Folayan AJ, Anawe PAL, Aladejare AE, Ayeni AO (2019) Experimental investigation of the effect of fatty acids configuration, chain length, branching and degree of unsaturation on biodiesel fuel properties obtained from lauric oils, high-oleic and high-linoleic vegetable oil biomass. Energy Reports 5:793-806.

Giakoumis EG (2018) Analysis of 22 vegetable oils' physico-chemical properties and fatty acid composition on a statistical basis, and correlation with the degree of unsaturation. Renew Energy 126:403-419.

Gutierrez-Lopez AN, Mena-Cervantes VY, García-Solares SM, Vazquez-Arenas J, Hernandez-Altamirano R (2021) $NaFeTiO_4/Fe_2O_3$-$FeTiO_3$ as heterogeneous catalyst towards a cleaner and sustainable biodiesel production from *Jatropha curcas L*. oil. Journal of Cleaner Production 304:127106.

Ha J, Shim S, Lee T, Kang YJ, Hwang WJ, Jeong H, Laosatit K, Lee J, Kim SK, Satywan D, Lestari P, Yoon MY, Kim MY, Chitikineni A, Tanya P, Somta P, Srinives P, Varshney RK, Lee S-H (2019) Genome sequence of *Jatropha curcas L*., a non-edible biodiesel plant, provides a resource to improve seed-related traits. Plant Biotechnology Journal 17:517-530.

Ho CK, McAuley KB, Peppley BA (2019) Biolubricants through renewable hydrocarbons: A perspective for new opportunities. Renewable and Sustainable Energy Reviews 113:109261.

Hoong SS, Arniza M, Mariam NMDNS, Armylisas AHN, Yeong SK (2019) Synthesis and physicochemical properties of novel lauric acid capped estolide esters and amides made from oleic acid and their evaluations for biolubricant basestock. Industrial Crops and Products 140:111653.

Jafarihaghighi F, Ardjmand M, Hassani MS, Mirzajanzadeh M, Bahrami H (2020) Effect of fatty acid profiles and molecular structures of nine new source of biodiesel on combustion and emission. ACS Omega 5:16053-16063.

Jafarihaghighi F, Bahrami H, Ardjmand M, Mirzajanzadeh M, Haghighi BJ, Mahdavi A (2022) Comparing among second, third, and fourth generations (genetically modified) of biodiesel feedstocks from the perspective of engine, exhaust gasses and fatty acid: Comparative assessment. CLCE 2:100025.

Jonas M, Ketlogetswe C, Gandure J (2021) Quantification of Phorbol-12-myristate 13-acetate in Jatropha seed oil and cake at different stages of fruit maturity. International Journal Environmental Studies:1-11.

Jumat S, Said M, Ramli S, Mat Lazim MAS (2006) Oils and fats analysis. Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia 43600 UKM Bangi.

Khethiwe E, Clever K, Jerekias G (2020) Effects of fatty acids composition on fuel properties of jatropha curcas biodiesel. Smart Grids Renewable Energy 11:165-180.

Koh MY, Ghazi TIM (2011) A review of biodiesel production from Jatropha curcas L. oil. Renew Sustainable Energy Reviews 15:2240-2251.

Kumar S, Singh S (2014) Variability assessment of seed traits in Jatropha curcas L. for improvement of oil yield. IJGMB 6:8-15.

Kuntom A, Lin SW, Ai TY, Idris NA, Yusof M, Sue TT, Ibrahim NA (2005) MPOB test method. Selangor, Malaysian Palm Oil Board.

Lama AD, Klemola T, Saloniemi I, Niemelä P, Vuorisalo T (2018) Factors affecting genetic and seed yield variability of *Jatropha curcas (L.)* across the globe: A review. Energy Sustainable Development 42:170-182.

Litinas A, Geivanidis S, Faliakis A, Courouclis Y, Samaras Z, Keder A, Krasnoholovets V, Gandzha I, Zabulonov Y, Puhach O, Dmytriyuk M (2020) Biodiesel production from high FFA feedstocks with a novel chemical multifunctional process intensifier. Biofuel Research Journal 26:1170-1177.

Maftuchah, Zainudin A, Winaya A, Rahmadesi Y (2020) Biodiesel generated from Jatropha (*Jatropha curcas Linn*.) seeds selected based on various genotypes crossbred. Energy Reports 6:345-350.

Mendonça HVD, Otenio MH, Marchão L, Lomeu A, Souza DSd, Reis A (2022) Biofuel recovery from microalgae biomass grown in dairy wastewater treated with activated sludge: The next step in sustainable production. Science Total of the Environment 824:153838.

Mićić R, Tomić M, Martinović F, Kiss F, Simikić M, Aleksic A (2019) Reduction of free fatty acids in waste oil for biodiesel production by glycerolysis: investigation and optimization of process parameters. Green Process Synthesis 8:15-23.

Sanghamitra K, Oramas RV, Prasad RN (2014) Comparative yield and oil quality of toxic and non-toxic Mexican Jatropha curcas grown in the same agroclimatic conditions. American Journal of Plant Sciences 5:230-234.

Satar I, Isahak WNRW, Salimon J (2015) Characterization of biodiesel from second generation gamma-irradiated Jatropha curcas. JTICE 49:85-89.

Sayyar S, Abidin ZZ, Yunus R, Muhammad A (2009) Extraction of oil from jatropha seeds-optimization and kinetics American Journal of Applied Sciences 6:1390-1395.

Siew WL, Tang TS, Tan YA (1995) PORIM test methods. Kuala Lumpur, Palm Oil Research Institute.

Silitonga AS, Atabani AE, Mahliaa TMI, Masjuki HH, Badruddin IA, Mekhilef S (2011) A review on prospect of Jatropha curcas for biodiesel in Indonesia. Renewable and Sustainable Energy Reviews 15:3733-3756.

Subhalaxmi PNK, Sahoo SNN, Sahoo PK (2010) Estimation of toxic phorbol ester in jatropha curcas oil, cake and biodiesel. Asian Journal of Chemistry 22:4069-4074.

Tan SX, Lim S, Ong HC, Pang YL, Kusumo F, Hoe BGH, Chong CT (2019) Two-step catalytic reactive extraction and transesterification process via ultrasonic irradiation for biodiesel production from solid Jatropha oil seeds. Chemical Engineering and Processing 146:107687.

Valdes-Rodriguez OA, Sanchez O, Perez-Vazquez A, Caplan J (2013) The Mexican non-toxic *Jatropha curcas L.*, food resource or biofuel? Ethnobotany Research and Applications 11:1-8.

Van Gerpen JH (2005) Biodiesel processing and production. Fuel Processing Tecnhnology 86:1097-1107.

Vidal NP, Adigun OA, Pham TH, Mumtaz A, Manful C, Callahan G, Stewart P, Keough D, Thomas RH (2018) The effects of cold saponification on the unsaponified fatty acid composition and sensory perception of commercial natural herbal soaps. Molecules 23:1-20.

Woo DG, Kim TH (2020) Effect of kinematic viscosity variation with blended-oil biodiesel on engine performance and exhaust emission in a power tiller engine Environmental Engineering Research 25:946-959.

Yang C, Fang Z, Li B, Liu G, Li J (2010) Breeding of high-oil Jatropha curcas L for biodiesel production. Plant Biotechnology Journal 26:1514-1525.

Yusuff AS (2021) Parametric optimization of solvent extraction of Jatropha curcas seed oil using design of experiment and its quality characterization. South African Journal of Chemical Engineering 55:60-68.

Zulqarnain, Ayoub M, Yusoff MHM, Nazir MH, Zahid I, Ameen M, Sher F, Floresyona D, Nursanto EB (2021) A comprehensive review on oil extraction and biodiesel production technologies. Sustainability 13:1-28.