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# Biodiesel production and de-oiled seed cake nutritional values of a Mexican edible *Jatropha curcas*

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ABSTRACT

The scarcity of fossil fuels, in addition to environmental damage due to fossil fuel use and exploration, promotes research into alternative energy sources such as biofuels. Biodiesel has attracted considerable attention in recent years as an alternative to fossil fuels, since it is renewable, biodegradable and non-toxic. Biodiesel can be obtained from animal fat, vegetable oils including cooking oil. In this work, a method of producing biodiesel from seed cake waste from the edible *Jatropha curcas* L. plant was developed. Oil extraction using hexane gave the best oil quality. Transesterifications of approximately 95% were obtained by alkali or acid catalysis, and the obtained biodiesel products were successfully corroborated with NMR techniques. Since *J. curcas* is a non-toxic plant, the remaining de-oiled cake was tested for its nutritional properties. Nutritional analysis showed a content of 43% and 33% of protein and carbohydrate, respectively; suggesting that this waste can be used as an attractive protein and carbohydrate source for fermentation processes and/or for formulations for animal feeding. In conclusion, this work provides evidence that the oil from an edible and non-toxic species of *J. curcas* is an attractive option for biodiesel production with nutritional applications and negligible wasting.

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## 1. Introduction

Towards what seems to be the end of the petroleum age, biodiesel has emerged as a biodegradable, non-toxic and environmentally friendly renewable alternative to conventional petroleum-derived diesel [1]. In the past, several organic sources have been tested for biodiesel production including vegetable oils, animal fats, and used cooking oils [2–6]. Despite the wide variety of sources, vegetable edible and non-edible oils appear to be the most promising materials for biodiesel production [2].

*Jatropha curcas* L. (Euphorbiaceae) is a drought-resistant shrub widely distributed in Central and South America, Africa and South-East Asia. This species is able to grow in wastelands and due to its high oil content; it serves as raw material for the production of soap, candles, cosmetics, and paraffins [3].

In general, seeds from different varieties of *J. curcas* are considered toxic to human and animals because of the presence of curcin and phorbol esters [3]. Accidental ingestion of these seeds produces giddiness, vomiting, diarrhea, and even death [4]. However, ionizing radiation [5] and chemical treatments [6,7] have been used to reduce the levels of these toxic compounds with a moderate success at an extremely high cost [8]. Interestingly, species of *J. curcas* from México lack the toxic compounds and are consumed as roasted seeds in the Mexican cuisine [9,10]. As a renewable source of energy, large extensions of land are necessary to assure a proper exploitation of the Mexican *J. curcas*. According to the Mexican Secretary of Agriculture, Livestock, Fishing, Feed and Rural Development (SAGARPA), the state of Puebla possesses a cultivable area of $6.2 \times 10^7$ acres, which can produce up to 3 kg of seeds/plant at a density of 2000 plants/2500 acre [11]. This extensible land area predicts that the state of Puebla will be able to produce enough biodiesel with an estimated yield of 324 g $kg^{-1}$ of seed.

The objective of this study was to generate biodiesel from an endemic, non-toxic, and edible variety of *J. curcas* in Puebla, México. After oil extraction, the generated waste (de-oiled cake) was analyzed as a potential source of nutritional compounds. In this work, we report the production of biodiesel using conventional

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processes, which was confirmed by structure elucidation of the final compounds. In addition, the nutritional value of the de-oiled cakes as protein and carbohydrate source was confirmed.

## 2. Experimental

### 2.1. Plant material

Seeds from a non-toxic, edible species of *J. curcas* were collected in Hueytamalco, a community in the state of Puebla, México, in August 2011. A voucher specimen numbered 53203 was deposited in the Herbarium of the Benemérita Universidad Autónoma de Puebla, México. As a result of the high content of moisture in the seeds and to avoid their deterioration by fungi during storage, the whole and undamaged seeds were cleaned, peeled, and dried at 60 °C for 12 h until the moisture content was below 5%. Dried seeds were crushed to improve oil extraction.

### 2.2. Oil extraction and characterization

All chemicals used in this work were of reactive grade (Fermot, Baker and Sigma–Aldrich). 100 g of crushed seed were sequentially extracted (×3) by reflux with *n*-hexane, ethyl ether, and ethyl acetate over 4 h using a Soxhlet apparatus. The solvent was evaporated to dryness under reduced pressure in a rotator evaporator (at 40 °C) and a yellow viscous oil was obtained. To avoid decomposition, the oil was stored at −20 °C for subsequent physicochemical analysis and transesterification reactions, whereas the de-oiled seed cake was stored at 5 °C for further bromatological analyses.

Physico-chemical properties such as moisture, refraction index, saponification number, pH, specific gravity, and iodine value (an indirect measurement of the level of unsaturated fatty acids) were evaluated following standards from the American Oil Chemists' Society (AOCS) methods [12]. Acidity, a measurement of the extent to which esteric bonds of fatty acids are hydrolyzed into the parent glyceride molecule and expressed as the content of free fatty acids [13], and viscosity were determined following the ASTM methods D-664 and D-445, respectively.

### 2.3. Oil transesterification

To optimize oil transesterification, several alcohols such as methanol, ethanol, 1-propanol or 1-butanol under alkaline or acid catalyzed conditions were tested. Under alkaline conditions, one of the following catalysts was present: NaOH, KOH, LiOH, $Ba(OH)_2$, $Ca(OH)_2$, CaO, $Na_2CO_3$, or $K_2CO_3$, whereas in the acid conditions $H_2SO_4$, HCl, or $H_3PO_4$ were assayed. Reactions were carried out in a 50 ml round bottom flask connected to a reflux condenser and a magnetic stirrer. Alkaline reactions commenced when 30 g of *J. curcas* oil was poured into the 50 ml flask supplemented with one of the alcohols in a molar ratio of 9:1 and the amount of basic catalyst was 1.3 wt% of the oil. In the case of acidic reactions, a molar ratio of 30:1 and the amount of acid catalyst was 3 wt% of the oil. Although other molar ratios were tested, the efficiencies of transesterification were much lower (data not shown).

Transesterification process was catalyzed at 5 °C below the boiling point of each alcohol used in the reaction for 1 h and 24 h for the alkaline and acid reaction. Then, the catalysis with methanol was performed at 62 °C (FAME), ethanol at 73 °C (FAEE), propanol at 92 °C (FAPE), and butanol at 112 °C (FABE). In order to compare the yields obtained for each of the alcohols used in the reaction, the alkaline catalyzed using NaOH was done at 62 °C.

After the transesterification reactions, mixtures were allowed to separate into two phases. The bottom phase containing the impurities and crude glycerol coming from the oil triglycerides were drawn off. The biodiesel present at the top phase was then purified by distillation and hot water washing (×3) to remove the excess of alcohol and dissolved glycerol, respectively. Finally, the biodiesel was dried with $Na_2SO_4$ anhydrous.

### 2.4. Fatty acid profile

The profile of the fatty acids in the oil was determined by gas chromatography using a Varian Star 3400 equipped with a mass detector (Varian Saturn 2000) and a silica column (30 m × 0.322 mm) containing nitroterephthalic-acid-modified polyethylene glycol as the stationary phase (DB-FFAP 123-3232, Agilent Tech). Helium was used as the carrier gas with a flow rate of 1 ml/min at an initial temperature of 140 °C (conditioned for 1 h). The temperature of the detector and the injector was set at 250 y 280 °C, respectively. The separation of the fatty acid methyl esters was performed by injecting 1 μl of the sample into the column at 140 °C for 1 min followed by an increase of 5 °C/min until a temperature of 190 °C was reached. Samples were run in triplicate.

Compounds were identified by comparison of the retention times and mass spectra of the methilic esters used as reference standards. Standard solutions of methyl esters of 1% (w/v) of capric, myristic, palmitoleic, palmitic, linoleic, oleic and stearic acids (Sigma–Aldrich) were used, and the relative percentage was determined by the total area of the peaks.

### 2.5. Structure elucidation

NMR spectroscopic measurements were recorded in a Varian Associates Gemini spectrometer operating at 200 MHz for hydrogen and 50.3 MHz for carbon, using 5 mm sample tubes in a solution of deuterated chloroform containing tetramethyl silane (TMS) as an internal standard [14]. Transesterification yields and efficiencies were determined by $^1$H NMR using published methodologies [14,15].

### 2.6. Bromatologic analysis of seed cake

The de-oiled seed cake was analyzed with routine bromatologic tests for crude protein, crude fiber, moisture, ash, ether extract and carbohydrates, following published methodologies [16].

### 2.7. Biodiesel properties

Fuel properties such as viscosity, specific gravity, flash point, cloud point, acid value, copper strip corrosion; and free and total glycerol contents were determined using the ASTM methods D445, D287, D93, D2500, D664, D874, and D6584; respectively.

## 3. Results

### 3.1. Oil extraction

Polar (ethyl acetate) and non-polar (*n*-hexane and ethyl ether) solvents have been used at laboratory scale to extract oil from seed [17]. Nowadays however, *n*-hexane is the only solvent used commercially in large-scale production of biodiesel [18]. In this study we compare the yields of oil extraction from seeds using various organic solvents, such as *n*-hexane, ethyl ether, and ethyl acetate. The best yield was obtained with ethyl acetate (54.3%), followed by *n*-hexane (47.7%) and ethyl ether (45.9%). However, the ethyl acetate extract showed an intense yellow color suggesting that, in addition to triglycerides, the solvent was able to extract other compounds. This finding is in agreement with the results obtained in the oil extraction of *Lotus plumule*, which contained

**Table 1**
Physico-chemical properties of *Jatropha curcas* oil.

| Parameter | Value ± SD |
|---|---|
| Acidity (mg KOH $g^{-1}$ oil) | 0.85 ± 0.002 |
| Iodine value [g $I_2$ (100 $g^{-1}$ oil)] | 102.6 ± 0.21 |
| Moisture (%)[a] | 4.72 ± 0.07 |
| Refractive index (20 °C) | 1.473 ± 0.00 |
| Saponification value (mg KOH $g^{-1}$ oil) | 211.9 ± 1.83 |
| Specific gravity (20 °C) | 0.91 |
| Viscosity (cP) (20 °C) | 44.42 ± 1.53 |

[a] *Jatropha curcas* seed moisture.

**Table 2**
Transesterification efficiencies[a] obtained in the alkaline reaction.

| Catalyst | Methanol | Ethanol | Propanol | Butanol |
|---|---|---|---|---|
| NaOH | 95.5 | 83.6 | 83.9 | 83.2 |
| KOH | 93.2 | 87.4 | 84.1 | 85.9 |
| LiOH | 90.1 | 82.9 | 79.6 | 71.1 |
| $Ba(OH)_2$ | 82.2 | 77.9 | 43.6 | 41.6 |
| $K_2CO_3$ | 78.0 | 82.4 | 86.8 | 85.8 |
| CaO | NR | NR | NR | NR |
| $Ca(OH)_2$ | NR | NR | NR | NR |
| $Na_2CO_3$ | NR | NR | NR | NR |

[a] In percentages. NR = no reaction observed.

unsaponifiable matter using the same solvent [19]. In contrast, the oil obtained with *n*-hexane, showed a clear yellowish color and this oil was chosen for testing the physicochemical properties and biodiesel generation.

### 3.2. Physico-chemical properties

The physico-chemical characterization of the *J. curcas* oil, such as moisture content, refractive index, specific gravity, viscosity and the iodine value; are shown in Table 1.

### 3.3. Oil transesterification

To explore the conditions by which the highest transesterification yield can be obtained, various reaction conditions using different alcohols were tested. These conditions included alcohol:oil ratio, catalyst concentration, reaction temperature, and reaction time. For the alkaline transesterification, a yield of 95.5% was obtained with methanol in presence of NaOH as a catalyst (Table 2), whereas for the acid transesterification, a yield of 94.4% was obtained with ethanol in presence of $H_2SO_4$ (Table 3).

To corroborate that the transesterification occurred, NMR spectral analyses of the final reaction were carried out. $^1H$ NMR spectra of pure *J. curcas* oil showed two doublets of doublets signals at 4.14 and 4.29 ppm corresponding to the glycerol moiety. This signal was used as a reference to confirm that transesterification occurred because it is missing in the biodiesel spectra [14]. Additionally, a signal at 3.65 ppm corresponds to the methyl group of the methanol was observed. In the case of the $^{13}C$ NMR, the signals corresponding to the fatty acid residues are as follows: at 172.7 ppm there is a signal corresponding to a carbonyl group, whereas at 128.9, 128.7, 128.5, 126.8 and 126.7 ppm there are signals

**Table 3**
Transesterification efficiencies[a] obtained in the acidic reaction.

| Catalyst | Methanol | Ethanol | Propanol | Butanol |
|---|---|---|---|---|
| $H_2SO_4$ | 71.5 | 94.4 | 82.4 | 81.3 |
| HCl | NR | 87.2 | 76.9 | 71.5 |
| $H_3PO_4$ | NR | NR | 34.9 | 58.4 |

[a] In percentages. NR = no reaction observed.

**Table 4**
Fatty acid composition[a] of *Jatropha curcas* seed oil.

| Fatty acid | | Area [%±SD] |
|---|---|---|
| Capric | C10:0 | 0.91 ± 0.14 |
| Myristic | C14:0 | 0.36 ± 0.01 |
| Palmitoleic | C16:1 | 0.46 ± 0.03 |
| Palmitic | C16:0 | 16.09 ± 0.52 |
| Linoleic | C18:2 | 27.60 ± 1.26 |
| Oleic | C18:1 | 47.20 ± 1.15 |
| Stearic | C18:0 | 7.08 ± 1.09 |
| Others | | 0.30 |
| ∑Saturated fatty acid | | 24.54 |
| ∑Unsaturated fatty acid | | 75.46 |

[a] At a detection limit of 0.01%.

**Table 5**
Bromatological analysis of the *Jatropha curcas* seed cake.

| Parameter | Value ± SD |
|---|---|
| Crude protein (%) | 43.48 ± 0.83 |
| Crude fiber (%) | 5.73 ± 0.07 |
| Moisture (%) | 8.21 ± 0.47 |
| Ash (%) | 8.50 ± 0.07 |
| Ether extract (%) | 1.54 ± 0.09 |
| Carbohydrates (%) | 32.54 ± 0.12 |

corresponding to double bonds of carbons. Signals corresponding to $CH_2$ can be observed at 33.8, 31.6, 31.2, 29.4, 29.3, 29.1, 28.8, 26.9, 25.4, 24.7, 22.5 and 22.4 ppm, whereas the signal for a methyl group is at 13.9 ppm. The methyl corresponding to the alcohol group appears at 50.9 ppm.

Based on the GC–MS analysis, the fatty acid profile of *J. curcas* seed oil contained capric, myristic, palmitoleic, palmitic, linoleic, oleic and stearic acids with their relative abundance are detailed in Table 4.

### 3.4. Bromatologic analyses of seed cake

After oil extraction, the seed cake was subjected to bromatological analyses. Results of the measured parameters are listed in Table 5.

### 3.5. Biodiesel properties

The fuel properties measured after the transesterification process, such as viscosity, specific gravity, flash point, cloud point, acid value, copper strip corrosion, and free and total glycerol are described in Table 6.

## 4. Discussion

The production of fuels from renewable sources is a growing research theme as most of the fuels used today are obtained from

**Table 6**
Properties of fuel obtained after transesterification

| Property | Method | Limits | FAME | FAEE | FAPE | FABE |
|---|---|---|---|---|---|---|
| Kinematic viscosity, 40 °C ($mm^2$ $s^{-1}$) | D445 | 1.9–6.0 | 4.35 | 4.72 | 5.27 | 5.62 |
| Specific gravity, 15 °C ($kg$ $m^{-3}$) | D287 | 860–900 | 876.8 | 877.1 | 890.2 | 929.5 |
| Flash point (°C) | D93 | 93 min | 163.6 | 172.6 | 173.7 | 178.2 |
| Cloud point (°C) | D2500 | Report | 1.3 | 1.0 | −2.5 | −4 |
| Acid value (mg KOH $g^{-1}$) | D664 | ≤0.50 | 0.49 | 0.47 | 0.48 | 0.50 |
| Copper strip corrosion | D130 | ≤3 | 1a | 1a | 1a | 1a |
| Free glycerol (% mass) | D6584 | ≤0.020 | 0.018 | 0.018 | 0.019 | 0.020 |
| Total glycerol (% mass) | D6584 | ≤0.240 | 0.200 | 0.205 | 0.230 | 0.239 |

non-renewable sources, such as petroleum. Although several renewable sources have been tested for biodiesel production including animal fats, and used cooking oils, vegetable oils appear to be the most promising materials for biodiesel production. In this work, biodiesels produced from the seeds of an endemic species of *J. curcas* in Mexico was studied.

Measurements of the physico-chemical properties of the oil extracted from the seeds of *J. curcas* show: (1) an iodine value of 101.60 g of $I_2$ [100 $g^{-1}$], which represents an 84% of the standard iodine value of 120 accepted for biodiesel, according to the Europe's EN 14214 specifications [20]; (2) an acidity of 0.85 mg KOH $g^{-1}$, which represents a low content of fatty acids, which will favor a high biodiesel yield as the saponification is reduced [21]; (3) a saponification value of 211.9 mg KOH $g^{-1}$ oil was measured, which is approximately a 10% higher than the average of 193.55 mg KOH $g^{-1}$ oil measured in oils extracted from other *Jatropha* species [20].

We report an oil yield of 48% (using hexane), which represents an increase of 38% and 43% of oil extracted from another specie of *J. curcas* and *Euphorbia lathyris*, respectively [22]. Interestingly, a very similar yield of oil (51.5%) was obtained from a similar species from China, but in that case, the extraction was achieved using pressures of 20–50 MPa [17], whereas in our study, the high yield was obtained by extraction with simple methods and no high pressure.

The moisture content is one of the main parameters to monitor and control during oil extraction from seeds. This parameter is very important because under high moisture content, residual enzymes left after oil extraction can promote the breakdown of the oil into fatty acids [18]. In addition, molds may grow on oilseeds with high moisture content if improperly stored for long periods. Active enzymes and molds will lead to a reduction in the final oil product both in the quality and in the yield. Furthermore, it is well known that free fatty acids and moisture content have important effects on the transesterification process using catalysts [21,23]. To avoid this inconvenient, in this work the moisture of seed was targeted below 5%, and a final moisture value of 4.72% was measured.

The oil transesterification yield was optimized under alkaline and acid conditions in the presence of various alcohols and catalysts. We report optimal results when methanol in presence of NaOH and ethanol in presence of $H_2SO_4$ were used. In both cases, transesterification yields reached approximately 95% efficiency. These results are in agreement with other studies that reported conversions between 90 and 98% using NaOH as a catalyst [24–26] and 90–95% efficiency when other *Jatropha* varieties were tested [27,28]. Interestingly, a concomitant decrease of the yield during the alkaline transesterification with an increase of the number of carbon atoms present in the alcohol molecule was observed. This behavior is associated with the reversibility of the reaction, because all the reagents are in contact throughout the reaction in the same phase [29,30]. Also, the use of catalysts such as NaOH, KOH, or LiOH produced a better transesterification than $Ba(OH)_2$, $K_2CO_3$, CaO, $Ca(OH)_2$, and $Na_2CO_3$. This difference can be attributed to a more homogenous mixing of monovalent ions, which facilitate the formation of alcoxide groups that are fundamental in an alkaline catalysis [31]. To compare the yields of the different alcohols in an alkaline catalysis with NaOH, the temperature used for the methanol (62 °C) was used. Results showed a similar tendency in yield associated with the number of carbons in the alcohol molecule giving yields of 96%, 80%, 78% and 75% when methanol, ethanol, propanol or butanol were used, respectively.

Regarding the acid transesterification, most of the reactions yielded a low to moderate efficiency. This behavior is based on the reaction rates, which are approximately 4000 slower than alkaline catalysis [32]. No effect of the length of the carbon chain of the alcohol was observed as an increase in the efficiency was observed. For instance, when $H_2SO_4$ was used, a higher yield was obtained with butanol (81.3%) when compared to methanol (71.5%). This tendency can be explained as a result of phase miscibility, which is more critical in acid than in alkaline catalysis [30].

Analysis of the fatty acid composition after the reaction (Table 4) shows that the majority of the fatty acids present in the oil were *cis*-oleic (C18:1, 47.2%), linoleic (C18:2, 27.6%) and palmitic (C16:0, 16%). In addition, we report for the first time the presence of capric and myristic acids in *J. curcas* seeds at concentrations of 0.91 y 0.36%, respectively [33–35].

After oil extraction from the seed of *J. curcas*, an enormous generation of de-oiled seed cakes is expected. Since the species used in this study lack toxic compounds [30], we prompt to evaluate the potential use as protein and carbohydrate sources for animal food and industrial applications, such as fermentations. Bromatological analyses of the de-oiled seed cakes show a high protein (~45%) and carbohydrate (~33%) content (Table 5). A previous study reported that the proteins extracted from *J. curcas* show a good balance of essential amino acids, which is comparable to proteins extracted from soybean [36].

Analyses of the properties of the biodiesel obtained in this study are comparable to other biodiesels obtained from *Jatropha* species [28,37,38]. In addition, the parameters measured in our study fit the standards recommended by the ASTM-D6751 (Table 6), which indicates that the ester content in the biodiesels FAME, FAEE, and FAPE have the standard values to function as fuels; except for FABE, which showed values out of range [37].

## 5. Conclusion

Biodiesel productions with a high to moderate yield from the oil extracted from the seeds of *J. curcas* is reported after optimization using different alcohols, reaction times, molar ratios (alcohol:oil), and reaction temperature. Best results were obtained with methanol under alkaline conditions using NaOH. Bromatological analyses showed that the de-oiled seed cake present a high protein and carbohydrate source with potential applications as food supplement or industrial processes. In addition, the biodiesels FAME, FAEE and FAPE fit the minimal specification as fuels. Taking together, the biodiesels obtained from the Mexican *J. curcas* oil and the use of de-oiled seed cake as a protein and carbohydrate source makes this species a very attractive option as a biofuel crop because its negligible waste production.

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