Extraction and Characterization of Crop Oil from Seed Kernels of Feun Kase (*Thevetia peruviana*)

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Abstract. The seed kernel of *Thevetia peruviana* is a non-edible material but a potential resource to produce bulb lights in local society of West Timor. The light produced by the seeds makes it a probable feedstock for biodiesel production. In this report, the optimum condition for extraction of the crop oil from the seed kernel of Feun Kase (*Thevetia peruviana*) using conventional soxhlet technique was studied. The solvent used were chloroform, water, and a chloroform-water binary solvent. The parameters investigated effecting the oil yield involved various solvent polarities, extraction time, and temperature. Each experiment was conducted in 250 mL soxhlet apparatus. The extract was analyzed to examine the physicochemical characteristics including density, kinematic viscosity, acid value, iodine value, saponification value, and water content. The optimum conditions were found after 4.0 h extraction time, extraction temperature of 75 °C and a chloroform-water ratio of 100:0 (polarity index 4.10). The oil extract was found to be 52.43 ± 1.73%. These results revealed that the crop oil from the seed kernel of Feun Kase is a potential feedstock for biodiesel production.

Keywords: *Thevetia peruviana*, crop oil, seed kernel, biodiesel production.

1. Introduction

*Thevetia peruviana* is a small plant globally known as yellow oleander, lucky nut, bush milk, orange oleander or kolkaphul. In Indonesia, this plant is more popularly known as ginje, but in some regions, it is given different names, e.g. Nagasari (East Java) and Ki Hujan (West Java). On Timor Island, the community calls this plant as Feun Kase, meaning *Kemiri Kota* (City Candlenut). Traditionally, in West Timor Island, this plant was used as a fuel source of lamps, but this habit disappeared with the development of technology and modernization. This species of plant grows widely in the tropics and subtropics. All parts of this plant are toxic [1]. The toxic properties of these plants are reported to come from the apigenin-5-methyl-ether (flavonoid) compounds and several other compounds such as triterpenoids and glycosides [2]. Feun Kase stars flowering after one and a half year after planted and it blooms thrice every year. In a hectare, 3000 saplings can be planted and out of which 52.5 tons of seed can be collected [3]. It has been reported [4] that Feun Kase seed kernels have a high level of oil content (about 60-65%) and a valuable protein content (30-37%), without fat. It remains non-edible because of the presence of toxins [5]. The high oil content of Feun Kase seeds indicates potentiality of the plant as a raw material for a biodiesel production.
The problems related to raw materials and extracting methods are still the major challenges in a biodiesel production because 60-75% of the total cost of biodiesel production is the cost of providing raw materials and the remaining 25-40% processing costs [6]. Recently, the development and research on biodiesel is 95% statically directed to biofuel sources, such as edible oil or conventional sources, using a transesterification method [7]. This condition is very unfavorable because of the food competition and the high cost of processing problems which make the price of biodiesel more expensive than the fossil fuel. One of the potential plants that could be developed as a raw material for a biodiesel production is Feun Kase.

In addition, the production of biodiesel from vegetable materials is done through several stages, namely oil extraction, transesterification process, and purification of products. In obtaining the crop oil with high yield, the oil must be extracted using a high-performance extraction method. The choice of solvent type (polarity), extraction time, and temperature are also the main factors determining the number of isolation products [8-10]. The inability to control these factors can lead to failure to achieve the high yields and a good oil quality. Several studies of reported extraction methods also support the empirical fact of the importance of solvent selection [8,11]. The objective of this works was to investigate the effects of chloroform-water solvent with different polarities, extraction time, and temperature for the crop oil extraction from the seed kernels of Feun Kase using a conventional soxhlet technique to obtain maximum oil yield.

2. Materials and Methods
The seeds of Feun Kase that were used in this work was obtained from Soba Village, West Amarasi, Kupang District of East Nusa Tenggara Province in Indonesia. After the seeds of Feun Kase were collected, the seeds were carefully separated from the shells and removed from unwanted ingredients, such as remnants of the outer shell, twigs, and fine pebbles. The seeds of Feun Kase were opened manually to obtain the Feun Kase kernels. The kernels were ground using a sieve plate and shaker grinder to reduce it into a size of range 0.5 – 0.75 mm. The entire chemical used in this work, such as chloroform, iodine, ethanol, potassium hydroxide, hydrochloric acid, glacial acetic acid, sodium thiosulfate, and potassium iodide, were of analytical reagent grade obtained from Cica-Merck (Kantako Chemical).

The effects of solvent type (polarities), extraction time, and temperature were studied in order to obtain the optimum conditions for the extraction to achieve maximum oil yield. The crop oil from seed kernels of Feun Kase was extracted with a soxhlet method. Thirty-seven (37) extractions with different combinations of solvent types, extraction time, and temperature were performed. The extraction of the oils was carried out with a soxhlet apparatus of 250 cm³ capacity using chloroform and water. A sample of 25 g of seed kernels was extracted with non-polar and polar solvents, with different ratios of chloroform to water (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50 v/v) to give the solvent polarity of 4.10, 4.59, 5.08, 5.57, 6.06, and 6.55, respectively. Next, five different temperatures of 70 °C, 75 °C, 80 °C, 85 °C, and 90 °C were investigated to obtain the optimum extraction temperature, while the extraction time was varied between 3.0 to 5.5 h. At the end of the extraction process, the solvent was removed by a vacuum evaporator at 40 °C for 1 h. Furthermore, the oil obtained was filtered and then mixed with 2% of distilled water (degumming process). Subsequently, the mixture was heated at 70° C and stirred for about 30-60 minutes, added magnesium sulfate anhydrous and filtered to obtain the oil extract. The oil obtained after evaporation was weighed and the oil yield was calculated. All experiments were repeated at least twice and the final value was the average of all. The oil yield was calculated by using equation 1 [5].

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\text{Oil yield} \, (\%) = \frac{W_o}{W_s} \times 100\%
\]

Where
\( W_o \) = mass of oil extracted (g)
\( W_s \) = mass of the seed kernels (g)
3. Results and Discussion

The successful separation of oil components in Feun Kase seed kernels by the soxhletation technique is highly dependent on the level of solvent polarity and separated components. The difference in solubility of each component into the solvent, then the selectivity of the solvent is crucial to succeeding in the extraction or isolation process.

In principle, the extraction of Feun Kase seed kernels oil was done by weakening the attraction between the oil molecules in a material so that the oil molecules can separate from the material and assemble to form bonds or new interactions. The occurrence of this event can be done by dissolving the mixture in a suitable solvent. The interaction between the solvent molecule and the material molecule produces events such as the occurrence of penetration of solvent molecules into the material, on the contrary, the molecules of the ingredients penetrate into the solvent. The easier a solvent penetrates into the material or otherwise, the attraction between the host molecules gets faster so that the molecules of the material (oil) are more easily extracted from the material and drawn into the solvent phase.

The polarity of the solvent is measured from the dipole moment. Water is the most polar solvent and has a polarity index or Snyder index (SI) of 9.0, whereas n-hexane has a Zero-Snyder index, including very non-polar solvents [8]. The polarity of other solvents lies between the polarity of water and n-hexane. Chloroform classified as a semi-polar solvent with a Snyder index of 4.1. The physical properties of the solvents used are listed in Table 1. The similarity or closeness of the polarity index between the solvent molecules with the building block molecules of a material, allows the shift or displacement of building block molecules into the solvent, resulting in separation.

The physicochemical properties of the oil yield, namely: density, viscosity, acid value, iodine value, saponification value, and water content of the extracted oil were determined using the ASTM method.

| Physical Properties       | Unit   | Name of Solvent | Chloroform | Water |
|---------------------------|--------|-----------------|------------|-------|
| Polarity (Snyder index)   | -      |                 | 4.1        | 9.0   |
| Dipole moment             | Debye  |                 | 1.04       | 1.87  |
| Boiling point             | °C     |                 | 61         | 100   |
| Dielectric constant       | -      |                 | 4.71       | 78.36 |
| Cohesive energy density   | J mol / mL |             | 332.00     | 2095.93 |
| Viscosity                 | mPa    |                 | 0.54       | 0.89  |
| Surface tension           | cal / mol A² |           | 38.39      | 104.70 |

The separation of the oil components from the building molecules using the chloroform-water solvent was also influenced by the formation of hydrogen bonds between the oxygen atoms contained in the oil molecule with the hydrogen atom contained in a water molecule or vice versa. The preferential of the solvent to donate or accept electrons (nucleophilic) and to donate or accept H⁺ (electrophilic) with the components in the material matrix also determines the ease of penetrating the oil component of the building block molecule [8]. The chloroform-water solvent composition of 100: 0; 90:10; 80:20; 70:30; 60:40; and 50:50 with a solvent polarity of 4.10 to 6.55 were tested for its ability to extract the oil from Feun Kase seed kernels. The amount of oil extraction was calculated in % of total oil present in the seed kernels of Feun Kase. The effect of the solvent composition on oil yield was shown in Figure 1.
The combination of chloroform-water solvent actually decreases the percentage of oil extracted (Figure 1). The maximum oil yield of 51.82% was obtained when using a single chloroform solvent. The increase of solvent polarity from 4.59 to 6.55 due to an increase in the water composition resulted in a decrease of oil yield by 4.9 - 67%. These results indicate that the percentage of non-polar lipid in Feun Kase seed kernels was greater than that of a polar lipid content. The solvent boiling point, surface tension, and viscosity decreased at lower temperatures, and then the solvent was able to reach the active sites in the matrix more easily. The water with higher viscosity and surface tension than that of chloroform (Table 1) was found to be inappropriate in oil extraction of Feun Kase seed kernels because it would hinder the solvent absorption into the active sites inside the matrix, however, the dielectric constant and cohesive energy are significantly higher, then the water molecules can strongly bond to the polar oil components in the matrix [8]. Therefore, the ratio of chloroform and water composition of 100: 0 (polarity 4.1) which gave the highest oil yield was considered the best solvent choice and to be studied further.

The extraction results are not only influenced by the type and polarity of the solvent, but also by extraction conditions such as extraction temperature and extraction time. The temperature may affect the rate of extraction and oil yield because the temperature change affects the kinetic energy of the solvent molecules and the collision frequency between the solvent molecules and the target molecules. The effect of the extraction temperature on the amount of extracted oil on Feun Kase seed kernels using the chloroform solvent was shown in Figure 2.

The increase in the extraction temperature can decrease the surface tension and solvent viscosity further increases the oil yield. The amount of extracted oil increased by 6.8% with an increase in the extraction temperatures from 70-75°C. Therefore, the selected extraction temperature was the temperature capable of producing the highest oil yield which was 75 °C. The results indicated that the increase of the extraction temperature will increase the kinetic energy of the solvent so that the penetration of the solvent into the material becomes larger in order the oil molecules are more easily extracted from the material and drawn into the solvent phase. The increase in temperature also affects the solvent's diffusivity into the inner part of the material and increases the solubility of the oil in the solvent, thus improve the extraction rate of oil from the seed kernels of Feun Kase. However, the extraction at 80-90 °C temperatures, the oil yield decreased by 9 to 22%. These results were in accordance with those reported by [13-15] as explained that the reduction of the oil yield would be at a high temperature. An increase in temperature reduced the oil kinematic viscosity with an increased mobility of biopolymers in cellular walls. Therefore, it can be deduced that 75 °C was adequate to give the highest yield of oil from the seed kernels of Feun Kase.
The extraction time is an important factor determining the efficiency of an oil extraction from vegetable materials. An appropriate timing of extraction directly affects the oil yield, due to the maximum interaction between the solvent and the oil molecules when the contact time and residence time of the solvent in the extractor were optimum. Fig. 3 showed the total amount of oil extracted from the seed kernels of Feun Kase at different extraction times. The extraction times in this work were within the range of 3.0 h-5.5 h. The results showed that oil extracted from seed kernels of Feun Kase increased by 20% due to the increase of extraction time from 3.0 h to 4.0 h, but the subsequent increase in extraction time did not affect oil yield. It also observed that the rate of extraction was very high during the first 30 minutes of extraction and afterward it tapered off. The initial high rate of extraction may be due to the quick solubility of the oil present at the solid surface and higher mass transfer driving force provided by low oil concentration in the fresh solvent [16]. This shows that the optimum residence time or contact time required by the extractant (oil) in the chloroform solvent was 4.0 h. Hence, 4.0 h was chosen as the optimum time for Feun Kase seed kernels oil yield.

**Figure 2.** The effect of temperatures on oil yield at the reaction condition of chloroform to water ratio of 100:0 extraction time 4 h and various temperatures in °C

**Figure 3.** The effect of extraction time on oil yield at the reaction condition of chloroform to water ratio of 100:0, extraction temperature 75 °C
An oil quality analysis was performed to determine the physicochemical characteristics of the oil produced from the Soxhlet extraction process using chloroform solvent at 75°C and 4.0 h of extraction time. The results of analysis of physicochemical properties of Feun Kase seed kernels oil are needed to determine the stage of transesterification process that would be used in the production of methyl ester and determining the required methanol and catalyst requirements. The parameters analyzed were density, viscosity, acid value, iodine value, saponification value, and water content. The physicochemical properties of the extracted oil at the optimum conditions are shown in Table 2. These properties were comparable to those of the National Standard of Indonesia (SNI) for biodiesel production.

Table 2. The physicochemical properties of crop oil from seed kernels of Feun Kase

| Properties             | Unit      | Values   | SNI Specification |
|------------------------|-----------|----------|-------------------|
| Density                | kg/m³     | 1066     | 850 – 890         |
| Viscosity              | mm²/s     | 2.18     | 2.3 – 6.0         |
| Iodine value           | g I₂/100 g oil | 43.56 | 115 max            |
| Acid value             | mg KOH/g oil | 1.86 | 0.8 max            |
| Saponification value   | mg KOH/g  | 140.52   | 500 max           |
| Water content          | %         | 0.032    | 0.05 max          |

Based on the analysis results, it was found that the density of oil before being processed into biodiesel was 1066 kg/m³, this value was higher than the value of SNI (850-890 kg/m³) and the value set by Pertamina for the diesel oil, which was 820 - 870 kg/m³. The value of oil density is influenced by the fatty acid composition and the purity of the raw materials. A high-density value indicated that in the Feun Kase seed kernels oil contains many unsaturated fatty acids. Feun Kase seed oil contained predominantly 4 types of fatty acids, namely palmitic acid (17.02%), stearic acid (6.23%), oleic acid (41.91%) and linoleic acid (11.89%) [4,17]. Oleic acid (C18:1) and linoleic acid (C18:2) with the composition of 41.91% and 11.89%, respectively, included unsaturated fatty acids which predominantly affected the high density of Feun Kase seed oil. The more the number of double bonds with the same length of the carbon chain, the greater the oil density. Since it contained 53.80% unsaturated fatty acids composition, its effect on the density was very dominant. The density will increase as the carbon chain length decreases and the increase in the number of double bonds in fatty acids. The high density of the extracted oil can be caused by the increasing number of unsaturated oil components in the extraction product. Therefore, before being used as a raw material for a biodiesel production, this extracted oil needs to be done degumming or bleaching process to decrease the oil density.

Viscosity is a measure of a fluid's resistance to flow. Overly high viscosity will cause smoke because the fuel is slow to flow and more difficult to atomize, as well as incomplete combustion and carbon deposition in the injector. The viscosity of a fuel is very influential on the performance of the engine so it becomes one of the important parameters. If the viscosity is high then the fuel will flow slowly and the degree of atomization of fuel will become more difficult, so the resulting energy becomes not optimal. The viscosity as a measure of oil resistance to fluid obtained in this work was 2.18 mm²/s, so that the viscosity of oil from Feun Kase seed kernels fulfilled the standard of SNI. These results are in line with the findings [18] that the unsaturated fatty acids composition of the Feun Kase seed kernels oil would contribute mostly to the viscosity value. The more the number of double bonds with the same length of the carbon chain, the smaller the viscosity. The saponification value indicated its average molecular weight of triglycerides in the oil. It was determined by the molecular weight of the constituent fatty acids. The saponification value obtained in this work was 140.52 mg KOH/g of oil. A low saponification value of the oil indicated that the triglyceride has a long carbon chain. The longer the carbon chain of fatty acid, the less fatty acid content in the oil. The oil composed of long-chain fatty acids indicated that a high molecular weight
will have a small saponification value. On the contrary, low molecular weight oils will have high saponification value.

The acid value showed free fatty acid levels in the oil. It is indicated the amount of milligram KOH required to neutralize free fatty acids in one gram of oil. The presence of free fatty acids in biofuel products can lead to the formation of ash at the time of combustion, clogging the filter with precipitate and corroding the diesel engine. The acid value can be an indicator of damage occurring to the oil, due to oxidation activity. The results showed that the acid value in Feun Kase seed oil was 1.86 mg KOH/g oil. It was higher than the SNI specification for biodiesel product with the maximum of 0.8 mg KOH/g oil. A high acid value indicated a high fatty acid (FFA) derived from oil hydrolysis. The higher the acid value the lower the quality, because oils containing free fatty acids more than 1% will form emulsion formulations that make it difficult at the time of biodiesel separation. The water content is one important factor determining the oil quality. The high water content can lead to a blockage of fuel flow, the occurrence of hydrolysis reactions that will increase the acid value, decrease the pH and improve the corrosive properties. The water content of the Feun Kase oil was 0.032%. This value was relatively lower and met the specified quality standard. The iodine value indicates the degree of unsaturation or number double bonds contained in oils expressed in I₂/100 g oil. The iodine value obtained in this work was 34.56 g I₂/100 g of oil and fulfilled the standard of SNI. This indicates that the iodine value attached to the double bond was relatively low so that the degree of unsaturation of the fatty acids was low. The amount of unsaturated fatty acid compounds in the oil allows the compound to react with oxygen at the time of combustion, forming a chain with a very large molecular weight. The iodine value has a direct relationship to viscosity and cetane numbers. The higher the iodine value the lower the cetane number and vice versa. The decrease in viscosity and cetane numbers will lead to an increase in oil unsaturation. The maximum iodine value for biodiesel feedstock was 70-100. The physical and chemical properties of crop oil from seed kernels of Feun Kase in this study compared with previous studies to determine its suitability as a feedstock for biodiesel production was shown in Table 3.

### Table 3. The comparison of physicochemical properties of the oil in this study to other studies

| Properties        | Unit       | [17] | [19] | [20] | [21] | [22] | This study |
|-------------------|------------|------|------|------|------|------|-----------|
| Density           | kg/m³      | 40.42| 39.84| 47.0 | 4.70 | 2.18 | 1066      |
| Viscosity         | mm²/s      | -    | 494  | 899  | 921  | -    | 412.30    |
| Iodine value      | g I₂/100 g oil | -   | 71.20| 12.60| 71.40| 43.56| 47.03    |
| Acid value        | mg KOH/g oil | -   | 0.568| 4.70 | 0.658| -    | 1.86      |
| Saponification value | mg KOH/g | -   | -    | 412.30| 121.00| 140.52| 52.43    |
| Oil yield         | %          | -    | 48.82| -    | -    | -    | 52.43     |

The viscosity obtained in this study was lower than the previous research by [17, 19, 21]. The lower the viscosity of the oil, the better the oil to be used as a feedstock for biodiesel production. The density of the oil was higher than the previous study by [19, 20, 21]. The high content of oleic acid and linoleic acid with a double bond and unsaturation level contributed to the high of the density in this study. The iodine values obtained in this study was better than the previous studies by [20, 22], it fulfilled the standard of SNI. The saponification value of the oil met with the standard of SNI indicated that the oil can be used as an effective feedstock for biodiesel production. The non-edible property and oil content of a seed are the first factor to be considered among others when large-scale extraction of the oil is to be considered [7, 21]. Any non-conventional and non-edible seeds with their oil contents more than 40% are the most as suitable feedstock to produce biodiesel. The oil yield obtained in this study (52.43%) was higher than that reported by [19] (48.82%), and exceeded the value for *Pongamia glabra* seed oil (33%)[23], *Moringa oleifera* seed oil (35%)[24], as well as
Jatropha curcas seed oil (30 – 50%) [25,26]. Prospectively, based on the physicochemical properties and oil yield, Feun Kase seed oil has a potential for a feedstock in biodiesel production

4. Conclusion
The crop oil from seed kernels of Feun Kase can potentially be utilized as a feedstock of biodiesel production. Therefore, it can reduce the dependency on using edible oil feedstock. The optimum conditions were obtained at chloroform to water ratio of 100:0 (polarity 4.10), extraction temperature of 75 °C and extraction time of 4.0 h. The optimized oil yield was found to be 52.43 ± 1.73% (weight basis). It had been observed that polarity of the solvent and extraction time was the most crucial parameters that affecting the oil yield. The oil yield and physicochemical analysis of the extracted oil further suggests its potential application as a feedstock for a biodiesel production.

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References
[1] S. Kishan, K.K. Agrawal, V. Mishra, S.M. Uddin and A. Shukla, International research Journal of Pharmacy 3, 74-77 (2012).
[2] D. Patel, S. Shukla and S. Gupta, International Journal of Oncology 30, 233-245 (2007).
[3] T. Balusamy and R. Marappan. Journal of Scientific and Industrial Research 66, 1035-1040 (2007).
[4] S.A. Ibiyemi, V.O. Fadipe, O.O. Akinremi and S.S. Bako, Journal Applied Science and Environment 6, 61-65 (2002).
[5] Ana Godson R.E.E and G.B. Udofia, International Journal of Sustainable and Green Energy 4, 150-158(2015).
[6] Ma Fangrui, A. H. Milford. Bioresource Technology 70, 1 – 115 (1999).
[7] S. Basumatary, Research Journal of Chemical Sciences 3, 99-103 (2013).
[8] M. Markom, M. Hasan, W.R.W. Daud, H. Singh and J.M. Jahim, Separation Purification Technology 52, 487-496 (2007).
[9] M.C. Straccia, F. Siano, R. Coppola, F.L. Cara and M.G. Volpe, Chemical Engineering Transactions 27, 391-396 (2012).
[10] A.G. Sicaire, M. Vian, F. Fine, F. Joffre, P. Carre, S. Tostain and F. Chemat, International Journal of Molecular Sciences 16, 8430-8453 (2015).
[11] T.P.L. Ferraz, M.C. Fiuza, M.L.A. Dos Santos, L.P. de Carvalho and N.M. Soares, Journal of Biochemical and Biophysical Methods 58, 187-193 (2004).
[12] C.H. Gu, H. Li, R.B. Gandhi, K. Raghavan, International Journal of Pharmaceutics 283, 117-125 (2004).
[13] S. Sayyar, Z.Z. Abidin, R. Yunus and A. Muhammad, American Journal of Applied Sciences 6, 1390-1395 (2009).
[14] O.S. Lawson, A. Oyewumi, F.O. Ologunagba and A.O. Ojomo, Journal of Engineering and Applied Sciences 5, 51-55 (2010).
[15] B. Panchal, S. Deshmukh and M. Sharma, International Journal of Oil, Gas and Coal Engineering 2, 1-6 (2014).
[16] D.K. Saxena, S. K. Sharma and S. S. Sambi, Journal of Engineering and Applied Sciences 6, 84-89 (2011).
[17] M.I. Oseni, S.E. Obetta and F.V. Orukotan, Journal of Scientific and Industrial Research 3, 62-68 (2012).
[18] Joeilangshih, A.H. Tambunan, T.H. Soerawidjaya, S. Yasuyuki and A. Kamaruddin, Journal
keteknikan Pertanian 22, 1-6 (2008).

[19] S.B. Dhoot, D.R. Jaju, S.S. Deshmukh, B.M. Panchal and M.R. Sharma, Journal of Alternative Energy Sources & Technologies 2, 8-16 (2011).

[20] D.C. Deka and S. Basumatary, Biomas Bioenergy 35, 1797-1803 (2011).

[21] C.I. Yarkasuwa, D. Wilson and E. Michael, Journal of the Korean Chemical Society 57, 377-381 (2013).

[22] M.M. Bora, D.K. Kakati, P. Gogoib and D.C. Deka, Industrial Crops and Products 52, 721-728 (2014).

[23] A.K. sarma, D. Konwer and P.K. Bordoloi, Energy Fuels 19, 656-657 (2005).

[24] U. Rashid, F. Anwar, B.R. Moser and G. Knothe, Bioresource Technology 99, 8175-8179 (2008).

[25] Y.H.T. Yap, M.Z. Hussein and R. Yunus, Biomass Bioenergy 35, 827-834 (2011).

[26] K. Permanik, Renewable Energy 28, 239-248 (2003).