Optimization of lipids extraction from Moringa oleifera seeds for biodiesel feedstock

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Abstract. This paper explores the mechanism of lipid extraction efficiency on Moringa oleifera seeds using Soxhlet extraction method. This present study essential to determine the effect of particle size of the sample, extraction time and type of solvent applied towards the efficiency in extracting the lipids from the material. Soxhlet extraction method utilizing Buchi B118 was used in this study and response surface method was applied to analyse the data and determine the optimum parameter condition to obtain the highest yield of Moringa oil extraction. Moringa oil derived from Moringa oleifera seeds was converted into biodiesel (FAME) via Transesterification process. Conversion of Moringa FAME was observed using three different alcohol oil to molar ratio by based-catalysed. This study shows significant strong correlations between particle size of the sample, extraction time and type of solvent use towards extraction yield. The Response surface analysis shows that 1.3611 mm particle size of sample, 3 hours of extraction time and hexane as extraction solvent was the optimum condition in order to get the highest yield of lipids extract from both Moringa oleifera samples. Authentication extraction based on RSM recommendation showed an average Moringa oil yield of 39.75 % by weight.

Keywords: Moringa oleifera, Soxhlet Extraction, Lipids, FAME

1. Introduction
Biodiesel or also known as fatty acid methyl ester (FAME) is an alternative to conventional diesel or petroleum diesel used in compression ignition engines [1]. It is derived from different types of feedstock such as vegetable oils, animal fats, waste oils or microalgae [2]. Basically, biodiesel or FAME is obtained when feedstock such as vegetable oil or animal fat is reacted chemically with alcohol such as methanol with the presence of a catalyst to produce [3]. The use of biodiesel is important as it lower the dependency on fossil fuels, which will deplete in the future and pose harmful threats towards the environment. Biodiesel can be used to improve diesel in terms of lubricity, performance of fuel and lengthen engine life due to its properties which is similar to conventional diesel [4]. This is achieved by blending the biodiesel with conventional diesel based on different ratio depending on the usage [5]. The advantage of biodiesel is its high cetane number which gives better quality in terms of fuel ignition [6]. The disadvantages of biodiesel are its lower heating value, higher density and higher viscosity.
compared to conventional diesel which makes it unsuitable for usage in cold climate region [3]. Biodiesel quality is highly dependent on the quality of feedstock utilized and parameters involved during production [4].

1.1. Moringa oleifera

Moringa oleifera commonly known as drumstick tree (English), horseradish tree, ben oil tree, benzoil tree, benzolive (French), kelor, marango, mlonge, mulangay, saijhan, sajna (India) and munggai in Malaysia is a species of oilseed tree from Moringaceae family [7]. The tree is native to northwest India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean Island and South America [1]. Moringa oleifera is regarded as an important commodity due to its nutritional value and medicinal uses [1]. The leaves are used traditionally to treat fever, indigestion and eyes problems [8]. All parts of Moringa oleifera which includes the leaves, flowers immature pods and fruit are used to produce dehydrated Moringa, Moringa powder, Moringa pulp and Moringa seed oil [9]. Moringa fruit has the length range of 25-40 cm. It contains about 20 seeds which are globular in three-angled shape with average weight of 0.3 g and has a diameter of about 1 cm where the seed account to 70-75 % of the weight of the seeds [10]. The seeds contain about 19-47% of oil and the oil is rich in palmitic, oleic, stearic and behenic acids [11]. The seed is rich in oil and protein and can be used in various applications from cosmetic product to environmental water treatment [12]. several researchers have proposed Moringa Oleifera as a potential feedstock in biodiesel production [1, 6, 13]. The focus of this study is to identify the optimize condition for the extraction of Moringa oil based on particle size of seeds, the extraction time and the type of solvent.

![Figure 1. Moringa Oleifera seed.](image)
2. Material and Methods

2.1. Sample
Moringa oleifera seeds were obtained from a local supplier. Good seeds were selected and separated from the damaged seeds. The shells were cracked, and the seeds are removed carefully from the shells. The seeds were dried in a drying oven to lower the moisture content to below 4%. The seeds were then grinded and sieved to selected particle sizes which were 0.85 mm, 1.0 mm and 2.0 mm using plates sieve shaker [14].

2.2. Soxhlet Extraction
Oil was extracted using Buchi B811 Soxhlet extractor. The oil was extracted based on different solvent, particle size and extraction time [14, 15]. The solvents for the extraction process are n-hexane and ethanol. Extraction time was set for 1, 2, and 3 hours. Three selected particle sizes was used in this study are between 2.0 mm, 1.0 mm and 0.85 mm. 15 g of ground samples were placed into a thimble and put into the extraction chamber. 200 mL of n-hexane/ethanol was poured into the solvent flask. The ratio of solvent to samples was kept constant for 13.33:1 w/w. Soxhlet warm was chosen as the extraction method and suitable process parameter was set. The upper heating is set at 4 and lower heating is set at 10. Extraction time was set at 1 to 3 hours. The extraction process for three consecutive variables was repeated for three times. The extraction process then continued with the other three consecutive variables until all extraction process variables are complete. The oil will be separated from the solvent using Buchi rotary evaporator. The pressure of rotary evaporator was set to 200 millibar to provide a condition under vacuum pressure. The temperature of the water bath was set above the boiling point of solvent under vacuum pressure. The water bath temperature was set to 60 °C [16]. The oil yield percentage was calculated using the formula shown below.

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\text{Yield of moringa oil (W/W\%)} = \frac{\text{Mass of moringa oil (g)}}{\text{Mass of moringa seeds used (g)}} \times 100\%
\]  

3. Results & Discussion

3.1. Soxhlet Extraction
Soxhlet extraction was performed utilizing three different particle sizes of the samples with three different extraction time were done to find out the effect of particle size of the sample and extraction time of Soxhlet extraction method towards the yield production of extracted oil. These two variables were extracted using two different solvents: hexane and ethanol. Figure 2 shows the data from the extraction process using hexane. The highest oil yield recorded for 1 hour extraction time was 35.47 % from 1.0 mm samples, followed by 28.34 % from 0.85 mm samples and 25.37 % from 2.0 mm samples. The highest oil yield recorded for 2 hours extraction time was 39.12 % from 1.0 mm samples, followed by 30.98 % from 0.85 mm samples and 29.63% from 2.0 mm samples. The highest oil yield recorded for 3 hours extraction time was 40.85 % from 1.0 mm samples, followed by 35.65 % from 0.85 mm samples and 33.62 % from 2.0 mm samples. The 1.0 mm samples yielded the highest oil percentage for each extraction time. 2.0 mm size samples yielded the lowest oil percentage for each extraction time.

Figure 3 shows the data from the extraction process using ethanol. The highest oil yield recorded for 1 hour extraction time was 16.40 % from 0.85 mm samples, followed by 15.15 % from 1.0 mm samples and 10.15 % from 2.0 mm samples. The highest oil yield recorded for 2 hours extraction time was 20.40 % from 0.85 mm samples, followed by 18.40 % from 1.0 mm samples and 17.85 % from 2.0 mm samples. The highest oil yield recorded for 3 hours extraction time was 27.20 % from 0.85 mm samples, followed by 26.50 % from 1.0 mm samples and 21.00 % from 2.0 mm samples. The 0.85 mm samples yielded the highest oil percentage for each extraction time. The 2.0 mm samples yielded the lowest oil percentage for each extraction time. N-hexane is known as a non-polar solvent meanwhile, ethanol is a polar solvent. The general rule of solvent is “like dissolves like” which means a polar solvent will more be attracted to polar molecules and vice versa [17, 18]. Since the candlenut oil consists of five major
fatty acids in its oil composition (palmitic acid, stearic acid, oleic acid, linoleic and linolenic acid), it is likely to be dissolved in n-hexane compared to ethanol. Previous study also shown that palmitic acid is more soluble in hexane compared to ethanol [19].

**Figure 2.** Percentage of Moringa oil extracted using hexane as solvent.

**Figure 3.** Percentage of Moringa oil extracted using ethanol as solvent.

### 3.2. Relations between Particle Size, Extraction Time and Solvent in Soxhlet Extraction

Figure 4 shows the data obtained from the first parameter of the extraction process, which was particle size, and its effect in terms of average oil yield obtained for two different solvent. The highest average oil yield obtained when hexane was used as extraction solvent was 38.48 % from 1.0 mm samples followed by 31.65 % from 0.85 mm samples and 29.54 % from 2.0 mm samples. The oil yield increased when the particle size of the samples was increased from 0.85 mm to 1.0 mm and decreased back when the particle size of the samples increased from 1.0 mm to 2.0 mm. The maximum oil yield obtained when hexane was used was not from the lowest particle size samples. The highest average oil yield obtained when ethanol was used as extraction solvent was 21.33 % from 0.85 mm samples followed by
20.02 % from 1.0 mm samples and 16.33 % from 2.0 mm samples. The oil yield continued to decrease when the particle size of the samples was increased from 0.85 mm to 1.0 mm until 2.0 mm. The maximum oil yield obtained when ethanol was used was from the lowest particle size samples.

Small particle size have a wider surface area in contact with the solvent compared to large particle size which increases the oil yield [20]. The data obtained from extraction using hexane showed otherwise which could be explained from the small particle size samples which sometimes has high chance of floating in the extraction solvent which reduce the contact with the solvent therefore affecting the extraction efficiency [21]. Masime et al. [20] find a similar finding where maximum oil yield was not obtained by the smallest particle size samples. Smaller particle size samples also have tendency to agglomerate which cause surface contact area between sample and solvent to decrease which results in decrease of oil yield [22]. Similar findings was recorded where the oil yield percentage decreased when the particle size of the sample were increased as there are less surface area contact with the solvent during the extraction process [10, 23]. This shows that the decreasing in particle size of sample in an extraction process helps in improving solid–fluid contact during the extraction process which in return increase the yield of oil [24].

![Moringa Oil Yields at Different Particle Sizes](image)

**Figure 4.** Percentage of Moringa oil extracted at different particle sizes.

Figure 5 illustrate the data obtained from the second parameter of the extraction process which was extraction time and its effect in terms of average oil yield obtained for two different solvent. The highest oil yield obtained for extraction using hexane was 36.7 % from 3 hours extraction time, followed by 33.24 % from 2 hours extraction time and 29.72 % from 1 hour extraction time. The highest oil yield obtained for extraction using ethanol was 24.9 % from 3 hours extraction time, followed by 18.88 % from 2 hours extraction time and 13.9 % from 1 hour extraction time. The same trend was observed in both extraction data where oil yield increased when the extraction time was increased. The increase in the oil yield over time is due to the increase in the diffusivities of oil and solvent over time [10]. Similar findings was recorded by several researchers which used the same extraction time [20, 25–27].
Figure 5 display data on the effect of type of solvent used in terms of average oil yield. The average oil yield obtained when using hexane as extraction solvent was 33.22 % while the average oil yield obtained when using ethanol as extraction solvent was 19.23 %. The average oil yield obtained when hexane was used as extraction solvent was relatively higher compared to ethanol. The difference in terms of average oil yield recorded between the two types of solvent was high. This might be explained from the characteristic of hexane which is a non-polar solvent compared to ethanol which is a polar solvent [28]. The difference in the oil yield between the two solvent could also be explained by the presence of different compound in the sample used [29]. Alkane compounds are non-polar and can easily be extracted by hexane while the polar compounds can easily be extracted by ethanol since they have O-H ends which enable them to react with hydrogen bond presence in the ethanol [29]. Similar finding was recorded by Zhao & Zhang [29] who used both type of solvents in extraction of Eucalyptus leaves oil. Since Moringa oil contain fatty acids which are non-polar, hexane is more suitable to be used as extraction solvent compared to ethanol [28].

Figure 6. Percentage of Moringa oil extracted at different extraction time.

Figure 6. Percentage of Moringa oil extracted using different solvent.

3.3. Optimization of Extraction (Response Surface Method)
Response surface plot for extraction of Moringa oil using hexane are shown in figure 7 and figure 8 below. The relationship of the two extraction parameters which are particle size and extraction time with
the oil yield is shown. The highest oil yield from hexane extraction was obtained from the highest extraction time which was 3 hours and the middle particle size used which was 1.0 mm. The highest oil yield was not obtained from the lowest particle size used in the extraction, which was 0.85 mm. Figure 8 shows that the highest oil yield from ethanol extraction was obtained from the highest extraction time as well which was 3 hours and the smallest particle size used which was 0.85 mm. The difference compared to extraction using hexane can be seen in which the highest oil yield from ethanol extraction was obtained was from the lowest particle size used in the extraction which was 0.85 mm. This can be explained by a few reasons. According to Yeop et al. [21] smallest particle size samples sometimes tend to float in the solvent of extraction which cause the contact of the samples with the solvent to be reduced and affect the diffusivity of the solvent into the samples to extract oil. Small particle size samples also tend to clump together and cause the surface area in contact with the solvent to be reduced which in return reduce the amount of oil extracted [22]. Similar finding also was recorded where the maximum oil yield from the smallest particle size used in the extraction [20]. The increase in the oil yield percentage alongside the decrease in particle size of the sample was also observed in few studies [10, 23]. Another set of extraction was performed to verify the optimize condition provided by the RSM simulation where 1.4 mm particle size sample, 3 hours of extraction time and hexane as extraction solvent were used. The average Moringa oil yield obtained from the extraction was 39.75 % by weight. This display a significant improvement compared to previous extraction where the average Moringa oil yield using hexane was to be at 33.22 % by weight.

![Surface Plot of Oil yield (%) vs Extraction time (hr), Size (mm)](image_url)

**Figure 7.** Response surface plot of oil yield at different particle size and extraction time using hexane.
Figure 8. Response surface plot of oil yield at different particle size and extraction time using ethanol.

4. Conclusions
For the conclusion of this study, it could be summarized that hexane produce more extraction yield compared to ethanol signifies that Moringa oil contained more non-polar compound. The extraction output illustrate that smaller particle size sample tend to clump together and cause the surface area in contact with the solvent to be reduced which in return reduce the amount of oil extracted. The optimized extraction conditions obtained based on the whole extraction data are using 1.36 mm particle size of sample, 3 hours of extraction time and hexane as extraction solvent which could give an oil yield of 42.50 %. Verification extraction was made using the condition provided by RSM displayed an average Moringa oil yield of 39.75 % which highly improved compared to previous extraction at an average of 33.22 % by weight.

Reference
[1] Rashid U, Anwar F, Moser B R, and Knothe G 2008 Moringa oleifera oil: A possible source of biodiesel Bioresour. Technol. 99 8175–9
[2] Issariyakul T and Dalai A K 2014 Biodiesel from vegetable oils Renew Sustain Energy Rev. 31 446–71
[3] Ali E N and Tay C I 2013 Characterization of biodiesel produced from palm oil via base catalyzed transesterification Procedia Eng. 53 7–12
[4] Blasio C D 2019 Biodiesel: Fundamentals of biofuels engineering and technology Green Energy and Technology. 253–6
[5] Talha N S and Sulaiman S 2016 Overview of catalysts in biodiesel production ARPN J. Eng. Appl. Sci. 11 439–42
[6] Azad A K, Rasul M G, Khan M M K, Sharma S C and Islam R 2015 Prospect of Moringa seed oil as a sustainable biodiesel fuel in Australia: A review Procedia Eng. 105 601–6
[7] Sagona W C J, Chirwa P W and Sajidu S M 2020 The miracle mix of Moringa: Status of Moringa research and development in Malawi South African J. Bot. 129 138–45
[8] Machado S D I, Gastênum J A N, Moreno C R, Wong B R and Cervantes J L 2010 Nutritional quality of edible parts of Moringa oleifera Food Anal. Methods. 3 175–80
[9] Daba M 2016 Miracle tree: A review on multi-purposes of Moringa oleifera and its implication for climate change mitigation J. Earth Sci. Clim. Change. 7 336
[10] Mani S, Jaya S and Vadivambal R 2007 Optimization of solvent extraction of Moringa oleifera
seed kernel oil using response surface methodology Food Bioprod. Process. 85 328–35

[11] Bhutada P R , JadHAV A J, Pinjari D V, Nemade P R and Jain R D 2016 Solvent assisted extraction of oil from Moringa oleifera Lam. seeds Ind. Crops Prod. 82 74–80

[12] Ndabigengesere A and Narasiah K S 1998 Quality of water treated by coagulation using Moringa oleifera seeds Water Res. 32 781–91

[13] Palafoux J O, Navarrete A, Rivero J C S, Atoche C R, P. Escoffie P A and Uribe J A R 2012 Extraction and characterization of oil from Moringa oleifera using supercritical co2 and traditional solvents Am. J. Anal. Chem. 3 946–9

[14] Zeković Z, Filip S, Vidović S, Jokić S and Svilović S 2014 Mathematical modeling of ocimum basilicum L. supercritical CO2 extraction Chem. Eng. Technol. 37 2123–8

[15] Ramluckan K, Moodley K G and Bux F 2014 An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method Fuel 116 103–8

[16] Cabral M R P, Santos S A L D, Stropa J M, Silva R C L, Cardoso C A L, Oliveira L C S, Scharf D R, Simionatto E L, Santiago E F and Simionatto E 2016 Chemical composition and thermal properties of methyl and ethyl esters prepared from Aleurites moluccanus (L.) Willd (Euphorbiaceae) nut oil Ind. Crops Prod. 85 109–16

[17] Guzman A 1962 Solubility of triglycerides in aqueous ethanol IOWA State Cap Dig Repo

[18] Harris D C 2015 Solvent Polarity Index Quantitative Chemical Analysis. 9 2015

[19] Calvo B, Collado I and Cepeda E A 2009 Solubilities of palmitic acid in pure solvents and its mixtures J. Chem. Eng. Data. 54 64–8

[20] Masime J O, Ogur E, MbAtia B, Aluoch A O and Otieno G 2017 Optimization and thermodynamics of the extraction of yellow oleander seed oil using soxhlet extractor Optimization 2 43–50

[21] Yeop A, Sandanasam J, Pan S F, Abdulla S, Yusoff M M and Gimbun J 2017 The effect of particle size and solvent type on the gallic acid yield obtained from Labisia pumila by ultrasonic extraction MATEC Web Conf. 111 1–5

[22] Shigidi I and Elkhaleefa A 2015 Original article parameters optimization, modelling and kinetics of balanites aegyptiaca kernel oil extraction Int. J. Chem. Eng. Appl. Sci. 5 1–4

[23] Reshad A S, Tiwari P and Goud V V 2015 Extraction of oil from rubber seeds for biodiesel application : Optimization of parameters Fuel. 150 636–44

[24] NguyEn H N, Gaspllo P D, Maridable J B, Malaluan R M, Hinode H, Salim C and Huynh H K P 2011 Extraction of oil from Moringa oleifera kernels using supercritical carbon dioxide with ethanol for pretreatment: Optimization of the extraction process Chem. Eng. Process. Process Intensif. 50 1207–13

[25] Alara O R, Abdurahman N H and Ukaegbu C I 2018 Soxhlet extraction of essential oil from neem seed by using soxhlet extraction methods Int. J. Adv. Eng. Manag. Sci. 3 646–50

[26] Gupta S P, Singh M, Ahmed I, Sharma D N and Kumar P 2018 Effect of temperature and time intervals on the solvent extraction of essential oil from azadirachta indica (neem) leaf powder by using soxhlet extraction method World. J. Pharma. Resch. 8 939–46

[27] Tesfaye B and Tefera T 2017 Extraction of essential oil from neem seed by using soxhlet extraction methods Int. J. Adv. Eng. Manag. Sci. 3 646–50

[28] Ghazali Q and Yasin N H M 2016 The effect of organic solvent, temperature and mixing time on the production of oil from Moringa oleifera seeds IOP Conf. Ser. Earth Environ. Sci. 36 0–7

[29] Zhao S and Zhang D 2014 Supercritical CO2 extraction of Eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods Sep. Purif. Technol. 133 443–51

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