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Research Paper

Extraction and Characterization of Oil from Moringa Stenopetala Seed as Potential Industrial Source

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Abstract

This study investigated the extraction and characterization of oil from *moringa stenopetala* seed oil. Oil was extracted using soxhlet extraction method at temperatures of 70 ^{0}C, 75 ^{0}C and 80 ^{0}C. The extraction time varied from 3 hours to 5 hours and the sample sieve size varied from 0.75 mm to 1.5 mm. The experimental result showed that the maximum oil yield was obtained at optimum process conditions of; temperature 80 °C, extraction time 5 hours and sample mesh size of 0.75 mm. The highest oil yield obtained was 39.86 %. This result agreed with the average oil content of *moringa stenopetala* which is 35 % to 45 %. Above 5 hours, the extraction was completed and no yield was obtained. Decreasing sample size below 0.75 mm led to lower yield. Hexane was the solvent used for the extraction. Experimental data was analyzed by Box Behnken design method and the significance of experimental results was determined from analysis of variance (ANOVA). The main physicochemical properties of oil were determined according to international ASTM and EN standards. It was found that pure extracted *moringa stenopetala* oil has moisture content 6.54 %, pH 6.7, specific gravity 0.84 g/mole, kinematic viscosity 9.4 mm2/s, acid value 1.6 mg KOH/g, saponification value 189 mg KOH/g, free fatty acid 0.8 % and molecular weight of 895.5 g/mol. These results agreed with international standards values and previous works.

1. Introduction

Moringa species are among the high value trees which belong to the family of *moringaceae* which consists of 13 species. Moringa species are highly distributed to Southern Asia and Africa. Moringa is a multipurpose tree which is used for medicinal, nutritional, industrial and social values (Hamza and Azmach, 2017).

Moringa tree is an evergreen, fast growing, deciduous and widely cultivated tree species. It has some other common English names such as, drumstick tree, horseradish tree and benzoic tree. It grows in tropical and subtropical areas. It requires rainfall about 250-2000 mm depending on soil condition. It grows best in dry sandy soil and tolerates poor soil with pH range 5 to 9. If water is available for irrigation, moringa trees can be seeded directly and grown anytime during the year. On the other hand, cuttings should be 45 cm to 1.5 m long and 10 cm thick can be planted directly or planted in sacks in the nursery. The tree can reach a height of 10 to12 m and the trunk can reach a diameter of 30 to 45 cm. When matured, each moringa tree can produce a high yield of seed pod from 300 up to 1000 seed pods (Azad et al., 2015).

In Ethiopia, Moringa has been promoted for long time. However, its utilization is limited to specific areas. *Moringa stenopetala* is native to Ethiopia. It is grown in southern parts of the country and in other areas. Moringa

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is a multipurpose and miracle tree. Its leaves are used for medicinal, food, cosmetics production and many other applications. The seed of moringa has high oil yield of 35-45 % (Ayerza, 2012). Moringa seed oil is not commercially utilized in Ethiopia currently however; moringa seed oil is used for energy (biodiesel production), cosmetics, and medicine as food in some areas.

Even though it has tremendous benefits, Moringa is a neglected, miracle and less utilized tree species in Ethiopia. Moringa particularly *Moringa stenopetala* is an indigenous, neglected, miracle and underutilized tree species in Ethiopia. *Moringa stenopetala* has been promoted for long period of time in different parts of the country mainly in drought prone areas. It has a wider adaptability and tolerance to drought and has high oil content.

The native Moringa species in Ethiopia is *Moringa stenopetala. Moringa stenopetala* is often referred as African Moringa tree because it is native to only Ethiopia and Northern Kenya. *Moringa stenopetala* belongs to the family of Moringa cease which is represented by single genus ‘’Moringa’’. The genus has 14 species to which *Moringa stenopetala* belongs (Mark, 1998). It can grow in elevations between 1000 – 1800 m in Ethiopia. It is also extremely a fast-growing tree and can continue growing during the exceptionally long dry season.

Moringa stenopetala is one of the most widely cultivated indigenous tree species in Ethiopia. It is widely distributed in southern parts of Ethiopia such as: Wolayta, Gamo Gofa, Derashe, Konso, Sidama, Bale, Arba Minch and Borana. Although MS is widely cultivated and has got research and development attention worldwide, it has introduced introduce to Ethiopia very recently and much more research has to be conducted to evaluate and investigate its species, benefits and biomass production (Petr, 2020).

There are different seed oil extraction methods. The common oil extraction methods are: mechanical extraction which is used for seeds with high oil yield. Its advantage is low operating cost and the absence of solvent separation method (Ong, 2013). The other method is enzymatic oil extraction which uses enzymes as to extract oil from crushed seeds. This method is expensive and it takes longer extraction time (Atabani, 2013).

The modern and current industrial oil extraction method is soxhlet oil extraction method. This method uses solvents such as hexane and petroleum ether. As the solvent evaporates and passes through the sample, the oil is extracted and goes to the flask (Nuratiqah, 2015). Soxhlet extraction method is selected due to various factors such as soxhlet extraction method results in high oil yield, it is simple and quick, solvent can be reused, and low cost (Bhuiya et al., 2016).

2. Materials and Methods

2.1 Sample collection and preparation

The sample of *moringa stenopetala* was collected from Arba Minch which is located in Southern Nations, Nationalities and Peoples of Ethiopia. Arba Minch is located in Gamo Gofa zone at an elevation of 1285 m and 434.6 km south of Addis Ababa. It has an average annual temperature of 25 °C and average annual rainfall of 476 mm. The external part of the seed was exposed to remove the kernel from the seed. Then, the seed was dried in the sun for three days so that the moisture content was reduced and the minimum value (3.12 %) which is suitable for oil extraction was obtained. The appropriate range of moisture content of feed stock is 0.5 to 10% according to Shaha (2011). The dried seed was milled to appropriate size and sieved to less than 1mm mesh size.

2.2 Extraction of seed oil

40 g of milled seed sample was placed in 500 mL capacity soxhlet apparatus. 200 mL hexane was used for the extraction of 3 to 5 hours. The extraction was conducted at 70, 75 and 80 °C by varying the extraction time to get the maximum possible oil yield. The oil was extracted for 3 to 5 hours by varying extraction temperatures.

The extracted crude oil has unwanted substance such as: hexane, wax, gum and moisture. These substances were removed by evaporation and degumming methods according to Akpan et al., (2006). Hexane was separated from oil by rotary evaporation at 80 °C. Gum and wax were separated from the oil by separatory funnel according to Orhevba et al. (2014).

Figure 1: Sample preparation.

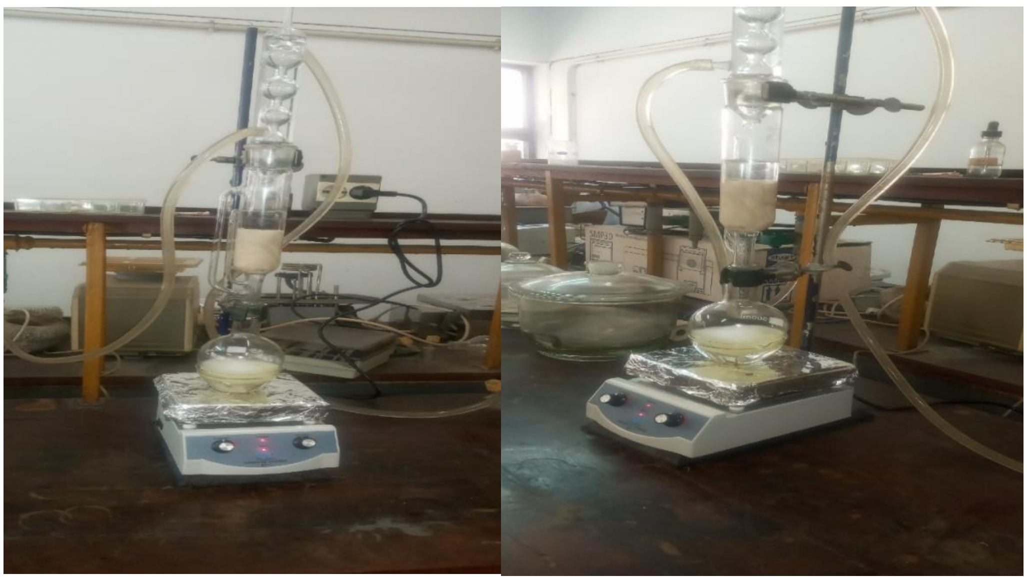

Figure 1: *Soxhlet oil extraction.*

2.3. Purification of the oil

The oil yield was calculated from the following equation according to Iloamaeke et al. (2016).

Oil yield (%) = $\frac{Mass\ of\ oil\ extracted}{Mass\ of\ sample\ used\ in\ feed}$ *100……(1)

2.4. Determination of physicochemical properties of the oil

a) Determination of moisture content

Moisture content of the sample was determined according to EN 14774-1 standard.

b) Determination of Acid value (A.V)

Acid value was determined according to EN 14104 standard method. 5 g of oil sample was dissolved in 250 mm conical flask with 25 mm ethanol. After adding few drops of phenolphthalein (2 drops) and boiled for few minutes (5 minutes), it was titrated with 0.1 N of KOH until the end point of colorless to pink was recognized. The acid value is calculated using the following equation according to Iloamaeke et al. (2016).

$$A.V = (V * N * 56.1)/W \text{……………..} \ (2)$$

Where, V is volume expressed in milliliter of 0.1 N ethanol KOH solution,

N is concentration of ethanol KOH solution, W is mass of oil taken in gram.

c) Determination of specific gravity

Specific gravity of oil was determined according to EN 14214 standard method.

d) Determination of kinematic viscosity

Kinematic viscosity of oil was determined according to ASTM D445 standard method.

e) Determination of density

Density of oil was determined from the following relation:

*Density of oil (ρ) = (specific gravity*density of water) . (3)*

f) Determination of saponification value (S.V)

Saponification value was determined according to ASTM D5558 standard method.

g) Determination of pH

Standard pH meter was used to determine the pH of the oil

h) Determination of free fatty acid (FFA)

The content of free fatty acid of the oil was determined from acid value using the following equation

$$\% FFA = (A.V)/2 \quad \ldots\ldots\ldots\ldots\ldots (4)$$

Where, A.V is acidic value of oil

i) Determination of molecular weight

Molecular weight of oil was determined according from the following equation according to Indhumathi et al. (2014).

$$Mwt = 168300/((S.V - A.V)) \quad \ldots\ldots\ldots (5)$$

Where, S.V = Saponification value of oil

A.V = Acid value of oil

2.5. Statistical analysis of the experimental results

Data analysis of this study was carried out by DESIGN EXPERT 11.0.0 software using Box-Behnken standard design method with two factors, three levels and three center points per block to determine the experimental results that maximize extraction of oil. The significance of experimental results was determined from analysis of variance (ANOVA).

3. Results and Discussions

3.1 Yield of oil

The maximum oil yield was obtained at 80 °C and 5 hours extraction time and the yield was 39.86 %. This oil yield is in the range of moringa stenopetala oil content (35 – 45%) and it agrees with the range of oil content of moringa stenopetala which was 35% - 45 % oil yield according to Ayerza (2012). The average oil yield 39.86 % obtained also agreed with 37 % which was reported by Orhevba et al. (2013) and 38 % moringa oil yield which was reported by Azad *et al*. (2014).

3.2 Physical and chemical properties of oil

Moisture content

The result of moisture was 6.54 %. This result agreed with the previous work of Schinas (2008) which was 5 %. Higher moisture content forms more soap during transesterification reaction and it is difficult for separation process. Moreover, higher moisture of oil decreases the yield of biodiesel produced.

pH

The pH of oil was determined with pH meter and the result was 6.7. This agreed with the range of MSSO pH which is 5 – 7. Lower pH value makes the oil more acidic which creates corrosion and higher pH value makes the oil more basic and this makes soap during transesterification reaction. Formation of more soap on the other hand decreases yield and quality of biodiesel. The result obtained was good since it meets the requirement for production of quality biodiesel and also agreed with the previous report of Orhevba et al.(2013) which was 5.9.

Kinematic viscosity

Kinematic viscosity of oil was 9.4 mm2. This result agreed with the previous report of Mahamudul (2017) which was 9 mm2/s. Viscosity o oil affects the storage and handling of fuel oil. Highly viscous oil is difficult for pumping and to operate in CI engine. In this study, the result showed that moringa stenopetala oil has lower viscosity and it can be used in CI engines.

Acid value

The acid value of oil was 1.63 mg KOH/g oil. This result agreed with literature value. The maximum acid value for alkaline transesterification of oil is two. If acid value is greater than this value, it needs further acid treatment method since it affects the fuel quality of biodiesel produced from it. Acid value is the measure of number of carboxylic acid groups such as free fatty acid.

Saponification value

The saponification value of oil was 189.2 mg KOH/g oil. Higher saponification value of oils is good for production of quality biodiesel. The saponification value of moringa stenopetala oil is 175 -195 mg alcohol/g oil. This result agreed with literature value.

Table 1: Analysis of variance.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	428.17	3	142.72	55.60	< 0.0001	significant
A-Temperature	66.59	1	66.59	25.94	0.0003	
B-Time	353.65	1	353.65	137.76	< 0.0001	
C-Sieve size	7.94	1	7.94	3.09	0.1064	
Residual	28.24	11	2.57			
Lack of Fit	27.68	9	3.08	10.98	0.0862	not significant
Pure Error	0.5600	2	0.2800			
Cor Total	456.41	14				

Factor coding is **coded**.

Sum of squares is **Type III - Partial**

Molecular weight

The molecular weight of oil was 895.5 g/mol. This result meets the range of molecular mass of vegetable oils (600 -900 g/mol) according to literature value.

3.3. Statistical analysis of experimental results

Analysis of variance (ANOVA) for linear model

The significance of the experimental results was determined from analysis of variance.

The **Model F-value** of 55.60 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 10.98 implies there is 8.62% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.

3.4. The effect of extraction parameters on yield of oil

Temperature

Temperature is the main parameter that affects yield of oil extracted. As extraction temperature increased from 70°C to 80 °C, the yield of oil increased and maximum yield (39.86 %) was obtained at optimum temperature of 80 °C. Increasing temperature beyond 80 °C led to lower yield and extraction stops finally since the solvent completely evaporates. The boiling point of solvent used (Hexane) varies from 70 °C to 80 °C. Oil starts to be extracted when hexane starts to boil. Fig (1) showed that oil yield increased from 27 % to 39 % as extraction temperature increased from 70 °C to 80 °C.

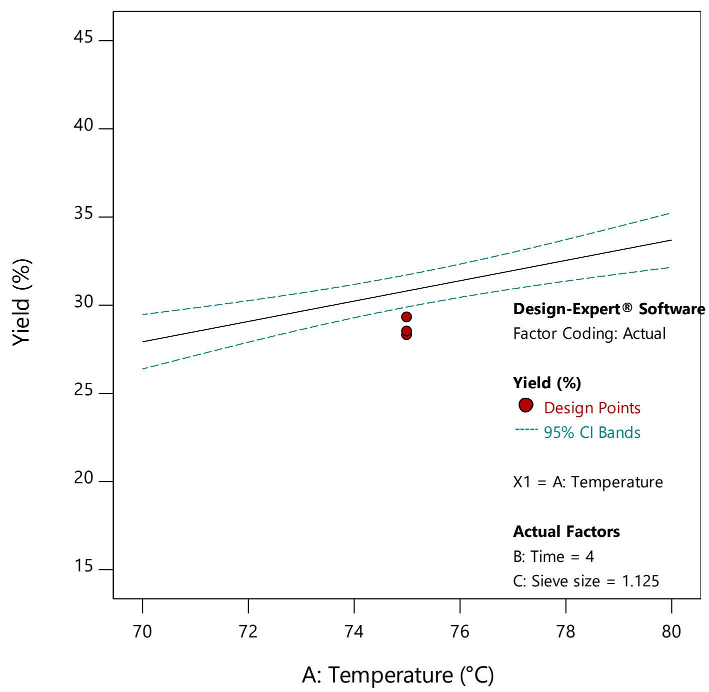

Figure 3: Effect of extraction temperature on oil yield

Time

As extraction time increases, the oil yield increased and the maximum yield was obtained at optimum time of 5 hours. Above 5 hours, the extraction stopped and no oil yield obtained. Fig (2) showed that oil yield increased from 26 % to 39.8 % as time increased from 3 hours to 5 hours. This showed that the maximum and optimum oil yield was 3.9.8 %.

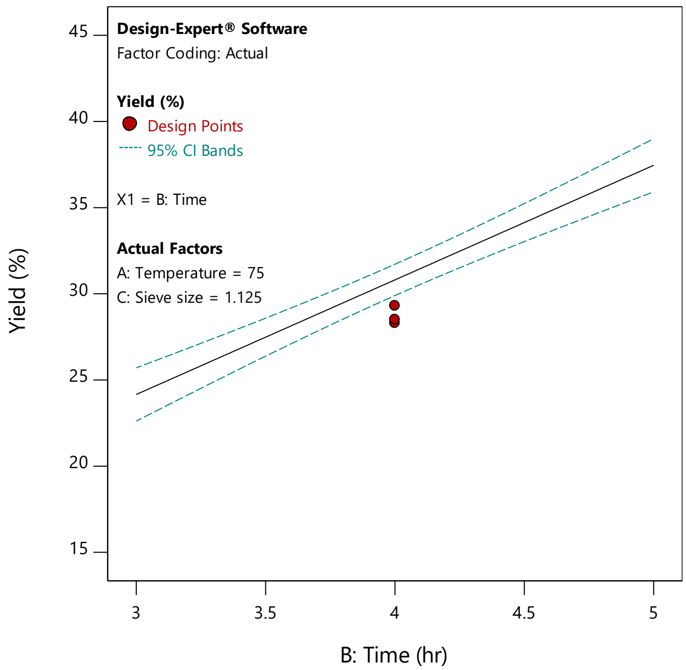

Figure 4: Effect of extraction time on yield of oil

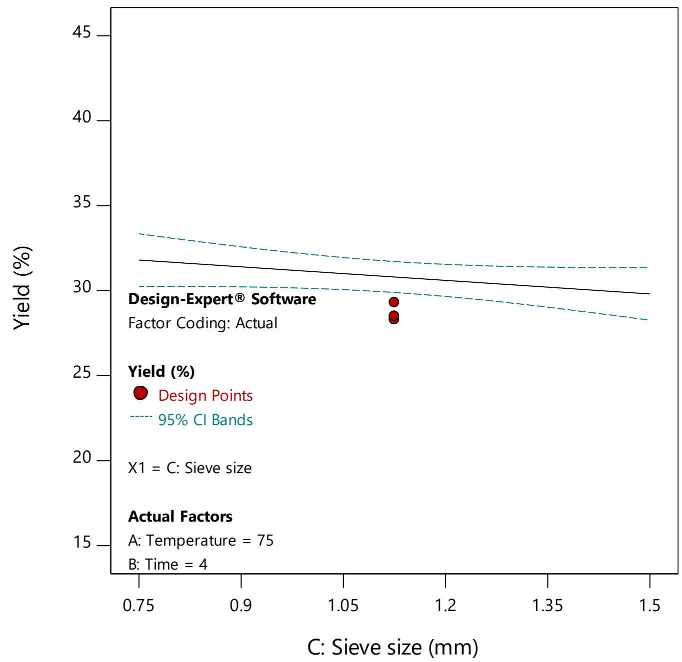

Figure 5: Effect of sample sieve size on oil yield

Sieve size

The mesh size of sample is another factor that affected oil yield. High oil yield was obtained from smaller size of sample. Fig (3) showed that as the size of sample increased from 0.75 mm to 1.5 mm, the oil yield decreased from 34 to 31 % and optimum yield of oil was obtained at optimum sample size of less than 1mm. This result showed that sieve size of the sample has lower effect on the yield of the sample. In this study, the sample sizes used are close to each other and this made the effect very lower.

4. Conclusion

In this research oil was extracted from moringa stenopetala seed by soxhlet extraction method. The statistical analysis of experimental results and experimental design was done using Design expert 11.0 software. The physicochemical properties of oil were determined according to international standard methods and all the results obtained meet these standards. The experimental result showed that the highest yield of oil was 39.86 % and this value was obtained at optimum value of temperature 8°C and extraction time of 5 hours. The statistical analysis done by Box-Behnken method showed that increasing extraction temperature and time up to the optimum point increased the yield of oil. However, further increase of the parameters above the optimum point decreased the yield. Generally, it can be concluded that moringa stenopetala is a promising alternative feed stock for oil extraction which has a wide industrial and social applications. Moringa stenopetala has good oil content and can be harvested throughout the year. The oil extracted meet the standard oil quality and international standard specifications. The novel finding of this research is the new discovered moringa stenopetala seed feed stock for multipurpose oil extraction.

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