

Extraction and Physicochemical Characterization of Oil from Moringa Stenopetala Seeds

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Abstract: The moringa stenopetala seed used for extraction of oil were collected from Arbaminch town in southern and south western of Ethiopia. The seed were prepared for use by removing the pods, drying with oven and milled to flour. A soxhlet extraction was used for extraction of the oil with petroleum; the oil was recovered by simple distillation using Rota evaporater. The residual oil obtained was characterized by investigating physico-chemical parameters and result shows that the extracted oils were liquid at room temperature, pale yellow colour and odorless. the results were average Seed Weight (g) (0.42), moisture content (5.7%), average density (0.907), fibre (7.50), protein (30.90), viscosity (10%), PH (7.53), acid value (1.68), saponification value (178), iodine value (70.2), peroxide value (9.45), yield of oil (47.8 %). This result of analysis confirms the standard specification and reveals that the extracted oil has good quality and food additives as well as industrial purposes and commercial applications.

Key Words: moringa stenopetala, soxhlet extraction, residual oil and physicochemical

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I. Introduction

1.1. Back Ground of the Study

Moringa is tropical plant belonging to the family moringa cease and growth throughout the tropics. Moringa is multipurpose tree of significant economic important as it has vital nutritional, industrial and medicinal application [1]. The genus's moringa consists of 13 species. Among these, moringa *oleifera* is relatively studied than *M. olifera* which is particularly easy to produce and its growth is very fast. It originates from India and is found in most tropical counties (Africa, Asia and America). The numerous economic uses of *M. olifera* together with its easy propagation have raised growing international interest for these trees [2].

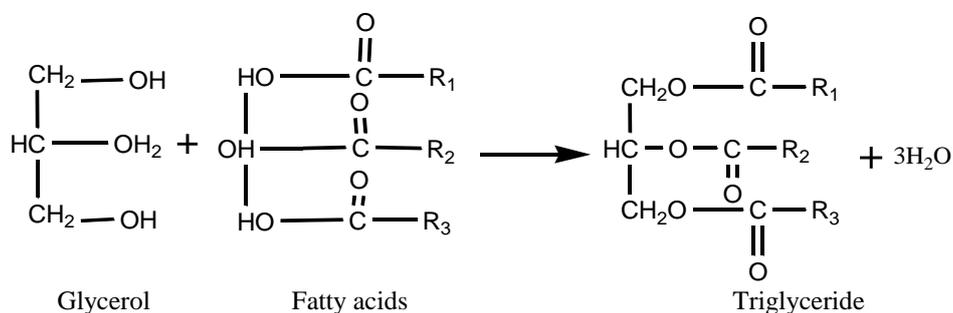
The species moringa *stenopetala* is known by different names with different language such as shiferaw (amharic), halako (*Gamo/wolayita*), kallanki (*Benishangul*), Shalchade (*Konso*), Haleko (*Burji*) and cabbage tree (English) [3]. Moringa *stenopetala* was domesticated in East Africa low lands and indigenous to southern and south western Ethiopia. Its leaves are consumed by Gofa, Konso, Burji and Gamo tribes as vegetable especially during the dry season [4].

The seeds of the moringa *species* plant are among the most nutritious and useful botanical and herbal remedies, as nutritional supplements and for industrial and agricultural purposes. Moringa seeds are edible in both fresh and dried forms and, along with the seed pods that contain them, can be prepared in numerous ways as both food and medicinal therapeutic purposes [5, 6]. The medicinal properties of the moringa seed are well documented in the scientific literature and are further supported by the experiences of generations of traditional Ayurvedic practitioners [7]. While many parts of moringa *species* trees are deemed useful, the seeds are especially prized for their medicinal powers. The seeds have valuable properties that enable them to treat a wide array of illnesses and conditions. The National Charity for Organic Growing has studied the efficacy of moringa *species* seeds as a medical treatment and found that they provide legitimate relief for many medical problems. These include rheumatism, gout, sexually transmitted diseases, urinary infections, boils, and even epilepsy [8]. Moringa seeds are also used as primary coagulate in drinking water classification and waste water treatment due to the presence of water soluble cationic coagulant protein able to reduce turbidity of the water treated [9].

Shiferaw/Haleko leaves contain high contain of essential amino acids and vitamin A and C [10]. Besides the industrial uses such as fine lubricant and perfumery, the fatty acids profile of the oil with its very high content of oleic acid may make it oil with high potential for further industrial application [11]. Oleic acid is an essential omega-9 fatty acid that was found to be responsible in hindering the occurrence of adrenoleukodystrophy (ADL), a fata disease that affects the brain and adrenal glands [12].

Moringa *stenopetala* seed powder can be used as effective heavy metal purifying from water and that moringa *stenopetala* is more effective than *oleifera* in removal of heavy metals [13].

Petrochemical based resin such as epoxy, polyester and vinyl ester find more engineering application because of their advantageous material properties such as high stiffness and strength [14]. However these resins have serious drawbacks in terms of biodegradability, initial processing cost, energy consumption, health hazards and for our everyday food products. Consequently there is a requirement to develop novel biobased product from renewable feedstock and finding an alternative source of cooking oil and as we all know, the price of commodities such as cooking oil keep an increasing every year. Therefore a number of researchers have been studied vegetable oils as alternative feedstock to substitute for petroleum.



However, some vegetable oil is not standards to it consumer satisfaction in terms of their physicochemical properties or for the texture and stability of the food product. The food value of the edible lipids also depend on chemical properties like iodine value, peroxide value, acidity etc, as well as on some physical properties like solidification temperature, color, appearance etc. Therefore, the study tried to analysis some important physicochemical properties o f moringa *stenopetala*.

General objective

- ❖ To Extract oil by soxhlet extractor and study the physico-chemical properties of moringa *stenopetala* seeds.

Specific Objectives

- To extract oil from moringa *stenopetala* seeds by soxhlet extraction method
- To determine the physicochemical properties of newly extracted oil

II. Experimental Sections

Materials and Methods

2.1. Apparatus

Hot tube, Rota evaporator, Measuring cylinder, Triple beam balance, Burette, Conical flask, Standard flask, Round bottom flask, Pipette, Heater, Boiling chip, Grinding mille, Water bath, Magnetic stirrer, Beaker, Sucker, Condenser, Soxhlet tube, Viscometer, Dropper and PH meter.

2.2. Chemicals

Moringa *Stenopetala* seed powder, petroleum, Diethyl ether, Ethanol, Phenolphthalein indicator, Alcoholic NaOH/KOH, HCl, Chloroform, Iodine, Glacial acetic acid, Na₂SO₃, KI, Tape and distilled Water, Na₂S₂O₃, Starch indicator, NH₄Cl, NH₄OH were analytical reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), BDH, CDH (India) and (Aldrich Chemical Company, Germany).

2.2.1. Reagents

0.5 Normality (N) HCl, accurately standardized Alcoholic KOH: 40gm of KOH was dissolved in 1L of distilled alcohol keeping the temperature below 15.5⁰C while the alkaline was dissolved and this solution was remained clear. Phenolphthalein indicator: 1%

Hanus iodine solution: 1.4 g of iodine was weighed and 82.5 ml of glacial acetic acid was dissolved by heating and cooling, then 25 ml of this solution was titrated against with 0.1N of Na₂SO₃.

Sodium hydroxide-sodium thiosulfate solution: 600gm sodium hydroxide and 50gm Na₂S₂O₃.5H₂O was dissolved in distilled water it was made to one liter.

2.3. Sample Collection

First, *M. stenopetala* seed was collected from *M. stenopetala* tree from surrounding of Arbaminch town, southern Ethiopia. Then, the seeds was removed from the pods, sorted, seeds was cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff as well as immature and broken seeds and oven dried and

stored until needed. Then the powder was obtained by in a grinding mille. Finally the seed powder was kept for extraction.



Figure 1. *Moringa stenopetala* tree



Figure 2. *Moringa stenopetala* undehulled seeds Figure 3. *Moringa stenopetala* kenguel seeds

All figure are taken from Eyasu Sefu report

2.4. Procedure of Oil Extraction

About 50g of *moringa stenopetala* crushed seeds was fed to lab-scale Soxhlet extractor fitted with a 1 liter round bottom flask and a condenser (weighed by using triple beam balance and was put in a tumble tube then, it was placed in distillation apparatus and oil was extracted by using 200ml of petroleum).The extraction was executed for 3 hours. Then, the solvent was separated from oil by using Rota evaporator (Evaporator N.N.Series equipped with an Aspirator and a Digital Water Bath SB-651, Japan). The experiment was repeated in triplicate. Finally the oil was kept for different analysis [15].

2.4.1. Determination of moisture content of seeds

Moringa stenopetala seeds were weight primarily, and it was dried in an oven at 109°C for 7 hours and the final weight was taken. The procedure was repeated in triplicate and recorded. The percentage moisture in the seed was calculated using the following equation [15].

$$\text{Moisture content} = [(w_1 - w_2) / w_2] \times 100$$

Where, w_1 = original weight of sample (before drying)

w_2 = Weight of sample after drying

2.4.2. Oil yield and density

The oil was extracted using soxhlet extraction method (*petrolleum*) as described by A.O.A.C. (1990) and density was also measured using pyconometer [16].

2.4.3. Determination of the seed oil content

The weight of oil of each replicated extracted was determined and mean value was recorded and the percentage of oil extracted was determined using below equation [18].

$$\text{Seed oil content} = (W_0/W_s) \times 100$$

Where, W_0 = weight of oil extracted
 W_s = Weight of sample (dry base)

2.4.4. Determination of acidic value

10g of the given oil was weighed in 250ml conical flask and it was dissolved in alcohol (mixture of 25ml of diethyl ether and 25ml of ethanol). Then, two drops of phenolphthalein indicator was added. The contents was titrated with 0.1M of alcoholic NaOH/KOH to the end point with consistent shakes until a dark pink color was observed and the volume of 0.1M NaOH/KOH (V_0) was recorded [17]. Finally the acid value of the oil was calculated as:

Where, 56.1 = equivalent weight of KOH

V = Volume of KOH

W_0 = Weight of oil

$$[56.1V \times 0.1] / W_0$$

2.4.5. Determination of saponification value

5g of oil was weighed in to conical flask and 50ml of alcoholic 0.1N KOH was added from burette by allowing it to drain for a definite period of time and a blank was also prepared by allowing it to drain at the same duration of time with continuous stirring. The flask was connected to the condenser and it was boiled gently for about 1 hour. Then the flask and condenser was cooled, the condenser was rinsed with a little distilled water and then the condenser was removed. Finally 1ml of phenolphthalein indicator was added and it was titrated against 0.5N HCl until the pink color was disappeared. Same procedures were used for other samples and blank [18]. The expression for SV was given by: $SV = [56.1 \times N (B-S)] / W_0$

Where, B = volume of solution for blank test

S = volume of solution uses for determination

N = actual normality of HCl uses and

W_0 = weight of oil.

2.4.6. Determination of peroxide value

1gm of *Moringa stenopetala* seed oil was weighed in to clean dry boiling tube and 3ml of solvent (12 ml of chloroform and 18 ml of glacial acetic acid mixture) was added, after this the tube was boiled for 30 seconds. The contents was transferred quickly to conical flask containing 20 ml of 5 % KI solution and the tube was washed twice with 25 ml of water and collected in to conical flask, then, the solution was titrated against 0.01N $Na_2S_2O_3$ solution until yellow color disappeared. 0.5 ml of starch was added with vigorous shaking and titrated carefully until the blue color just disappeared. The blank was also prepared with the same procedure [19]. Then the calculation as:

$$\text{Peroxide value} = [1000(S-B) N] / W$$

Where, S = Volume, in ml of $Na_2S_2O_3$ solution used up by the sample

B = Volume, in ml of $Na_2S_2O_3$ solution used up by the blank

N = Normality of $Na_2S_2O_3$ solution and

W = Weight, in g of the oil taken.

2.4.7. Determination of iodine value

0.5gm of oil was weighed into a conical flask and 10ml of chloroform was dissolved and 25ml of hanus iodine solution was added by using a pipette and was drained in a definite time to dissolve the oil. Then it was mixed well and was allowed to stand in a dark place for exactly 1hr with occasional shaking and 20ml of 15% KI was added. Then the solution was shaken thoroughly and 100ml of freshly boiled and cooled water was added and the stopper was washed down by any free iodine solution. Then the solution was titrated against 0.1N of $Na_2S_2O_3$ until yellow solution turns almost colorless and a few drop of 1% starch indicator was added. Then the solution was titrated until the blue color completely disappears. The flask was stopped towards the end titration and was shaken vigorously so that any iodine remaining in $CHCl_3$ was taken up by KI solution [4]. Finally the blank was run without the sample [2]. IV expression was given by:

$$IV = 12.69 \times C (V_1 - V_2) / w_0$$

Where, C = conc. of sodium thiosulphate, V_1 = volume of sodium thiosulphate use for blank, V_2 = volume of sodium thiosulphate use for determination, w_0 = Weight of oil

2.4.8. Determination of viscosity

A clean, dry Viscometer with a few times above 3 and ½ minutes for the fluid to be test was selected. The sample was filtered to eliminate dust and other solid material in the oil. The viscometer was turned to its normal vertical position. The viscometer was placed in to a holder and was inserted to a constant temp. The suction force was applied to the thinner arm draw sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flow the upper timing mark was recorded. And this procedure was repeated in triplicate [6]. Then the viscosity was calculated as:

$$\text{Viscosity} = (\text{average time}) / (\text{density of sample})$$

2.4.9. Determination of pH value

3gm of sample was taken to a clean dry 25ml of beaker and 15ml hot distilled water was added to the sample into the beaker and was stirred slowly. It was cooled in a cold water bath. The PH electrode was standardized with (NH₄Cl+NH₄OH) buffer solution and immersed in to the sample and the PH value was recorded [19].

2.4.10. Analysis of Oil seed waste

The oil seed residues remaining after the extraction of oil were analyzed for protein, fiber and ash contents. Protein content was determined as samples of residue were digested for 11 minutes with a digestion mixture of sulphuric acid/ hydrogen peroxide, using mercuric oxide as a catalyst. The final end point in the ammonia titration was measured photometrically. Fiber content was determined as 2.0-g of finely ground defatted sample were weighed and boiled with a sulfuric acid solution (0.255 mol/L) for half an hour followed by separation and washing of the insoluble residue. The residue was then boiled with a sodium hydroxide (0.313 mol L⁻¹) solution followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace at 600°C and the loss in mass was determined. Ash content was determined as two grams of the test portion were taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace at 550°C until a constant mass was achieved according to [11].

III. Results and Discussion

The data from the analyses of *M. Stenopetala* oil seeds and extracted oils have been summarized in Table 1-5. Values for the present analyses are given as mean ± SD, for three *M. Stenopetala* oil seed samples in triplicate. Table 1 below shows the proximate analyses of *M. Stenopetala* oil seeds.

The physical properties of moringa *stenopetala* seeds such as size, shape and bulk density and chemical composition of seeds are needed for the design of equipments to handle, transport, process and store for asserting product quality. Moreover, unit operation for preparation of seeds for oil extraction very slightly depending up on the physical properties and oil contents of the seed. Undehulled moringa *stenopetala* seeds examined are triangular, have tree wings and covered with spongy thick yellowish seed coat. The kernel has whitish gray color and oval shape and its thickness decrease from the center towards either end along the length of seed. The average weights of undehulled seeds is much higher than that moringa *oleifera* seeds and the average weights of kernel of moringa *stenopetala* seed is more than double to moringa *oleifera* seed. The extracted oils were liquid at room temperature. The oil content of Moringa *Stenopetala* seeds. The oil extraction with lab-scale Soxhlet extractor had the highest yield, due to the increased ability of the polar solvent to overcome forces that bind lipids within the sample. The lowest yield due to losses during the separation of the oil from the water.

Table 1. Analysis of *Moringa stanopetala* seeds

Constituents	Results
Average Seed Weight (g)	0.42± 0.40
Moisture content (%)	5.7± 0.65
Average oil Content (%)	39.18 ± 0.70
Fiber (%)	7.50 ± 0.55
Protein (%)	30.90 ± 0.85

Values are mean ± SD of three seeds from each region, analyzed individually in triplicate Average Seed Weight *M. Stenopetala* in this study agrees with reported result of Ethiopian agricultural research (0.5g)[1]. The average moisture content of moringa *stenopetala* seed in this study is 5.7%. This value is comparable to the corresponding value reported [6]. The moisture content of oil seeds can determine the storage condition and influence the duration of storage and subsequently the gravity of the oil and meal. Whole, intact, low-moisture oilseeds (about8-10% moisture) can be stored for an extended time without significant change on the quality of oil. High moisture content (about14-15% moisture) in seeds has an adverse effect on oil and meal quality [1].

The range of oil content ($39.18 \pm 0.70\%$) of *M.stanopetala* seeds in the present analyses was found to exceed those of cotton seed (15.0-24.0 %) and soybean (17.0-21.0 %) and comparable with those of safflower (25.0-40.0 %). The high oil yield allows the possibility of economical exploitation which results in lower operation costs compared to some other oil seeds. The high oil yield from this region might be accredited to the sandy soil texture and favorable environment for Moringa growth because report revealed that the Moringa tree is suitable with sandy soil streams. The results of the *M.stanopetala* oil seed wastes indicate high protein content in the seeds, ranging from 29.6 to 31.3% the results agrees with the literatures. The result revealed that Fiber contents 7.50 ± 0.55 . The result is comparable to those reported for *M.stanopetala* seeds [5].

Table 2. Physical and chemical Characteristics of the extracted Oil

Physical property	Value	Chemical property	Value
Viscosity	10	Seed oil content	46.18 %
Density at 24°C	0.907	Acidic value	1.68 mg KOH/g
pH	7.53	Saponification value	178 mg KOH/g
Color	Pale yellow	Iodine value	70.2 g I ₂ /100g
Odor	odorless	Peroxide value	9.45 meq/kg

The average density of Moringa oil 0.907 obtained in this study was consistent with the reported reviews [19]. In this manner the oil from moringa *stenopetala* seed using soxhlet apparatus and petroleum as a solvent were liquid at room temperature, pale yellow, odorless and its P^H value was 7.53 (Table 2). Average acid value; in this study is 1.68 mg KOH/g. This value agrees with previous literature 1.42 mgKOH/g [6]. The acceptable acid value limit for edible oil is less than 10 [13]. From this the oil from moringa *stenopetala* seed used for edible oil.

Table 3. Comparison of Saponification value (SV) and Iodine value (IV) of common edible oils

Type of oil	SV	IV	Type of oil	SV	IV
<i>M. stenopetala</i> oil	176.5-178	69-70.2	Present work		
Coconut oil	250-264	7.5-10.5	Safflower oil	189-195	138-146
Cottonseed	190-198	98-115	Sunflower oil	188-194	100-140
Groundnut oil	188-195	87-98	Soybean oil	189-195	125-140
Mustard oil	169-177	98-110	Rice bran oil	180-195	90-105
Sesame oil	188-193	103-115	Palm oil	195-205	44-58

Moringa *stenopetala* oil has higher saturation than soybean, sunflower and rape seed oil, but lower saturation than plam oil, it is important also to remarkable that, the absence polysaturated fatty acid in moringa *stenopetala* oil in comparison to that of soybean, sunflower and rape seed oil. Another interesting fact is they high contain of behenic (docosnic) acid in moringa *stenopetala* oil possesses significant resistance to oxidative degradation due to it contain of behenic acid [6].

Saponification value of moringa *stenopetala* seed oil in this study is 178 (mg koH/g). This result agrees with 178.23(mg koH/g) [6]. This indicates the presence of high percentage of fatty acid in moringa *stenopetala* oil and there for implies the possible tendency to soap formation.

Iodine value of moringa *stenopetala* seed oil in this study is 70.2 gI₂/100g, and this result indicates in agreement with previous literature 69.21gI₂/100g [6]. Iodine value is the measure of the unsaturation fats and oils in which high iodine value indicates high unsaturation of fats and oils. Oils with iodine value above 125 are classified as drying oils. Those with iodine value 110-140 are classified as semidrying oil and oil with iodine value less than 110 are considered as non drying oil.

The peroxide value of fresh edible oil is usually within 10meq/kg and the result from peroxide value of moringa *stenopetala* seed oil in this study is 9.4 meq/kg which is approximately agree with this fresh edible oil. This result is higher than that of jatropha oil seed (1.93meq/kg) and shea-nut oil (0.2meq/kg). The oil having higher percentage of peroxide is unstable and grows rancidity easily. This indicates that if the oil is to be used for the purpose of biodiesel production [13]. Generally this result have some variation, this may be due to the difference in variety of plant, cultivation climate, ripening stage, harvesting time of the seed, extraction method used.

IV. Conclusion

The extraction of oil from moringa *stenopetala* seed has been successfully taken place by using Soxhlet extractor and the properties of the extracted oil were studied by physico-chemical determination. The results of extracted oil from the seeds of *M.stenopetala* in this study have showed that this oil could be utilized successfully as source of edible oil for human consumption. In this manner, the result from moisture content of the seed is low which shows as the extracted oil is advantageous in terms of storage stability since the moisture content, the better the storability and suitability to be preserved for a longer period of time. The result from

Iodine value of moringa *stenopetala* seed oil in this study indicates a relatively low ratio of unsaturated which might be an acceptable substitute for less unsaturated oils such as olive oil in diets. Therefore, moringa *stenopetala* seed oil has high stability to oxidative rancidity. The oil can be classified as non-drying and edible based on value obtained for iodine and acid values. additionally, the result obtained for the saponification value portend that oil is good for soap production, thus the oil has great prospects in terms of its edibility and also suitability for soap production which also supported by the result of acidity value.

In addition to its promising result *M. stenopetala* have high nutritional value, medicinal value, drought tolerance, fast growth, and many of its potential uses have great impact on economic and social values so need better attention of researchers.

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