

Extraction and Characterization of *Moringa oleifera* Seed Oil

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ABSTRACT

Moringa Oleifera seed oil was extracted using the solvent extraction method. The proximate analysis of the oil was carried out. The physicochemical properties of the oil were also determined. The parameters determined were: moisture content, ash, crude protein, crude fat and carbohydrate for proximate analysis; and pH, saponification value, iodine value, free fatty acid and specific gravity for physicochemical properties. The values of moisture content, ash, crude protein, crude fat and carbohydrate were 0.60%, 1.50%, 2.19%, 39.3% and 56.42% respectively. While the values obtained for pH, saponification value, iodine value, free fatty acid and specific gravity were 5.96, 164.09mg/g, 68.23g/mol, 8.27mgKOH/g and 0.86 respectively. The results showed that *Moringa oleifera* seed oil is a good source of Carbohydrate, also the saponification values and iodine values obtained shows that it is a good raw material for both home and industrial consumption.

INTRODUCTION

Moringa oleifera, commonly referred to as "*Moringa*" (from Tamil: Muringa and Malayalam: Murunggi,) is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family *Moringaceae*. *Moringa*, which is the only genus in the family *Moringaceae*, is an exceptionally nutritious vegetable tree with a variety of potential uses. Every part of *Moringa oleifera* such as the seed, root and stem are useful. The tree itself is rather slender, with drooping branches that grow to approximately 10m in height ^[1]. *Moringa oleifera* is called different names worldwide ^[2].

Moringa oleifera is now grown widely throughout the tropics of which Nigeria is a part. It is sometimes known as the 'drumstick' or 'horseradish' tree. Ranging in height from 5 to 12m with an open, umbrella-shaped crown, straight trunk and corky, whitish bark, the tree produces a tuberous tap root. The evergreen or deciduous foliage (depending on climate) has leaflets 1 to 2 cm in diameter; the flowers are white or cream coloured. The fruits (pods) are initially light green, slim and tender, eventually becoming dark green, firm and up to 120cm long, depending on the variety. Fully mature, dried seeds are round or triangular, the kernel being surrounded by a lightly wooded shell with three papery wings ^[3].

In the tropics, it is used as forage for livestock; in many countries, *Moringa* is used as a micronutrient powder to treat diseases. The green pods, fresh and dried leaves are used as vegetable. The seeds contain up to 40% of oil by weight which is used for cooking, soap manufacture, cosmetic base and in lamps. All parts of the plant are used in a variety of traditional medicines. The press cake, obtained following oil extraction, is useful as a soil conditioner; the plants are grown as live fences and windbreaks. It is also used as fuel wood source after coppicing (cutting back the main stem to encourage side shoots); as an intercrop with other crops and the wood pulp may be used for paper-making. In the tropics, it is used as forage for livestock; in many countries, *Moringa* is used as a micronutrient powder to treat diseases. The green pods, fresh and dried leaves are used as vegetable ^[3,4].

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. *Moringa*

is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce [5,6]. It is commonly said that *Moringa* leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas,” and that the protein quality of *Moringa* leaves rivals that of milk and eggs. However, the leaves and stem of *M. oleifera* are known to have large amounts of their calcium bound in calcium oxalate crystals [7].

The tree's bark, roots, fruit, flowers, leaves, seeds, and gum are also used medicinally. The uses include as an antiseptic and in treating rheumatism, venomous bites, and other conditions. The flowers, leaves and roots are widely used as remedies for several ailments. The bark of the *moringa* root should be scraped off because of its toxicity and the flesh of the root should be eaten sparingly [8]. *Moringa* seeds are effective against skin-infecting bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* [8]. They contain the potent antibiotic and fungicide terygospemin. *Moringa* seem to have most of the food nutrients required by the body to replenish its defensive mechanisms. The Tonga people of Binga District in Zimbabwe use the root powder as an aphrodisiac and when it is mixed with milk, it is considered useful against asthma, gout, rheumatism and enlarged spleen or liver. It also helps in the removal of wind from the stomach and as a snuff and can be used to alleviate ear and toothache [8,9].

The leaf juice has a stabilizing effect on blood pressure. The leaf juice controls glucose levels in diabetic patients. Fresh leaves and leaf powder are recommended for tuberculosis patients because of the availability of vitamin A that boosts the immune system. If leaf juice is used as diuretic, it increases urine flow and cures gonorrhoea. Leaf juice mixed with honey treats diarrhoea, dysentery and colitis (colon inflammation). Fresh leaves are good for pregnant and lactating mothers; they improve milk production and are prescribed for anaemia. Paste made from bark treats boils. Paste from ground bark can be applied to relieve pain caused by snake, scorpion and insect bites. Oil is sometimes applied externally for skin diseases [9].

Fully mature, dried seeds are round or triangular in shape, where the kernel is surrounded by light wooded shell with three papery wings. When mature, the seeds from the pods can be extracted and treated like green peas and can be fried or roasted and eaten like peanuts. It also contains oleic acid-type oil. The seeds contain up to 40% of oil by weight which is used for cooking, soap manufacture, cosmetic base and in lamps. All parts of the plant are used in a variety of traditional medicines. The press cake, obtained following oil extraction, is useful as a soil conditioner; the plants are grown as live fences and windbreaks. It is also used as fuelwood source after coppicing (cutting back the main stem to encourage side shoots); as an intercrop with other crops and the wood pulp may be used for paper-making [3].

Due to ever diminishing sources of fats and oils, there is the growing need for the search of new sources of oil as well as exploiting sources that are currently unexploited in order to supplement the existing ones [1]. *Moringa oleifera*, a very rapid growing tree found growing in a varying range of climatic condition is a promising tree and has the potential to become a new source of oil for Nigeria. It has been reported that some 3000 kg of seeds could be obtained from one hectare, equivalent to 900 kg oil/hectare, comparable to soybean which also yields an average of 3000 kg seeds/hectare but with only 20% oil yield. The oil is edible, and closely resembles olive oil in its fatty acids composition. According to Sengupta and Gupta [10], *M. oleifera* seeds contain between 33 and 41% w/w of vegetable oil. Anwar *et al.*, [11] also investigated the composition of *M. oleifera*, including its fatty acid profile and showed that *M. oleifera* oil is high in oleic acid (>70%). *M. oleifera* is commercially known as “ben oil” or “behen oil”, due to its content of behenic (docosanoic) acid, it possesses significant resistance to oxidative degradation [12]. *M. oleifera* has many medicinal uses and has significant nutritional value [11]. A recent survey conducted on 75 indigenous (India) plant-derived non-traditional oils concluded that *M. oleifera* oil, among others, has good potential for biodiesel production [11].

Various extraction methods can be used in the extraction of oil and the method is normally dependent on what type of botanical material is been used. These methods include mechanical, traditional and solvent extraction. Solvent extraction method involves the counter – current flow of solvent and out bearing materials in the extraction vessel. It is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feed stocks or reaction products. Some extraction techniques involve partition between two immiscible liquids; others involve either continuous extractions or batch extractions. Because of environmental concerns, many common liquid/liquid processes have been modified to either utilise benign solvents, or move to more frugal processes such as solid phase extraction. The solvent can be a vapour, supercritical fluid, or liquid, and the sample can be a gas, liquid or solid [13]. The yield using this process is usually higher than that of mechanical method; and the residue usually contains less than 2% oil. Common solvents used are hexane and benzene (hydrocarbons) both of which are petroleum derivatives. Solvent extraction plants are becoming more as processing industries now aim to produce meals with minimum oil contact for commercially acceptable production levels without impairing oil or meal quality.

Usually solvents are used to extract fats and oils. Solvents, however, are not environmentally friendly. Enzymes, on the other hand, are green catalysts, and are often used to improve the efficiency of oil extraction. Rosenthal *et al* [14] have reviewed the use of

enzymes in the extraction of oil, protein, and other components from oil-containing seeds and fruits. This technology has been developed to extract oil on a laboratory and pilot scale from many oil-bearing materials.

The characterisation of essential oils through chemical analysis is a mandatory step in the production chain, to be carried out by both researchers and quality control laboratories. Essential oils are primarily composed of terpenes and their oxygenated derivatives. Conventionally, gas chromatography methods are used to perform overall analytical cycle of the essential oil due to its high performance and high level of accuracy. The colour, viscosity, solubility and smell of the extract also can be tested to ascertain the quality of an optimum grade of the extract [15]. The index of refraction is related to the physical structure of the medium through which light is passing. For this reason, the index of refraction is a characteristic of substances that can be employed in identifying unknown compounds. The higher the refractive index, the greater the amount of dispersion which increase the brilliance of a material. Acid value is measure of the amount of free acid present in fat. Some of the deterioration that takes place during storage in the raw material from which the fat is obtained results in hydrolysis of fatty acids. Fatty acids must be removed in the preparation of insecticides. Specific gravity is the density of a substance divided by the density of water. Since water has a density of 1 g/cm^3 , and since all of the units cancel, specific gravity is the same number as density but without any units. For example, the specific gravity of leached solution (i.e. ethanol and the solute from *Moringa oleifera*) is a function of the specific gravity of the oil and that of the organic solvent i.e. ethanol. The specific gravity of the oil is about 0.93 and that of the solids not fat is 1.5. Hence as the oil content of solution increases, the specific gravity decreases and, conversely as the solid (solute) increases, the specific gravity of the milk also increases. Iodine value is also called iodine number which is the measure of the proportion of the unsaturated acid present. There is no iodine present in oils and fats, but the test measures the amount of iodine which can be absorbed by the unsaturated acids. As the concentration and types of unsaturated acids present are fairly constant, the iodine value will give a figure for the total degree of unsaturation [15].

Due to high dependency of humans on oil for both domestic and industrial uses. There is need to look for another source of oil with better method of extraction to give a higher yield. This study focuses on the extraction and characterization of *moringa oleifera* seed oil.

MATERIALS AND METHODS

Some samples of *Moringa oleifera* were obtained from maizube farms at Garatu Village in Bosso LGA of Niger State. A Picture of the seeds is shown below.



Plate 1: *Moringa oleifera* seeds

Pre-treatment procedure

This is the initial stage of sample preparation and it includes the following:

Seed collection

This involves the collection of the fruits of *Moringa oleifera* and drying it. The drying process stimulates the opening of the fruits to release seeds embedded inside. The seeds were separated from the chaffs and other impurities. This Preparation is very important since any impurity in the seeds will eventually reflect on the oil extracted.

Drying

After the seeds had been cleaned thoroughly; they were dried in an electric oven to reduce the moisture content of the seeds.

Moringa oleifera seeds were milled into a paste using thermal Willey mill. This operation ruptures the cell wall and releases the solute for direct contact with the solvent during the contact equilibrium process.

Weighing

This was done before and after the seeds were dried. The weight was taken and recorded using electronic weighing balance.

Experimental Procedure

The samples collected were washed with distilled water. These were then air dried in the laboratory and grinded. 10.0g of this was weighed and placed on a filter paper which was folded carefully. The filter paper containing the sample was then inserted into the Soxhlet apparatus. The weight of the filter paper and sample was recorded. 200ml of the solvent (hexane) was measured using a measuring cylinder and then poured into a 500ml round bottom flask with the sample and heated at 60°C for 5 hours after which the sample was removed and transferred into the air oven to dry at 105°C for 15 minutes. This sample was then weighed and the difference was calculated as: weight of sample before extraction - weight of sample after extraction, divided by the initial weight of sample, and multiplied by 100 to give the percentage yield oil. The oil was recovered by solvent evaporation. It was heated at a low temperature until the solvent finally evaporates leaving behind the oil extracted. Figure 3.1 shows the flow chart for the solvent extraction of *Moringa oleifera* oil from Moringa seeds.

Proximate Analysis

Proximate analysis was carried out at the Chemistry Laboratory, Federal Polytechnic, Bida, Niger State, Nigeria. The proximate composition of the material was determined by the method described by the Association of Analytical Chemists^[16]. The proximate analyses carried out are moisture content, crude fat, ash, crude protein and carbohydrate.

Determination of Physicochemical Properties

The following physicochemical properties were determined;

Saponification Value

The sample was melted and filtered to remove any impurities and the last traces of moisture. 5g of the sample was weighed into a conical flask. 50ml of alcohol KOH was added from burette by allowing it to drain for a definite period of time. A blank sample was prepared using the same method. Then a reflux condenser was connected to the flask and it was boiled gently for about 1hr. The flask and condenser were allowed to cool and rinsed with little distilled water and the condenser was removed. 1ml of indicator was added and 0.5M HCl was used for titration until the pink colour disappeared.

Iodine Value

0.5g of oil was weighed into iodine flask and it was dissolved into 10ml of chloroform. 25ml of Wiji's iodine solution was added to it using pipette for a certain period of time. The solution was mixed well and allowed to stand in a dark corner for exactly 30 minutes with occasional shaking. 50ml of 15% KI was added and shaken thoroughly. 100ml of freshly boiled and cooled water was used to wash down free iodine on a stopper. It was titrated against 60 normal sodium trioxothiosulphate (VI) until the yellow solution turned almost colourless. Little starch was added as an indicator and titrated until blue colouration completely disappeared.

Free Fatty Acid

5g of oil or melted fat was dissolved in 50ml of the neutral solvent in 250ml conical flask. 3 - 4 drops of phenolphthalein indicator was added. The sample was titrated against 0.1KOH and shaken constantly until it changed to pink colour.

Specific gravity

A stopper density bottle was filled with cold distilled water and kept in a water bath at 100°C for 30 minutes. The weight was taken after losing away any water drops on the bottle. After drying the bottle was filled with the extracted oil and the process was repeated to get the final weight.

2g of the sample was poured into a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold water bath to 25°C. The pH electrode was standard with buffer solution and the electrode immersed into the sample and the pH value was read and recorded.

RESULTS AND DISCUSSIONS

The results of the proximate composition of *Moringa oleifera* oil are presented in Table 1.

Table 1: Proximate composition of *Moringa oleifera*

Nutrient	Composition (%)
Moisture content	0.60± 0.07
Ash	1.50±0.01
Crude protein	2.19±0.21
Crude fat	39.30±1.00
Carbohydrate	56.42±0.72

Values are mean ±Standard deviation of triplicate determinations

The results of the physicochemical properties of *Moringa oleifera* oil are presented on Table 2.

Table 2: Physicochemical properties of *Moringa oleifera* oil

Characteristics	<i>Moringa oleifera</i>
pH	5.96±0.03
Saponification value	164.09±1.58mg/g
Iodine value	68.23±0.60g/mol
Free fatty acid	8.27±0.19mgKOH/g
Specific gravity	0.86±0.01

Values are mean ±Standard deviation of triplicate determinations

The oil yield was between 33 – 37% for the replicates, this was in agreement with Solade [17] which got 35 – 40% oil yield in palm kernel. This is also in line with the results given by Abdulkarim *et al* that gave the value of oil yield from mature seeds of any plant to be between 22 – 43%. Variation in oil yield may be due to differences in variety of plant, cultivation climate, ripening stage and the method of extraction used. The value of 39.30% obtained for crude fat agrees with the 39.80% reported by Nzikou *et al*. [18] for crude fat. The high potential of oil makes this seed a distinct potential for the oil industry. Jamieson reported 40% crude fat from castor oil seed which in agreement or close to the value obtained in this research. The saponification value of 164.09mg/g and iodine value of 68.23g/mol agrees slightly with the values of 166.0 09mg/g and 67.40g/mol obtained by Nzikou *et al*. However, the values obtained for moisture content, ash content and crude protein were contrary to what Nzikou *et al*. reported. Nzikou *et al*. reported 5.3% for moisture, 37.6% for crude protein, and 4.2% for ash content. Also, the value of 8.27mg/KOH/g obtained for fatty acid is contrary to the value of 1.08mgKOH/g reported by Nzikou *et al*. The difference in value may be attributed to the different methods of extraction used [18].

CONCLUSIONS

Moringa oleifera seed has the potential to become a new source of oil and its full potential should be exploited. It contains high unsaturated to saturated fatty acids ratio due to its high iodine and saponification values and might be an acceptable substitute for high saturated oil such as olive oil in diets. The production of useful oil from the seeds could be of economic importance of benefit to the areas where the tree is cultivated.

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