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Extraction of Oil, Biogas and Biodiesel from *Moringa oleifera* Seeds.

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Abstract

The present work was on the extraction of the oil from the *Moringa* (*Moringa oleifera*) kernel as a source for biodiesel and biogas production. *Moringa* is an indigenous tree in India. Several extraction methods are used to determine the extraction yields, solvent extraction (*n*-hexane and ethanol), and supercritical extraction (Sc-CO_2) respectively. Supercritical extraction pressures of 200 to 400 bar and temperatures of 40°C and 60°C were tested. Gas Chromatography analysis revealed that the main fatty acids in *Moringa* oil are oleic acid (69%), palmitic acid (10%) and stearic acid (8%).

Keywords: (*Moringa oleifera*, CO_2 Supercritical Extraction, Solvent Extraction, Ben Oil)

Introduction

Moringa oleifera Lam belonging to the family *Moringaceae* is a handsome softwood tree, native of India and now grown world-wide in the tropics and sub-tropics. In India, it is grown all over the subcontinent for its tender pods and also for its leaves and flowers. The pods of *moringa* are very popular vegetables and valued for its distinctly inviting flavour.

Moringa oleifera is considered as "miracle tree" because all its parts are used, especially for their pharmacological, nutritional and purifying water properties. Leaves are eaten as vegetables and pressed; they are used in traditional pharmacology to treat many aliments. The fruits are mainly used in condiments or cooked as vegetables. Flowers produce nectar and have anti-inflammation properties. Moringa seeds are rich in proteins and oil, traditionally are used for beauty care. Seeds are also used for water purification. The wood provides a blue dye and it is used for live fences. Medicinal qualities offer to treat diabetes, to enrich anaemic blood, to staunch a skin infection, to be an antibiotic, to heal gastric ulcers and to care eyes. Thus, this tree offers very interesting opportunities as food supplement, nutrition, vegetable, oil, water treatment, green manure, foliar spray, natural fertilizer, livestock feed, fodder, medicine, cosmetic and care products.

The oil extracted from *Moringa* is known as ben oil and reportedly contains 70% of oleic acid, an 18-carbon long monounsaturated fatty acid (MUFA). Since the oleic acid has good oxidative stability when compared with polyunsaturated fatty acids (PUFAs), it is useful in the food industry, as it encourages longer storage and high-temperature frying processing. The oil content of de-hulled seed (kernel) is approximately 42%. The oil is yellowish in colour. It is also used as a lubricant for fine machinery because of its little tendency to deteriorate, become rancid and sticky (Ahaotu, 1997). In some areas, it serves as vegetable cooking oil. The oil is known for its capacity to absorb and retain volatile substances and is therefore valuable in the perfume industry for stabilizing scents. The free fatty acid content varies from 0.5 to 3 %. The seed oil of *Moringa* contains approximately 13 % saturated fatty acids and 82 % unsaturated fatty acids. It has a particularly high level of oleic acid (70 %) (Abdulkarim *et al.*, 2007).

Ben oil is more stable than canola oil, soybean oil and palm oil when used in frying. Blending ben oil with sunflower oil and soybean oil enhances the oxidative stability of the mixture. Mani *et al.* (2007) and Ahaotu *et al.*, (2013b) stated that comparing its chemical properties, *Moringa* seed oil is considered equivalent to olive oil and may be used for human consumption. Also, the oil from *Moringa* seeds has shown the strongest anti- fungal activity against a zoophilic dematophyte caused marked inflammatory reactions in humans (Chuang *et al.*, 2007; Ahaotu *et al.*, 2013a).

Solvent extraction has been reported by Mani *et al.* (2007) using *n*-hexane, petroleum ether and acetone. Experimental Soxhlet extraction using *n*-hexane and ethanol, and also supercritical extraction with CO_2 on Moringa seeds have been reported by Nguyen *et al.* (2011). Sovova and Stateva (2011) recently review the field of supercritical extraction of vegetable materials and reported appreciations in industrial applications.

Moringa seeds as biosorbent could be used as a less expensive biosorbent for the removal of cadmium (Cd) from aqueous media (Sharma *et al.*, 2006). The amino acids found in moringa seeds constitute a physiologically active group of binding agents, which has the ability to interact with metal ions to increase the sorption of metal ions (Sharma *et al.*, 2006; Brostlap and Schuurmans, 1988).

Moringa plants (approximately 30 days old) were milled together with water. The fibre was separated by filtration through a mesh with 5 mm pores and the liquid fraction produced was then added. With an average feed of 5.7 g of volatile solids the gas production was 580 liters of gas per 1 kg of volatile solids. The average methane content of the gas was 81 % (Broin *et al*., 2002).

Materials and method

Collection of *Moringa* seeds

The *Moringa oleifera* seeds used for this work were obtained from a *Moringa* plantation at the Forestry unit, Imo State Polytechnic Umuagwo, Nigeria.

Processing of Moringa seeds

Moringa oleifera kernels were cleaned to remove stones, dirt, sand and other extraneous materials. The cleaned kernels were cracked by hand to remove the shell from the nuts. The seeds were divided into three equal parts; a part of the seeds were dried in the cabinet oven at 60^0C for 2 hours; after which it was milled to flour in an attrition mill to obtain a smooth *Moringa oleifera* seeds flour. Another portion was sundried at the normal atmospheric temperature for 4 days and milled to obtain the flour while the last part was not subjected to any drying method (serves as control sample).

Extraction of *Moringa* seed oil

The smooth flours (500g each) were transferred inside a jar bottle and 400ml of hexane was poured inside the jar for 24hours. The samples were mixed together by shaking and was turned into a round bottom flask of the Soxhlet extractor and covered with the reflux condenser subjecting the bottom of the flask to heat. The solvent was allowed to boil gently and left to siphon over a period of 2 hours. The boiling point of hexane is lower than that of the oil, hence hexane was evaporated leaving the oil; and hexane was collected. The oil obtained from the extract was allowed to dry in an oven at 105^{o}C for 2 hours and cooled in a desiccator before using for experimental work. The oil obtained is called crude oil because it has not been refined. Oil sample was also extracted from freshly harvested *Moringa oleifera* seeds without subjecting it to any processing as the control sample.

Table 1. Proximate Analysis of *Moringa oleifera* Seed Oil

Characteristics	Obtained Values
Moisture	10.50±0.71
Ash	5.00±0.00
Crude fiber	5.00±0.00
Crude protein	39.57±3.23
Fat	32.50±7.78
Carbohydrate	7.44±10.30

The GC and GC-MS analysis of the seed oil of *M. oleifera* was performed using a multi-dimensional gas chromatography coupled with gas chromatography-mass spectrophotometer. (Shimadzu Japan) equipped with non-polar and polar double capillary columns (25.0m×0.25µm i.d., 0.25µm). High purity helium was used as the carrier gas at a constant flow rate of 0.99ml/min. 1 µl sample was injected (split ratio 100:1) into GC and GCMS using AOC-2Oi; auto injector for analysis. The initial temperature was set at 60°C, heated at a rate of 3°C/min to 280°C and held isothermally for 6minutes. Ion source temperature was set to 200°C while the interface was set at 250°C; solvent cut time was 3 minutes. Electron impact (EI) ionization mode was 70ev and the linear velocity of the column was 36.8cm/sec.

Plate 1. Seeds of *Moringa oleifera*

Plate 2. *Moringa oleifera* Seed and Powder

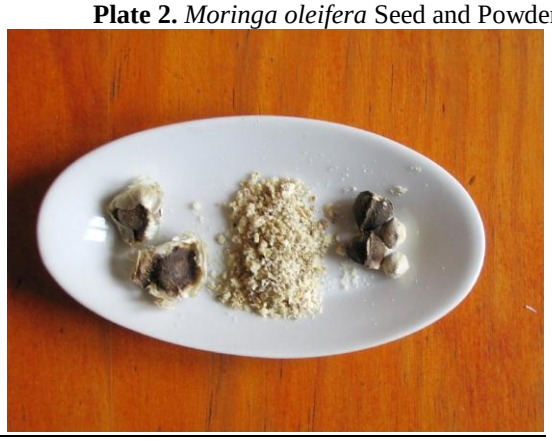

Plate 3. Ben oil extracted from *Moringa oleifera* seeds.

Table 2. Proximate Composition of Ben Oil

Components	Percentages (%)
Myristic acid	0.1
Palmitic acid	9.3
Stearic acid	7.4
Behenic acid	8.6
Oleic acid	65.5
Palmitoleic acid	1.4
Linoleic acid	1.5
Arachidic acid	3.7
Eicosenoic acid	2.3
Cerotic acid	1.3
Phytosterols	9.0

Results and discussions

The oil extracted from *M. oleifera* seed has an agreeable odour and the colour is cream-yellow, the percentage oil yield is 38%. This percentage yield was higher' than that reported by (AOAS, 2001), where the seed oil yield of *M. oleifera* was 34.50. The 38% yield of *M. oleifera* was consistent with that of Literature (Lalas and Tsaksins, 2002). The specific gravity of *M. oleifera* seed oil was 0.9050 and this value is in agreement with the FAO/WHO (2009) international standard for edible oil. The refractive index 1.4559 was in agreement with the FAO/WHO (2009) international standard for edible oil. The physical properties of the oil extracted from *M. oleifera* seed were in conformity with the FAO/WHO (2009) standard. On the other hand, the chemical properties of the oil are shown also in table 1. An acid value of 6.73 mg/ KOHg-1, this value is higher than the acid value specified for edible oil by FAO/WHO (2009) but this value was almost in agreement with Literature (5.0386 mg KOH/g) reported by (AOAC, 2006)

Result of the proximate composition of *M. oleifera* seed oil is shown in table 1. The moisture content is 10.50%, ash content, 5.00%, crude fiber 5.00%, crude protein 39.57%, fat content 32.50% and carbohydrate (by difference) 7.44%. The observed low moisture content in *M. oleifera* seed in this study serve as an indication that the activities of the micro-organisms would be reduced and thereby increases the life of *M. oleifera* sample. The observed moisture content value of 10.50% is higher than the value (9.40%) reported by (Aja *et al.*, 2013). The ash content is 5.00% which is higher than the value (3.87%) reported by (Aja *et al.*, 2013) but in agreement with the value (5.00%) reported by (Peter and Philip, 2014). Ash is an incombustible residue left after complete combustion of any substance. The crude fiber content of 5.00% obtained in our sample was higher than 2.87% reported by (Aja *et al.*, 2013) but lower than 20.00% reported by (Peter and Philip, 2014) from Ebonyi State, Nigeria, crude fiber content has been established to help in bowel movement. Adequate intake of dietary fiber can lower cholesterol level, risk of coronary heart diseases, constipation, hypertension, diabetes, colon and breast cancer (Adegbe *et al.*, 2016). Crude protein and fat content are 39.57% and 32.50% respectively. The crude protein content is higher than 35.97% and 9.98% reported by (Masurekar *et al.*, 2015) while the observed fat content in this study is lower than 38.62% and 40.00% reported by (Aja *et al.*, 2013). Adegbe *et al.*, (2016) stated that plant food provide more than 12% of its calorific value from protein, is considered good source of protein. Therefore, *M. oleifera* is a good source of protein. Carbohydrate content is 7.44% which is lower compared to the value 18.00 reported by (Peter and Philip, 2014).

The chemical components of the fixed oil *M. oleifera* seed oil was analyzed using multi-dimensional gas chromatography coupled with gas-chromatography-mass spectrophotometer (GC-MS). Twenty four (24) components amounting to 96.81% were identified in the seed oil. The identified components, their retention indexes and percentage composition of each component are shown in the table 2 above. The major constituents found in the fixed oil of *M. oleifera* seed oil are: Oleic acid (22.51%), Palmitic acid (10.64%), Stearic acid (6.07%), 9-octadecenal (12.76%) and Phenyl but-3-1-yne (5.79%). Other noticeable constituents found in the oil were o-Ethyltoulene (4.64%), m-Propyltoulene (3.56%), 4-methylindin (2.35%), 2-phenyl-2-pentane

(2.47%), p-mentha -1, 3, 8-triene (2.36%) and Arachidic acid (2.21%). It is worth mentioning that the main compounds characterizing the fixed oil of *M. oleifera* are qualitatively and quantitatively different. The fixed oil of *M. oleifera* is rich in fatty acid (44.93%) followed by hydrocarbons (32.95%), others are aldehyde (12.76%), esters (3.55%) and Oxygenated hydrocarbons (2.62%). The following fatty acids were identified from the GC-MS analysis: Oleic acid (22.51%) was the major component of the fixed oil. Oleic acid is a mono-saturated omega-9-fatty acid with many health benefits and is safe in present practices for use and concentrations in cosmetics (Liebert 1987). Oleic acid prevents ulcerative colitis (De Silver *et al.,* 2014), protects cell from free radical damage (Haug *et al.,* 2007), reduces blood pressure (Ruiz-Gutirrez *et al*., 1996) and increases fat burning (Lim *et al.,* 2013). Palmitic acid is a saturated long chain fatty acid with sixteen carbon backbone. It is one of the most abundant and wide spread natural saturated acids present in plants like palm oil, palm kernel oil, *M. oleifera* seed oil, in animals and animal-derived foodstuffs like cheese, milk, meat and microorganisms (Lim *et al.,* 2013). It is among the fatty acid that is used as concentration in cosmetics (Adegbe *et al.*, 2016).

Arachidic acid (icosanoic acid C_20H40O_2) is a saturated long-chain fatty acid with 20-carbon backbone found naturally as a minor component of peanut oil, also found in *M. oleifera* seed oil. It is used in the industry as component of adhesive, sealant and lubricants, as lubricant additive or in agricultural products. Stearic acid (octadecanoic acid), a saturated fatty acid having 18-carbon chain was formed in *M. oleifera* seed oil. Stearic acid is mainly used in the production of detergent, soaps and cosmetics such as shampoos and sharing cream products. Soap is made saponification of triglycerides consisting of stearic acid esters. Surfactants, cosmetics and personal hygiene products are in fact prospects of stearic acid (Gunstone, 2014).

Myristic acid was also found as one of the constituents of *M. oleifera* seed oil. It is a saturated fourteen (14) carbon fatty acid found naturally in palm oil, coconut oil and butter fat. Myristic acid is used as a flavoring agent in food (Morton, 1991 and Adegbe *et al.*, 2016) and also used as emulsifiers, emollient and lubricants in variety of cosmetics, creams, cake, soaps and pastes (Farooq and Umar, 2007). It is an important fatty acid which the body uses to stabilize many different proteins, including protein in the immune system. Erucic acid also known as (Z)-docos-13-enoic acid was also present in *M. oleifera* seed oil. It is a monounsaturated omega-9-fatty acid with twenty-two carbon atoms.

Docosanoic acid (Behenic acid) is a major component of Ben oil which is extracted from the seed of *M. oleifera* (Quattrocchi and Umberto, 2000) and found in the seeds of *M. oleifera* sample of this present study. It is used to give hair conditioners and moisturizers their smoothing properties (Bulus, 2000).

Conclusion

Moringa seed oil could be utilized successfully as a source of edible oil for human consumption. The physio-chemical parameters of the oil are comparable to those of other edible oil, therefore flour from *M. oleifera* seeds could be employed in the fortification of other food materials. The results from the study also showed that the properties of *M. oleifera* oil in Nigeria could be employed for edible and cosmetics application. The seed oil exhibited good physio-chemical properties and could be useful for industrial applications.

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