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Physico-chemical and Antioxidant Properties of *Moringa oleifera* Seed Oil

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Abstract: Oil was extracted from *Moringa oleifera* Lam (Moringaceae) seeds collected from Enugu, South-East Nigeria and evaluate its physico-chemical and antioxidant properties in comparism to palm oil. *M. oleifera* seeds gave oil yield of 41.47%. Refractive index, melting point (^{0}C) and acid value (mg KOH g^{-1}) of *M. oleifera* oil were 1.471±0.00, 28±0.00, 3.80±0.28 while palm oil had 1.473±0.00, 31±0.00, 6.20±0.35 respectively. Similarly, iodine (I_2 100 g^{-1}), saponification (mg KOH g^{-1}) and peroxide (mMol kg^{-1}) values obtained for *M. oleifera* oil were 85.30±0.25, 171.90±0.56 and 8.10±0.07 whereas palm oil had 34.70±0.13, 210.50±0.00 and 13.40±0.28 respectively. Total phenol (mg Gallic Acid Equivalent g^{-1}), total flavonoids (mg Rutin Equivalent g^{-1}) and total antioxidant capacity (mg Ascorbic Acid Equivalent g^{-1}) were 40.17±0.01, 18.24±0.01, 37.94±0.02 for *M. oleifera* oil and 62.32±0.04, 33.13±0.03, 68.27±0.02 for palm oil respectively. *M. oleifera* oil and palm oil showed a concentration dependent DPPH free radical scavenging and reducing power capabilities. This study has shown that *Moringa Oleifera* gave high oil yield, which has good antioxidant capacity with potential for industrial, nutritional and health applications, therefore large scale cultivation of this economic plant could be used as poverty alleviation strategy in Nigeria.

Key words: *Moringa oleifera*, seed oil, physico-chemical, antioxidant

INTRODUCTION

Moringa oleifera L (Moringaceae) known commonly as Ben oil tree or drumstick tree in English language, *'Okwe oyibo'* in Igbo, *'Gawara' or 'Habiwal'* in Hausa and*'Adagba maloye'* or '*Ewe Igbale*' in Yoruba grows rapidly in most regions and climatic conditions of Nigeria. *M. oleifera* is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics' (Anwar *et al*., 2007). A number of medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia (Fahey, 2005). It has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Morimitsu *et al*., 2000; Siddhuraju and Becker, 2003). Most of plants uses for medicinal purposes have been correlated to their possession of antioxidant activity (Middleton *et al.*, 2000, Sofidiya *et al.*, 2006). Antioxidants quench, scavenge and suppress the formation of reactive oxygen species and free radicals or oppose their actions (Adesegun *et al*., 2008).

Furthermore, *M. oleifera* has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/fats derived fuels (Biodiesel) all over the world (Anwar and Rashid, 2007). However, the plant has been identified as one of the under explored plants and there is dearth information on physico-chemical properties of the seed oil which has limited its applications.

Therefore, the need for cultivation of commercially viable crops for diversification of economy and poverty alleviation strategy of developing countries like Nigeria has made it imperative to take advantage of this economically important plant. To date, a detailed physico-chemical and antioxidant properties of the oil produced from seeds of *M. oleifera* plants native to the South-Eastern part of Nigeria has not yet been reported. The present research was therefore undertaken with the main objective of investigating the physico-chemical and antioxidant properties of the oil extracted from seeds of *Moringa* plants native to South-Eastern Nigeria and compared with that of palm oil from obtained from the same region.

MATERIALS AND METHODS

Chemicals: Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, potassium ferricyanide, trichloroacetic acid, potassium hydroxide, phenolphthalein, potassium iodide and starch were purchased from Sigma Chemical Company (Germany). Ascorbic acid, solvents and all other chemicals were of analytical grade from BDH Chemical Laboratory (England, UK).

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Plant seeds collection and identification: The seeds of *M. oleifera* were collected from the Enugu *M. oleifera* plantation, South-Eastern part of Nigeria by Dr. A.N. Ozumba, chairman of the Moringa Development Association of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The seeds were harvested between February and March, 2010. They were identified and authenticated at the Department of Botany, University of Nigeria, Nsukka, by a taxonomist Mr. E.C. Ekekwe. Voucher specimen No. 8257 were prepared and deposited at the herbarium of the International Centre for Ethno medicine and Drug Development (INTERCED), Nsukka. Palm oil was purchased from local market in Enugu, Nigeria. This oil was derived from the flesh of the fruit of the oil palm species *Elaeis guineensis*.

Oil extraction from *M. oleifera* seeds: *M. oleifera* whole seeds (123.49 g) were dehusked and 86.71 g of seeds crushed with mortar and pestle. Oil was extracted from the ground seeds with n-hexane (0.5 L) for 16 h by Soxhlet extraction method. The solvent of extraction (n-hexane) was evaporated over water bath for 12 h or until completely evaporated.

Physicochemical properties determination: The physicochemical properties of oil from *M. oleifera* seeds and palm oil were determined.

Refractive index determination: The refractive index of oil sample was determined using Abbe's Refractometer 300918. The sample chamber containing the lens was opened and cleaned with acetone then plugged to source of light. The equipment was calibrated with a drop of water, after which a drop of the sample was added into the sample chamber and closed. The adjustment knob was turned until the light and dark field crossed the cross bar then readings were taken.

Melting point determination: The melting point of the oil sample was determined using WRR melting point apparatus (Model J400118). One end of a capillary tube was sealed by flaming, then tube filled with pre-frozen sample and inserted into the melting point chamber and a thermometer inserted into the thermometer chamber of the apparatus. The equipment was switched on and the temperature at first and at complete melting of the samples was recorded.

Determination of saponification value: Ethanolic potassium hydroxide (0.5 N) was pipetted into conical flasks containing 1.0 g of sample. The content of each flask was reflux for 45 min or until clear with occasional shaking, then cooled to room temperature, after which it was titrated with sulfuric acid (0.5 N) using phenolphthalein as indicator. A blank was subjected to the same treatment. Results were expressed as mg $KOHg^{-1}$. Calculation of the saponification value of the oil sample was as follows:

$$\text{Saponification value} = \frac{(Vb - Vs) \times 28.05}{W}$$

Where, Vb = Titre for blank; Vs = Titre for sample; W = Weight of sample in gram.

Determination of peroxide value: Oil sample (0.5 g) was added into a boiling tube containing 1 g powered potassium iodide. Glacial acetic acid/chloroform mixture (20 mL; 2:1) was added, the boiling tube was placed in boiling water for 1 min after which its content was poured into conical flask containing potassium iodide solution (20 mL; 5 %). The boiling tube was rinsed twice with distilled water (25 mL) and content added into the conical flask. The whole content was titrated with sodium thiosulphate (0.002 M) solution to colourless end point using starch as indicator. Results are expressed as mMol/kg. Peroxide value of the oil sample was calculated as follows:

$$\text{Peroxide value} = \frac{(Vs - Vb) \times \text{molarity of titrant} \times 103 \text{ g kg}^{-1}}{W}$$

Where, Vb = Titre for blank; Vs = Titre for sample; W = Weight of sample in grams.

Determination of iodine value: The sample (2%) was prepared in chloroform, titrated with Wij's solution (5 mL) mixed thoroughly and allowed to stand in the dark for 5 min. Potassium iodide solution (5 mL; 7.5%) was added and titrated to a light straw colour using 0.1 N sodium thiosulphate solution. Starch indicator (3 drops) was thereafter added and titration continued to a colourless (white or milky) end point. Results are expressed as I_2 100 g^{-1}. Iodine value of the oil sample was calculated as follows:

$$\text{Iodine value} = \frac{(Vb - Vs) \times 1.269}{W}$$

Where, Vb = Titre for blank; Vs = Titre for sample; W = Weight of sample in grams.

Determination of acid value: The number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the sample was determined by placing 0.5 g of sample in conical flask containing mixture of ether and ethanol (50 mL; 95% v/v). The resulting solution was titrated with 0.1 N potassium hydroxide solution using phenolphthalein as indicator. The acid value was expressed as KOH g^{-1} and calculated as follows:

$$\text{Acid value} = \frac{(Vb - Vs) \times 5.61}{W}$$

Where, Vb = Titre for blank; Vs = Titre for sample; W = Weight of sample in grams.

Antioxidant assay: The antioxidant properties of *M. oleifera* oil were also determined comparatively with palm oil.

Estimation of total phenol content: The total phenol content of the oil sample was determined using Folin-Ciocalteu reagent according modified method of Khanahmadi *et al*. (2010). A 1 mL aliquot of oil sample (100 μg mL^{-1}) was put in testtube, 2.5 mL Folin-Ciocalteu reagent (0.2 M) and 2 mL sodium carbonate (7.5%) was added, allowed to stand in the dark for 20 min at room temperature, thereafter the absorbance was read at 765 nm. The amount of total phenolic component in the oil sample was determined from gallic acid calibration curve and expressed as mg of Gallic Acid Equivalent per gram sample (mg GAE g^{-1}).

Estimation of total flavonoid content: Total flavonoid of the oil sample was determined by aluminum trichloride colorimetric method using rutin as standard (Nile and Khobragade, 2010). The method was based on formation of a flavonoid-aluminum complex. The sample (0.1 mL) in methanol (100 μg mL^{-1}) was mixed with 0.2 mL of 5% sodium nitrate, then allowed to react for 5 min thereafter 0.2 mL aluminum trichloride in methanol (10%) and 1 mL of sodium hydroxide (1 M) were added, then allowed to stand at room temperature for 15 min. The absorbance was read at 510 nm against reagent blank. The amount of flavonoid was calculated from rutin calibration curve, results expressed as mg of Rutin Equivalent per gram of sample (mg RE g^{-1}).

Total antioxidant capacity: The total antioxidant capacity was evaluated by phosphomolybdenum method as described by Nabasree and Bratati (2007). The assay was based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Sample solution in ethanol (0.3 mL; 100 μg mL^{-1}) was mixed reagent solution (3 mL; 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). A blank composed of 3 mL of reagent solution and methanol was also prepared. All tubes were capped and incubated in boiling water bath at 95°C for 90 min. Absorbance of samples were read against blank at 695 nm. The antioxidant capacities of samples were expressed as mg Ascorbic Acid Equivalent per gram of sample (mg AAE g^{-1}).

Determination of free radical scavenging activity: The free radical scavenging activity of oil samples against by 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Adesegun *et al*. (2008). Various concentrations of the oil samples were prepared in methanol (25, 50, 75, 100 μg mL^{-1}), 1 mL of oil was added to 1.0 mL of methanolic solution of DPPH (1 mM). The mixture was shaken vigorously and allowed to stand at room temperature in dark for 20 min. The absorbance was read against reagent blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated as follows:

$$I\ (\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) x\ 100$$

Where, A_{blank} = Absorbance of blank; A_{sample} = Absorbance of sample.

Determination of reducing power: Reducing power ability of the samples was determined using the method of Adesegun *et al*. (2008) by mixing 2.5 mL of oil sample of various concentrations (25, 50, 75, 100 μg mL^{-1}), with 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added and centrifuged (1000 x g, 10 min). The supernatant (2.5 mL) was mixed with equal volume of distilled water and ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a reagent blank.

Statistical analysis: Analyses were carried out in triplicates of each set up. Results are expressed as Mean±SEM. Statistical significant difference ($p<0.05$) was determined using student's t-test. All data were analyzed using Statistical Package for the Social Science 15.0 for windows (SPSS 15.0).

RESULTS

Oil yield and physicochemical properties: The oil yield from *M. oleifera* seeds was 41.47%. The *M. oleifera* oil was light golden yellow in color, liquid at room temperature with palatable flavor while palm oil was bright orange-red, semi-solid at room temperature and also with palatable flavor. The physico-chemical properties of the oils revealed that there was significant difference ($p<0.05$) between *M. oleifera* oil and palm oil in terms of their melting point, saponification, peroxide, iodine and acid value. *M. oleifera* oil had higher iodine value while palm oil had higher melting point, saponification, peroxide and acid value (Table 1). However, there was no significant difference in their refractive indices.

Table 1: Oil yield and physico-chemical properties of *Moringa oleifera* seed oil from South-Eastern, Nigeria comparatively with palm oil

Parameter	*M. oleifera* oil	Palm oil
Oil yield	41.47%	
Refractive index	1.4713±0.00	1.4725±0.00
Melting point (°C)	28.0±0.00*	31.0±0.00*
Saponification value (mg KOH g^{-1})	171.9±0.56*	210.5±0.06*
Peroxide value (mMol kg^{-1})	8.1±0.07*	13.4±0.28*
Iodine value (I_2 100 g^{-1})	85.3±0.25*	34.7±0.13*
Acid value (KOH g^{-1})	3.8±0.28*	6.2±0.35

*Values are ±SEM; *Significant at $p<0.05$

Table 2: Total phenol, flavonoid and antioxidant capacity of *Moringa oleifera* seed oil and palm oil

Parameter	*M. oleifera* oil	Palm oil
Total phenol (mg GAE g^{-1})	40.17±0.01*	62.32±0.04*
Total flavonoid (mg RE g^{-1})	18.24±0.01*	33.13±0.03*
Total antioxidant capacity (mg AAE g^{-1})	37.94±0.02*	68.27±0.02

*Values are ± SEM; *Significant at p<0.05

Table 3: Free radical scavenging activity expressed as % inhibition (I %) of DPPH with different concentration of *Moringa oleifera* seed oil and palm oil

	Concentration of extract (µg/mL)			
Test sample	25	50	75	100
M. oleifera oil (I %)	48.18±0.01*	52.06±0.06*	60.21±0.02*	70.15±0.03*
Palm oil (I %)	59.02±0.01*	70.16±0.01*	82.06±0.02*	88.16±0.03*

Values are ± SEM; *Significant at p<0.05

Table 4: Reducing power potential of different concentrations of *Moringa oleifera* seed oil and Palm oil expressed as absorbance at 700 nm

	Concentration of extract (µg/mL)			
Test sample	25	50	75	100
M. oleifera oil	0.531±0.002*	0.626±0.004*	0.677±0.003*	0.798±0.003*
Palm oil	0.663±0.001*	0.810±0.007*	0.929±0.006*	0.955±0.001

*Values are ± SEM; *Significant at p<0.05

Total phenolic, flavonoid and antioxidant capacity: Total phenolic, flavonoid and antioxidant capacity of *M. oleifera* oil and palm oil (Table 2) showed that there was significant difference (p<0.05) in these characteristics of both *M. oleifera* oil and palm oil with palm oil having higher values.

Free radical scavenging activity and reducing power potential: Oil of both *M. oleifera* seed oil and palm oil had free radical scavenging activity; this was evident in a concentration-dependent manner (Table 3). There was decrease in absorption of DPPH as concentration of the oils increased. The reductive capacity of the both oil samples showed increased absorbance with increasing amounts of both oil respectively (Table 4). There was significant difference (p<0.05) in free radical scavenging activity and reducing power potential of both oil samples at the various concentrations tested.

DISCUSSION

Over the years, exploration of natural plants products has been on the increase leading to the identification and improvement of plant products beneficial to man-kind. *Moringa oleifera L (Moringaceae)* has been identified as a multifunctional versatile plant with enormous economic, nutritional and health potentials. In this study, oil was extracted from *Moringa oleifera* seeds and compared to palm oil in terms of their physico-chemical and antioxidant characteristics. The choice of palm oil for this comparative study was because it is the widely processed oil in the South-Eastern Nigeria, household cooking oil and has been shown to have high antioxidant capability.

The high oil yield of the seed of *Moringa oleifera* from Enugu, South-Eastern, Nigeria corroborates with findings from other regions of the world having yield range of 32-40% (Lalas and Tsaknis, 2002). Refractive index (1.474) of the *M. oleifera* oil from South-Eastern Nigeria was similar to those from other regions of the world; which include the breed from Pakistan, Periyakulan 1 (1.457) and Kenya, Mbololo (1.454) (Lalas and Tsaknis, 2002). Similarly, the saponification value of the present *M. oleifera* oil was comparable to those from Pakistan and Kenya but not in their peroxide and acid values (Lalas and Tsaknis, 2002). These differences indicated that *M. oleifera* oil from South-Eastern, Nigeria has its unique characteristics. This is in agreement with previous findings that *Moringa* seed oil content and its properties show a wide variation depending mainly on the species, environmental conditions and geographical location (Lalas and Tsaknis, 2002). This therefore substantiates the need to establish the physio-chemical properties of varieties of the plant to enhance its economical, health, nutritional and environmental uses. Furthermore, the lower peroxide value (8.1±0.07 mMol kg^{-1}) compared to that of palm oil (13.40 mMol/kg) indicated that the oil may be more stable to oxidative degradation (Manzoor *et al.*, 2007). The higher iodine value of the *Moringa* oil compared to the palm oil suggested that the former may have greater unsaturated fatty acid than the later. This support previous studies which suggested that *Moringa* oil is composed of highly unsaturated fatty acids containing 80.4% polyunsaturates mainly oleic acids (67.9%) (Anwar and Rashid, 2007). The lower acid value of *Moringa* oil compared to palm oil indicated a possible low free fatty

acid composition which suggest lesser susceptible to rancidity (Li *et al*., 2007). In addition, oil of low acidity has been considered acceptable for edible application. Previous studies have suggested that the high oleic acid content of the *Moringa* oil coupled with its highly unsaturated nature and low peroxide, may qualify the *Moringa* oil for use in industries, such as food, biofuel generation, agrochemicals, paint and varnishes manufacturer (Anwar and Rashid, 2007; Manzoor *et al*., 2007).

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and also involved in retardation of oxidative degradation of lipids (Pourmorad *et al*., 2006). The observed higher phenolic content of palm oil compared to *Moringa* oil is reflected by the intense bright orange-red colour of the former which is contributed by phenols known to be responsible for most of plants colouration (Gupta *et al*., 2010). In addition, Odukoya *et al*. (2005) has reported a strong relationship between phenolic content and antioxidant activity in selected fruits and vegetables. Thus, the presence phenolic compounds in *Moringa* seed oil is an added value to its nutritional and health potential. Furthermore, the occurrence of flavonoids in the *Moringa* oil which are also phenolic compounds, similarly improves the economic and health potential of the oil. This is in agreement with previous findings which suggested that flavonoids carry out antioxidant action through scavenging or chelating process and are reported to play a preventive role in cancer and heart disease (Middleton *et al*., 2000). Therefore, the importance of the antioxidant constituents of *Moringa* oil in the maintenance of health is strengthened as trend of the future is moving toward using foods as medicine in the management of various chronic diseases.

Moreover, the antioxidant potential of *Moringa* oil was further highlighted by the quenching of DPPH free radicals, which is a proton free radical commonly used to determine the free radical scavenging power of antioxidants (Adesegun *et al*., 2008). The decrease in absorbance of DPPH-oil mixture in this study which measured the extent of radical scavenging potential of the oils supported the findings of AsokKumar *et al*. (2009). In addition, reducing power of a compound is related to electron transfer ability of the compound which could lead to the neutralization of free radical (Zhu *et al*., 2001). The observed increase in reducing power of the oils which was concentration dependent suggested that they are good electron donors. Studies have shown that the reducing power capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Sofidiya *et al*., 2006; Adesegun *et al*., 2008). This suggestion was confirmed in this present study whereby palm oil with more phenolic content exhibited greater antioxidant constituent and activity than *Moringa* oil. Nevertheless, the *Moringa* oil exhibited remarkable physico-chemical and antioxidant properties which need to be explored for economic, nutritional and health applications.

Conclusion: In conclusion, this study has highlighted the unique characteristics of *M. oleifera* seed oil from South-Eastern Nigeria and the results indicated good quality oil comparable to oil from different breeds from other parts of the world. The detailed scientific knowledge regarding the physico-chemical properties of the seed oil is of considerable importance for the development and commercialization of this potential seed oil.

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