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Characterization of *Moringa oleifera* Seed Oil Variety Congo-Brazzaville

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Abstract: The oil from *Moringa oleifera* seeds variety Congo-Brazzaville was extracted using two oils extraction methods with petroleum ether (Soxlhet) and extraction with a mixture of chloroform:methanol (1:1) (Blye and Dyer). The oils were compared of *Moringa oleifera* other countries. The oil concentration ranged from 38.5% (Soxlhet) to 40% (Blye and Dyer). The minerals, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, unsaponifiable matter content, peroxide value, activation energy and differential scanning calorimetry were determined. *Moringa oleifera* seeds have ash content of 4.2% (with the presence of following minerals: Ca, K, Na and Mg). The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 74.93%). The dominant saturated acids were palmitic (up to 6.44%) and behenic (up to 5.33%). *Moringa oleifera* seeds were also founded to contain high levels of crude protein (37.6%). The oil extracts exhibited good physicochemical properties and could be useful as edible oils and for industrial applications.

Key words: Nutritive values, viscosity, *Moringa oleifera* seeds, behenic acid, essential fatty acid, activation energy

INTRODUCTION

Moringa oleifera belongs to the Moringaceae family and Moringa genus, the best known and most widely distributed species (Morton, 1991; Sengupta and Gupta, 1970). There are a few known varieties namely Jaffna, Chauakacheri Murunga, Chem, Kadu, Palmurungai, Periyakulam 1 (PKM 1) (Tsaknis *et al*., 1998) and Peregrina (Somali *et al*., 1984). The edible oil was extracted, where the tree is cultivated by boiling the seeds with water and collecting the oil from the surface of the water (Somali *et al*., 1984). The seed oil contains all the fatty acids contained in olive oil, except linoleic and was used as its acceptable substitute (Morton, 1991). *Moringa oleifera* Congo, Brazzaville is a selection of local types and is propagated only by seed. Until now a full characterization of the oil produced from the seeds of *Moringa oleifera* Congo-Brazzaville has not been reported. Additionally, the use of different methods of extraction and their effect on the composition and the characteristics of the oil has not been investigated. The oil was compared to virgin olive oil.

Also, the characteristics of *Moringa oleifera* seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids (Corbett, 2003). High oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying (Warner and Knowlton, 1997). The press cake obtained after oil extraction has positively charged protein molecules that have coagulant properties (Sutherland *et al*., 1994). These properties have been exploited in water clarification and wastewater treatments. Previous studies on *Moringa oleifera* have been focused on its medicinal uses and nutritional aspects of the tree parts (Lowell, 1999) and on the use of the seed in the clarification of waste-water during treatment (Folkard *et al*., 1993); however, little or no studies have been done on the oil properties, such as the triacylglycerol profiles and other physico-chemical properties apart from the fatty acid composition. In this study, some physical and chemical properties such as thermal behavior and triacyglycerol profile were determined following extraction using soxlhet and methanol-chloroform methods.

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MATERIALS AND METHODS

Mature *Moringa oleifera* pods were collected from neighborhood gardens around University Campus Marien Ngouabi of Brazzaville. The seeds were removed from the pods, sorted and sun dried. Only seeds that were not damaged were chosen and stored under cool dry storage conditions until needed.

Proximate analysis of *Moringa oleifera* seed Moisture, crude protein (micro-Kjeldahl), crude fiber and oil (Soxhlet) contents were determined using the methods described by Pearson (1976), whereas, the ash content was determined using the method of Pomeranz and Meloan (1994) and total carbohydrate was determined by difference. All determinations were done in triplicate.

Oil extraction: Dried *M. oleifera* seeds were ground in a Moulinex model SeB PREP'LINE 850 (Moulin cafe). For solvent extraction (Soxlhet method), 50 g of ground seeds were placed into a cellulose paper cone and extracted using light petroleum ether (bp 40-60°C) in a 5 L Soxhlet extractor for 8 h (Pena *et al*., 1992). The oil was then recovered by evaporating off the solvent using rotary evaporator model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60°C for 1 h and flushing with 99.9% nitrogen. For methanol/chloroform extraction, 100 g of the ground seeds were homogenized with a chloroform mixture:methanol (1:1) and water. Two phases was obtained, aqueous layer (methanol-water) and organic layer (chloroform). Oil was recovered by evaporating off the solvent (chloroform) using rotary evaporator model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60°C for 1 h and flushing with 99.9% nitrogen All experiments were done in triplicates and the mean and standard deviations were calculated.

Physical and chemical analysis of crude oil

Thermal behaviour: The thermal property of the oil samples was investigated by differential scanning calorimetry using a Perkin-Elmer Diamond DSC (Norwalk, USA). The instrument was calibrated using indium and zinc. The purge gas used was 99.99% nitrogen with a flow rate of 100 mL min^{-1} and a pressure of 20 psi. Sample weights ranged from 5-7 mg and were subjected to the following temperature program: frozen oil sample was heated at 50°C in an oven until completely melted. Oil sample was placed in an aluminum volatile pan and was cooled to -50°C and held for 2 min, it was then heated from -50 to 50°C at the rate of 5°C min^{-1} (normal rate) (Che Man and Swe, 1995) and 10°C min^{-1} (past rate) and held -50°C isothermally for 2 min and cooled from -50 to 50°C at the rate of 5°C min^{-1}. The heating and cooling thermogram for the normal and the fast (hyper DSC) scan rates were recorded and the onset, peak and offset temperatures were tabulated. These values provide information on the temperature at which the melting process starts, the temperature at which most of the TAG have melted and the complete melting temperature of the oil, respectively.

Viscosity measurements: A rheometer as described by Nzikou *et al*. (2007) was used to measure the different oil viscosities. By this procedure, a concentric cylinder system is submerged in the oil and the force necessary to overcome the resistance of the viscosity to the rotation is measured. The viscosity value (mPas) is automatically calculated on the basis of the speed and the geometry of the probe. Temperature (20°C) was controlled with a water bath connected to the rheometer. The experiment was carried out by putting 3 mL of sample in a concentric cylinder system using 100 sec as shear rate.

Chemical analysis: Determinations for peroxide, iodine and saponification values, unsaponifiable matter and free fatty acid contents were carried out using Pena *et al*. (1992) standard analytical methods. The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 μL of n-hexane 50 mg of oil followed by 50 μL of sodium methoxide using the method of Cocks and Van Rede (1966). The mixtures were vortex for 5 sec and allowed to settle for 5 min. The top layer (1 μL) was injected into a gas chromatograph (model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector and a polar capillary column (BPX 70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 μm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240°C and column temperature was 110°C held for 1 min and increased at the rate of 8°C min^{-1} to 220°C and held for 1 min. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample. The minerals were determined by atomic absorption spectrophotometry. One gram samples in triplicate, were dry ashed in a muffle furnace at 550°C for 8 h until, a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20.0 mL of 2.5% HCl, heated in a steam bath to reduce the volume to about 7.0 mL and this was transferred quantitatively to a 50 mL volumetric flask. It was diluted to volume (50 mL) with

deionised water, stored in clean polyethylene bottles and mineral contents determined using an atomic absorption spectrophotometer (Perkin-Elmer, model 2380, USA). These bottles and flasks were rinsed in dilute hydrochloric acid (0.10 M HCl) to arrest microbial action, which may affect the concentrations of the anions and cations in the samples. The instrument was calibrated with standard solutions.

Statistical analysis: Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Excel version 8.0 software. Significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Proximate analysis of *Moringa oleifera* seed oil: Results obtained showed that the seeds contained 5.3% moisture, 39.3% crude oil, 37.6% crude proteins, 13.6% carbohydrate (by difference), 3.2% crude fiber and 4.2% ash (Table 1). The high percentage of oil makes this seed a distinct potential for the oil industry. According to Benthall (1946), Burkill (1966), Irvine (1961), Makkar *et al.* (1997), Duke and Atchley (1984) and Abdulkarim *et al.* (2005), the mature seed yields 22-38% oil. Jamieson (1939) reported a 40% yield by weight of the seed. Variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used.

Minerals: It is of interest to note that the most prevalent mineral element in *M. oleifera* seeds is magnesium, which is a high as 251.30±0.02 mg/100 g dry mater (Table 2). Mg plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Russel, 1973). Calcium (83.75±0.01 mg/100 g dry matter) is also the major component of bone and assists in teeth development (Brody, 1994).

Oil extraction: Characteristics of the oil were compared with *M. oleifera* varieties others country, described by Tsaknis *et al.* (1998), Dahot and Memon (1985), Ferrao and Ferrao (1970) and Abdulkarim *et al.* (2005). The extracted oils were liquid at room temperature. The oil content of *M. oleifera* Congo-Brazzaville seeds and the level at which, the differences are significant are shown in Table 3. The oil extraction with the Soxlhet method had the highest yield, due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley and Colwell, 1991). The Blye and Dyer method, showed the low yield due to losses during the separation of the two phases, aqueous layer (methanol-water) and organic layer (chloroform). The results of the above researchers agree with those of the present research.

Table 1: Proximate analysis of *Moringa oleifera* oil seed

		Reported values[b]		
Characteristic	Obtained values[a]	1	2	3
Moisture content (%)	5.3±1.05	ND	4.1	7.9
Crude protein[c] (%)	37.6±1.07	36.7	38.4	38.3
Fats/oils (%)	39.3±1.06	41.7	34.7	30.8
Crude fibre (%)	3.2±0.80	4.8	3.5	4.5
Ash content (%)	4.2±0.30	3.8	3.2	6.5
Total carbohydrate[d] (%)	13.6	17.8	17.1	16.5

ND: Not Determined; [a]:Mean±SD; [b]: Abdulkarim *et al.* (2005), [c]Crude protein = N (%)×6.25; [d]Carbohydrate obtained by difference

Table 2: Mineral elemental composition of *Moringa oleifera* seeds

Mineral elements	Composition (mg/100 g) of seed
Calcium (Ca)	83.75±0.01
Magnesium (Mg)	251.30±0.02
Potassium (K)	36.53±0.02
Sodium (Na)	22.50±0.01

Values are mean±SD of triplicate determinations

Table 3: Physical and chemical properties of *Moringa oleifera* seed oil extracted using solvent process

	Obtained values		Reported values[a]
Properties	Blye and dyer	Soxlhet	Solvent extract
Oil[b] (%)	38.5 ± 1.350^{B}	40.0 ± 2.340^{A}	30.8
PV	0.89 ± 0.42^{A}	1.67 ± 0.84^{A}	ND
FFA (as % oleic acid)	1.08 ± 0.24^{A}	2.10 ± 0.10^{B}	2.48
IV (wijs)	67.4 ± 0.300^{A}	66.2 ± 1.120^{A}	65.4
Saponification value	166.0 ± 1.240^{A}	16.07 ± 0.81^{A}	164
Unsaponifiable matter content (%)	0.65 ± 0.02^{A}	0.87 ± 0.07^{B}	0.74
Viscosity (mPa.s) at 20°C	52.46 ± 0.18^{B}	49.96 ± 0.20^{B}	ND
Ea (KJ mol^{-1})	6.80	6.57	ND

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p<0.05$); [a]:Abdulkarim *et al.* (2005); [b]Oil = Weight of extracted oil×100/weight of seed; PV: Peroxide Value; FFA: Free Fatty Acid; IV: Iodine Value

Physical and chemical properties of oil

Physical properties

Differential Scanning Calorimetry (DSC): DSC is suitable to determine these physical properties. Results obtained from the heating with the DSC showed slight differences in both melting behaviour for the two oil samples when temperatures scanning (5°C min^{-1} and 10°C min^{-1}) were used. The heating profiles using the scan rate (5 and 10°C min^{-1}) for the 2 extractions methods showed that there is two major peaks (2) and (2'), 4 small shoulder peaks 1,1' and 3, 3', respectively (Fig. 1 and 2). The shoulder peaks 1 and 1' represented the melting temperature of unstable crystals of the low melting TAG that pre-maturely melted. The more stable low melting unsaturated TAG crystals melted at a higher temperature

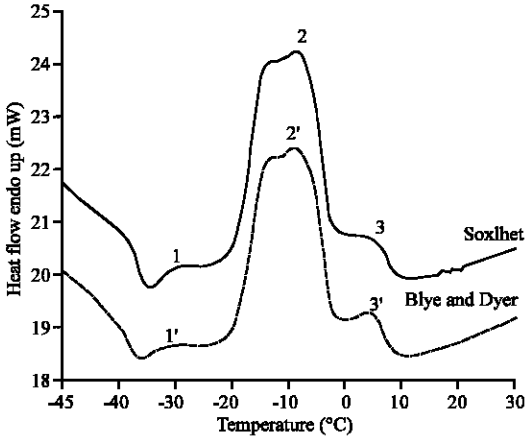

Fig. 1: Heating profiles of 2 *M. oleifera* oils extracted by two methods (Blye and Dyer; Soxlhet), at 5°C min^{-1} scan rate

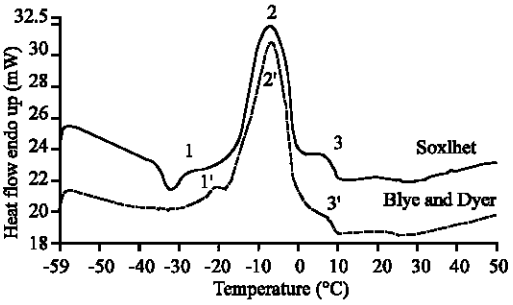

Fig. 2: Heating profiles of two *Moringa oleifera* oils extracted by two methods (Blye and Dyer;Soxlhet), at 10°C min^{-1} scan rate

shown as peaks 2 and 2'. The higher melting, more saturated TAG peaks (3 and 3') appeared at higher temperatures. According to cooling/heating rates, the DSC makes it possible to highlight the existence of various crystalline forms called polymorphism. According to cooling/heating rates, the DSC makes it possible to highlight the existence of various crystalline forms called polymorphism. However in the case of the study, of mixed triglycerides saturate-unsaturated, at the speed of 5°C min^{-1}, this polymorphism can be particularly rich since at the same temperature corresponding to the two major peaks 2 and 2' (Fig. 1), it seems to have the existence of another peak on the peaks 2 and 2' (Fig. 1), which is probably due to the coexistence of two crystalline varieties: the forms α and β, which is thermodynamically unstable. This crystalline form β' disappears, when the speed increased at 10°C min^{-1} (Fig. 3). The form β', existing like a state of transition.

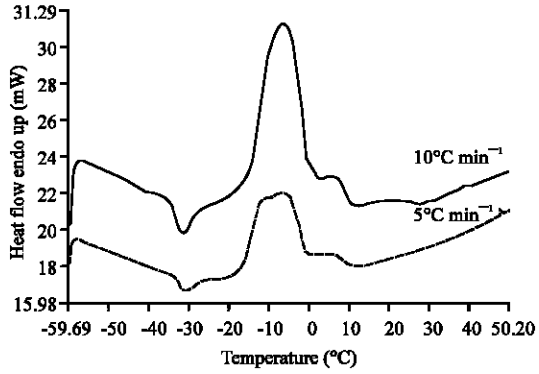

Fig. 3: Heating profiles of *M. oleifera* oil extracted by soxlhet method, at 5 and 10°C min^{-1} scan rate

Viscosity: Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring. Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. In optics to know, the rheological proprieties of these oils, we studied the influence of temperature on viscosity. Activation energies of the various classes of fatty acids contained in these oils were shown Table 3. When, the temperature increases, viscosity decreases exponentially (Table 4) some is the extraction method (Arslan *et al*., 2005; Nzikou *et al*., 2007). Viscosity varies between 66.82 and 46.08 mPa.s when temperature decreases of 45-5°C by Blye and Dyer method. By Soxlhet method, the viscosity of oil decreases of 62.51-43.78 mPa.s (Table 4). The viscosity of the oil obtained by Blye and Dyer method was highest, possibly because of the water that was absorbed by the gums (phospholipid) during extraction. This calculator calculates the effect of temperature on reaction rates using the Arrhenius equation.

$$\eta = A \times \exp^{(-E_a/R \times T)}$$

Where:

η = The viscosity
A = Constant
E_a = The activation energy (KJ moL^{-1})
R = The universal gas constant
T = The temperature (°C)

R has the value of 8.314×10^{-3} KJ moL K^{-1}. We should use this calculator to investigate the influence of temperature on viscosity. Linear regression analysis was applied to the logarithmic form of Arrhenius equation in order to determine the parameters of the relation (Table 5). ln η against $1/T, -E_a/RT$ is the slope from, which E_a was evaluated. Activation energies of oils are shown in

Table 4: Oil viscosity at various temperature in degree celsuis

	η (mPa.s)	
Temp. (°C)	Blye and Dyer	Soxlhet
05	66.82	62.51
10	61.08	57.25
15	56.00	52.84
20	52.46	49.96
25	49.66	47.24
30	48.15	45.27
35	47.00	44.52
40	46.56	44.14
45	46.08	43.78

Table 5: Energie plot derived from the Arrhenius equation

	Lnη (mPa.s)	
1/T (K^{-1})	Blye and Dyer	Soxlhet
0.00359518	4.20200244	4.13532654
0.0035317	4.11218448	4.04742764
0.00347041	4.02535169	3.96726848
0.00341122	3.96005097	3.91122269
0.00335402	3.90519978	3.85524099
0.0032987	3.87432114	3.81264456
0.00324517	3.85014760	3.79593853
0.00319336	3.84074180	3.78736640
0.00314317	3.83037902	3.77917709

Table 3. The highest value of activation energy is obtained by Blye and Dyer method (6.80 KJ moL^{-1}) and 6.57 KJ moL^{-1} by Soxlhet method.

Chemical properties: The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The free fatty acid and the unsaponifiable matter content of the Soxlhet method were significantly higher ($p<0.05$) than those of the Blye and Dyer method (Table 3). There was no significant difference in the iodine and saponification values in the two extraction methods ($p>0.05$). The slightly higher value of unsaponifiable matter in the Soxlhet method may be due to the ability of the Solvent to extract other lipid associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic *et al*., 1978; Salunke *et al*., 1992).

Fatty acid composition: The major saturated fatty acids in *Moringa oleifera* seed oil were palmitic, stearic, arachidic and behenic acids and the main unsaturated fatty acid is oleic acid (74.68%) with small amounts of palmitoleic, linoleic, linolenic and eicosenoic acids (Table 6 and 7). There was no significant difference ($p>0.05$) in the amounts of the major fatty acids in the two oil samples. The two oil samples of *Moringa oleifera* contained a substantial amount of behenic acid (5.22 and 5.33%), respectively. The oil can, therefore, be used as a natural source of b ehenic acid, which has been used as an oil

Table 6: Relative percent composition of fatty acid in *Moringa oleifera* seed oil

	Determined values		Reported values [a]			
Fatty acid	Blye and Dyer	Soxlhet	1	2	3	4
C14:0	-	-	-	1.4	-	0.1
C16:0	6.44 ± 1.23^{A}	6.24 ± 1.32^{A}	6.9	3.5	6.7	7.8
C16:1	1.67 ± 0.22^{A}	1.6 ± 0.25^{A}	1.1	-	-	2.2
C18:0	4.73 ± 0.18^{A}	4.71 ± 0.20^{B}	8.3	8.3	4.3	7.6
C18:1	74.43 ± 0.35^{B}	74.93 ± 0.31^{A}	67.7	67.3	76.5	67.9
C18:2	1.02 ± 0.10^{A}	0.72 ± 0.12^{A}	0.4	3.5	0.7	1.1
C18:3	-	-	-	-	-	0.2
C20:0	3.04 ± 0.18^{A}	3.09 ± 0.15^{A}	4.7	2.7	2.7	4.0
C20:1	2.43 ± 0.34^{B}	2.32 ± 0.33^{A}	2.3	-	-	1.5
C22:0	5.22 ± 0.12^{A}	5.33 ± 0.1^{A}	7.4	5.6	4.6	6.2
C24:0	1.03 ± 0.42^{A}	1.05 ± 0.46^{A}	0.4	3.2	1.1	1.3

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p<0.05$), [a]Sunga and Whitby (1995), Dahot and Menon (1985), Ferrao and Ferrao (1970) and Abdulkarim *et al*. (2005)

Table 7: Melting behaviour of *Moringa oleifera* seed oil using different scan rates. Experimental conditions: temperature program set at -50°C for 10 min, rising to 50°C at rate of 5 and of 10°C min^{-1}

	5°C min^{-1}		10°C min^{-1}	
Thermogram	Blye and Dyer	Soxlhet	Blye and Dyer	Soxlhet
Peak 1 (°C)	-32.52	-31.10	-33.52	-31.54
ΔH (J g^{-1})	-4.47	-5.36	-2.16	-5.91
Peak 2 (°C)	-7.12	-7.03	-6.23	-6.71
ΔH (J g^{-1})	44.06	49.56	63.10	52.51
Peak 3 (°C)	6.21	6.30	11.13	10.64
ΔH (J g^{-1})	+0.89	+0.55	-2.43	-2.02

structuring and solidifying agent in margarine, shortening and foods containing semi-solid and solid fats, eliminating the need to hydrogenate the oil (Abdulkarim *et al*., 2005). The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil. Many circumstances have focused attention on high-oleic vegetable oils. It has been demonstrated that a higher dietary intake of bad fats (saturated and trans fatty acids) is associated with an increased risk of coronary heart disease caused by high cholesterol levels in the blood (Mensink and Katan, 1990; Siguel and Lerman, 1993) whereas, a higher intake of good fats (monounsaturated/oleic) is associated with decreased risk (Corbett, 2003). High oleic-acid vegetable oils such as high-oleic corn, sunflower and canola have been found to have enough oxidative stability to be used in demanding applications such as frying (Petukhov *et al*., 1999; Warner and Knawlton, 1997). In addition, high-oleic oils have low saturated fatty acid levels. Therefore, high-oleic oils can be viewed as a healthy alternative to partially hydrogenated vegetable oils (Abdulkarim *et al*., 2005).

CONCLUSION

Moringa oleifera seed oil has the potential to become a new source of high-oleic acid oil and its full

potential should be exploited. It contains high monounsaturated to saturated fatty acids ratio and might be an acceptable substitute for highly monounsaturated oils such as olive oil in diets. *Moringa oleifera* is a tree growing rapidly even in poor soil and is little affected by drought (Sengupta and Gupta, 1970; Morton, 1991) and can be easily grown in poor third world countries. The production of useful oil from its seeds could be of economic benefit to the native population of the areas, where the tree is cultivated.

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