

## Research Article

### Characteristic Physicochemical of Oil Extract from *Moringa oleifera* and the Kinetics of Degradation of the Oil during Heating

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**Abstract:** The aim of the study was to investigate the physicochemical properties and the kinetics of degradation of *Moringa oleifera* seed oil during heating. The seed is a good source of oil (40%). The physical properties of the oil extracts showed the state to be liquid at room temperature and indicated that the oil had refractive index, 1.4680; the peroxide value, 1.67 (meq O<sub>2</sub>/kg oil); free fatty acid, 2.10%; iodine value, 66.2%; saponification value, 167; unsaponifiable matter content, 0.87% and viscosity, 47.24 (mPa.s at 25°C). Gas liquid chromatography technique has been developed for identification and quantitative determination of total unsaturated and saturated fatty acids shows that the crude oil had 79.57 and 20.42% respectively. Differential Scanning Calorimetry (DSC) indicates the presence of three components in oil extracted. The first peak at low melting point appears at -31.10°C ( $\Delta H_f = -5.36$  J/g), the second peak appears to -7.03°C ( $\Delta H_f = +49.56$  J/g) and the last peak appears to +6.30°C ( $\Delta H_f = +0.55$  J/g). The degradation kinetic of the oil was also investigated. The thermal oxidation of the double bonds of the oil showed a first-order thermal oxidation kinetic and the Arrhenius plot yielded a straight line with a slope equivalent to activation energy of 1.593 KJ/mol. There is the possibility of considering the seed as feed supplement and its oil for industrial application.

**Keywords:** Activation energy, DSC, half-life, kinetics, *Moringa oleifera* seed, viscosity

## INTRODUCTION

Seed oils are important sources of nutritional oils, industrial and pharmaceutical importance.

The characteristics of oils from different sources depend mainly on their compositions and no oil from a single source can be suitable for all purposes (Ramadan and Mörsel, 2003). The study of these constituents is important for their effective uses.

Seed oils are known to deteriorate when processed inadequately with the principal decomposition reaction being oxidation. Oxidation of seed oil occurs through a free radical mechanism, initially characterized by the emergence of a sweetish and unpleasant odour which becomes progressively worse until it attains a characteristic smell of rancid fat (Gouveia de Souza *et al.*, 2004). Heating is one of the most commonly used methods of food preparation in the home and industries and prolong use of oil for this purpose causes change in

its physical and chemical properties (Morette and Fett, 1998).

Under the influence of temperature, fat and oils are susceptible to oxidation primarily leading to the formation of hydroperoxides. Due to their high reactivity, these hydroperoxides especially at high temperatures rapidly react with secondary oxidative products e.g., aldehydes, ketones, peroxides, hydrocarbons as well as cyclic compounds that exhibits very different possible toxic or carcinogenic properties (Kowalki, 1995). The products formed during this oxidative process can be determined by chemical analysis and one of the frequently used tests employed to predict the quality of seed oils is the determination of peroxide value and iodine value. A number of seed oils have been characterized but the vast majority have not been adequately evaluated. *Moringa oleifera* belongs to the Moringaceae family and *Moringa* genus, the best-known and most widely distributed species (Morton,

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1991; Sengupta and Gupta, 1970). There are a few known varieties namely Jaffna, Chaukacheri Murunga, Chem, Kadu, Palmurungai and Periyakulam 1 (PKM 1) (Tsaknis *et al.*, 1998). 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). Some physico-chemical properties of *Moringa oleifera* seed oil have been studied by Abdulkarim *et al.* (2005). Characterization of *Moringa oleifera* Seed Oil Variety "Periyakulam 1" was studied by Lalas and Tsaknis (2002). However the kinetics of degradation of the oil during heating has not yet been studied. Therefore, the objectives of this study were to investigate the kinetics of degradation of the *Moringa oleifera* seed oil during heating.

## MATERIALS AND METHODS

This study was led to the laboratory of Engineering and Biomolecule of the ENSAIA-INPL, Vandoeuvre-lès-Nancy (France) for the period of Oct. 10, 2010 to Jan. 10, 2011.

**Materials:** *Moringa oleifera* seeds, grown in Diata district (Brazzaville-Congo), were bought from Total market of Bacongo. The seeds were dried in an oven at 60°C. The dried seeds were stored at -10°C until required for analysis.

### Methods:

**Oil extraction:** For solvent extraction (soxhlet method), 50 g of *Moringa oleifera* seed flour were placed into a cellulose paper cone and extracted using light petroleum ether (b.p 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. All experiments were done in triplicates and the mean and standard deviations were calculated.

### Physico-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 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 aluminium 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 (normal rate) (Che Man and Swe, 1995) and held -50°C isothermally for 2 min and cooled from -50 to 50°C at the rate of 5°C/min. The heating and cooling thermo grams 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.* (2009) 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, in mPa.s, 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 s<sup>-1</sup> as shear rate.

**Refractive indices:** The refractive indices of the oil (at room temperature) were determined with Abbe refractometer (Pearson, 1976) and the specific gravity measurement (also carried out at room temperature) using specific gravity bottle. The state and color of the oil were noted using visual inspection at room temperature. The mean molecular mass was estimated from the relation (56/SV) × 1000 (Akintayo and Bayer, 2002).

**Chemical analysis:** Determinations for peroxide, iodine and saponification values 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) and Mensink and Katan (1990). 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-ionisation detector and a polar capillary column (BPX70 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 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 drying 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.

**Proximate analysis:** Proximate analysis was carried out as described by the Association of Official Analytical Chemists (AOAC, 1995).

**Heat treatment:** Thermal degradation of *Moringa oleifera* oil was carried out by heating the oil up to 200°C for a period of 0-240 min. The peroxide value, refractive index and iodine values were determined respectively at 100, 150 and 200°C, respectively using the Association of Official Analytical Chemists method.

**Kinetic calculations:** A general reaction rate expression for the deterioration kinetic can be written as follows:

$$-d [C] / dt = k [C]^m$$

where, 'C' is the quantitative value of the concentration of the molecule under consideration, 'k' is the reaction rate constant and 'm' is the order of the reaction. For first order reaction where  $m = 1$  the equation can be written as:

$$\ln ([C_t] / [C_0]) = -kt$$

where,  $[C_0]$  is the concentration of the reactants under consideration at time zero and  $[C_t]$  is the concentration of the reactants at time 't'. Arrhenius relationship of the reaction rate to temperature is generally given as:

$$K = A_0 \exp (-E_a/RT)$$

where, 'E<sub>a</sub>' is the activation energy of the reaction, 'R' the gas constant, 'T' is absolute temperature and 'A<sub>0</sub>' is a pre-exponential constant. Each experiment was performed in triplicates and the average values were taken for the parameters determined. Kinetic data were analysed by regression analysis using MS Excel 8.

**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:** The oil content is high, it was found to be  $40 \pm 2.34\%$  (Table 1) which shows that the processing of the oil for industrial or edible would be economical. The obtained fat content agree with the value (40%) reported by Jamieson (1939). slight 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.

State of *Moringa oleifera* seed oil at room temperature is liquid.

### Physico-chemical properties of oil:

#### Physical properties:

**Differential Scanning Calorimetry (DSC):** DSC is suitable to determine these physical properties. The results of thermal analysis of oils are presented in Table 2. The obtained peaks were asymmetries and may indicate the presence of three components in oil extracted from Soxhlet method. The first peak at low melting point appears at  $-31.10^\circ\text{C}$  ( $\Delta H_f = -5.36 \text{ J/g}$ ). This peak corresponds to triglycerides formed by Polyunsaturated Acids (PUFA). The second melting point is at  $-7.03^\circ\text{C}$  ( $\Delta H_f = +49.56 \text{ J/g}$ ), this is a

Table 1: Physical and chemical properties of *Moringa oleifera* seed oil extracted using solvent process

Properties	Obtained values Soxhlet
Colour	Red/yellow
Oil <sup>a</sup> (%)	$40.00 \pm 2.34^A$
PV	$1.67 \pm 0.84^A$
FFA (as % oleic acid)	$2.10 \pm 0.10^B$
IV (wijs)	$66.20 \pm 1.12^A$
Saponification value	$167 \pm 0.81^A$
Unsaponifiable matter content (%)	$0.87 \pm 0.07^B$
Refractive index (at 25°C)	1.4680
Viscosity (mPa.s) at 25°C	$47.24 \pm 0.20^B$
Mean molecular mass	335.32
E <sub>a</sub> (KJ/mol)	1.593

Means for the determined values in the same row followed by the same superscript letter are not significantly different ( $p < 0.05$ ); <sup>a</sup>: Oil = weight of extracted oil  $\times 100$ /weight of seed; PV: Peroxide value; FFA: Free fatty acid; IV: Iodine value

Table 2: Melting behaviour of *Moringa oleifera* seed oil using different scan rates

Thermogram	5°C/min soxhlet
Peak 1 (°C)	-31.10
$\Delta H_f$ (J/g)	-5.36
Peak 2 (°C)	-7.03
$\Delta H_f$ (J/g)	+49.56
Peak 3 (°C)	6.30
$\Delta H_f$ (J/g)	+0.55

Experimental conditions: Temperature program set at  $-50^\circ\text{C}$  for 2 min, rising to  $50^\circ\text{C}$  at rate of  $5^\circ\text{C}/\text{min}$

characteristic of Mono Unsaturated Fatty Acids (MUFA). The last peak appears to  $+6.30^{\circ}\text{C}$  ( $\Delta H_f = +0.55 \text{ J/g}$ ) suggests the presence of Saturated Fatty Acids (SFA).

**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. The viscosity at  $25^{\circ}\text{C}$  of this oil is given Table 1. This result ( $47.24 \text{ mPa}\cdot\text{s}$ ) is in agreement with that found by Nzikou *et al.* (2009).

**Chemical properties:** Iodine value is a measure of the degree of unsaturation in oil and it is an identity characteristic of native oil. It indicates the degree of unsaturation in the fatty acids of triacylglycerol. This value could be used to quantify the amount of double bonds present in the oil, which reflects the susceptibility of oil to oxidation. The iodine value obtained is high which suggest the presence of unsaturated fatty acid and this places the oil in the drying groups. This oil may find application as a raw material in industries for the manufacture of vegetable oil-based ice cream (Ibiyemi *et al.*, 1992). The free fatty acid value is on the low side ( $2.10 \pm 0.9$  as % oleic acid). This value shows that this oil is stable. The saponification value is high and this suggests the use of the oil in production of liquid soap, shampoos and lather shaving creams. The peroxide value is  $1.67 \pm 0.81 \text{ mg/g}$  oil, this value is lower than that expected of rancid oil, which ranges from  $20.00$  to  $40.00 \text{ mg/g}$  oil (Oderinde and Ajayi, 1998). This shows that the oil is not rancid and considered stable (Ajayi *et al.*, 2002). The refractive index, which is the ratio of the velocity of light in a medium, was found to be  $1.4652$ . This refractive index is an indication of the level of saturation of the oil.

**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 ( $74.93\%$ ), with small amounts of palmitoleic, linoleic, linolenic and eicosenoic acids (Table 3). The oil sample of *Moringa oleifera* contained a substantial amount of behenic acid ( $5.33\%$ ). The oil can, therefore, be used as a natural source of behenic acid, which has been used as an oil structuring and solidifying agent in margarine, shortening and foods containing semi-solid and solid fats, eliminating the need to hydrogenate the oil (FDA, 2001). 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

Table 3: Relative percent composition of fatty acid in *Moringa oleifera* seed oil

Fatty acid	Determined values Soxhlet	Reported values <sup>a</sup>		
		1	2	3
C14: 0	-	-	1.4	-
C16: 0	$6.24 \pm 1.32^A$	6.9	3.5	6.7
C16: 1	$1.60 \pm 0.25^A$	1.1	-	-
C18: 0	$4.71 \pm 0.20^B$	8.3	8.3	4.3
C18: 1	$74.93 \pm 0.31^A$	67.7	67.3	76.5
C18: 2	$0.72 \pm 0.12^A$	0.4	3.5	0.7
C18: 3	-	-	-	-
C20: 0	$3.09 \pm 0.15^A$	4.7	2.7	2.7
C20: 1	$2.32 \pm 0.33^A$	2.3	-	-
C22: 0	$5.33 \pm 0.10^A$	7.4	5.6	4.6
C24: 0	$1.05 \pm 0.46^A$	0.4	3.2	1.1

Means for the determined values in the same row followed by the same superscript letter are not significantly different ( $p < 0.05$ ); <sup>a</sup>: (1) Sunga and Whitby (1995), (2) Dahot and Memon (1985), (3) Ferrao and Ferrao (1970)

Table 4: Effect of heating on Peroxide Value (PV) and Iodine Value (IV) of *Moringa oleifera* seed oil

Temp. (°C)	Time (min)	PV (mg/g)	IV (mg/iodine)	RI (25°C)
100	30	$2.01 \pm 0.12$	$66.02 \pm 0.27$	1.4309
	60	$2.42 \pm 0.18$	$65.74 \pm 0.30$	1.3710
	120	$2.82 \pm 0.20$	$65.45 \pm 0.50$	1.3108
	180	$3.25 \pm 0.25$	$65.22 \pm 0.12$	1.2509
	240	$3.71 \pm 0.42$	$65.03 \pm 0.18$	1.1711
150	30	$2.30 \pm 0.22$	$65.90 \pm 0.44$	1.3587
	60	$2.62 \pm 0.31$	$65.50 \pm 0.12$	1.3179
	120	$3.02 \pm 0.18$	$65.10 \pm 0.28$	1.2568
	180	$3.43 \pm 0.24$	$64.12 \pm 0.21$	1.1847
	240	$3.78 \pm 0.13$	$63.64 \pm 0.15$	1.1651
200	30	$2.90 \pm 0.12$	$65.43 \pm 0.24$	1.2412
	60	$3.17 \pm 0.20$	$64.92 \pm 0.15$	1.2189
	120	$3.31 \pm 0.32$	$64.33 \pm 0.22$	1.1941
	180	$3.53 \pm 0.14$	$63.98 \pm 0.38$	1.1849
	240	$3.82 \pm 0.28$	$63.49 \pm 0.28$	1.1808

Values are mean  $\pm$  standard deviation of triplicate determinations; RI: Refractive index

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 Knowlton, 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).

#### Kinetic data for the degradation of *Moringa oleifera*

**oil:** The rate of production of peroxide in *Moringa oleifera* oil increases as the temperature increases as shown in Table 4. This shows that the prolong heating of this oil makes it to undergo thermal degradation resulting in oxidative rancidity, formation of hydroperoxides and other products of degradation that can liberate volatile compounds.

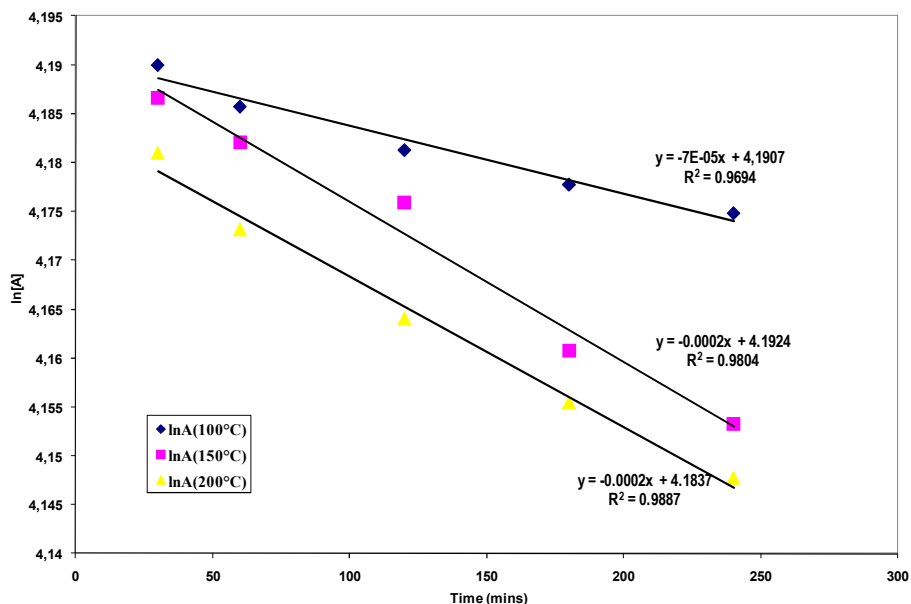


Fig. 1: Graph of rate of change in concentration of iodine value against time  
 $\ln [A]$ : Napierian logarithm of  $[A]$ ;  $[A]$ : Iodine value

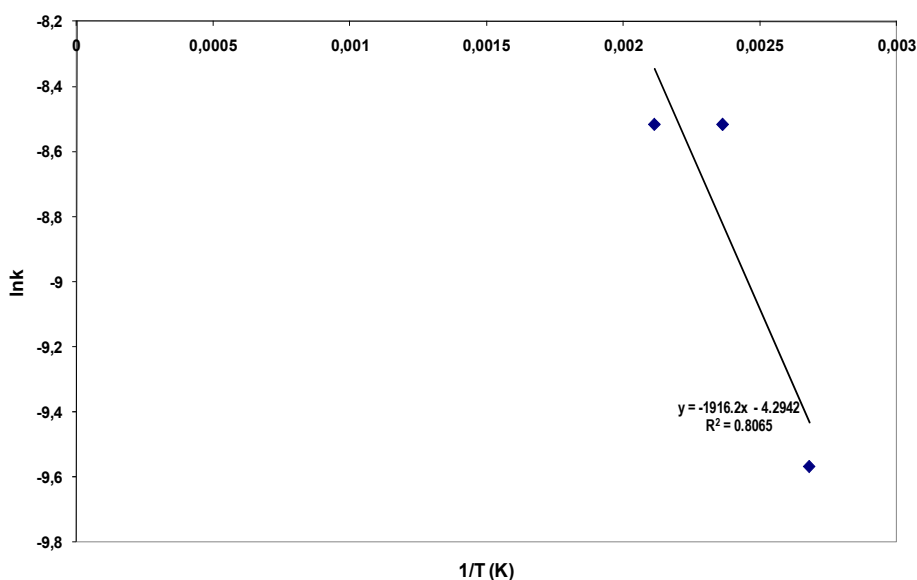


Fig. 2: Arrhenius plot for oxidation rate of the double bonds of fatty acids in *Moringa oleifera* seed oil  
 $\ln k$ : Napierian logarithm of  $k$ ;  $k$ : Reaction rate constant;  $1/T$  (K): The reverse of the temperature

Table 5: Kinetic parameters for degradation of *Moringa oleifera* seed oil

Kinetics parameters	100°C	150°C	200°C
$k_1$ ( $s^{-1}$ )	0.00007	0.0002	0.0002
$t_{1/2}$ (s)	9900	3465	3465

$k_1$ : Reaction rate constant;  $t_{1/2}$ : Half-life or time of half-reaction

Table 4 also shows that the iodine value of the oil decreases as it was heated over a period of time. This suggests the loss of unsaturation in the fatty acids of the triacylglycerols. This loss of unsaturation was also reflected by the decrease in the value of refractive

index, since double bonds (unsaturation) increase the refractive index of organic compounds by reducing the angle of refraction in relation to the angle of incidence and the higher the unsaturation the greater the effect of reducing the refraction angle (Oderinde *et al.*, 2009).

In order to obtain the reaction rate constant (' $k$ '), a first order degradation of the oil was presumed (Labuza and Riboh, 1982). Accordingly ' $\ln [C]_t / [C]_0$ ' was plotted against ' $t$ ' (time) from which rate constant ' $k$ ' was calculated from the slope of the line (Anthon and Barrett, 2002) as shown in Fig. 1. A correlation

coefficient >0.9 in all the cases confirmed the assumption of the degradation (loss of unsaturation) to follow the first order kinetic. The half-life or time of half-reaction for the degradation was calculated from the rate constant as '0.693/k' and is given Table 5.

Figure 2 shows the Arrhenus plot of ln k versus 1/T for the reduction of unsaturation (Iodine value) in *Moringa oleifera* oil. The linear nature of the plot obtained gave the activation energy of the reaction to be 1.593 KJ/mol (Table 1).

## 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. The thermal oxidation of *Moringa oleifera* seed oil follows a first order reaction. This oxidation is temperature and time dependent. The process quality assurance of this oil can be monitored using iodine value, refractive index and peroxide value.

## ABBREVIATIONS

PV : Peroxide Value  
FFA : Free Fatty Acid  
IV : Iodine Value  
DSC : Differential Scanning Calorimetry  
RI : Refractive Index

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