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Proximate Composition And Physico-chemical Characteristics Of Seed And Seed Oil From *Terminalia Catappa L* And The Kinetics Of Degradation Of The Oil During Heating

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Abstract: The seeds and seed oil of *Terminalia catappa* were analyzed to establish their chemical compositions and nutritional properties in order to investigate the possibility of using them for human and/or animal consumption. The seed is a good source of oil and protein; these were found to be 51.80% and 23.78% respectively. The seeds were found to be good sources of minerals. Potassium (9280 ± 0.14 mg/100g) was the highest, followed in descending order by Calcium (827.20 ± 2.18 mg/100g), Magnesium (798.6 ± 0.32 mg/100g) and Sodium (27.89 ± 0.42 mg/100g).The physical properties of the oil extracts showed the state to be liquid at room temperature. The saponification value suggest the use of this oil in liquid soap, shampoo and oil based ice cream production. The moisture content is also low (4.13%) which indicates the possibility of long shelve-life. 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 7.752 KJ.mol^{-1}.There is the possibility of considering the seed as feed supplement and its oil for industrial application.

Key words: *Terminalia catappa*, oil yield, proximate composition, Kinetics, DSC, Degradation, activation energy.

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 (Mohammed and Jorg, 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 characterised by the emergence of a sweetish and unpleasant odour which becomes progressively worse until it attains a characteristic smell of rancid fat (Gouveia *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

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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 characterised but the vast majority have not been adequately evaluated. *Terminalia catappa* L. believed to have originated in Malaysia, this tree is generally confined to mesic and wet coastal habitats and is distributed throughout the Old World tropics and tropical America (Morton, 1985). Reaching heights of 15 to 25 m, *T. catappa* shows strong salt-, drought- and wind-tolerance and produces fruit (5-10 cm long) with a thin flesh surrounding a large fibrous nut. While the fleshy fruit is the target of larval infestation, *T. catappa* leaf extracts have also been shown to preferentially attract female oriental fruit flies (Chen and Dong, 2000). Clarke *et al.* (2001) found that *T. catappa* along with *Psidium guajava* L. constituted the major hosts for *B. dorsalis* in a survey of Thailand and Malaysia. In addition, *T. catappa* reared a particularly high number of larvae in proportion to the weight and number of fruit sampled, leading to the suggestion that it is a "primary native host" in the surveyed areas (Clarke *et al.*, 2001). No study has been conducted in these areas. Even, there are limited informations on the physicochemical and proximate composition of the seed and seed oil of this plant. In view of this, the aim of this work is to characterize, correlate parameters such as peroxide value and iodine value in quality assurance and analyze the stability of this oil during thermal treatment.

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 Jan. 15, 2010 to Feb. 29, 2010.

Materials:

The fruits containing seeds of *Terminalia catappa L.* were obtained with the feet trees of *Terminalia catappa L.* which are in the centre town of Brazzaville (Congo). Then, they were further dried in our Laboratory at about 40°C and then crushed in Moulinex coffee blender (type Prep' line 850). Powdered *Terminalia catappa L.* seeds were kept at 5°C in polyethylene bag before analysis.

Methods:

Proximate analysis of *Terminalia catappa L.* 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 *et al*. (1994), and total carbohydrate was determined by difference. The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57 respectively) used in vegetable analysis (Asibey-Berko and Tayie, 1999). All determinations were done in triplicate.

Oil Extraction:

Dried *Terminalia catappa L* seeds were ground in a Moulinex Model SeB PREP'LINE 850 (Moulinex coffee). For solvent extraction (soxlhet method), 50g of ground seeds 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 of 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.

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 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^{-1} (normal rate) (Che Man *et al*., 1995), and held - 50 °C isothermally for 2 min and cooled from -50 to 50 °C at the rate of 5 °C per minute. The heating and cooling thermograms 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, 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 2 ml of sample in a concentric cylinder system using 100 s^{-1} 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 *et al*., (1966). The mixtures were vortex for 5 s 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 one minute and increased at the rate of 8 °C/min to 220 °C and held for one minute. 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 atomicabsorption 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.

Proximate Analysis:

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

Heat Treatement:

Thermal degradation of *Terminalia Catappa L* oil was carried out by heating the oil up to 200°C for a period of 0 – 240 min. The peroxide and the iodine values were determined respectively at 100°C, 150°C and 200°C using the Association of Official Analytical Chemists method.

Kinetic Calculations:

A general reaction rate expression for the deterioration kinetic can be written as follows (Ramaswami *et al*., 1989 ; Van, 1996):

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

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:

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

Where $[C_o]$ 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 =Ao exp (-Ea/ RT). Where ‘Ea’ is the activation energy of the reaction, ‘R’ is the gas constant, ‘T’ is absolute temperature and ‘A_o’ 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 Of Terminalia catappa L. Seed Oil:

Results obtained showed that the seeds contained 4.13% moisture, 51.80% crude oil, 23.78% crude proteins, 16.02% carbohydrate (by difference), 4.94% crude fibre, 4.27% ash and 548.78 Kcal calorific value (Table1). The high percentage of oil makes this seed a distinct potential for the oil industry. According to Omeje *et al*. (2008) and Guillermo Arrázola *et al*. (2008). 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:

The *Terminalia catappa L* seeds contained significant amount of important minerals (Table 2). The Potassium concentration (9280.0 ± 0.14 mg/100g dry mater) was the highest, followed in descending order by Calcium (827.20 ± 2.18 mg/100g dry mater), Magnesium (798.6 ± 0.32 mg/100g dry mater) and Sodium (27.89 ± 0.42 mg/100g dry mater). Potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins (Malik, 1982). Calcium and Magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Russel, 1973). Calcium assists in tech development (Brody, 1994). Magnesium is essential mineral for enzyme activity, like calcium and chloride; magnesium also plays a role in regulating the acid-alkaline balance in the body. High magnesium levels in drinking water have been linked to resistance to heart disease (Fallon, 2001).

Oil Extraction:

Characteristics of the oil were compared with *Terminalia Catappa L* varieties described by Dos Santos *et al* (2008). The extracted oils were liquid at room temperature. The oil content of *Terminalia Catappa L* "Congo-Brazzaville" seeds is high, it was found to be 56.30 ± 2.35% which shows that the processing of the oil for industrial or edible purposes would be economical. This value is slightly lower than the value reported for the seed of *Sesamum indicum* L (57.0 ± 1.27%), Nzikou *et al*., (2009), but higher than that of *Moringa oleifera* (40.0 ± 1.34%) by Nzikou *et al*., (2009).

Physical And 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 4. The obtained peaks were asymmetries and may indicate the presence of two components. The first peak at low melting point appears at – 19.75 °C (H_f = +1.47 $J.g^{-1}$). This peak corresponds to triglycerides formed by poly unsaturated acids (PUFA). The second melting point is at +4.56 °C (H_f = +8.64 $J.g^{-1}$). This is a characteristic of saturated 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 38°C of this oil is given Table 3. This result (32.92 ± 0.17 mPa.s) is in agreement with that found by Dos Santos, 2008.

Chemical Properties:

Iodine value is a measure of the degree of unsaturation in an oil and it is an identity characteristic of native oil. It indicates the degree of unsaturation in the fatty acids of triacyglycerol. 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

Table 1: Proximate analysis of *Terminalia catappa oil* seed

Characteristic	Obtained values[a] (M ± S.D.)	Reported values[b]	
		1	2
Moisture content (%)	4.13 ± 0, 24	1.54	4.5
Crude protein[c](%)	23.78 ± 0.15	26.30	24
Ether extract (%)	51.80 ± 0.21	56.71	54
Crude fibre (%)	4.94 ± 0.32	4.40	12
Ash content (%)	4,27 ± 0,74	4.55	4.0
Total carbohydrate[d] (%)	16.02	10.9	13.5
Calorific value (Kcal/100g)	548.78	577.75	588.74

[a] M ± S.D. mean ± standard deviation.
[b] (1) Omeje *et al*. (2008). (2) Guillermo Arrázola *et al*. (2008)
[c] Crude protein = N (%) x 6.25
[d] Carbohydrate obtained by difference

Table 2: Mineral elemental Composition of *Terminalia catappa* seeds

Mineral Elements	Composition (mg/100g) of Seed
Calcium, Ca	827.20± 2.18
Magnesium, Mg	798. 6 ± 0.32
Potassium, K	9280.0 ± 0.14
Sodium, Na	27.89 ± 0.42

Values are mean ± S.D of triplicate determinations

Table 3: Physical and chemical properties of *Terminalia catappa* seed oil extracted using solvent process

Properties	Reported values[a]	
	Soxlhet process	Solvent extract
Oil[b] (%)	56.30 ± 2.35^{A}	49
PV	0.51 ± 0.35^{A}	0.5
FFA (as % oleic acid)	2. 42 ± 0.27^{B}	ND
IV (wijs)	82.43 ± 1.10^{A}	83.92
Saponification value Unsaponifiable matter	207 ± 0. 13^{A}	ND
Content (%)	0.50 ± 0.07^{B}	ND
Viscosity (mPa.s) at 38°C	32.92 ± 0.17^{B}	39.8
E_a (KJ. mol^{-1})	7.752	ND

ND: not determined.
Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$).
[a] Dos Santos *et al*. (2008).
[b] Oil = weight of extracted oil x 100/weight of seed.
Abbreviations: PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value

Table 4: Melting behaviour of *Terminalia catappa* seed oil. Experimental conditions: temperature program set at -50 °C for 2 min, rising to 50 °C at rate of 5°C.min^{-1} .

Thermogram	5 °C.min^{-1}
	Soxlhet Process
Peak 1 [°C]	-19.75
H [J.g^{-1}]	+1.47
Peak 2 [°C]	+4.56
H [J.g^{-1}]	+8.64

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 (2.42± 0.41 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 0.51 ± 0.35 mg/g oil, this value is lower than that expected of rancid oil which ranges from 20.00 to 40.00 mg/g oil (Oderinde *et al*., 1998).This shows that the oil is not rancid and considered stable (Ajayi *et al*., 2002).

Fatty Acid Composition:

The major saturated fatty acids in *Terminalia catappa L.* seed oil were palmitic (35.81%), stearic (4.14%) acids. The main unsaturated fatty acids are linoleic (29.40%) and oleic (31. 65%) acids (Table 5). *Terminalia catappa L.* oil contained saturated and unsaturated acids (39.95% and 60.05%) respectively. *Terminalia catappa L* oil can be classified in the oleic-linoleic acid group. Linoleic acid which is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart vascular diseases (Boelhouwer, 1983). A part from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, linoleic acid also prevents high blood pressure. Also linoleic derivatives serve as structural

components of the plasma membrane and as precursors of some metabolic regulatory compounds (Vles, 1989). *Terminalia capatta L* oil is predominantly made up of palmitic (35.81%), oleic (31.65%) and linoleic (29.40%) acids respectively (Table 5). The results obtained are in agreement with those of the literature Dos santos *et al*., (2008).

Kinetic Data For The Degradation of Terminalia capatta L Oil:

The rate of production of peroxide in *Terminalia capatta L* oil increases as the temperature increases as shown in Table 6. 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.

Table 6 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.

In order to obtain the reaction rate constant ('k'), a first order degradation of the oil was presumed (Labuza and Riboh, 1982). Accordingly 'ln $[C]_t/[C]_o$ 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 above. 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 for the degradation was calculated from the rate constant as '0.693/k' and is given Table 7.

Fig 2 shows the Arrhenus plot of ln k versus 1/T for the reduction of unsaturation (Iodine value) in *Terminalia capatta L* oil. The linear nature of the plot obtained gave the activation energy of the reaction to be 7.752 $KJ.mol^{-1}$(Table 3).

Table 5: Relative percent composition of fatty acid in *Terminalia catappa* seed oil

Fatty acid	Reported values[a]	
	Soxlhet Process	1
C16 :0	35. 81 ± 1.41 ^{A}	35.0
C18 :0	4.14 ± 0.25 ^{A}	5.0
C18 :1	31.65 ± 0.48 ^{B}	32.0
C18 : 2	29.40 ± 0.37 ^{A}	28.0
C18 :3	-	-
Saturated	39.95	40.0
Unsaturated	60.05	60.0

ND: not determined.
Means for the determined values in the same row followed by the same superscript letter are not significantly different (P < 0.05). [a] (1) Dos Santos *et al*., (2008)

Table 6: Effect of heating on PV and IV of *Terminalia catappa* oil

Temp. (°C)	Time (mins)	PV (mg/g)	IV (mg/iodine)
100	30	0.85±0.24	82.24±0.18
	60	1.24±0.31	81.99±0.51
	120	1.66±0.22	81.68±0.12
	180	2.06±0.14	81.47±0.41
	240	2.54±0.42	81.24±0.72
150	30	1.16±0.23	82.12±0.16
	60	1.48± 0.27	81.32±0.35
	120	1.86±0.12	81.15±1.01
	180	2.18±0.08	80.12±0.81
	240	2.53±0.15	79.62±0.45
200	30	1.76±0.03	81.63±0.24
	60	1.97±0.32	81.15±0.17
	120	2.16±0.31	80.54±0.20
	180	2.39±0.17	80.19±0.32
	240	2.63±0.15	79.69±0.52

Values are mean ± standard deviation of triplicate determinations
PV: Peroxide Value IV: Iodine Value

Table 7: Kinetic parameters for degradation of *Terminalia catappa* oil

Parameters	100°C	150°C	200°C
k (s^{-1})	0.00006	0.0001	0.0001
t ½ (s)	11550	6930	6930

k: reaction rate constant
t ½ : time of half-reaction or half life

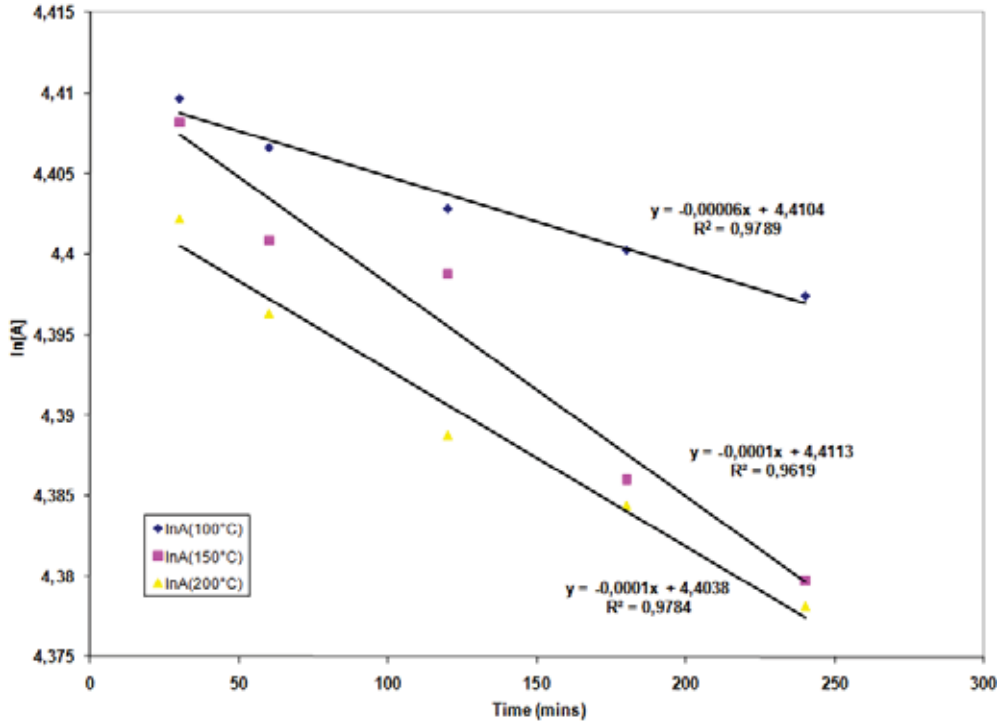

Fig. 1: Graph of rate of change in concentration of iodine value against time. [A] Represents iodine value

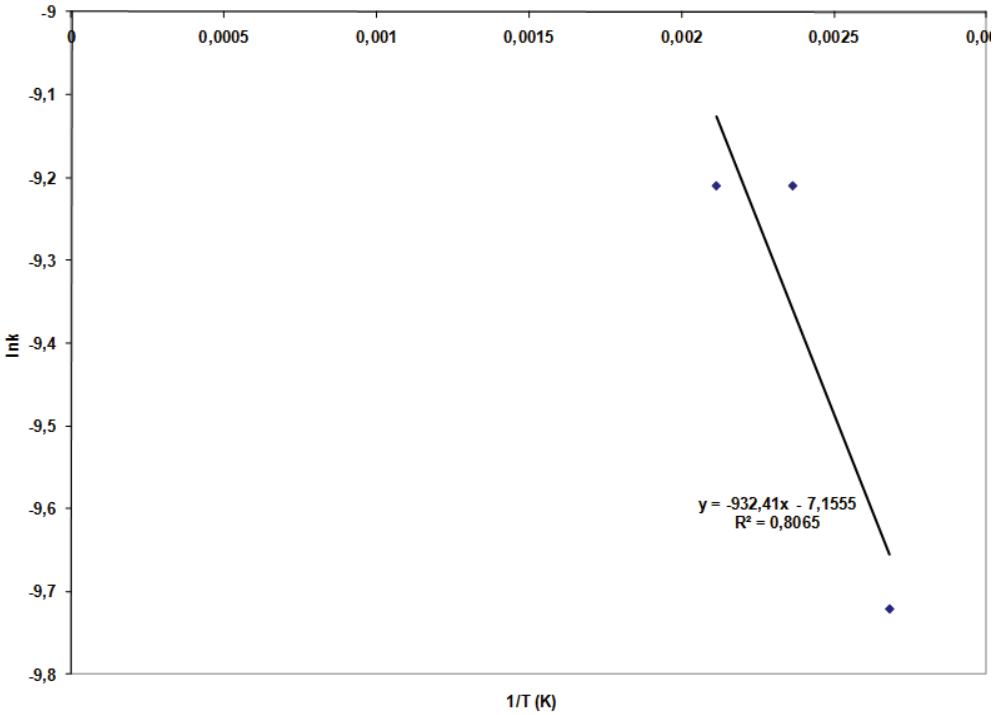

Fig. 2: Arrhenius plot for oxidation rate of the double bonds of fatty acids in *Terminalia catappa*. oil

Conclusion:

This study showed that the *Terminalia catappa L.* seed is a good source rich in protein, minerals and oil. *Terminalia catappa L.* seed oil was obtained from the kernels with good yield (51.80%), allowing the possibility of economical exploitation, and its fatty acid composition is comparable to that of some conventional oils. *Terminalia catappa L.* seed oil is of unsaturated type and contains mainly the fatty acids oleic C18:1(31.48%) and linoleic C18:2 (28.93%). The oil can be classified in the oleic-linoleic acid group. The oil extracts exhibited good physicochemical properties and could be useful for industrial applications. The thermal oxidation of *Terminalia capatta L* 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 and peroxide value.

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