Physicochemical Properties of Oil Extract from Gumbo (*Abelmoschus Esculentus L.*) And the Kinetics of Degradation of the Oil During Heating

[1]G. Bouanga-Kalou, [1]J.G. Nitou, [1,4]J.M. Nzikou, [1]F.B. Ganongo-Po,
[1]C.H. Hounounou, [1]B. Diabangouaya, [1]J. Mpika-Bouesso, [3]Th. Silou and [4]S. Desobry

[1]ENSP-UMNG, Laboratory of Food Physicochemistry and Biotechnology, Pole of Excellence in Nutrition and Food, P.0. box 69 Brazzaville-Congo
[2]Faculty of Science of Health -University Marien Ngouabi P.0. box 69 Brazzaville-Congo
[3]Multi-field Team of Research in Food and Nutrition, Center IRD.
P.O. box 1286 Pointe-Noire (Congo)
[4]ENSAIA-INPL, Laboratory of Engineering and Biomolecule, 2, avenue de la forêt de Haye,
54505 Vandoeuvre-lès-Nancy (France)

Abstract: The aim of the study was to investigate the physicochemical properties and the kinetics of degradation of gumbo seed oil during heating. The seed is a good source of oil (23.27%). The physical properties of the oil extracts showed the state to be liquid at room temperature and indicated that the oil had refractive index, 1.4689; the peroxide value, 3.21 (meq O_2/kg oil); free fatty acid, 3.74%; iodine value, 127.2%; saponification value, 196.3; unsaponifiable matter content, 1.58% and viscosity, 35.2 (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 69.94% and 29.53% respectively. DSC indicates the presence of three components in oil extracted. The first peak at low melting point appears at – 23.28 °C (H_f = +6.05 J.g^{-1}), the second peak appears to -1.14°C (H_f = +6.87 J.g^{-1}) and the last peak appears to +2.93°C (H_f = +2.18J.g^{-1}). 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 8.646 KJ.mol^{-1}.There is the possibility of considering the seed as feed supplement and its oil for industrial application.

Key words: *Abelmoschus esculentus L.* seed, kinetics, essential fatty acid, viscosity, DSC.

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 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. Gumbo (*Abelmoschus esculentus L.*) is widely consumed as a fresh vegetable in both temperate and tropical countries. Although the seed pods are most often used (Camciuc *et al.,* 1998), the mature seed is

---

Corresponding Author: J.M. Nzikou, ENSP-UMNG, Laboratory of Food Physicochemistry and Biotechnology, Pole of Excellence in Nutrition and Food, P.0. box 69 Brazzaville-Congo

known to have superior nutritional quality. Rubatzky and Yamaguchi (1997) reported that the seed is a rich source of protein and oil.

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 Sept. 20, 2010 to Dec. 10, 2010.

Materials:

Abelmoschus esculentus L. 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 (soxlhet method), 50g of gumbo 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^{-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 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 and Swe, 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*. (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^{-1} 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) x 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 *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^{-1} 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 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.

Proximate Analysis:

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

Heat Treatement:

Thermal degradation of *Abelmoschus esculentus L.*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 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:
$K = A_o \exp (-E_a/ RT)$. Where 'E_a' 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:

The oil content is high, it was found to be 23.44± 0.12% (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 (23.85%) reported by Ndangui *et al*., (2010). 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 gumbo 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 Soxlhet method. The first peak at low melting point appears at -23.28 °C ($H_f = +6.05$ J.g^{-1}). This peak corresponds to triglycerides formed by poly unsaturated acids (PUFA). The second melting point is at -1.14 °C ($H_f = +6.87$ J.g^{-1}), this is a characteristic of mono unsaturated fatty acids (MUFA). The last peak appears to $+2.93$°C ($H_f = +2.18$J.g^{-1}) suggest 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°C of this oil is given Table 1. This result (35.2 mPa.s) is in agreement with that found by Ndangui *et al.*, 2010.

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 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 (3.74± 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 3.21 ± 0.14 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). 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 acid in *Abelmoschus esculentus L.* seed oil was palmitic acid (25.85%); the main unsaturated fatty acid were oleic acid (25.18%) and linoleic acid (42.15%) with small amounts of linolenic acid (2.25%) and arachidic acid (0.51%). The oil sample of *Abelmoschus esculentus L.* contained saturated and unsaturated acids (29.53% and 69.94%) respectively. *Abelmoschus esculentus 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).The results obtained are in agreement with those of the literature (Camciuc, 1998).

Kinetic data for the degradation of Abelmoschus esculentus L.oil:

The rate of production of peroxide in *Abelmoschus esculentus L.* 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.

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 $[C]_t/[C]_o$ 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 5.

Fig 2 shows the Arrhenus plot of ln k versus 1/T for the reduction of unsaturation (Iodine value) in *Abelmoschus esculentus L.* oil. The linear nature of the plot obtained gave the activation energy of the reaction to be 8.646 KJ.mol^{-1}(Table 1).

Table 1: Physical and chemical properties of *Abelmoschus esculentus L.* seed oil extracted using soxlhet process

Properties	Obtained values
	Soxlhet
Colour	yellow-greenish
Oil[b] (%)	24.90 ± 0.55^A
PV	3.21 ± 0.14^A
FFA (as % oleic acid)	3.74 ± 0.9^B
IV (wijs)	127.2 ± 3.12^A
Saponification value	196.3 ± 5.3^A
Unsaponifiable matter content(%)	1.58 ± 0.07^B
Viscosity (mPa.s) at 25°C	35.2 ± 0.12^B
Refractive index (at 25°C)	1.4689
Mean molecular mass	285.28
E_a (KJ. mol^{-1})	8.646

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).
[b] Oil = weight of extracted oil x 100/weight of seed.
Abbreviations: PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value.

Table 2: Melting behaviour of *Abelmoschus esculentus L.* 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 °C.min^{-1}

Thermogram	5 °C.min^{-1}
	Soxlhet
Peak 1 [°C]	-23.28
H_f [J.g^{-1}]	+6.05
Peak 2 [°C]	-1.14
H_f [J.g^{-1}]	+6.87
Peak 3 [°C]	+2.93
H_f [J.g^{-1}]	+2.18

Table 3: Relative percent composition of fatty acid in *Abelmoschus esculentus* seed oil

Fatty acid	Determined values	Reported values[a]
	Soxlhet	1
C14:0	0.38 ± 1.45^A	0.25
C16 :0	25.85 ± 0.72^A	26.13
C16:1	0.36 ± 1.12^A	0.41
C17:0	-	0.15
C17:1	-	0.32
C18 :0	2.79 ± 0.28^A	2.86
C18 : 1	25.18 ± 0.38^B	16.23
C18 :2	42.15 ± 3.21^A	50.62
C18 :3	2.25 ± 1.85^A	-
C19:1	-	1.40
C20:0	0.51 ± 0.85^A	0.62
Saturated	29.53	ND
Unsaturated	69.94	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] (1) Camciuc *et al*. (1998).

**Table 4:** Effect of heating on PV and IV of *Abelmoschus esculentus L.* seed oil

Temp. (°C)	Time (mins)	PV (mg/g)	IV (mg/iodine)	RI (25°C)
100	30	3.55±0.23	127.02±0.21	1.4311
	60	3.96±0.10	126.76±0.19	1.3712
	120	4.36±0.24	126.45±0.15	1.3110
	180	4.79±0.35	126.24±0.33	1.2511
	240	5.25±0.23	126.01±0.13	1.1713
150	30	3.84±0.25	126.9±0.24	1.3589
	60	4.16±0.17	126.3±0.42	1.3181
	120	4.54±0.12	126.13±0.20	1.2570
	180	4.86±0.18	125.10±0.21	1.1849
	240	5.21±0.31	124.60±0.15	1.1653
200	30	4.44±0.25	126.41±0.31	1.2414
	60	4.65±0.17	125.93±0.12	1.2191
	120	4.84±0.21	125.32±0.42	1.1943
	180	5.07±0.34	124.97±0.23	1.1851
	240	5.36±0.21	124.47±0.21	1.1810

Values are mean ± standard deviation of triplicate determinations
RI: Refractive Index

Table 5: Kinetic paramaters for degradation of *Abelmoschus esculentus L.* seed oil

Kinetics parameters	100°C	150°C	200°C
k_1 (s $^{-1}$)	0.00004	0.00008	0.00007
t ½ (s)	17325	8662.50	9900
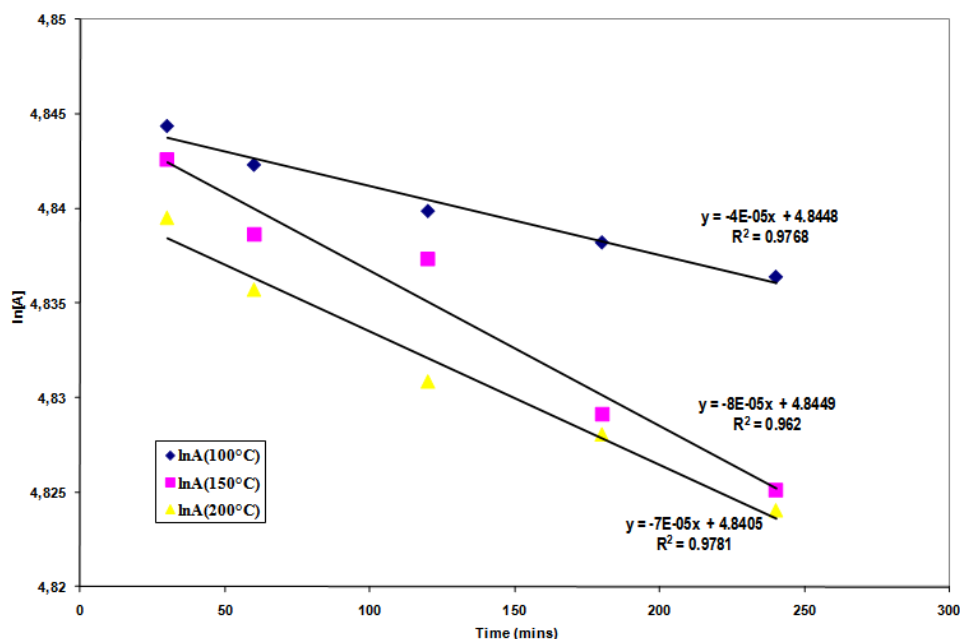
Fig. 1: Graph of rate of change in concentration of iodine value against time. [A] Represents iodine value
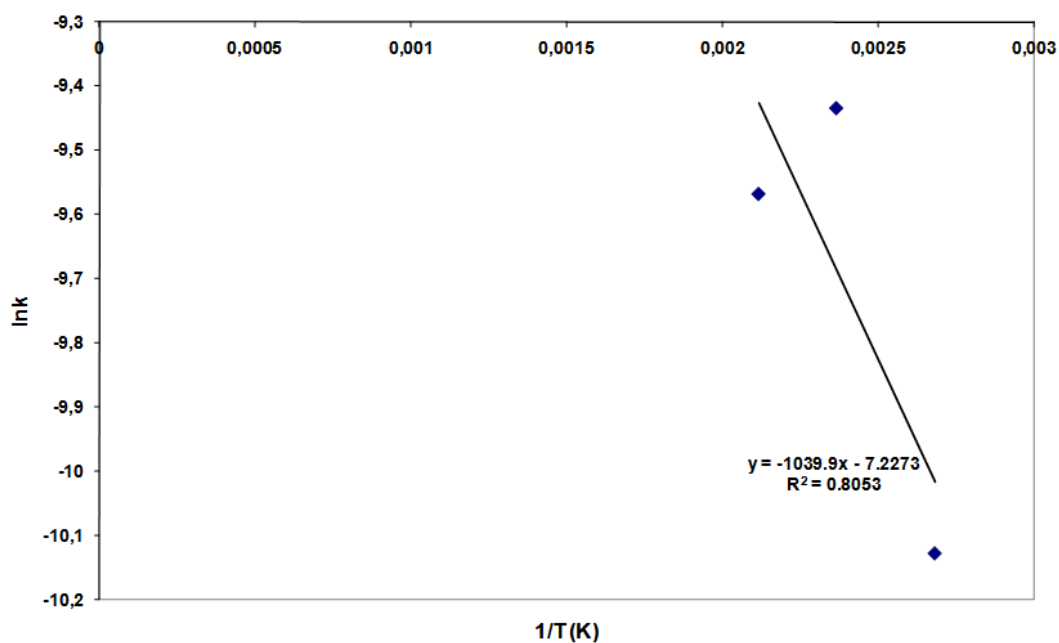
Fig. 2: Arrhenius plot for oxidation rate of the double bonds of fatty acids in *Abelmoschus esculentus L.* seed oil.

Conclusion:

This study showed that the *Abelmoschus esculentus L.*seed is a good source rich in oil. *Abelmoschus esculentus L.* seeds oil have high contents of unsaturated fatty acids, it is more balanced that some edible oils such as grape seed oil, corn oil and sunflower oil. High unsaponifiable matter content guarantees the use the oils in cosmetics industry and we suggest the application of these oils for soap making and manufacture of

lather shaving creams. Free fatty acids content and peroxides value were low indicating that the oils could qualify as good edible oils. The thermal oxidation of *Abelmoschus esculentus 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, refractive index and peroxide value.

ACKNOWLEDGEMENTS

REFERENCES

Akintayo, E.T. and E., Bayer. 2002. Characterization and possible uses of *plukenatia_conophora_and Adenopus_breviflorus* seed and seed oil. Bores. Technol., 85: 95-97.

Ajayi, I.A, F.A. Dawodu, K.O. Adebowale and R.A. Oderinde, 2002. Chemical composition of Pentaclethra macrophylla seed and seed oil grown in Nigeria. Riv.Ital.Sostanze Grasse., 74: 183-185.

Anthon, G.E. and D.M. Barrett, 2002. Kinetic parameter for the thermal inactivation of quality-related enzymes in carrots and potatoes.Journal of Agriculture and Food Chemistry, 50: 4119-4125.

AOAC, Official Methods of Analysis, 1995. 14th edn., Association of Official Analytical Chemist, Arlington, VA, 67: 1-45.

Bailey, E.A., 1954. Industrial oil and fat products. Edn 3rd , Interscience publishers, London.

Boelhouwer, C., 1983. Trends in chemistry and technology of lipids. J. Am. Oil Chem. Soc., 60(2): 457-462.

Che Man, Y.B. and P.Z. Swe, 1995. Thermal analysis of failed-batch Palm oil by differential scanning calorimetry. J. Am. Oil Chem. Soc., 72(12): 1529-1532.

Camciuc, M., M. Deplagne, G. Vilarem and A. Gaset, 1998. Okra—*Abelmoschus esculentus* L. (Moench.) a crop with economic potential for set aside acreage in France. Industrial   Crops and Products., 7: 257-264

Cocks, L. V. and C.Van Rede, 1966. Laboratory handbook for oil and fats analysts. London: Academic Press. pp: 88

Gouveia. A, De Souza, OLivera, J.C., Santos, M.M., Conceicae, M.C., Dantas Silva,

PRASAD, S., 2004. A thermoanalytic and kinetic study of sunflower oil. Brazilian Journal of Chemical Engineering, 21(2): 265-273.

Ibiyemi, S.O., T.O. Adepoju, S.O. Okanlawon and V.O Fadipe, 1992. Roasted Cyperus esculentum(Tiger nut): Emulsion preparation and stability.Nigeria Journal of Nutritional Science, 13(1-2): 31-34.

Kowalki, B., 1995. Determination of oxidative stability of edible vegetable oil by Pressure differential scanning calorimetry. Thermo chim. Acta, 250: 197-205.

Labuza, T.P. and D. Riboh, 1982. Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. Food Technology, 36: 66-74.

Mohammed, R.F. and M. Jorf-Thomas, 2003. Determination of the lipid classes and fatty acid profile of Niger seed (Guizotia abyssinica Cass). Phytochem. Anal., 14: 366-370.

Morette, E. and R. Fett, 1998. Tecnologia de oles e Gorduras na Indutria de Alimentos. Sao Paulo: Varele.

Ndangui, C.B., A. Kimbonguila, J.M. Nzikou, L. Matos, N.P.G. Pambou-Tobi, A.A. Abena, Th. Silou, J. Scher and S. Desobry, 2010. Nutritive Composition and Properties Physico-chemical of gumbo (Abelmoschus esculentus L.) Seed and Oil. Res. J. Environ. Earth Sci., 2(1): 49-54.

Nzikou, J.M., L. Matos, G. Bouanga-Kalou, C.B. Ndangui, N.P.G. Pambou-Tobi, A. Kimboguila, Th. Silou, M. Linder and S. Desobry, 2009. Chemical Composition on the Seed and Oil Sesame (*Sesamum indicum* L.) Grown in Congo-Brazzaville. Advance Journal of Food Science and Technology, 1(1): 6-11.

Oderinde, R.A and I.A. Ajayi, 1998. Metal and oil characteristics of Terminalia catappa.Riv.Ital.Sostanze Grasse., 75: 361-362.

Oderinde, R.A., I.A. Ajayi and A. Adewuyi, 2009. Characterization of seed and seed oil of *Hura crepitans* and the kinetics of degradation of the oil during heating. EJEAFChe, 8(3): 201-208.

Pearson, D., 1976. The Chemical Analysis of Foods. 7rd Edn. ChurchillLivingstone, Edinburgh, U.K., pp: 488-496.

Pena, D.G., R.G.L. Anguiano and J.J.M. Arredondo, 1992. Modification of the method 1 AOAC (CB-method) for the detection of aflatoxins, Bull. Environ. Contam. Toxicol., 49: 485-489.

Pomeranz, Y. and C. Meloan, 1994. Food analysis: Theory and practice, 3rd Edn., pp: 778. New York: Chapman & Hall.

Ramaswami, H.S., F.R. Van De Voort and S. Ghazada, 1989. Analysis of TDT and Arrhenius methodfor handling process and kinetic data.Journal of Food Science, 54: 1322-1326.

Rubatzky, V.E. and M. Yamaguchi, 1997. World vegetables. Principles, production and nutritive values. 2nd Edn. Inter. Thomson Publ., New York. pp: 681-686.

Van Boekel, M.A., 1996. Statistical aspect of kinetic modeling for food science problems. Journal of Food Science, 61: 477-485.