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# Chemical Composition of Seed Oil from Roselle (*Hibiscus sabdariffa* L.) and the Kinetics of Degradation of the Oil During Heating

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**Abstract:** The aim of the study was to investigate the chemical composition and the kinetics of degradation of roselle 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, 1.4652; the peroxide value, 3.15 (meq $O_2$/kg oil); free fatty acid, 0.82%; iodine value, 97.78%; saponification value, 198.45 and viscosity, 15.15 (mPa.s at 25ºC). 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 73.4 and 26.57%, respectively. DSC indicates the presence of two components in oil extracted. The first peak at low melting point appears at -20.53°C ($H_f$ = +3.00 J/g) and the second peak appears to -2.17°C ($H_f$ = +0.49 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 9.041 KJ/mol. There is the possibility of considering the seed as feed supplement and its oil for industrial application.

**Key words:** Essential fatty acid, DSC, *Hibiscus sabdariffa* L. seed, kinetics, 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 (Mohammed and Jorg-Thomas, 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. Roselle, *Hibiscus sabdariffa* L. (family *Malvaceae*) is one of the most important and popular medicinal plants. Roselle is native from India to Malaysia, where it is commonly cultivated, and must have been carried at an early date to Africa. In some parts of Africa, the seeds are reported to be used for its oil. The seeds are reported to be rich in proteins, dietary fiber, carbohydrates and fats (Abu-Tarboush *et al*., 1997; Rao, 1996; El-Adawy and Khalil, 1994).

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The chemical composition of *Hibiscus sabdariffa* L. seed oil in Congo Brazzaville, as well as their kinetics of degradation, has not yet been studied. Therefore, the objectives of this study were to investigate the chemical composition of seed oil from *Hibiscus sabdariffa* L. and the kinetics of degradation of the 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 Sept. 20, 2010 to Dec. 10, 2010.

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

**Methods:**

**Oil extraction:** For solvent extraction (soxlhet method), 50 g of roselle seed flour were placed into a cellulose paper cone and extracted using light petroleum ether (b.p 40-60ºC) 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 60ºC 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 50ºC in an oven until completely melted. Oil sample was placed in an aluminium volatile pan and was cooled to -50ºC and held for 2 min, it was then heated from -50 to 50ºC at the rate of 5ºC/min (normal rate) (Che Man and Swe, 1995), and held -50ºC isothermally for 2 min and cooled from -50 to 50ºC at the rate of 5ºC/min. 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 (20ºC) 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 and Van Rede (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 240ºC and column temperature was 110ºC held for 1 min and increased at the rate of 8ºC/min to 220ºC 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 550ºC 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 *Hibiscus sabdariffa* L. oil was carried out by heating the oil up to 200ºC for a period of 0-240 min. The peroxide value, refractive index and the iodine values were determined respectively at 100, 150 and 200ºC 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 Boekel, 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:** The oil content is high, it was found to be 23.27±1.1% (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 (20.02%) reported by Cissé *et al*. (2009). 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 roselle seed oil at room temperature is liquid.

Table 1: Physical and chemical properties of roselle (*Hibiscus sabdariffa* L.) seed

| | Obtained values |
|---|---|
| Properties | Soxlhet |
| Colour | Yellow-greenish |
| Oil[a] (%) | $23.27 \pm 1.1^{A}$ |
| PV | $1.15 \pm 0.44^{B}$ |
| FFA (as % oleic acid) | $0.82 \pm 0.21^{A}$ |
| IV (wijs) | $97.78 \pm 0.25^{A}$ |
| Saponification value Unsaponifiable matter | $198.45 \pm 0.15^{A}$ |
| Content (%) | $1.6 \pm 0.37^{B}$ |
| Refractive index (at 25ºC) | 1.4652 |
| Mean molecular mass | 282.19 |
| Viscosity (mPa.s) at 25ºC | 15.15 |
| Ea (KJ/mol) | 9.041 |
| State at room temperature | Liquid |

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

Table 2: Melting behaviour of roselle (*Hibiscus sabdariffa* 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

| | 5 °C/min |
|---|---|
| Thermogram | Soxlhet |
| Peak 1 [°C] | -20.53 |
| $\Delta H_f$ [J/g] | +3.00 |
| Peak 2 [°C] | -2.17 |
| $\Delta H_f$ [J/g] | +0.49 |

**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 two components. The first peak at low melting point appears at -20.53ºC ($H_f$ = +3.00 J/g). This peak corresponds to triglycerides formed by Poly Unsaturated Fatty Acids (PUFA). The second melting point is at -2.17°C ($H_f$ = +0.49 J/g). This is suggesting the presence of mixed triglycerides groups with different melting points.

**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 25ºC of this oil is given Table 1. This result (15.15 mPa.s) is in agreement with that found by Cissé *et al*. (2009).

Table 3: Relative percent composition of fatty acid in roselle (*Hibiscus sabdariffa* L.) seed oil

| | Determined values |
|---|---|
| Fatty acid | Soxlhet |
| Myristic acid (C14:0) | 0.26 ± 0.5 [B] |
| Palmitic acid (C16:0) | 20.52 ± 0.12 [B] |
| Stearic acid (C18:0) | 5.79 ± 0.35 [A] |
| Oleic acid (C18 : 1) | 32.28 ± 0.24[A] |
| Linoleic acid (C18 :2) | 39.46 ± 0.17[A] |
| Linolenic acid | 1.69± 0.19[A] |
| Saturated acid | 26.57 |
| Unsaturated acid | 73.4 |

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p<0.05$)

Table 4: Effect of heating on PV, IV and RI of roselle (*Hibiscus sabdariffa* L.) seed oil

| Temp. (ºC) | Time (mins) | PV (mg/g) | IV (mg/iodine) | RI (25ºC) |
|---|---|---|---|---|
| 100 | 30 | 1.49±0. 24 | 97.59±0.22 | 1.4274 |
| | 60 | 1.90±0.12 | 97.35±0.28 | 1.3674 |
| | 120 | 2.30±0.18 | 97.02±0.17 | 1.3073 |
| | 180 | 2.73±0.10 | 96.81±0.21 | 1.2473 |
| | 240 | 3.19±0.24 | 96.57±0.23 | 1.1673 |
| 150 | 30 | 1.78±0. 42 | 97.46±0.21 | 1.3552 |
| | 60 | 2.10±0.22 | 96.88±0.19 | 1.3142 |
| | 120 | 2.48±0.31 | 96.70±1.20 | 1.2533 |
| | 180 | 2.80±0.28 | 95.66±0. 15 | 1.1812 |
| | 240 | 3.15±0.32 | 95.18±0.20 | 1.1612 |
| 200 | 30 | 2.38±0.25 | 97.33±0.14 | 1.2373 |
| | 60 | 2.59±0.29 | 96.85±0.18 | 1.2153 |
| | 120 | 2.78±0.20 | 96.25±0.21 | 1.1904 |
| | 180 | 3.07±0.23 | 95.80±0.12 | 1.1813 |
| | 240 | 3.30±0.17 | 95.40±0.10 | 1.1773 |

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

**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 (0.82± 0.21 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). 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.

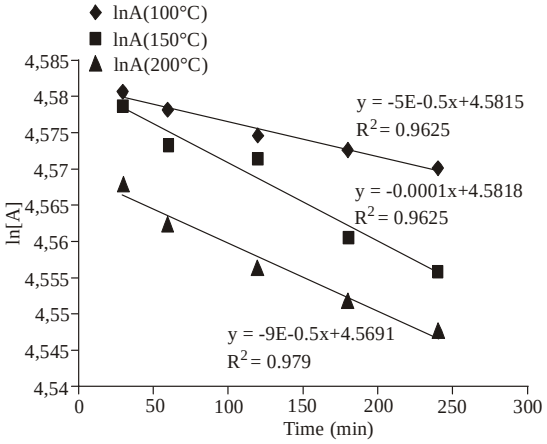


Fig. 1: Graph of rate of change in concentration of iodine value against time

**Fatty acid composition:** The major saturated fatty acids in *Hibiscus sabdariffa* L. seed oil were palmitic (20.52%) and stearic (5.79%) acids and the main unsaturated fatty acids are linoleic (39.46%) and oleic (32.28%) acids (Table 3). The oil sample of *Hibiscus sabdariffa* L. contained saturated and unsaturated acids (26.57% and 73.40%) respectively. The proportion of unsaturated fatty acids was greater than the saturated fatty acids. One notes 0.26 % of myristic acid C14:0. The results obtained are in agreement with those of the literature (Cissé *et al*., 2009).

**Kinetic data for the degradation of *Hibiscus sabdariffa* L. oil:** The rate of production of peroxide in *Hibiscus sabdariffa* 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. A correlation

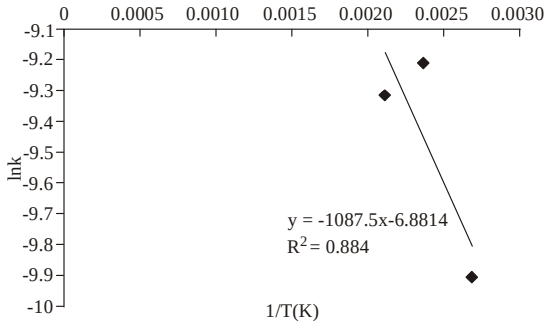


Fig. 2: Arrhenius plot for oxidation rate of the double bonds of fatty acids in roselle (*Hibiscus sabdariffa* L.) seed oil

Table 5: Kinetic paramaters for degradation of roselle (*Hibiscus sabdariffa* L.) seed oil

| Kinetic parameters | 100ºC | 150ºC | 200ºC |
|---|---|---|---|
| $k_1$ ($s^{-1}$) | 0.00006 | 0.0001 | 0.00009 |
| $t_{½}$ (s ) | 13860 | 6930 | 7700 |

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.

Figure 2 shows the Arrhenus plot of ln k versus 1/T for the reduction of unsaturation (Iodine value) in *Hibiscus sabdariffa* L. oil. The linear nature of the plot obtained gave the activation energy of the reaction to be 9.041 KJ/mol (Table 1).

## CONCLUSION

This study showed that the *Hibiscus sabdariffa* L. seed is a good source rich in oil. *Hibiscus sabdariffa* L. seed oil was obtained from the seed with good yield (23.27%), allowing the possibility of economical exploitation, and its fatty acid composition is comparable to that of some conventional oils. *Hibiscus sabdariffa* L. seed oil is of unsaturated type and contains mainly the fatty acids oleic C18:1(32.28%) and linoleic C18:2 (39.46%). 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 *Hibiscus sabdariffa* 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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