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Characteristics and Nutritional Evaluation of seed oil from Roselle (*Hibiscus sabdariffa L.*) in Congo-Brazzaville

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Abstract: The aim of the study was to clarify the characteristics and nutritional evaluation of roselle seed oil. The seed is a good source of oil and protein; these were found to be 27.78 and 21.85%, respectively. The seeds were found to be good sources of minerals. Potassium (1329±1.47 mg/100 g) was the highest, followed in descending order by Sodium (659±1.58 mg/100 g), Calcium (647±1.21 mg/100 g), Phosphorus (510±1.58 mg/100 g) and Magnesium (442.8±1.80 mg/100 g).The physical properties of the oil extracts showed the state to be liquid at room temperature and indicated that the oil had refractive index, 1.467. The saponification value suggests the use of this oil in liquid soap, shampoo and oil based ice cream production. The moisture content is also low (6.48%) which indicates the possibility of long shelve-life. Suggest that the roselle seeds could be novel and economic source of healthy edible fat and other food industry applications.

Key words: Essential fatty acid, *hibiscus sabdariffa* L. seed, minerals, nutritive values, viscosity

INTRODUCTION

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). Plant proteins are widely recognized as an important source of affordable protein. The use of different sources of protein can vary widely from one country to another depending on food habits and traditions of the community. Nowadays, the rapid rate of population growth in underdeveloped and third world countries has increased the demand for food supply and food production. This has led to an increase in the incidence of malnutrition problems like marasmus and kwashiorkor or Protein-energy Malnutrition (PEM) especially in young children. Roselle plants are abundant in Africa and India. Previous studies showed that the seeds can be used as a potential source of proteins (El-Adawy and Khalil, 1994; Al-Wandawi *et al*., 1984). The characteristics, nutritional properties of *Hibiscus sabdariffa* L. seed oil in Congo Brazzaville, as well as their functional properties, have not yet been studied. Thus, this study was conducted to investigate the characteristics and nutritional evaluation from Roselle seeds oil before that it be utilized and applied for human consumption or industrial uses.

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 Apr. 1, 2010 to Jun. 30, 2010.

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. Proximate analysis of *Hibiscus sabdariffa* L. seed Moisture, crude protein (micro-Kjeldahl), crude fiber, oil (Soxhlet) contents and refractive index of the oil (at room temperature) were determined using the methods described by Pearson (1976), whereas the ash content was

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determined using the method of Pomeranz and Meloan (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: 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. For methanol/chloroform extraction (Bligh and Dyer, 1959), 100g of the papaya seeds flour were homogenised with a chloroform mixture methanol (1:1) and water. Two phases was obtained, aqueous layer (methanol-water) and organic layer (chloroform). Oil was recovered by evaporating off the solvent (chloroform) using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60ºC 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 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.

Chemical analysis: Determinations for peroxide, iodine, and saponification values, unsaponifiable matter and free fatty acid contents were carried out using Pena *et al*. (1992) standard analytical methods. The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 μL of n-hexane 50 mg of oil followed by 50 μL of sodium methoxide using the method of Cocks and Van Rede (1966). The mixtures were vortex for 5 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 one minute and increased at the rate of 8ºC/min to 220ºC 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 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.

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 $p<0.05$.

Table 1: Proximate analysis (g/100 g dry weight) of roselle (*Hibiscus sabdariffa* L.) seed

Characteristic	Obtained values[a] (M±S.D.)	Reported values[b] 1	Reported values[b] 2
Moisture Content (%)	6.48±0.21	5.57	9.93
Crude Protein[c](%)	27.78±0. 42	25.20	33.45
Ether Extract (%)	21.85±0. 15	21.10	22.13
Crude Fiber	16. 44 ±0.27	16.30	nd
Ash Content (%)	6.2±0.20	5.19	7.47
Total Carbohydrate[d] (%)	21.25	26.64	Nd
Calorific Value (Kcal/100g)	326.53	nd	nd

[a]: M±S.D. mean±standard deviation; [b]: (1) Al-Wandawi *et al*. (1984). (2) Emmy Hainida *et al*. (2008); [c]: Crude protein = N (%) × 6.25; [d]: Total carbohydrate was estimated by difference of mean values i.e 100-(sum of percentages of moisture, ash, fiber, protein and lipid); nd : not determined

Table 2: Mineral elemental composition of roselle (*Hibiscus sabdariffa* L.) seed

Mineral elements	Composition (mg/100 g) of seed
Calcium, Ca	647±1. 21
Magnesium, Mg	1329±1.47
Potassium, K	1329±1.47
Sodium, Na	659±1.58
Phosphorous, P	510±1.25

Values are mean±S.D of triplicate determinations

RESULTS AND DISCUSSION

Proximate analysis of roselle seed: Results obtained showed that the seeds contained 6.48% moisture, 21.85% crude oil, 27.78% crude protein, 21.25% carbohydrate (by difference), 16.44% crude fibre, 6.2% ash and 326.53 Kcal calorific value (Table 1). The seed is a rich source of proteins (21.25%), lipids (27.78%) and crude fiber (16.44%).

The obtained fat content agree with the value (21.10%) reported by Al-Wandawi *et al*. (1984).These high values for proximate analysis make the roselle seeds a rich source of nutrients. 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. Meanwhile, the relatively high fat and protein contents indicate that these seeds could become an excellent economic source for edible oil production, and the meal could be more efficiently in animal feeding and/or possible human use.

Minerals: It is of interest to note that the most prevalent mineral element in *Hibiscus sabdariffa* L. seeds is Potassium which is a high as 1329±1.47 mg/100 g dry mater (Table 2), followed in descending order by Sodium (659±1.58 mg/100 g dry mater), Calcium (647±1.21 mg/100 g dry mater), Phosphorus (510±1.25 mg/100 g dry mater) and Magnesium (442.8±1.80 mg/100 g dry mater). Potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins (Malik and Srivastava, 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 teeth 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. Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Fallon and Enig, 2001).

Oil extraction: Characteristics of the oil were compared with *Hibiscus sabdariffa* L. varieties described by Al-Wandawi *et al*. (1984) and Hainida *et al*. (2008).The extracted oils were liquid at room temperature. The oil content of *Hibiscus sabdariffa* L. "Congo-Brazzaville" seeds and the level at which the differences are significant are shown in Table 3. The oil extraction with the Soxlhet method had the highest yield, due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley and Colwell, 1991). The Bligh and Dyer method, showed the low yield due to losses during the separation of the two phases, aqueous layer (methanol-water) and organic layer (chloroform). The results of the above authors agree with those of the present study.

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 in oil extracted from the two methods. The first peaks at low melting points appear at -21.36ºC (ΔH_f=+2.14 J/g) for Bligh and Dyer method and -20.53ºC (ΔH_f=+3.00 J/g) for Soxlhet method. These first peaks (at -21.36 and -20.53ºC, Bligh & Dyer and Soxlhet methods respectively), correspond to triglycerides formed by poly unsaturated acids (PUFA) and the last peaks appear to -2.92ºC (ΔH_f = +0.19 J/g) for Bligh & Dyer method and -2.17ºC (ΔH_f = +0.49 J/g) for Soxlhet method, suggest 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. In optics to know the rheological proprieties of these oils, we studied the influence of temperature on viscosity. Activation energies of the various classes of fatty acids contained in these oils were given Table 3. When the temperature increases, viscosity decreases exponentially (Fig. 1) some is the extraction method (Arslan *et al*., 2005; Nzikou *et al*., 2009). Viscosity varies between 42.40 and 6.40 mPa.s when temperature decreases of 50 to 5ºC by Soxlhet

Table 3: Physical and chemical properties of roselle (*Hibiscus sabdariffa* L.) seed

	Obtained values		
	----------		Reported values[a]
Properties	Bligh & Dyer (1959)	Solvent extract	Solvent extract
Oil[b] (%)	20.43± 0.24[A]	23.27±1.1 [A]	20. 02
PV	2.03±0. 72[B]	3.15±0.44[B]	8.63
FFA (as % Oleic Acid)	0. 88±0.14[A]	0.82±0.21[A]	2.24
IV (Wijs)	98.32±0.31[A]	97. 78±0.25[A]	81.45
Saponification value	197.32±0.22[A]	198.45±0. 15[A]	194.95
Unsaponifiable matter content (%)	1.49±0. 21[A]	1.6±0. 37[B]	nd
Refractive Index (at 25ºC)	1.4697	1.4652	1.48
Viscosity (mPa.s) at 25ºC	25.30	15.15	15.85
Ea (KJ/mol)	23.28	34.08	nd

Nd: not determined; Means for the determined values in the same row followed by the same superscrip letter are not significantly different ($p<0.05$); [a]: Cissé *et al*. (2009); [b]: Oil = weight of extracted oil × 100/weight of seed; Abbreviations: PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value

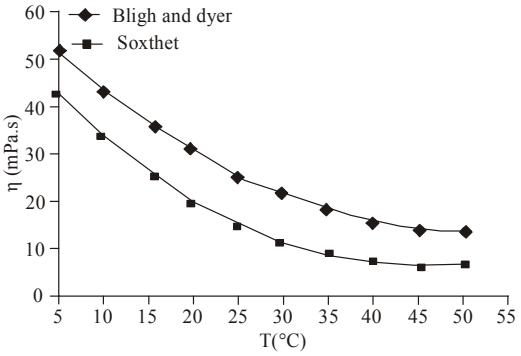

Fig. 1: Effet of roselle (*Hibiscus sabdariffa* L.) temperature on seed oil

Table 4: Melting behaviour of roselle (*Hibiscus sabdariffa* L.) seed oil using different scan rates. Experimental conditions: temperature program set at -50ºC for 10 min, rising to 50ºC at rate of 5ºC/min

	5ºC/min	

Thermogram	Bligh & Dyer (1959)	Soxlhet
Peak 1 [ºC]	-21.36	-20.53
ΔH_f [J./g]	+2.14	+3.00
Peak 2 [ºC]	-2.92	-2.17
ΔH_f [J/g]	+0.19	+0.49

Table 5: Oil viscosity at various temperature in degree celsuis

	η (mPa.s)	

T (ºC)	Bligh & Dyer (1959)	Soxlhet
5	51.70	42.40
10	42.90	33.40
15	35.60	25.60
20	30.70	20.00
25	25.30	15.15
30	21.40	11.30
35	18.20	8.80
40	15.70	7.30
45	14.10	6.50
50	13.60	6.40

Table 6: Energie plot derived from the Arrhenius equation

	Lnη (mPa.s)	

1/T (K^{-1})	Bligh & Dyer (1959)	Soxlhet
0.00359712	3.94545778	3.74714836
0.00353357	3.75887183	3.50855590
0.00346380	3.57234564	3.24259235
0.00341297	3.42426265	2.99573227
0.00335570	3.23080440	2.71800053
0.00330033	3.06339092	2.42480273
0.00324675	2.90142159	2.17475172
0.00319489	2.53660710	1.98787435
0.03146500	2.64617480	1.87180218
0.00309157	2.61006979	1.85629799

method. By Bligh and Dyer method, the viscosity of oil decreases of 51.70 to 13.60 mPa.s (Table 5). The viscosity of the oil obtained by Bligh and Dyer method was highest, possibly because of the water that was absorbed by the gums (phospholipids) during extraction. This calculator calculates the effect of temperature on reaction rates using the Arrhenius equation.

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

where, η is the viscosity, A is constant, E_a is the activation energy (in KJ/mol), R is the universal gas constant and T is the temperature (in degrees Kelvin). R has the value of 8.314×10^{-3} KJ/mol.K. We should use this calculator to investigate the influence of temperature on viscosity. Linear regression analysis was applied to the logarithmic form of Arrhenius equation in order to determine the parameters of the relation (Fig. 2, Table 6). lnη against 1/T, -Ea/RT is the slope from which Ea was evaluated. Activation energies of oils are given in Table 3. The highest value of activation energy is obtained by Soxlhet method (34.08 KJ/mol) and 23.28 KJ/mol by Bligh and Dyer method. The higher the activation energy, the more stable the fatty acid is:

Chemical properties: The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine

Table 7: Relative percent composition of fatty acid in roselle (*Hibiscus sabdariffa* L.) seed oil

	Determined values		
Fatty acid	Bligh & Dyer (1959)	Soxlhet	Reported values[a] solvent extract
Myristic acid (C14:0)	0.31±0. 42[A]	0.26 ±0.5[B]	0.31
Palmitic acid (C16:0)	21.15±0.15[A]	20.52±0.12[B]	21.65
Palmitoleic(C16:1)	-	-	0.44
Stearic acid (C18:0)	5.97±0. 21[A]	5.79±0.35[A]	5.47
Oleic acid (C18 : 1)	31.84±0.12[B]	32.28±0.24[A]	30.90
Linoleic acid (C18 :2)	39.16±0.34[A]	39.46±0.17[A]	39.16
Linolenic acid (C18:3)	1.57±0.28[A]	1.69±0.19[A]	0.57
Arachidic acid (C20:0)	-	-	0.72
Eicosenoic acid (C20:1)	-	-	0.08
Eicosatrienoic acid (C20:3)	-	-	0.34
Behenic acid (C22:0)	-	-	0.37
Saturated acid	27.43	26.57	nd
Unsaturated acid	72.57	73.4	nd

Nd: not determined; a: Cissé *et al*. (2009); Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p<0.05$)

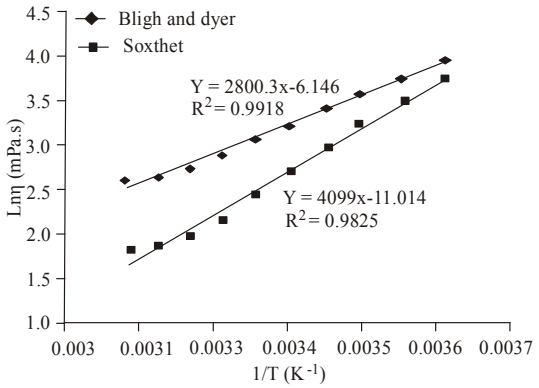

Fig. 2: Relationship between viscosity and temperature of roselle (*Hibiscus sabdariffa* L.) seed oil extracted by Bligh Dyer (1959) and Soxlhet

value is the measure of the degree of unsaturation of the oil. The free fatty acid and the unsaponifiable matter content of the Soxlhet method were significantly higher ($p<0.05$) than those of the Bligh and Dyer method (Table 3). There was no significant difference in the iodine and saponification values, in the two extraction methods ($p>0.05$). The low free fatty acids content is indicative of low enzymatic hydrolysis. This could be an advantage asoil high free fatty acids develop off flavour during storage (Bailey, 1954). The refractive index

reflects the degree of unsaturation and chain length. Values obtained here (1.4697 for Bligh & Dyer and 1.4652 for Soxlhet methods) are expected of on oils with low iodine value and the presence of Oleic acid and linoleic acid fatty in the proportion observed (Table 3). This is a good oil property sought after for both nutritional and industrial purposes. The free fatty acid value is on the low side (0.85±0.13 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 2.6±0.75 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 and Ajayi, 1998). This shows that the oil is not rancid and considered stable (Ajayi *et al*., 2002). The slightly higher value of unsaponifiable matter in the Soxlhet method may be due to the ability of the solvent to extract other lipid associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic *et al*., 1978; Salunke *et al*., 1992).

Fatty acid composition: The major saturated fatty acids in *Hibiscus sabdariffa* L. seed oil were palmitic (20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids are linoleic (39.31%) and oleic (32.06%) (Table 7). There was no significant difference ($p>0.05$) in the amounts of the major fatty acids in the two oil samples. In the two oil samples of *Hibiscus sabdariffa* L. contained saturated and unsaturated acids (27 and 73%) respectively. The proportion of unsaturated fatty acids was greater than the saturated fatty acids. One notes 0.29% of myristic acid C14:0. The results obtained are in agreement with those of the literature (Cissé *et al*., 2009).

CONCLUSION

This study showed that the roselle seed is a rich source of nutrients. *Hibiscus sabdariffa* L. seed oil is of unsaturated type and contains mainly the fatty acids oleic C18:1 (32.06%) and linoleic C18:2 (39.31%). The oil can be classified in the oleic-linoleic acid group. High unsaponifiable matters content (1.55%) guarantees the use the oils in cosmetics industry.

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