

## Nutritive Composition and Properties Physico-chemical of gumbo (*Abelmoschus esculentus* L.) Seed and Oil

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**Abstract:** Chemical and physical properties of mature gumbo (*Abelmoschus esculentus* L.) seeds from Impfondo, in North Congo-Brazzaville were evaluated. The chemical properties evaluated were moisture, crude protein, crude oil, crude ash, crude fiber, and crude energy. The oil from *A. esculentus* seeds was extracted using two oils extraction methods with petroleum ether (Soxhlet) and extraction with a mixture of chloroform:methanol (1:1) (Blye and Dyer). The oil concentration ranged from 24.90% (Soxhlet) to 21.98% (Blye & Dyer). The minerals, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, unsaponifiable matter content, peroxide value, activation energy and differential scanning calorimetry were determined. *Abelmoschus esculentus* L. seeds have ash content of 5.68% (with the presence of following minerals: Ca, Mg, K and Na). The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 24.89%) and linoleic (up to 42.78%). *Abelmoschus esculentus* L. oil can be classified in the oleic-linoleic acid group. The dominant saturated acid was palmitic (up to 25.79%). *Abelmoschus esculentus* L. seeds were also founded to contain high levels of crude protein (24.85%). The content of insaponifiables is 1.53%. Taking into account these results, the gumbo (*Abelmoschus esculentus* L.) finds its applications in the food and cosmetic industry.

**Key words:** Activation energy, DSC, fatty acid, *Abelmoschus esculentus* L. seeds, nutritive values and viscosity

### INTRODUCTION

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 known to have superior nutritional quality. Rubatzky and Yamaguchi (1997) reported that the seed is a rich source of protein and oil; contains cyclopropenoid fatty acids which cause some toxicity concerns and is used as a substitute for coffee in some countries. In an earlier study, Karakotssides and Constantinides (1975) found that the Protein Efficiency Ratio (PER) of Gumbo seed flour heated at 130°C for 3hr was not different from the nonheated flour, indicating the absence of anti-nutritional factors. According to these authors, the amino acid composition of gumbo seed protein is similar to that of soybean and the PER is higher than that of soybean. The high percentage of linoleic acid (42%) makes okra seed

oil desirable and the amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets (Savello *et al.*, 1982). Recently, the seed has been utilized as a source of oil and protein in the United states of America (Jambhale and Nerkar, 1998). Although mature gumbo seed has a harsh flavour, it can be improved by processing. Okra cheese prepared from mature gumbo seed and bakery products in which wheat flour was substituted with gumbo seed meal at replacement levels of 25 – 100% had acceptable sensory properties (Martin and Ruberte, 1979). Gombo seed is a potential source of protein. The objective of this study was therefore to improve the Nutritive Composition and Properties Physicochemical of gumbo Seed 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 Jan. 5, 2009 to Feb. 27, 2009.

The *A. esculentus* seeds were obtained from cultivated farmlands located at Impfondo, in North Congo. The seeds were removed from the pods, sorted and sun dried. Only seeds that were not damaged were chosen and stored under cool dry storage conditions until needed.

Proximate analysis of *A. esculentus* 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 *A. esculentus* seeds were ground in a Moulinex Model SeB PREP'LINE 850 (Moulin cafe). For solvent extraction (soxhlet 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. For methanol/chloroform extraction (Bligh *et al.*, 1959), 100g of the ground seeds 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 of 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 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<sup>-1</sup> 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<sup>-1</sup> (normal

rate) (Che Man *et al.*, 1995) and 2.5 °C.min<sup>-1</sup> (past rate), 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 (hyperDSC) 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 mPas, is automatically calculated on the basis of the speed and the geometry of the probe. Temperature (20 °C) was controlled with a water bath connected to the rheometer. The experiment was carried out by putting 3 ml of sample in a concentric cylinder system using 100 s<sup>-1</sup> as shear rate.

**Chemical analysis:** Determinations for peroxide, iodine, 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<sup>-1</sup> 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.

**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 *Abelmoschus esculentus* L. seed oil:** Results obtained showed that the seeds contained 9.45% moisture, 23.85% crude oil, 24.85% crude proteins, 36.83% carbohydrate (by difference), 9.7% crude fiber, 5.68% ash and 385.13 Kcal calorific value (Table 1). The moisture, ash, crude oil and protein content were high compared with that of Turkey okra seeds reported by Çalışır *et al.* (2005). Those Variations 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 gumbo (*A. esculentus*) seeds also contained significant amount of important minerals (Table 2). The magnesium concentration (3259.64±7.4 mg/100 g dry matter) was the highest, followed in descending order by phosphorus (1450±5.12 mg/100 g dry matter), potassium (109.76± 4.84 mg/100 g dry matter), calcium (78.65 ± 3.25 mg/100 g dry matter) and sodium (54.78 ± 0.58 mg/100 g dry matter). Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins (Malik, 1982). Ca and Mg play a significant role in photosynthesis, carbohydrate metabolism, nucleic acids (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. 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, 2001).

**Oil extraction:** Table 3 show the results of physical and chemical properties of oil extracted from two methods. The extracted oils were liquid at room temperature. The oil extraction with the Soxhlet method had the highest yield, due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley *et al.*, 1991). The Blye and Dyer method, showed the low yield due to losses during the separation of the two phases, aqueous layer (methanol-water) and

Table 1: Proximate analysis of *Abelmoschus esculentus* oil seed

Characteristic	Obtained values <sup>a</sup>	Reported values <sup>b</sup>
	(M±S.D.)	1
Moisture content (%)	9.45±0.8	6.35
Crude protein <sup>c</sup> (%)	24.85±1.5	19.10
Ether extract (%)	23.44±0.12	20.00
Crude fiber (%)	9.7±0.17	26.34
Ash content (%)	5.68±0.12	4.63
Total carbohydrate <sup>d</sup> (%)	36.83	ND
Calorific value (Kcal/100g)	385.13	25

ND: not determined.

<sup>a</sup> M ± S.D. mean ± standard deviation.

<sup>b</sup> (1) Çalışır *et al.* (2005)

<sup>c</sup> Crude protein = N (%) x 6.25

<sup>d</sup> Carbohydrate obtained by difference

Table 2: Mineral elemental Composition of *Abelmoschus esculentus* seeds

Mineral Elements	Composition (mg/100g) of Seed
Phosphorus , P	1450±5.12
Calcium, Ca	78.65±3.25
Magnesium, Mg	3259.64±7.4
Potassium, K	109.76±4.84
Sodium, Na	54.78±0.58

Values are mean ± S.D of triplicate determinations

Table 3: Physical and chemical properties of *Abelmoschus esculentus* seed oil extracted using solvent process

Properties	Obtained values	
	Blye & Dyer	Soxhlet
Oil <sup>b</sup> (%)	21.98±0.35 <sup>B</sup>	24.90±0.55 <sup>A</sup>
PV	4.13±0.28 <sup>A</sup>	3.21±0.14 <sup>A</sup>
FFA (as % oleic acid)	2.37±0.18 <sup>A</sup>	3.74±0.9 <sup>B</sup>
IV (w/w)	126.4±4.23 <sup>A</sup>	127.2±3.12 <sup>A</sup>
Saponification value	183.1±4.8 <sup>A</sup>	196.3±5.3 <sup>A</sup>
Unsaponifiable matter	1.47±0.02 <sup>A</sup>	1.58±0.07 <sup>B</sup>
Content (%)		
Viscosity (mPa.s) at 20°C	43.5±0.32 <sup>B</sup>	38.2±0.29 <sup>B</sup>
E <sub>a</sub> (KJ. mol <sup>-1</sup> )	12.75	14.07

ND: not determined.

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

<sup>b</sup> Oil = weight of extracted oil x 100/weight of seed.

Abbreviations: PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value.

organic layer (chloroform). The results of this work are in agreement with those of the literature (Camciuc, 1998).

### 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 three components in oil extracted from the two methods. The first peaks at low melting points appear at  $-21.54\text{ }^{\circ}\text{C}$  ( $H_f = +6.16\text{ J.g}^{-1}$ ) for Blye and Dyer method and  $-23.28\text{ }^{\circ}\text{C}$  ( $H_f = +6.05\text{ J.g}^{-1}$ ) for Soxhlet method. Those peaks correspond to triglycerides formed by poly unsaturated acids (PUFA). The second melting points are at  $-2.81\text{ }^{\circ}\text{C}$  ( $H_f = +5.09\text{ J.g}^{-1}$ ) for Blye and Dyer method and  $-1.14\text{ }^{\circ}\text{C}$  ( $H_f = +6.87\text{ J.g}^{-1}$ ) for Soxhlet method. This is a characteristic of mono

Table 4: Melting behaviour of *Abelmoschus esculentus* 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<sup>-1</sup>

Thermogram	5 °C.min <sup>-1</sup>	
	Blye and Dyer	Soxhlet
Peak 1 [°C]	-21.54	-23.28
?H <sub>f</sub> [J.g <sup>-1</sup> ]	+6.16	+6.05
Peak 2 [°C]	-2.81	-1.14
?H <sub>f</sub> [J.g <sup>-1</sup> ]	+5.09	+6.87
Peak 3 [°C]	+4.88	+2.93
?H <sub>f</sub> [J.g <sup>-1</sup> ]	+1.83	+2.18

Table 5: Oil viscosity at various temperature in degree celsius

T (°C)	η (mPa.s)	
	Blye and Dyer	Soxhlet
5	66.35	57.42
10	59.70	49.82
15	54.00	43.92
20	49.10	39.10
25	44.40	35.00
30	40.60	31.92
35	37.40	29.46
40	34.70	27.62
45	32.70	25.92
50	31.50	26.82

unsaturated acids (MUFA). The last peaks appear to +4.88°C (H<sub>f</sub> = +1.83J.g<sup>-1</sup>) for Blye and Dyer method and +2.93 °C (H<sub>f</sub> = +2.18 J.g<sup>-1</sup>) for Soxhlet 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.*, 2007). Viscosity varies between 66.35 and 31.50 mPa.s when temperature decreases of 50 to 5 °C by Blye and Dyer method. By Soxhlet method, the viscosity of oil decreases of 57.42 to 26.82 mPa.s (Table 5). The viscosity of the oil obtained by Blye 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, Ea is the activation energy (in KJ mol<sup>-1</sup>), R is the universal gas

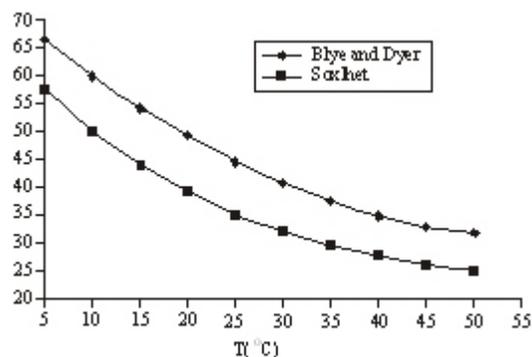


Fig. 1: Effect of temperature on *A. esculentus* seed oil viscosity

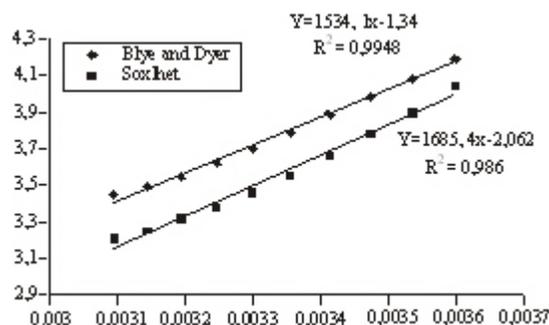


Fig. 2: Relationship between viscosity and temperature for *A. esculentus* seed oil extracted by Blye and Dyer and Soxhlet. Solid line Arrhenius model

Table 6: Energie plot derived from the Arrhenius equation

1/T (K <sup>-1</sup> )	Lnη (mPa.s)	
	Blye and Dyer	Soxhlet
0.00359712	4.19494376	4.05039267
0.00353357	4.08933202	3.90841651
0.00347222	3.98898405	3.78236980
0.00341297	3.89385903	3.66612247
0.00335570	3.79323947	3.55534806
0.00330033	3.70376807	3.46323277
0.00324675	3.62167070	3.38303341
0.00319489	3.54673969	3.31854015
0.00314465	3.48737508	3.25501487
0.00309598	3.44998755	3.21164978

constant and T is the temperature (in degrees Kelvin). R has the value of 8.314 x 10<sup>-3</sup> KJ mol<sup>-1</sup> K<sup>-1</sup>. 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, -E<sub>a</sub>/RT is the slope from which E<sub>a</sub> was evaluated. Activation energies of oils are given in Table 3. The highest value of activation energy is obtained by Blye and Dyer method (12.75 KJ mol<sup>-1</sup>) and 14.07 KJ mol<sup>-1</sup> by Soxhlet method.

**Chemical properties:** The chemical properties of oil are amongst the most important properties that determines the

Table 7: Relative percent composition of fatty acid in *Abelmoschus esculentus* seed oil

Fatty acid	Determined values		Reported values <sup>a</sup>
	Blye & Dyer	Soxhlet	1
C14:0	0.34±1.13 <sup>A</sup>	0.38±1.45 <sup>A</sup>	0.25
C16:0	25.72±0.45 <sup>A</sup>	25.85±0.72 <sup>A</sup>	26.13
C16:1	0.38± 1.18 <sup>A</sup>	0.36±1.12 <sup>A</sup>	0.41
C17:0	-	-	0.15
C17:1	-	-	0.32
C18:0	2.64±0.42 <sup>B</sup>	2.79±0.28 <sup>A</sup>	2.86
C18:1	24.61±0.23 <sup>A</sup>	25.18±0.38 <sup>B</sup>	16.23
C18:2	43.40± 1.53 <sup>A</sup>	42.15± 3.21 <sup>A</sup>	50.62
C18:3	1.86±0.38 <sup>A</sup>	2.25± 1.85 <sup>A</sup>	-
C19:1	-	-	1.40
C20:0	0.42± 1.32 <sup>B</sup>	0.51± 0.85 <sup>A</sup>	0.62
Saturated	29.12	29.53	ND
Unsaturated	70.25	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 ( $P < 0.05$ ).

<sup>a</sup>(1) Camciuc *et al.* (1998).

present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The free fatty acid and the unsaponifiable matter content of the Soxhlet method were significantly higher ( $P < 0.05$ ) than those of the Blye 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 slightly higher value of unsaponifiable matter in the Soxhlet 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:** Fatty acid composition of the two studied seed oils is shown in Table 7. The most abundant fatty acids of gumbo (*A. esculentus*) seed oil were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) which together composed about 93.18% of the total fatty acids. The major saturated fatty acid in *A. esculentus* seed oil was palmitic acid (25.79%); the main unsaturated fatty acid were oleic acid (24.89%) and linoleic acid (42.78%) with small amounts of palmitoleic acid (0.37%). There was no significant difference ( $P > 0.05$ ) in the amounts of

the major fatty acids in the two oil samples. The two oil samples of *A. esculentus* contained saturated and unsaturated acids (29.33% and 70.10%) respectively. *A. esculentus* 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). Unsaturated total fatty acids are prevalent (70.10 %) (Table 7). This prevalence of the unsaturated fatty acids and the high values of the iodine index indicate that the oil of *A. esculentus* in Impfondo (Congo-Brazzaville) is of the unsaturated type. The comparison of the composition in fatty acids of *A. esculentus* seed oil with that of vegetable oils (Table 8) indicates that this plant is rich in acids oleic (C18:1), linoleic (C18:2) and palmitic (C16:0). Table 8 shows that, Gumbo (*A. esculentus*) seed oil has a similar composition to that of cotton seed oil and it may be used as a substitute for cotton seed oil in pharmaceutical formulations (Sengupta *et al.*, 1974). Its high  $\gamma$ -tocopherol content (twice that of soybean oil) favors stability (Karakotssides and Constantinidis, 1975). This oil represents a potential source of palmitic acid, a chemical imported into the European Community, and an important raw material for soaps, esters and plasticizers. It could also improve the quality of soybean oil, which has limitations as a shortening as it only contains around 11% palmitic acid (Karakotssides and Constantinidis, 1975). In addition, the linoleic acid, once separated could be utilized for producing dyes, plastics and resins.

## CONCLUSION

Gumbo (*A. esculentus*) seeds could be considered as good sources of protein and minerals such as phosphorus, magnesium, calcium and potassium. *A. esculentus* seeds oil have high contents of unsaturated fatty acids, it is more balanced than some edible oils such as grape seed oil, corn oil and sunflower oil. High unsaponifiable matters content

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Table 8: Comparison of the profile in fatty vegetable oil acids

Huiles	C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0
Palm	1.0	44.5	0.2	-	4.6	38.7	10.5	0.3	0.3
Safou	-	45.5	-	-	2.8	28	24.9	1.24	-
Maize	-	10.5	-	-	2.5	28	58.5	1.0	0.5
Groundnut	-	10.0	-	-	2.0	46.0	31.0	-	-
Cotton	0.9	23.0	-	-	2.2	17.7	55.8	-	-
Hazel nut	-	7.0	0.1	-	2.0	74.5	16.5	-	-
Soybean	-	11.0	-	-	4.0	22.0	54.3	7.5	-
Jatropha	-	15.6	1.0	-	5.8	40.1	37.6	-	-
Gumbo	0.36	25.79	0.37	-	2.7	24.89	42.78	2.06	0.4

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