



**NUTRITIONAL EVALUATION AND PHYSICO-CHEMICAL  
CHARACTERIZATION OF VEGETABLE OIL EXTRACTED FROM  
*HIBISCUS SABDARIFFA SABDARIFFA* SEEDS PRODUCED IN BENIN**

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**ABSTRACT**

**Background and aim:** *Hibiscus sabdariffa sabdariffa* is a non-wood forest product found in sub-Saharan Africa for its use in pharmacopoeia and food. By the determination of the physical and chemical characteristics of the vegetable oil extracted from the seeds of *Hibiscus sabdariffa sabdariffa*, this study aims to contribute to the establishment of a viable food chain through the food valuation of the seeds of *H. sabdariffa sabdariffa* produced in Benin. **Methods:** Several quality indices were evaluated according to specific standards and the fatty acids profile was established. **Results:** The results of the physicochemical characterization of the vegetable oil extracted from the seeds of *H. sabdariffa sabdariffa* revealed an insoluble impurities content of 1.185% and unsaponifiable matter content of 0.67%. The quality indices of the vegetable oil extracted from the seeds were respectively: 15.87 mg/g of oil for the acid value; 212.08 mg KOH/g

for the saponification value; 5.82 mEq of O<sub>2</sub>/kg for the peroxide value and 52.57 g/100g for the iodine value. In addition, this oil density is 0.887 kg/l and its refractive index is 1.482. Moreover it was noticed a richness in fatty acids such as palmitic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) with cover together 98.2% of total fatty acids content with un higher content in unsaturated fatty acids which allow its use both in cosmetics and food industries.

**KEYWORDS:** Physicochemical characteristics, *Hibiscus sabdariffa sabdariffa*, vegetable oil, fatty acids profile, Benin.

## 1. INTRODUCTION

Vegetable oils are an important part of humans diet and contain many essential nutrients namely vitamins and Essential Fatty Acids (EFA).<sup>[1]</sup> Humans being unable to manufacture them, they must be brought by food, in particular by the vegetable oils.<sup>[2]</sup> 90% of the world production of vegetable oils are obtained from a small number of oilseeds such as palm, soybean, rapeseed, sunflower, cotton, peanut, olive etc. that the producers and markets are actively looking for their nutritional properties, their textures, their heat resistance and their economic importance.<sup>[3]</sup> Moreover, the evolution of knowledge about the impact of diets on health, the need to conserve biodiversity are among other reasons that lead us to adapt, change diets and take new eating habits. Since the oilseed market is continuously growing, the seeds of *Hibiscus*, *Hibiscus sabdariffa* presents a good scientific interest because of their availability and their underutilization. Indeed global oilseed consumption increased from 392.0 Mt in 2013 to 444.5 Mt in 2018 with an increase of 13.4% over the 2013 level and a forecasts of 489.6 Mt for 2022 indicate an increase in world oilseed demand of 24.9% compared to the 2013 level.<sup>[4]</sup> In view of the above, the main objective of this study is to propose an alternative to oilseed market through the characterization and the valorization of the oil extracted from the seeds of this non-timber forest product produced in Benin, in order to establish a viable food chain. The determination of the fatty acid profile of the vegetable oil extracted from the seeds of *H. sabdariffa sabdariffa* and the assessment of its edibility are prospects to be explored.



Figure 1: Chalices of *H. sabdariffa sabdariffa*.

## 2. EXPERIMENTAL

### 2.1. Materials

#### 2.1.1. Seeds of *Hibiscus sabdariffa sabdariffa*

The harvest of the analyzed of the chalices was carried out at Lobogo on the Valley farm in the Mono department of Benin in December 2017. The hulls were then removed from chalices and their seeds were then separated from the hulls and dried for one month at 25°C away from sun and humidity. These dried seeds were pulverized with an electric grinder and the resulting powder was stored in sealing bottles under a hood for later use.



Figure 2: Seeds of *H. sabdariffa sabdariffa*. Figure 3: Powder of *H. sabdariffa sabdariffa*.



**Figure 4: Vegetable oil of *H. sabdariffa sabdariffa*.**

### **2.1.2. Extraction of *Hibiscus sabdariffa sabdariffa* vegetable oil**

The extraction of the vegetable oil was carried following Benin standard NB ISO 659 2009. The extraction of the seed powder was performed with hexane using a Soxhlet for 6 hours at  $69^{\circ} \pm 1C$ . After the extraction, the solvent was recycled in a rotavapor and the crude vegetable oil obtained was dried in an oven for 20 min at  $103^{\circ}C$ , cooled in a desiccator for 30 min and weighed (Photo 2-3). The extracted oil was then packaged in dark bottles for later use.<sup>[5,7]</sup> The seed oil content expressed as a percentage (w/w) of dry matter was obtained by equation (1).

$$O_c = (W_2 - W_1) * 100 / W_0 \quad (1)$$

Where

$w_0$ : weight of the sample (g)

$w_1$ : weight of the empty vacuum flask (g)

$w_2$ : weight of empty flask + oil (g)

$w_2 - w_1$ : weight of oil obtained (g)

$O_c$ : vegetable oil content (% m/m)

## 2.2. Physico-chemical parameters of the vegetable oil extracted from the seeds of *H. sabdariffa sabdariffa*

### 2.2.1. Density

The density was evaluated using standard 6883:2017 (T60-214).<sup>[8]</sup> The principle is based on the measurement of the weight, at room temperature, of a volume of oil contained in a pycnometer which will be calibrated beforehand at the same temperature. It is expressed in grams per mL or kilogram per liter. The density is given by formula 2:

$$d = (W_3 - W_1) / (W_2 - W_1) \quad (2)$$

Where

d: Oil density (kg / L) à 30°C

W<sub>1</sub>: weight of the empty pycnometer (g).

W<sub>2</sub>: weight of the pycnometer filled with distilled water (g).

W<sub>3</sub>: weight of the pycnometer filled with oil (g).

W<sub>3</sub> - W<sub>1</sub>: weight of oil (g)

W<sub>2</sub> - W<sub>1</sub>: weight of distilled water (g)

### 2.2.2. Refractive index

The refractive index of the vegetable oil extracted from *H. sabdariffa sabdariffa* was determined following to the Beninese standard NB ISO 6320: 2006.<sup>[6]</sup> For this purpose the prisms of the refractometer are cleaned with petroleum ether and wiped with a clean and soft handkerchief. Then 2 to 3 drops of oil are deposited between the prisms. The telescope is then moved so that the separation line of the clear and the dark range is at the crossroads of the reticle wires. The refractive index of the oil at room temperature is then read. The refractive index at 20°C is obtained by formula 3.

$$RI_{20^\circ C} = I^t + 0.00035 (T - 20) \quad (3)$$

Where:

RI<sub>20°C</sub>: Refractive index at 20°C

I<sup>t</sup>: Index read on the refractometer

T: temperature at which the test was performed

### 2.2.3. Acid value ( $A_v$ )

The acid number was determined according to Beninese standard NB ISO 660: 2006.<sup>[10]</sup> 2g of powder of *H. sabdariffa sabdariffa* oil were weighed in a 250 ml flask and dissolved in 100 ml of neutralized a mixture of ethanol-diethyl ether (1:1 v/v). 1.0ml of a phenolphthalein solution was used as indicator. The mixture was titrated with 0.1N ethanolic potassium hydroxide solution until a persistent phenolphthalein rose color for at least 10 seconds. The acid value was determined by equation 4 and was expressed in mg KOH/g oil.

$$A_v = (V * 56.1 * N) / W \quad (4)$$

Where:

V: volume of KOH used (mL).

N: Normality of the KOH solution (0.1N).

W: weight of the sample (g)

W (KOH) = 56.1 (g/mol)

### 2.2.4. Peroxide value ( $P_v$ )

The peroxide number was determined according to standard 3960:2017 (NF T 60-220: 1995).<sup>[11]</sup> 2g of oil were weighed into a vial. 1ml of potassium solution and 25ml of a mixture of chloroform and glacial acetic acid (2:3 v/v) were added. After allowing the closed mixture to stand for exactly 5 minutes protected from light at room temperature (15-25°C), 75ml of distilled water was added. The liberated iodine was titrated slowly by a solution of sodium thiosulfate  $Na_2S_2O_3$  (0.01 N) by with continuous vigorous shaking. A starch paste solution was used as an indicator. A blank test was performed simultaneously.

The peroxide index (PI), expressed in milli-equivalents of active oxygen per kilogram of oil was obtained by formula 5:

$$P_v = 10 * N * (V_0 - V_1) / W \quad (5)$$

Where

N: normality of thiosulfate solution

$V_0$ : volume of sodium thiosulfate used for the blank test (ml),

$V_1$ : volume of sodium thiosulfate used for titration (ml),

W: weight of vegetable oil (g).

IP: peroxide index in milliequivalent of active oxygen/kg (mEq  $O_2$ /kg)

### 2.2.5. Saponification value

Saponification value was obtained according to Beninese standard NB ISO 3657: 2006.<sup>[12]</sup> 2g of oil and 25ml of alcoholic potassium hydroxide (0.5N) was boiled with stirring for one hour under a reflux condenser. 1ml of phenolphthalein was added to the mixture and the dosage of this mixture was carried out with a standard solution of hydrochloric acid (0.5 N) until the disappearance of the pink color of the indicator (titration of excess alcoholic KOH). A blank test was carried out under the same conditions to titrate the alcoholic liquor of potash.

The saponification value was determined by the following formula (6):

$$Sv = ((V_1 - V_2) * N * 56.1) / W \quad (6)$$

Where

V<sub>1</sub>: Volume of the standard solution of hydrochloric acid used for the blank (ml),

V<sub>2</sub>: Volume of the standard solution of hydrochloric acid required for the sample (ml),

N: Normality of the standard solution of hydrochloric acid.

W: Weight of the test sample (g).

Sv: Saponification value in mg KOH /g

W (KOH) = 56.1 g/mol.

### 2.2.6. Iodine value

Iodine value of the oil extracted from the seed of *H. sabdariffa sabdariffa* was evaluated according to Beninese standard NB ISO 3961: 2006.<sup>[11]</sup> For this purpose, to 0.10g of oil 15ml of chloroform and 25ml of Wijs reagent were added to dissolve the fat. The flask containing the mixture was capped, stirred gently and placed in a dark place for at least 2 hours with stirring. A blank was prepared in the same conditions but without the sample. Then, were added to the mixture 20ml of the 100g/L potassium iodide solution and 150ml of distilled water in each flask plus a little starch paste. Titration was carried out with the titrated solution of 0.1mol/l sodium thiosulfate contained in the burette until the mixture was discolored. Iodine value was obtained according to formula 7.

$$Iv = (12.69 * N * (V_1 - V_2)) / W \quad (7)$$

Where

N: normality of the sodium thiosulfate solution.

V<sub>1</sub>: volume of the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution for the blank (ml).

$V_2$ : volume of the  $\text{Na}_2\text{S}_2\text{O}_3$  solution used for the sample (ml).

W: weight of the sample (g).

### 2.2.7. Insoluble impurities content

Insoluble impurities content was evaluated according to Beninese standard ISO 663: 2006.<sup>[13]</sup>

To 10 g ( $W_0$ ) of sample, 200ml of hexane were added. Then the flask was capped, stirred and allowed to stand for 30 min at room temperature. A filter paper and a Buchner were pre-dried in an oven at  $103^\circ\text{C}$  for 10 min, desiccated and weighed ( $W_1$ ). The mixture was filtered using the filter paper placed in the Buchner. The filter paper was washed using hexane so that the last filtrate was free of fat. The filter paper was then dried in an oven for 1 hour at  $103^\circ\text{C}$ , cooled in a desiccator and weighed ( $W_2$ ).

The content of insoluble impurities, expressed as a percentage of weight, was obtained according to formula 8:

$$II_c = (W_2 - W_1) * 100 / W_0 \quad (8)$$

Where:

$W_0$ : mass of the test sample (g).

$W_1$ : mass of the filter paper (g).

$W_2$ : mass of the filter paper + the dry residue (g).

### 2.2.8. Unsaponifiable matters

The unsaponifiable matters was evaluated using Beninese standard NB ISO 3596, 2006.<sup>[14]</sup>

This assay is carried out by stirring a solution of alcoholic potash. 5 grams of oil are saponified for one hour boiling with 50ml of normal alcoholic potassium hydroxide (1mol/L). After cooling, an equal volume of water is added, the mixture is placed in a separatory funnel and stirred vigorously with boiling petroleum ether. After separating the two layers, the lower hydro-alcoholic layer is passed through a second funnel and stirred again with petroleum ether. It is repeated until the solvent leaves no more visible residue by evaporation.

The combined petroleum ether mixtures are stirred twice with  $20 \text{ cm}^3$  of 50% alcohol plus a little alkali. The majority of the petroleum ether mixture is then distilled, the residue is passed through a tared glass capsule and then evaporated at constant weight, and weighed. The titration is carried out with a solution of potassium hydroxide (0.1mol/L). The unsaponifiable content expressed as a percentage by weight of the sample was given by the formula 9.

$$I_m (\%) = 100(W_1 - W_2 - 0.28V * C) / W_0 \quad (9)$$

Where

$W_0$ : mass of the sample (g).

$W_1$ : mass in g of the residue (g).

$W_2$ : residue obtained with white (g).

$V$ : volume of potassium hydroxide used for titration (mL).

$C$ : concentration of potassium hydroxide (mol / L).

### 2.3. Determination of fatty acid composition by GC-MS

The fatty acid composition of the seed oils of the two varieties of *Hibiscus sabdariffa* has been determined by coupling gas chromatography with mass spectrometry (GC/MS). Chromatographic analysis was performed on Trace GC Ultra equipped with an ASI 3000 autosampler and with Polaris Q spectral mass detector, all from Thermo Fischer Scientific Ultra Brand. The coupling and the automatic control of the devices have been done by the software EXCALIBUR 2.0 Thermo Fisher. The Split/splitless injector was set at 250°C and the ion source temperature set at 250°C. Ultrapure Helium Alpha-gas 2 was the gas carrier set at 1ml/min constant flow with automatically adjusted pressure. Injections were on split mode. GC was fitted with a fused silica capillary column (DB-FFAP) 30m x 0.25mm inner diameter (ID) x 0.25µm film thickness (J & W Scientific, Agilent Technologies). Initial oven temperature was 130° C. The program temperature was as follows: equilibration time: 0.5 min; linear increase to 178°C at 4°C /min, followed by linear increase to 210°C at 1°C/min, followed by an increase to 245°C at 40°C/min and final 13min hold. The duration of the analysis was 60min. The injected volume was 1µL and the injected amount 10µg/mL. Positive ionisation of the FAMES was performed by electronic impact (EI), with 70 eV energy and full scan detection mod. Mass spectra range was 50-650 m/z; scan 0.58 sec. Precise identification of the analytes was achieved by their relative retention times and mass spectra on the spectral mass database NIST libraries for fatty acid composition.

## 3. RESULTS AND DISCUSSION

### 3.1. Vegetable oil content

The oil extracted from *H. sabdariffa sabdariffa* is greenish in color. The oil content (13.6%) of *H. sabadariffa sabdariffa* (Table 1) obtained in the current study is closed to the one from other sample from Benin (14.4%),<sup>[15]</sup> but is lower than that obtained with the West Africa samples originated respectively from Nigeria (17.8%)<sup>[16]</sup> and Côte d'Ivoire (24.5%),<sup>[17]</sup> with

samples from other African countries such as Sudan 21.1%<sup>[18]</sup> and Egypt 23.3%;<sup>[19]</sup> or from Saudi Arabia (19.4%) and India (23.8%). However, the oil content obtained in the current study is included in the range of 11.8 to 22.1% predicted by Holser and Bost (2004)<sup>[20]</sup> for natural *Hibiscus* species. Differences observed should be attributed to the soil characteristics, the harvest period or the type of plant material treatment before the extraction of the vegetable oil.

Compared to other oilseed oils, the present oil content is lower than the one obtained with dry soybeans (20.0%),<sup>[21]</sup> groundnut (41.0%),<sup>[22]</sup> sesame (45-52%)<sup>[23]</sup> and closed to cottonseed oil content which is 10-16%.<sup>[24]</sup> In view to its oil content, cottonseeds is at the penultimate position in the list of major oilseeds, just in front of corn. Despite the low global production of cottonseed oil (3.3%) and its weak international trade (only 3%), its price on the world market is roughly the same as those of sunflower and corn oils, and a little more expensive than palm oil and soy. As the prices of edible oils are a function of their availability on the market and their trade policies,<sup>[24]</sup> such as cottonseed oil, the underutilized seeds of *H. sabdariffa sabdariffa* could become an excellent source of edible oil production,<sup>[18]</sup> with a viable global agro-food chain, for this non-timber forest product.

### 3.2. Physico-chemical parameters of the vegetable oil

#### 3.2.1. Density

The density of an oil is one of the criteria of its purity which indicates the possible presence of foreign bodies.<sup>[2]</sup> The density of the vegetable oil extracted from the seeds of *Hibiscus sabdariffa sabdariffa* is 0.887 kg/L at 30°C (Table1). This density is lower than the one from Egypt sample (0.917 kg/L at 25°C). This value is lower than the requirements of groundnut oil (0.909-0.920), cottonseed oil (0.918-0.926) at 20°C and palm seed oil (0.889-0.895) at 50°C. The low density value obtained in the current study may be in accordance with the higher temperature at which it was determined in this study. That could also indicate the presence of impurities and therefore be an indicator of a high level of insoluble impurities when extraction of the vegetable oil is carried out with a solvent.<sup>[2]</sup> Therefore it will be better to favor softer extraction methods such as cold extraction to increase the oil quality and ensure quality requirement of non-conventional vegetable oils.

**Table No 1: Physicochemical properties of Roselle seed oil and some conventional and non-conventional oils.**

| Parameters   | HSS oil*      | Oils not covered by individual standards <sup>[25]</sup> | Groundnut oil <sup>[22,26]</sup> | Cottonseed oil <sup>[22,26]</sup> | Palm seed oil <sup>[22,26]</sup> |
|--|---------------|--|----------------------------------|-----------------------------------|----------------------------------|
| Oil content  | 13.6 %        | NA   | 41%                              | Unlimited <sup>[26]</sup>         | 21-23%                           |
| Density kg/l   | 0.887 at 30°C | NA   | 0.909-0.920 (20°C)               | 0.918-0.926 (20°C)                | 0.889-0.895 (50°C)               |
| Refractive index                                       | 1.482 (30°C)  | NA   | 1.460-1.465 (40°C)               | 1.458-1.466 (40°C)                | 1.454-1.456 (50°C)               |
| Acid value (mg KOH/g)                                  | 15.87         | 4.0  | NA                               | NA                                | NA                               |
| Peroxide value (meq d <sup>3</sup> O <sub>2</sub> /kg) | 5.82          | ≤10  | NA                               | NA                                | NA                               |
| Saponification value mg KOH/g                          | 212.08        | NA   | 187-196                          | 189-198                           | 190-209                          |
| Iodine value g/100 g                                   | 52.57         | NA   | 77-107                           | 100-123                           | 50.0-55.0                        |
| Insoluble impurities (% w/w)                           | 1.185         | 0.05   | NA                               | NA                                | NA                               |
| Unsaponifiable matter g/kg                             | 6.7           | NA   | ≤10                              | ≤15                               | ≤12                              |

\*HSS: *H. sabdariffa sabdariffa*; ND: value not available

### 3.2.2. Refractive index

The refractive index is an indicator of its purity and its fatty profile. The present study reveals a refractive index of 1.482 at 30°C (Table 1). This value is comparable to that found by Mohamed and al. on samples from Egypt (1.477) at 40°C,<sup>[28]</sup> Sudan (1.447)<sup>[18]</sup> and slightly higher than the refractive index of groundnut oil at 40°C (1.460-1.465).<sup>[26]</sup> This refractive index value demonstrates that the vegetable oil extracted from the seeds of *H. sabdariffa sabdariffa* is less drying than crude peanut and cottonseed oils and reflects its high degree of unsaturation.<sup>[29]</sup>

### 3.2.3. Acid value

The acid value defines the quality of the oil and is an indicator to its the purity and stability at room temperature.<sup>[2]</sup> The value of the acid value obtained (15.87 mg KOH/g of oil) is about 4 times higher than the recommended value for oils not covered by individual standards (4 mg of KOH/g of oil)<sup>[25]</sup> but lower than the one obtained by Akabassi *et al.* (2016) on *Hibiscus sabdariffa sabdariffa* seeds from Benin (47.88 mg KOH/g oil)<sup>[15]</sup> (Table 1). Samples from other countries leads to acid values respecting the rules of the oils not covered by the individual standards: Ivory Coast (2.80 mg KOH/g),<sup>[17]</sup> Sudan (3.57 KOH/g),<sup>[18]</sup> Nigeria (0.80 mg KOH/g).<sup>[16]</sup> This acid value is also higher than required value for cottonseed oil (10 mg KOH/g),<sup>[30]</sup> soybean oil (3-6 mg KOH/g)<sup>[21]</sup> and sesame oil (2-6 mg KOH/g).<sup>[23]</sup> This high acid number point out the possible alteration of the fruit on the tree after ripening, harvest conditions and seed storage. This value is also an indicator of the presence of a large quantity

of free fatty acids (Table 2) and would translate the low stability of this oil at room temperature, and it ease to rancidity especially during storage.

### 3.2.4. Peroxide value

The peroxide value of a vegetable oil is related to the conditions of conservation and the methods of extraction. It is a very useful criterion and of a satisfactory sensitivity to appreciate the first stages of an oxidative deterioration.<sup>[31]</sup> The peroxide value found in this study is 5.82 meq O<sub>2</sub>/kg oil (Table 1). This value is very closed to that obtained with samples from Sudan (4.6 meq O<sub>2</sub>/kg)<sup>[18]</sup> and Nigeria (5.0 meq O<sub>2</sub>/kg)<sup>[16]</sup> and Ivory Coast (5.33 meq O<sub>2</sub>/kg).<sup>[17]</sup> It meets the requirements for oils not covered by individual standards ( $\leq 10$  meq O<sub>2</sub>/kg)<sup>[25]</sup> and covered by individual standards ( $\leq 10$  meq O<sub>2</sub>/kg).<sup>[22]</sup> Since the solvent extraction was conducted with heating, hot inducing possible thermal oxidation reactions may occur with formation of peroxides. This low peroxide value is the indication to the absence of peroxide in this oil and its richness in natural antioxidant such as tocopherols, sterols and carotenoids.<sup>[28,32]</sup>

### 3.2.5. Saponification value

The saponification value is related to the length of the fatty acids profile of a vegetable oil and an indicator to their weights. The saponification value revealed by the present study (212.08 mg of KOH/g) (Table 1). This saponification value is higher than the results obtained by Zoué *et al.* (2012) (189.23 mg KOH/g); of Betiku *et al.* (2016) (1197.75 mg KOH/g) and Eltayeib *et al.* 2014 (189.7 mg KOH/g) respectively on samples from Ivory Coast, Nigeria and Sudan (Table 1). It is also higher than value required for palm oil (190-209 mg of KOH/g), groundnut oil (187-196 mg KOH/g) and soybean oil (189-198 mg KOH/g).<sup>[22,26,30]</sup> This high saponification value indicated that it contained a high concentration of triglycerides<sup>[16]</sup> and could be recommended for the manufacture of soap and shaving creams.

### 3.2.6. Iodine value

The iodine value shows the degree of unsaturation of the oil. It's an assessment of how easy it is to be rancid. More unsaturated an oil is, and higher is its iodine value.<sup>[18,33]</sup> The value of the iodine value found in this study (52.57 g/100g) (Table 1) is lower than values reported for with Sudan sample (119.00 g/100 g),<sup>[18]</sup> Ivory Coast sample (118.57 g/100 g)<sup>[17]</sup> and Nigeria sample (97.77 g/100 g).<sup>[16]</sup> However, this value is closed to value required for palm oil (50.0-55.0 g/100 g), Cottonseed oil (100-123 g/100 g) and groundnut oil (77-107 g/100 g)<sup>[34]</sup> requirements. This low iodine value indicates an unsaturated degree comparable to the one of

palm oil a lower food quality compared to groundnut and cottonseed oils. According to its iodine value it possible to classify the vegetable oil extracted from the seeds of *H. sabdariffa sabdariffa* as non-drying (value <95-100).<sup>[29,35]</sup> Indeed, a drying oil is able to dry in the open air when it is deposited in a thin layer, hence its use in the paint and varnish industry due to the presence of linolenic and linoleic acid.<sup>[33]</sup> On the contrary, non-drying oils such as the oil extracted from the seeds of *H. sabdariffa sabdariffa*, remain liquid at ambient temperature, form no film in contact with air, and can be used in cosmetics, specifically for normal and dry skins, and are the best base oils for massage.

### 3.2.7. Insoluble impurities content

The content of insoluble impurities is the amount of dust and other foreign matter insoluble in n-hexane or petroleum ether under specified conditions.<sup>[36]</sup> The value of the content of insoluble impurities found in this study 1.185% (Table 1), is higher than recommended values for olive oil (0.05% w/w),<sup>[37]</sup> edible fats and oils not covered by individual standard (0.05 % w/w),<sup>[25]</sup> and cottonseed oil (0.05-1.00% w/w). This high level of insoluble impurities confirms the low value (0.887 kg/L) of its relative density. Therefore for food use, the refining of this oil or a change in the extraction method such as cold extraction is mandatory. Since the refining of the oil involves the use of soda, it would be important to avoid traces of soap in the refined oil.<sup>[38]</sup>

### 3.2.8. Content of unsaponifiable matter

The unsaponifiable matter content (6.7 g/kg) (Table 1) is in accordance with the recommended values for soybean oil ( $\leq 15$  g/kg), sesame oil ( $\leq 20$  g/kg), groundnut oil ( $\leq 10$  g/kg) and cottonseed oil ( $\leq 15$  g/kg).<sup>[22,34]</sup> This relatively high value confirms the assumption made for the high value of the peroxide index (5.82 mEq of O<sub>2</sub>). Indeed, the vegetable oil of *H. sabdariffa sabdariffa* would be rich in antioxidant substances such as tocopherols and tocotrienols ( $\alpha$ ,  $\beta$  tocopherols), carotenoids ( $\beta$  carotene) ...which are part of unsaponifiable materials.<sup>[3,28]</sup>

### 3.3. Fatty acids composition

Fatty acids profiles of *H. sabdariffa sabdariffa* seeds oil (Figure 6, Table 2), highlighted the presence of four major fatty acids namely linoleic (45.66%), oleic (27.55%), palmitic (20.5%) and stearic acids (4.49%). The average of these four fatty acids were about 98.2% of total fatty acids in *H. sabdariffa sabdariffa* seeds oil. This fatty acids profile indicated that this oil is highly unsaturated, with a fatty acids profile very closed with the one from the

Nigeria sample<sup>[16]</sup> which fatty profile was as follows : linoleic (44.39%), oleic (32.06%), palmitic (12.68%) and stearic acids (10.87%). Indeed, in the current study, the total unsaturated fatty acid composition covered 74.02% with 27.74% for mono-unsaturated and 46.28% for poly-unsaturated fatty acids. This result is a good agreement with the previous data from Nigeria, Saudi Arabia and India, with a ratio of saturated and unsaturated fatty acids of 1:3.<sup>[16,39,41]</sup> In contrast with samples from Egypt<sup>[19]</sup> and ivory Coast<sup>[17]</sup> which ratio of saturated and unsaturated fatty acids is respectively 1:2 and 1:1. It follows that the high content of unsaturated fatty acids especially oleic acid and linoleic acid and the low saturated content of fatty acids classify this oil as healthy oil for human.<sup>[40,41]</sup> Moreover this oil is belonging to linoleic-oleic vegetable oil with a higher percentage in linoleic acid.

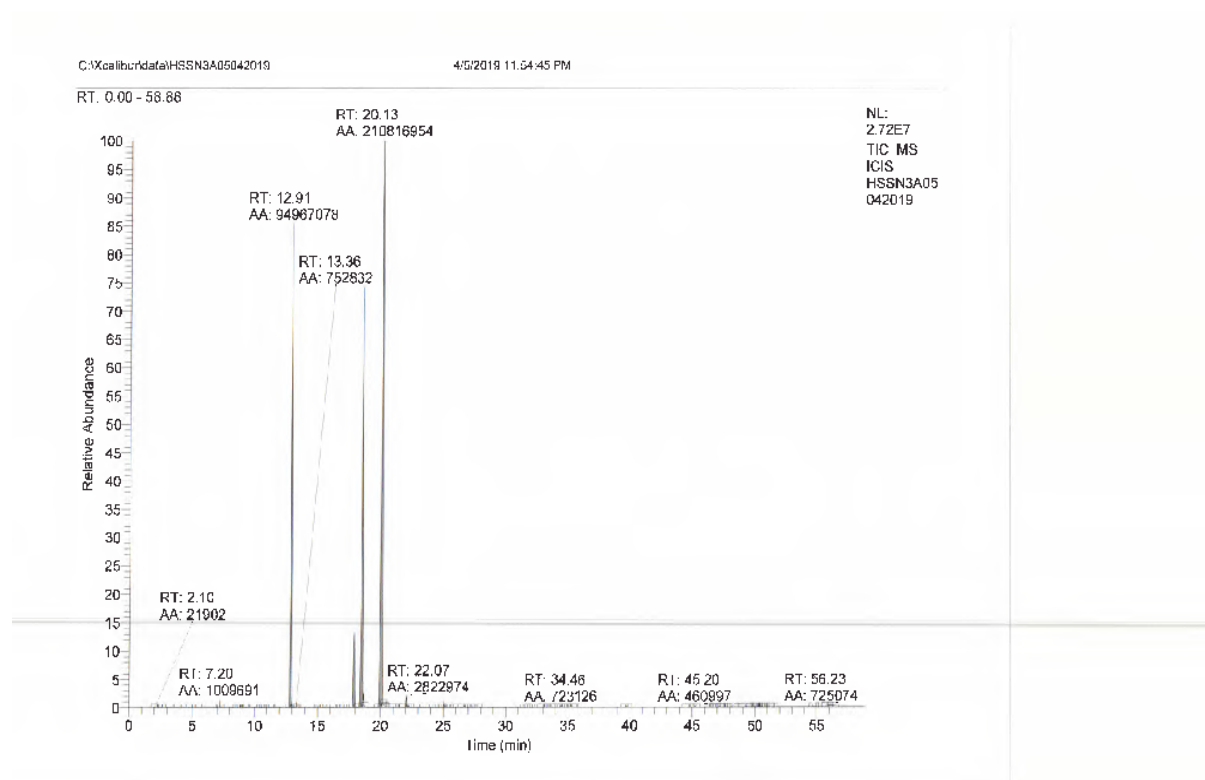


Figure 5: Gas chromatograms of fatty acids profile of *H. sabdariffa sabdariffa* seed oil.

**Table No 2: Fatty acids composition of *H. sabdariffa sabdariffa*, groundnut and cottonseed oils.**

| Fatty acids                    | HSS seed oil          |                    | Groundnut oil <sup>[34]</sup> | Cottonseed oil <sup>[34]</sup> |
|--------------------------------|-----------------------|--------------------|-------------------------------|--------------------------------|
|                                | Average (%), Mean n=3 | Standard deviation |                               |                                |
| Tridecanoic C13:0              | 0,21                  | 0,03               | ND                            | ND                             |
| C14:0                          | ND                    | ND                 | ND                            | 21.4-26.4                      |
| C15:1                          | 0.01                  | 0,01               | ND                            | ND                             |
| C16:0                          | ND                    | 0,48               | 5.0-14.0                      | ND                             |
| <b>Palmitic (C17:0)</b>        | <b>20,5</b>           | 0,48               | ND                            | ND                             |
| Palmitoleic (C17:1)            | 0,16                  | 0,01               | ND                            | ND                             |
| <b>Stearic (C18:0)</b>         | <b>4,49</b>           | 0,05               | <b>1.0-4.5</b>                | <b>2.1-3.3</b>                 |
| <b>Oleic (C18:1)</b>           | <b>27,55</b>          | 0,34               | <b>35.0-80.0</b>              | <b>14.7-21.7</b>               |
| <b>Linoleic (C18:2)</b>        | <b>45,66</b>          | 0,12               | <b>4.0-43.0</b>               | <b>46.7-58.2</b>               |
| Linolenic (18:3)               | 0,62                  | 0,02               | ND                            | ND                             |
| Arachidic (C20:0)              | 0,41                  | 0,03               | 0.7-3.7                       | 0.2-0.5                        |
| Gondoic (C20:1)                | 0,02                  | 0,03               | ND                            | ND                             |
| Béhénic (C22:0)                | 0,16                  | 0,01               | 1.5-4.5                       | ND                             |
| STD (C23:0)                    | 0,1                   | 0,01               | ND                            | ND                             |
| Lignoceric (C24:0)             | 0,1                   | 0,01               | 0.5-2.5                       | ND                             |
| <b>Total identified</b>        | <b>99,99</b>          |                    |                               |                                |
| <b>Saturated fatty acids</b>   | <b>25.97</b>          |                    |                               |                                |
| <b>Unsaturated fatty acids</b> | <b>74.02</b>          |                    |                               |                                |

ND - not détectable

#### 4. CONCLUSION

The present study focuses on the determination of the physico-chemical characteristics of the vegetable oil extracted from the seeds of *Hibiscus sabdariffa sabdariffa* from Benin and the determination of its fatty acids profile. Results obtained revealed the possibility to use this oil as a new alternative to conventional vegetable oil. Physico-chemical characterization have brought out its possible use in several industrial and food applications. The fatty acids profile revealed a very high percentage of unsaturated fatty acids which make it healthy for human.

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