

*Full Length Research Paper*

# **Comparative study of fatty acid composition and nervonic acid contents of four tropicals plants: *Ricinodendron heudelotii*, *Cyperus esculentus*, *Citrullus colocynthis* and *Irvingia gabonensis* from Côte d'Ivoire**

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This work was carried out to compare the potential applications of *Ricinodendron heudelotii*, *Cyperus esculentus*, *Citrullus colocynthis* and *Irvingia gabonensis* oil seeds by investigating their physicochemical parameters. Physicochemical parameters of the extracted oils were respectively as follow: Refractive index (1.49; 1.46; 1.45 and 1.44), acid value (1.35±0.02; 1.87 ± 0.45; 13.85 ± 0.02 and 39.04 ± 0.34 mg KOH/g), peroxide value (37.98 ± 0.01; 86.16 ± 0.32; 48.77 ± 0.22 and 2.34 ± 0.31 meq O<sub>2</sub>/kg), iodine value (142.12 ± 0.34; 75.46 ± 0.35; 120.45 ± 0.29 and 07.42 ± 0.3 g I<sub>2</sub>/100 g), saponification value (154.67 ± 0.28; 175.47 ± 0.30; 175.64 ± 0.28 and 176.03 ± 0.50 mg KOH/g), unsaponifiable matter (1.88 ± 0.04; 0.88 ± 0.03; 0.74 ± 0.03 and 1.26 ± 0.05%), vitamin A (0.00 ± 0.00; 2.78 ± 0.07; 3.66 ± 0.04 and 7.17 ± 0.32 µg/g), cholesterol (0.00 mg/g) for *R. heudelotii*, *C. esculentus*, *C. colocynthis* and *I. gabonensis*. P-anisidine value (301.17 ± 0.98; 24.47 ± 0.03; 29.04 ± 0.03 and 0.39 ± 0.00). *R. heudelotii* and *C. colocynthis* seeds showed relatively high content of linoleic acid (about 26.61 and 53.09% of total fatty acids). The nervonic acid content of the *R. heudelotii* seed is 45.24 ± 0.02 of total fatty acids. All these interesting characteristics should arouse attention for the usage of these oils seeds in food, pharmaceutical, cosmetic industries and biodiesel. These results suggest that they can be good for table, cooking and frying oils. The high linoleic acid level makes them good oils for the fight against cardiovascular illnesses. In addition, the high content of nervonic acid in *R. heudelotii* oil could be used in cases of diseases involving demyelination, such as adrenoleukodystrophy and multiple sclerosis where there is a considerable reduction in the levels of nervonic acid in the sphingolipids.

**Key words:** Acid nervonic, fatty acids, oils seeds.

## **INTRODUCTION**

The need to maintain good health is the driving force in the search for alternative oil seeds of spices with high

medicinal and nutritional potentials. Spices, depending on the part of the plant being used can be classified into

fruits, seed, leaves or floral parts and bulbs used to season food due to their distinctive flavor and aroma (Betti et al., 2009), as well as for therapeutic purposes. Many oils and fats for human consumption or for industrial purposes are derived from plants. Indeed, seeds constitute essential oil reserves of nutritional, industrial and pharmaceutical importance (Combe and Boue-Vaysse, 2004). Extracted oils from plant seeds are mainly composed of triacylglycerols (95 to 98%) which are esters of glycerol and complex mixtures (2 to 5%) of minor compounds (Aluyor et al., 2009). Those minor compounds include fat soluble vitamins, pigments such as chlorophylls and carotenoids, phenolic compounds, phospholipids, mono and diacylglycerols and free fatty acids (Kamal-Eldin, 2013). The fatty acids composition determine the physical properties, stability, and nutritional value of lipids (Hedren et al., 2002). This nutritional value is linked to essential fatty acids (EFA) that are polyunsaturated fatty acids (PUFA). These compounds are essential for the human nutrition because, they are unable to be physiologically synthesized. In this respect, diet must cover organism needs (Naudet et al., 1992). Among PUFA, the most important families are the well-known  $\omega_3$  (n-3) and  $\omega_6$  (n-6) ones (Alais and Linden, 2009). These families are similar as they both comprise a precursor, namely linoleic acid (LA) for the  $\omega_6$  and linolenic acid (ALA) for the  $\omega_3$  (Dubois et al., 2007). EFA are used as substrates to synthesize a number of biologically active compounds such as steroid hormones, prostaglandins and leukotrienes (Nukhet et al., 2001). A part from the leukotrienes and prostaglandins structuring, the ratio of n-6/n-3 fatty acids has an essential effect on cardiovascular health (Wijendran and Hayes, 2004). Also, PUFA are essential for highly excitable membranes such as the brain and nervous tissues because of their role in membrane fluidity (Anhwange et al., 2010). An  $\omega_6$  fatty acid deficiency is characterized by growth retardation in children, skin lesions, dry scaly dermatitis and reproductive failures (Anhwange et al., 2010). However, cognitive development and visual acuity may be impaired in children receiving inadequate intakes of  $\omega_3$  fatty acids (Nagakura et al., 2000). In view of the cardinal role of EFA in human health and diseases, characterization of the fatty acids composition of oils has become a current focus of lipid research (Savag et al., 1999; Anwer et al., 2006). It is from this perspective that a number of non-conventional oilseeds from several plants of sub-Saharan Africa such as *Raphia sese*, *Raphi laurentii*, *Canarium schweinfurthii*, *Dacryodes edulis*, *Coula edulis*, *Balanites egyptiaca*, *Vitellaria paradoxa*, *Telfairia occidentalis*, *Pentaclethra macrophylla*, *Parkia Africana*, *Cucumis melo*, *Tetracarpidium conophorum* and *Irvingia gabonensis* have been investigated (Kapseu et al., 2007).

These works have revealed the indisputable potentialities of most of these unexploited oils for food, pharmaceutical or cosmetic applications. Therefore, to contribute to non conventional oils promotion, we focused our attention on oilseeds of four tropical plants, namely *Ricinodendron heudelotii*, *Cyperus esculentus*, *Citrullus colocynthis*, and *I. gabonensis*.

These species are annual tree can grow between 20 to 50 m in height with a straight trunk that has a diameter of about 2.7 m for *R. heudelotii* and *I. gabonensis* (Kapseu et al., 2007). *C. esculentus* and *C. colocynthis* plants which produce seeds of about 1 and 3 to 5 mm length, respectively (Mbaye et al., 2001; Mahadevan et al., 2009). In most countries of tropical Africa and particularly in Côte d'Ivoire, leaves of these plants are widely consumed as green vegetables due to their richness in polysaccharides, vitamins and minerals (Leung et al., 1968) but, their seeds are underexploited. Vegetable oil is for the most part used as food and feed, but it is increasingly used as a biofuel and as feedstock by the chemical industry (Dyer et al., 2008). Expanding its usage for non food applications will require a substantial increase in the total production of vegetable oil. This increase has the potential to be met by increasing the oil content in presently used oil crops or introducing new high-oil-yielding crops. Much progress has been made in understanding how plants produce and accumulate oils. The specific enzymes involved in the metabolic pathway leading to triacylglycerols (TAGs) stored in the oil bodies, as well as the pathway that supplies the precursors generated from imported sucrose, are to a large extent known (Voelker and Kinney, 2001; Rawsthorne, 2002). However, we still have a poor understanding in key areas such as factors important for regulating the flux of photosynthates into storage compartments, the synthesis of fatty acids, or the level of oil content in storage tissues. Hence, research in these areas is of great importance to enable a substantial increase in vegetable oil production. Today the level of knowledge on oilseeds remains insufficient therefore, the aim of this work was to contribute to these tropical plants promotion by investigating their oilseeds properties in order to explore and discuss their nutritional and industrial potentiality.

## MATERIALS AND METHODS

### Plant materials

*R. heudelotii*, *C. esculentus*, *C. colocynthis* and *I. gabonensis* seeds were collected from Abidjan district (Côte d'Ivoire) in January 2014. Seeds were rinsed thoroughly with distilled water to remove dirt and dried at 40°C for 24 h in an electric oven (Memmert, Germany) according to Achu (2006).

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## Chemicals

Analytical grade solvents, standards, reagents and culture media were used to perform analysis. Solvents (n-hexane, chloroform, acetic acid, diethyl-ether, ethanol, methanol and n-heptane) put up were from Prolabo (France). Standards such as fatty acids, cholesterol, vitamin A, erucic acid and trifluoroacetic acid (TFA) were from Sigma-Aldrich (Germany). Wijs reagent was from Prolabo (France). The standard of nervonic acid has been provided by Merck (Germany).

## Oils seeds extraction

Oils were extracted from 50 g crushed seeds (Laboratory crusher, Culatti, France) with 300 ml of n-hexane (40 to 60°C) in a Soxhlet extractor. Then the solvent was removed (vacuum-packed) at 40°C with a rotary evaporator (Heidolph, Hei-Vap, Germany). The extracted lipid was weighed to determine the oil content of the seed. Crude oils were stored at 4°C in air tight brown sterile glass bottles (AFNOR ISO 734-1, 2006) until further use for physicochemical analysis.

## Physicochemical analysis

### Specific gravity and refractive index

Specific gravity and refractive index of oils seeds were determined at 20°C following the ISO 6320:2000(F) method by using a refractometer (Mettler RE 50), respectively. Viscosity of oils seeds was determined at 25°C by using a viscometer tube Anton SVM 3000 (AFNOR, 2000).

### Acid, peroxide, iodine, saponification and p-anisidine values

Acid, peroxide, iodine, saponification and p-anisidine values were determined following the AFNOR (2000) methods. pH value of oil samples was determined at 25°C according to Afane et al. (1997) by using a pH meter (Hanna, Hi 8915 ATC, Spain). 2 ml of each oil sample were dissolved in 15 ml of n-hexane. The pH-meter electrode was standardized with buffer solutions (pH 4.0 and 7.0) and then, immersed into the sample to record pH value. The determination of the p-anisidine number is based on the principle that in acetic acid medium, p-anisidine reacts with the conjugated aldehydes derived from lipid oxidation to form yellow compounds which absorb at 350 nm. We determined this index by the method described by Doleschall (2002). The p-anisidine index is a reliable indicator of lipid oxidative rancidity (Van Der Merwe, 2004).

### Unsaponifiable matter, vitamin A and vitamin E

Unsaponifiable matter content of oil samples was determined following the IUPAC (1979) method. The vitamin A and E was determined by High Performance Liquid Chromatography (HPLC) according to ISO 14565:2000(F). The sample is saponified with ethanolic potassium hydroxide solution and vitamin A is extracted into petroleum ether. The petroleum ether is removed by evaporation and the residue dissolved in methanol. The vitamin A concentration of the methanol extract is determined by reverse phase liquid chromatography under conditions giving a single peak for all the isomers of vitamin A. The sample injection is at 325 nm for the vitamin A and 292 nm for vitamin E. The flow rate is set at 0.25 ml/min at 70°C.

## Solid fat content (SFC)

The solids content was determined by nuclear magnetic resonance (ISO 8292: 1992 (F)). The solid content of a fatty phase at different temperatures is an important element for the knowledge of the rheological properties of fat. This method applies to fatty substances with pronounced polymorphisms. The preparation of test samples at specified temperatures makes it possible to measure magnetic decay signals emitted by liquid and solid fat protons by magnetic resonance with calculation and automatic display of the solid fat content.

## Cholesterol content

Cholesterol content was determined by using the enzymatic colorimetric method (Meiattini, 1978; Naito, 1988). About 100 mg of unsaponifiable fraction was dissolved in 1 ml of hexane. Then, 10 µl of mixture was added to 1 ml of the enzymatic reagent and the whole mixture was allowed to stand for 10 min at room temperature. The absorbance was measured at 505 nm using a spectrophotometer (T80+, PG instruments, England) against a blank. A standard cholesterol solution was tested as reference following the same procedure.

## Determination of fatty acid composition (FAC) by gaz

FAC of the oils samples was determined by GC analysis according to AFNOR ISO 5509 (2000). The fatty acids were converted to their methyl esters (FAMES) as described by AFNOR (ISO 5509-2000). About 0.4 g of oil sample was mixed with 5 ml of isooctane and 5 ml of a methanolic solution of potassium hydroxide (2N). The whole mixture was shaken up for 30 s and allowed to settle for 5 min. The top layer containing the FAMES was used for gas chromatography (GC) analysis. FAMES solution (1 µl) containing the internal standard (erucic acid) was injected into a gas chromatograph (Perkin Elmer, Clarus 580) equipped with a flame ionization detector (FID) and a capillary column Rt-2560 biscyanopropyl polysiloxane (100 m × 0.25 mm i.d. × 0.2 µm). The carrier gas was hydrogen and the flow rate was adjusted to 1.2 ml/min. Temperatures of detector and injector were 260°C. The initial column temperature was fixed to 140°C and programmed to increase by 4°C per min intervals until 240°C and, kept for 15 min at this temperature. The fatty acid methyl esters peaks were identified by comparing their retention times with those of standards. After adjusting areas with the internal standard (erucic acid), the yield of each fatty acid was calculated as follow: area of the fatty acid/areas of total fatty acids in the oil sample × 100 (%).

## Statistical analysis

In the experiment, each test for the sample was analyzed in triplicate. The data were expressed as means ± standard deviation (SD). Differences between means were analysed by analysis of variance (one way ANOVA) using GraphPad Prism 5 (Analyst soft Inc) software. Statistical significance was measured at  $p < 0.05$ .

## RESULTS

### Oil yield

The oil content of *R. heudelotii* seeds ( $54.27 \pm 1.73$ ), *I. gabonensis* seeds ( $64.98 \pm 0.03$ ), *C. esculentus* ( $20.18 \pm$

**Table 1.** Extraction yield and physicochemical properties of *R. heudelotii*, *I. gabonensis*, *C. esculentus* and *C. colocynthis* seeds.

Parameter	Oilseed			
	RH	IG	CE	CC
Extraction yield	54.27±1.73 <sup>a</sup>	64.98±0.00 <sup>b</sup>	20.18±0.66 <sup>c</sup>	51.70±0.00 <sup>d</sup>
Specific gravity at 25°C	0.94±0.00 <sup>a</sup>	0.85±0.00 <sup>a</sup>	0.88±0.00 <sup>a</sup>	0.88±0.00 <sup>a</sup>
Refractive index at 25°C	1.49±0.00 <sup>a</sup>	1.44±0.00 <sup>a</sup>	1.46±0.00 <sup>a</sup>	1.46±0.00 <sup>a</sup>
Dynamic viscosity (mPa.s)	596.88±0.40 <sup>a</sup>	502.20±0.23 <sup>b</sup>	519.81±0.33 <sup>c</sup>	517.85±0.01 <sup>c</sup>
kinetic viscosity (cm <sup>2</sup> /s)	632.85±0.08 <sup>a</sup>	547.38±0.23 <sup>b</sup>	554.99±0.33 <sup>c</sup>	553.03±0.01 <sup>c</sup>
pH	5.50±0.01 <sup>a</sup>	5.71±0.00 <sup>a</sup>	4.45±0.01 <sup>c</sup>	5.22±0.00 <sup>a</sup>
Unsaponifiable matter (%)	1.88±0.00 <sup>a</sup>	1.26±0.00 <sup>a</sup>	0.88±0.00 <sup>a</sup>	0.77±0.00 <sup>a</sup>
Cholesterol (mg/g)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
SMP (Sleep Melting Point) (°C)	0.00±0.00 <sup>a</sup>	38.13±0.08 <sup>b</sup>	9.75±0.03 <sup>c</sup>	0.00±0.00 <sup>a</sup>
SFC (Solid Fat Contain) at 0°C	65.26±0.02 <sup>a</sup>	70.42±0.24 <sup>b</sup>	63.22±0.02 <sup>c</sup>	61.22±0.02 <sup>d</sup>
SFC (Solid Fat Contain) at 5°C	64.19±0.18 <sup>a</sup>	57.72±0.35 <sup>b</sup>	54.19±0.02 <sup>c</sup>	54.35±0.02 <sup>d</sup>
SFC (Solid Fat Contain) at 10°C	41.20±0.00 <sup>a</sup>	38.12±0.02 <sup>b</sup>	31.19±0.00 <sup>c</sup>	29.50±0.00 <sup>d</sup>
SFC (Solid Fat Contain) at 15°C	19.13±0.02 <sup>a</sup>	35.15±0.08 <sup>b</sup>	10.13±0.02 <sup>c</sup>	12.64±0.01 <sup>d</sup>
SFC (Solid Fat Contain) at 20°C	0.18±0.00 <sup>a</sup>	34.67±0.04 <sup>b</sup>	0.10±0.00 <sup>a</sup>	0.20±0.00 <sup>a</sup>
SFC (Solid Fat Contain) at 25°C	0.02±0.00 <sup>a</sup>	30.41±0.23 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
SFC (Solid Fat Contain) at 30°C	0.02±0.00 <sup>a</sup>	25.25±0.03 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
SFC (Solid Fat Contain) at 35°C	0.02±0.00 <sup>a</sup>	17.92±0.09 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
SFC (Solid Fat Contain) at 40°C	0.00±0.00 <sup>a</sup>	10.58±0.04 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

<sup>a,b,c,d</sup> Means in line with no common superscript differ significantly ( $p < 0.05$ ).

0.66) and *C. colocynthis* (51.07 ± 0.7). These four oilseeds can be classified in descending order of their oil content: *I. gabonensis*, *R. heudelotii*, *C. colocynthis* and *C. esculentus* (Table 1).

### Physicochemicals characteristics

There was no significant difference ( $p < 0.05$ ) between most of the physicochemical parameters of the four seed oils except for dynamic viscosity (59.75 ± 0.39 for *R. heudelotii*, 50.22 ± 0.23 for *I. gabonensis*, 52.02 ± 0.23 for *C. esculentus* and 51.79 ± 0.005 for *C. colocynthis* mPa.s), iodine values (142.12 ± 0.34 for *R. heudelotii*, 7.42 ± 0.29 for *I. gabonensis*, 75.46 ± 0.35 for *C. esculentus* and 120.45 ± 0.29 for *C. colocynthis* I<sub>2</sub>/100 g), vitamin A (0.00 ± 0.00 for *R. heudelotii*, 7.17 ± 0.32 for *I. gabonensis*, 2.48 ± 0.07 for *C. esculentus* and 3.67 ± 0.04 for *C. colocynthis* µg/g), and p-anisidine value (301.17 ± 0.98 for *R. heudelotii*, 0.39 ± 0.00 for *I. gabonensis*, 24.47 ± 0.03 for *C. esculentus* and 29.09 ± 0.03 for *C. colocynthis* respectively. vitamin E (α-tocophérol 26.63 ± 0.44 and α-tocotriénol 94367.75 ± 1.91 10<sup>3</sup> for *R. heudelotii*, α-tocophérol 00.00 ± 0.00 and α-tocotriénol 19625.75 ± 1.13 10<sup>3</sup> for *I. gabonensis*, α-tocophérol 71.67 ± 0.47 and α-tocotriénol 00.00 ± 0.00 for *C. esculentus*, α-tocophérol 00.00 ± 0.00 and α-tocotriénol 19625.153 ± 1.90 10<sup>3</sup> for *C. colocynthis* ppm), and p-anisidine value (301.17 ± 0.98 for *R. heudelotii*, 0.39 ± 0.00 for *I. gabonensis*, 24.47 ± 0.03 for *C.*

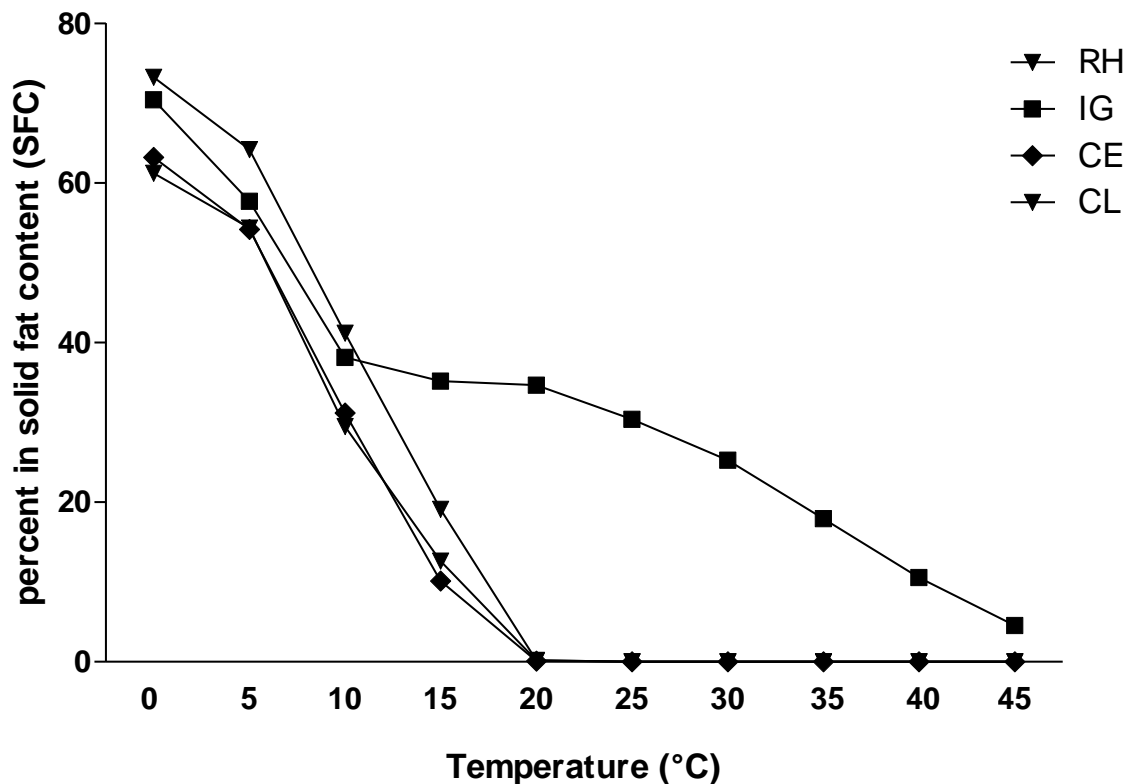
*esculentus* and 29.09 ± 0.03 for *C. colocynthis* respectively (Table 1). The values of specific gravity were 0.86 ± 0.01 while those of refractive index were about 1.46 ± 0.00. Quality parameters such as free fatty acids (FFA) and peroxide value (PV) were closed to 1.5% and 85 meq O<sub>2</sub>/kg, respectively. Oils extracted from *R. heudelotii*, *C. esculentus*, *C. colocynthis*, and *I. gabonensis* seeds were cholesterol free and their respective unsaponifiable matter contents were less than 1%. The saponification values of the four oilseeds were about 150 to 176 mg KOH/g (Table 1).

### Solid fat content (SFC)

The different solid fat content obtained show a higher value for *I. gabonensis*. Indeed, this oil remains practically solid until 20°C. The oil of *R. heudelotii*, *C. esculentus* and *C. colocynthis* are practically fluid at room temperature (Figure 1). The saponification values of the two oilseeds were about 150 to 180 mg KOH/g (Table 2).

### Characterization of FAC

Data presented in Table 3 show the FAC of the different oils analyzed. Total saturated fatty acid were in the range of 93.26% (IG) to 25.53% (CC). In these oils, the major saturated fatty acids were myristic (C14 :0) and lauric (C12 :0) in the oil of IG. Chromatographic profiles of the



**Figure 1.** Evolution curve of the solid fat content (SFC) as a function of the temperature of the four extracted oil samples. Percent change in solid as a function of temperature.

**Table 2.** Chemical parameters of four oils.

Parameter	Oilseed			
	RH	IG	CE	CC
Acidity ((%)	0.68±0.01 <sup>a</sup>	14.92±0.13 <sup>b</sup>	0.70±0.00 <sup>a</sup>	6.95±0.01 <sup>d</sup>
Acid value (mg KOH/g)	1.35±0.02 <sup>a</sup>	39.05±0.34 <sup>b</sup>	1.88±0.45 <sup>a</sup>	13.85±0.02 <sup>d</sup>
Saponification value (mg KOH/g)	154.67±1.82 <sup>a</sup>	176.03±0.50 <sup>b</sup>	175.47±0.30 <sup>c</sup>	175.64±3.04 <sup>d</sup>
Ester value	153.32±1.80 <sup>a</sup>	136.98±0.84 <sup>b</sup>	174.12±0.32 <sup>c</sup>	161.79±3.05 <sup>d</sup>
Iodine value (g I <sub>2</sub> /100 g)	142.12±0.34 <sup>a</sup>	7.42±0.29 <sup>b</sup>	75.46±0.35 <sup>c</sup>	120.45±0.29 <sup>d</sup>
Peroxide value (meq O <sub>2</sub> /kg)	37.98±2.03 <sup>a</sup>	2.34±0.32 <sup>b</sup>	86.16±1.06 <sup>c</sup>	48.77±0.22 <sup>d</sup>
Paraanisidin value	301.17±0.98 <sup>a</sup>	0.39±0.00 <sup>b</sup>	24.47±0.03 <sup>c</sup>	29.04±0.03 <sup>d</sup>
Totox	640.32±1.83 <sup>a</sup>	3.13±0.32 <sup>b</sup>	135.09±0.99 <sup>c</sup>	106.86±0.21 <sup>d</sup>
Vitamine A (µg/g)	nd	7.17±0.32 <sup>b</sup>	2.48±0.07 <sup>a</sup>	3.66±0.04 <sup>a</sup>

Mean ± SD (n=3), significant differences in the same row are shown by different letter (p < 0.05).

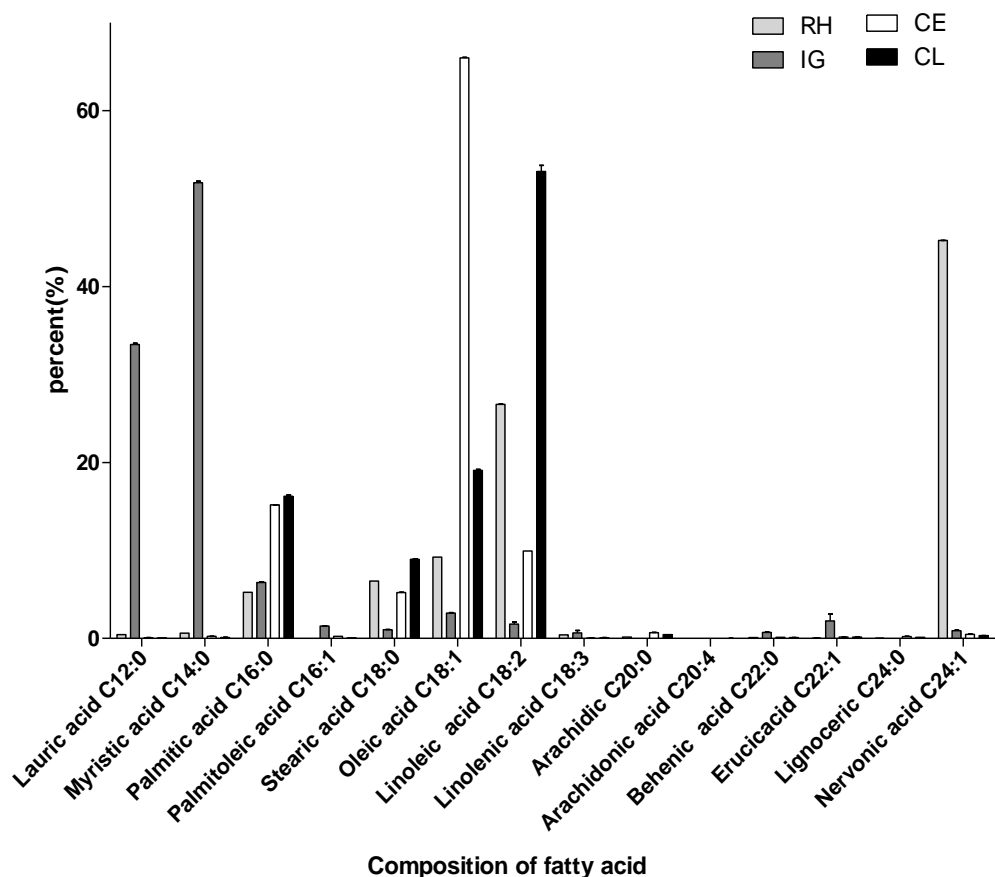
fatty acid composition and their relative amounts in the different oilseeds are shown in Figures 2 and 3. Proportion of fatty acids from the oilseeds studied highlighted the presence of six main compounds namely palmitic, stearic, oleic, linoleic, linolenic and nervonic acids in the oils of *R. heudelotii*, *C. Colocynthis* and *C. esculentus*. On the other hand, in *I. gabonensis* oil, we find the presence of saturated fatty acid, namely lauric,

myristic and palmitic acid. On average, these fatty acids were approximately 93.24, 96.29 and 97.35% of the total fatty acids in *R. heudelotii*, *C. esculentus*, and *C. colocynthis* respectively (Table 3). The four oilseeds were mainly composed of saturated fatty acids (SFA) (approximately 12 to 25% of total fatty acids) for *R. heudelotii*, *C. esculentus*, and *C. colocynthis* and unsaturated fatty acids (UFA) (approximately 75 to 80%).

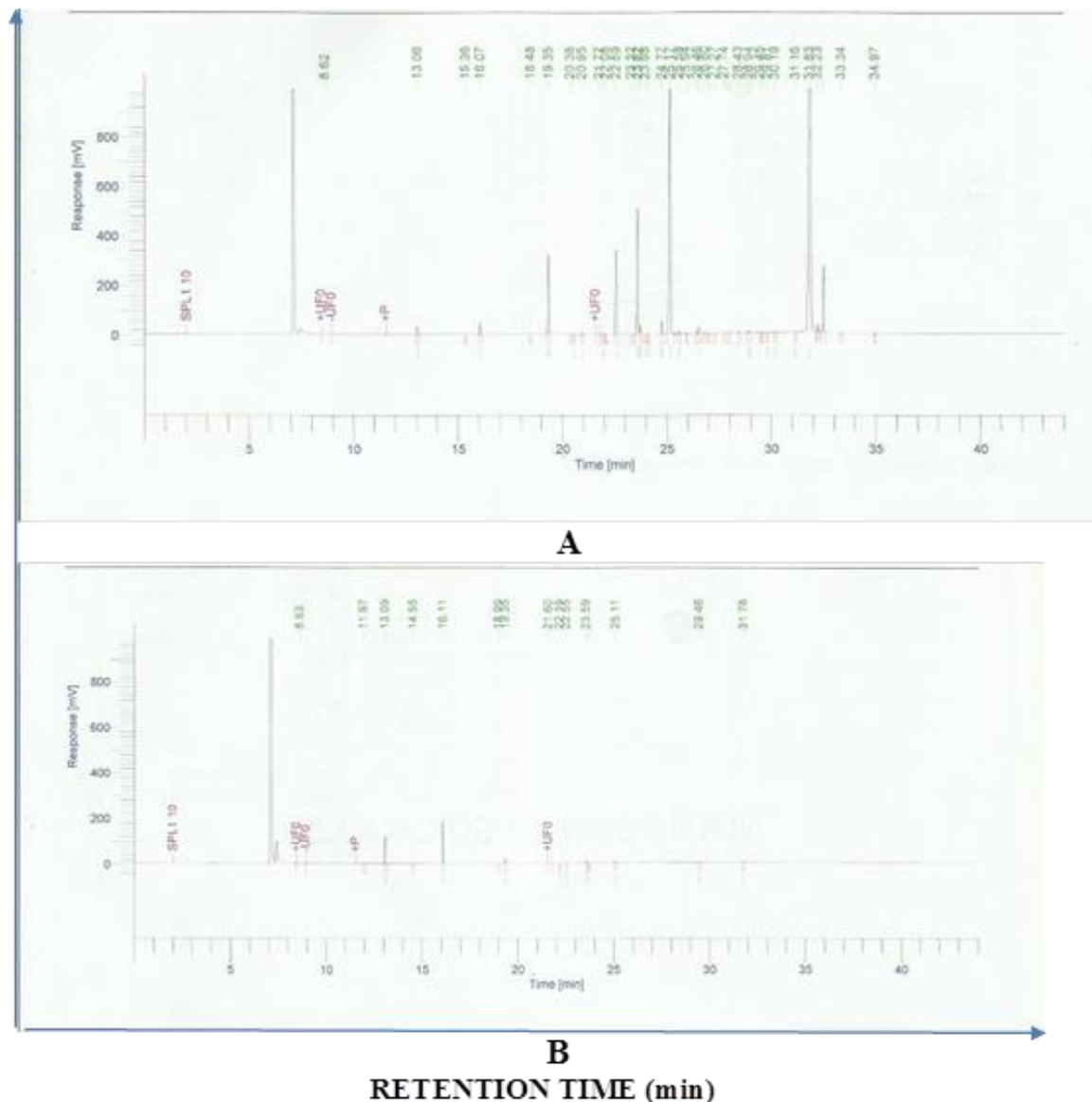
**Table 3.** Fatty acids composition (% of methyl fatty acids).

Fatty acids	RH	IG	CE	CC
Lauric acid C12:0	0.42±0.00 <sup>a</sup>	33.42±0.14 <sup>b</sup>	0.08±0.01 <sup>a</sup>	0.06±0.00 <sup>a</sup>
Myristic acid C14:0	0.61±0.00 <sup>a</sup>	51.80±0.19 <sup>b</sup>	0.23±0.01 <sup>a</sup>	0.11±0.01 <sup>a</sup>
Palmitic acid C16:0	5.24±0.01 <sup>a</sup>	6.36±0.07 <sup>b</sup>	15.18±0.02 <sup>c</sup>	16.16±0.14 <sup>d</sup>
Stéaric acid C18:0	6.53±0.00 <sup>a</sup>	1.00±0.01 <sup>b</sup>	5.22±0.01 <sup>c</sup>	8.99±0.05 <sup>d</sup>
Arachidic acid C20:0	0.15±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.66±0.01 <sup>a</sup>	0.43±0.00 <sup>a</sup>
Behenic acid C22:0	0.10±0.00 <sup>a</sup>	0.67±0.02 <sup>a</sup>	0.12±0.01 <sup>a</sup>	0.09±0.00 <sup>a</sup>
Lignoceric acid C24:0	0.03±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.22±0.01 <sup>a</sup>	0.13±0.00 <sup>a</sup>
SFA	12.92±0.02 <sup>a</sup>	93.26±0.44 <sup>b</sup>	21.04±0.05 <sup>c</sup>	25.53±0.20 <sup>d</sup>
Palmitoleic acid C16:1	0.02±0.00 <sup>a</sup>	1.41±0.01 <sup>b</sup>	0.25±0.00 <sup>a</sup>	0.07±0.01 <sup>a</sup>
Oleic acid C18:1	9.24±0.00 <sup>a</sup>	2.87±0.07 <sup>b</sup>	66.01±0.06 <sup>c</sup>	19.10±0.12 <sup>d</sup>
Erucic acid C22:1	0.04±0.03 <sup>a</sup>	1.98±0.78 <sup>b</sup>	0.14±0.01 <sup>a</sup>	0.16±0.01 <sup>a</sup>
Nervonic acid C24:1	45.24±0.02 <sup>a</sup>	0.87±0.06 <sup>b</sup>	0.46±0.02 <sup>b</sup>	0.31±0.00 <sup>b</sup>
MUFA	54.55±0.06 <sup>a</sup>	7.12±0.92 <sup>b</sup>	66.86±0.09 <sup>c</sup>	19.64±0.13 <sup>d</sup>
Linoleic acid C18:2	26.61±0.02 <sup>a</sup>	1.61±0.25 <sup>b</sup>	9.94±0.01 <sup>c</sup>	53.10±0.68 <sup>d</sup>
Linolenic acid C18:3	0.40±0.00 <sup>a</sup>	0.91±0.00 <sup>a</sup>	0.04±0.02 <sup>a</sup>	0.07±0.03 <sup>a</sup>
Arachidic acid C20:4	0.01±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
PUFA	27.06±0.05 <sup>a</sup>	4.50±1.04 <sup>b</sup>	10.12±0.04 <sup>c</sup>	53.33±0.72 <sup>d</sup>
Total UFA	81.61±0.11 <sup>a</sup>	11.62±1.96 <sup>b</sup>	76.97±0.12 <sup>c</sup>	72.97±0.86 <sup>d</sup>

Mean ± SD (n=3), significant differences in the same row are shown by different letter (p < 0.05) SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids ; UFA, Unsaturated fatty acid.



**Figure 2.** Fatty acids composition (% of methyl fatty acids).



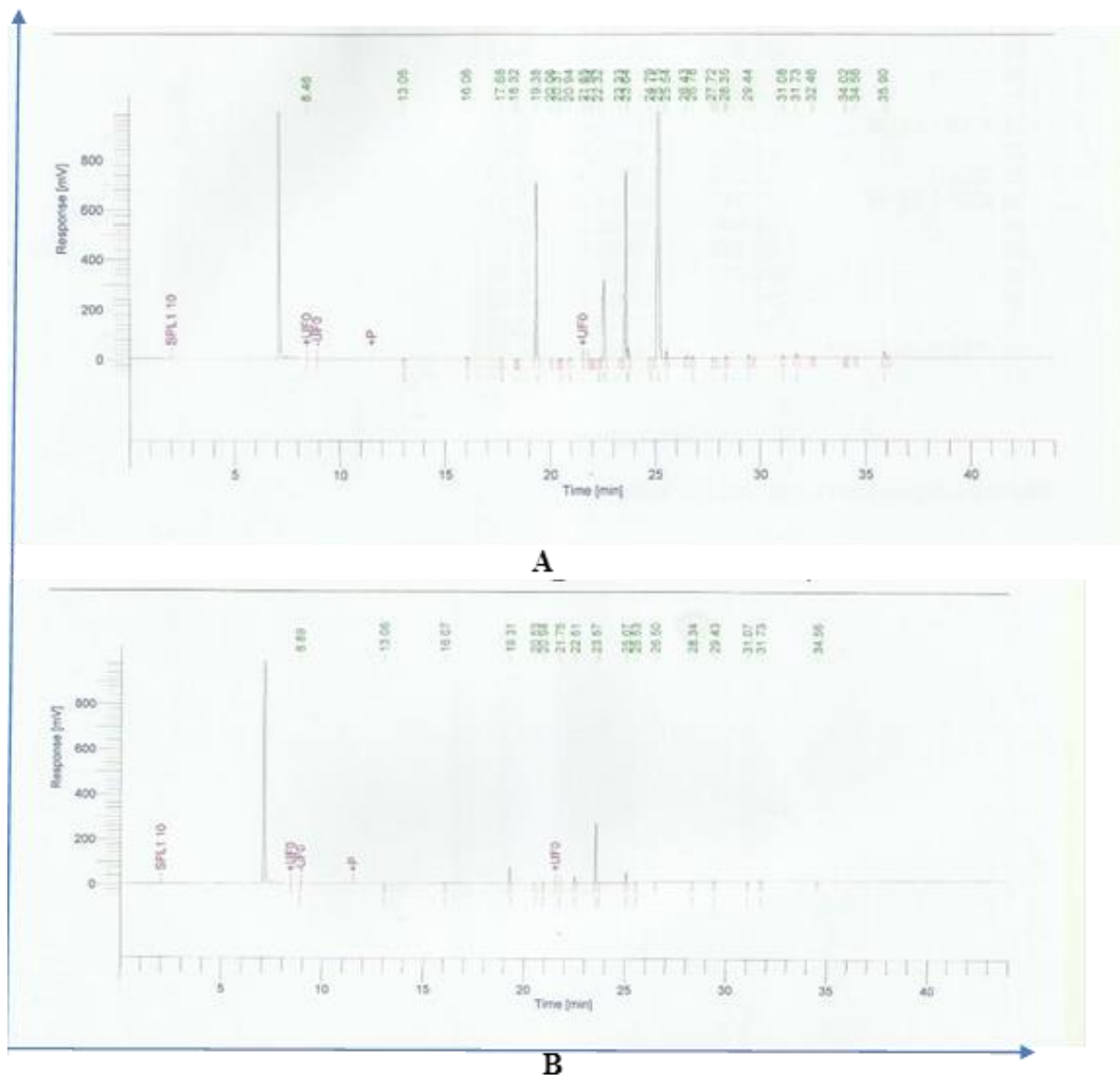
**Figure 3.** Gas chromatograms of fatty acids constituents of *R. heudelotii* (A) and *I. gabonensis* (B) oilseeds. Experiments were performed in triplicate by gas chromatographic analysis (GC-FID) of fatty acids methyl esters derived from *R. heudelotii* and *I. gabonensis* oilseeds. Data represents mean  $\pm$  SD.

The monounsaturated fatty acid (MUFA) consisted of oleic acid with a high content in *C. esculentus* oil of 66.01% and a rate of 19.10 in *C. colocyntis* (Table 3). These oilseeds were also composed of polyunsaturated fatty acids (PUFA) which was essentially linoleic acid with a value of 53.10% in *C. colocyntis* and 26.61% (Table 3). Nervonic acid accounts for the bulk of MUFA in *R. heudelotii* oil, 45.24%. The total unsaturated acid content in the four samples analyzed was significantly different. This content is higher in the *R. heudelotii* oil with a value of 81.61%, followed by *C. esculentus* is 76.97% and *C. colocyntis* with a content of 72.97%. These three oils generally remain fluid at room temperature. The density

of the oils analyzed showed that the oil of *R. heudelotii* at the highest density is 0.94 g/ml. The oils of *I. gabonensis*, *C. esculentus* and *C. colocyntis* have similar densities respectively  $0.85 \pm 0.00$ ,  $0.88 \pm 0.00$  and  $0.88 \pm 0.00$  (Figure 4).

#### Analytical characteristics of tocopherols and tocotrienols

Data presented in Table 4 show the tocopherol and tocotrienol contents (ppm) of the different oils analyzed. The range of total content of vitamin E was from 19695 to 110893 ppm and was higher than that found in some



**Figure 4.** Gas chromatograms of fatty acids constituents of *C. colocynthis* (A) and *C. esculentus* (B) oilseeds. Experiments were performed in triplicate by gas chromatographic analysis (GC-FID) of fatty acids methyl esters derived from *C. colocynthis* (A) and *C. esculentus* (B). Data represents mean  $\pm$  SD.

conventional oils such as palm oils reported by Monde (2011). Tocopherols and tocotrienol are major antioxidants of vegetable oils, and the main ones of these oils. Of these four oils the richest vitamin E is that of *R. heudelotii* with a content of 110893,29 ppm. *I. gabonensis* oil is the poorest vitamin E oil with a value of 19674,72 ppm. These results are significantly higher than those found by Anhwange et al. (2010).

#### Vitamine A content

Figure 5 shows the different chromatograms obtained during the analysis of vitamins A in the different oils.

It turns out that vitamin A was not detected in the *R. heudelotii* oil. In addition, the oil rich in vitamin A is oil of *I. gabonensis*.

#### DISCUSSION

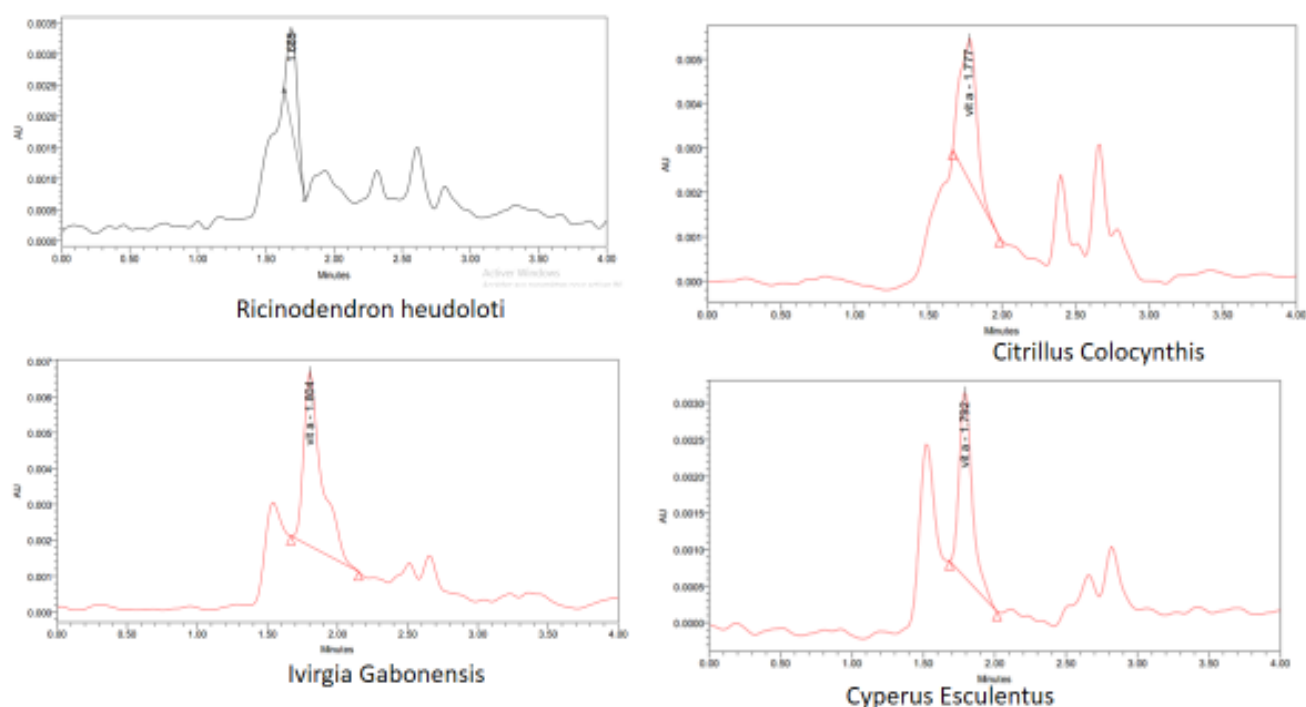
In regard to the oil content, *R. heudelotii*, *C. esculentus*, *I. gabonensis* and *C. colocynthis* seeds are lipidrich than most of the conventional oilseeds such as cotton (13%), soybean (14%) and palm fruit (20%) (Nzikou et al., 2011; Kapseu et al., 2005) and can be used as an alternative source of oil for lipid industries (Nzikou et al., 2009). The result shows also higher oil content compared to earlier



**Table 4.** Changes in the tocotrienol and tocopherol of different oils studied (ppm).

Vitamin E	RH	IG	CE	CC
α-Tocopherol (ppm)	26.63±0.44 <sup>a</sup>	0.00±0.00 <sup>b</sup>	71.67±0.47 <sup>c</sup>	0.00±0.00 <sup>a</sup>
β-Tocopherol (ppm)	80.61±0.04 <sup>a</sup>	12.01±2.30 <sup>b</sup>	11.70±0.62 <sup>c</sup>	32.37±1.64 <sup>d</sup>
δ-Tocopherol (ppm)	286.03±1.14 <sup>a</sup>	36.45±4.65 <sup>b</sup>	0.00±0.00 <sup>c</sup>	36.45±4.65 <sup>b</sup>
γ-Tocopherol (ppm)	16.77±1.24 <sup>a</sup>	1.11±0.10 <sup>b</sup>	22881.91±1527.11 <sup>c</sup>	1.14±0.09 <sup>b</sup>
α-Tocotrienol (ppm)	94367.75±1911.54 <sup>a</sup>	1962.15±1131.53 <sup>b</sup>	0.00±0.00 <sup>c</sup>	19625.15±1131.53 <sup>b</sup>
β-Tocotrienol (ppm)	nd	nd	nd	nd
δ-Tocotrienol (ppm)	16115.51±85.93 <sup>a</sup>	nd	nd	Nd
γ-Tocotrienol (ppm)	0.00±0.00 <sup>a</sup>	nd	nd	Nd
Vitamin E (ppm)	110893.29±1827.36 <sup>a</sup>	19674.72±1133.99 <sup>b</sup>	22965.28±1526.14 <sup>c</sup>	19695.11±1137.58 <sup>b</sup>

nd, not detected. Mean ± SD (n=3), significant differences in the same row are shown by different letter (p < 0.05).

**Figure 5.** Chromatogram of vitamin A.

reports (El-Adawy and Khalil, 1994; Nzikou et al., 2011). The oil content of *R. heudelottii* seeds did not show much variation compared to previous report (Ogunkano and Bravil, 2013). However, this oil content was lower than that (42.2%) reported by Silou et al. (1999). These variations between oil yields in seeds could be attributed to their cultivation climate, ripening stage, harvesting time and the extraction method employed (Egbekun and Ehieze, 1997). The specific gravity of *C. esculentus*, *C. colocynthis* and *I. gabonensis* oilseeds are lower than those reported for most conventional oilseeds which are about 0.9 (Codex, 1993). Among the four oils analyzed alone, the specific gravity of *R. heudelottii* oil is close to the norm of the Codex Alimentarius, which are

about 0.9. In addition, the viscosity dynamic values of both of the oilseeds were in the range (550 to 650 mPas) of most vegetable oils (Besbes et al., 2004). These results corroborate the fluid state of the studied oils at ambient temperature and this physical characteristic could be suitable for skin care products preparation (Reiger, 1989; Dhellot et al., 2006). Thirdly, these oils of *C. esculentus*, *R. heudelottii* and *C. colocynthis* oilseeds contain mostly polyunsaturated fatty acids, which easily undergo oxidation, raising peroxide values in these seeds. They are lower than those of *R. heudelottii* oils (19-114) (Aboubakar et al., 2000). Most of our values are lower than 15 m.equiv.g of O<sub>2</sub>/kg of oil (the maximum level for cold pressed and virgin oils, Codex Alimentarius, 1999),

showing that these oils are good edible oils. The relatively high peroxide values of *C. esculentus*, *R. heudelotii* and *C. lanatus* oilseeds indicate that they are less liable to oxidative rancidity at ambient temperature (DeMan, 1992). Therefore, these oilseeds could be suitable in combination with antioxidants for cosmetic formulations (Judde, 2004). In addition, the studied oilseeds could be recommended for soap making and in the manufacture of lather shaving creams due to their relatively higher saponification values (Wolf, 1968; Eka, 1980). Also, the unsaponifiable matter contents of *R. heudelotii* and *I. gabonensis* oilseeds are higher than those reported for other potential cosmetic oils such as cotton seed oil (0.52%), peanut oil (0.33%) and palm kernel oil (0.22%) (Kapseu and Parmentier, 1997). This lipid fraction is a good source of stabilizers and provides essential moisture to skin (Helme, 1990). The refractive indexes of the three oilseeds *R. heudelotii*, *C. esculentus*, *C. colocythis* are within the range of those reported for edible oils (Rossell, 1991). The study indicates that seed oils of both plants contain lower FFA and so, they can be recommended for salads seasoning and can be stored for longer period without deterioration (Anwar et al., 2007; Matos et al., 2009). The iodine values are approximately the same as those of other oils such as soybean (120 to 143 g I<sub>2</sub>/100 g) and sunflower (110 to 143 g I<sub>2</sub>/100 g) oils (Codex, 1993). However, these values are higher than those reported by Kapseu and Parmentier (1997) for other non-conventional oilseeds such as *Coula edulis* (90-95 g I<sub>2</sub>/100 g), *Dacryodes edulis* (60 to 80 g I<sub>2</sub>/100 g) and *Canarium schweinfurthii* (71.1 to 94.9 g I<sub>2</sub>/100 g). In view of the results above, the studied oilseeds could be categorized as semi-drying oils which consist predominately in polyunsaturated fatty acids (Anhwange et al., 2010). Therefore, these oils could be nutritionally beneficial to patients suffering from most of the lipid disorders (Njoku et al., 2001). PUFA amounts of *R. heudelotii* and *H. colocythis* oilseeds are higher than those reported for most of the nonconventional oilseeds as sheabutter (6.9%), avocado (15.5%), *D. edulis* (25.2%) and *Canarium schweinfurthii* (28.8%) (Chalon et al., 2001). The higher content of total PUFA observed in the studied oilseeds may confer flexibility, fluidity and selective permeability to cellular membranes and may also be beneficial for reducing cardiovascular disease risk (Das, 2006). The linoleic acid content of *R. heudelotii* and *C. colocythis* oilseeds are higher than those reported for sea buckthorn seed oil (28.8%) and raspberry seed oil (29.1%) which are considered as new sources of linoleic oils and could be used in human diet for antiinflammatory, anti-thrombotic, anti-hypertensive and antiarrhythmic actions (Kamal-Eldin, 2006; Nzikou et al., 2007).

Oilseed was higher than that (1.57%) reported by Nzikou et al. (2011) for the same specie. Due to their linoleic acid content which were less than 17%, the studied oils could be used in human diet to decrease

plasma LDLcholesterol also called "bad cholesterol" (Winjendran and Hayes, 2004; Mhanhmad et al., 2011). As regards palmitic and stearic acids, which are the main saturated fatty acids of the two oilseeds, previous studies have shown that they are free from deleterious effect on plasma cholesterol (Khosala and Sundram, 1996; Hunter et al., 2000). In addition, they are often used in food industries to provide texture and softness to products (Dubois et al., 2007). Vitamin A contents of *I.gabonensis*, *C. esculentus* and *C. colocythis* oilseeds were lower than that reported (1 mg/g) for palm oil (Codex Alimentarius, 1993). The consumption of these oilseeds could cover infants (0 to 6 months) needs, which are estimated at 0.375 mg per day for vitamin A (FAO, 2001). In addition, the studied oilseeds were cholesterol free and this property is advantageous for using these oils for human nutrition without fearing increase in plasma LDL-cholesterol which is positively correlated to cardiovascular diseases (Dubois et al., 2007; Maki et al., 2011).

The determination of the solids content was performed on all four oil samples. These results show that the SFC values decrease as a function of temperature with a variable dispersion according to the sample. In addition, the three oils *R. heudelotii*, *C. esculentus* and *C. colocythis* remain fluid and free of solids from 20°C. This result is in agreement with some conventional oil such as rapeseed and sunflower oil (Fokou et al., 2004). The characteristics of a ready-to-use plastic grease depend both on the composition of the mixture and on the thermal and mechanical treatments it has undergone. Among all the parameters likely to influence the rheological parameters, the composition of the fatty phase is both the most important and the one on which it is easier to act. This qualitative and quantitative composition of the fatty phase, in effect primarily affects any temperature on the solid / liquid ratio. *I. gabonensis* oil which remains solid at temperatures above 20°C can be used as concrete fat and gives it the rheological property of being used in margarine and cosmetics.

The nervonic acid content was measured. *R. heudelotii* oil has a significant content of nervonic acid with more than 45%. Nervonic acid (cis-tetracos-15-enoic acid, 24:1) is a mono-unsaturated omega-9 fatty acid with very long chain length (24 carbon). Nervonic acid plays a role in the biosynthesis of neuronal myelin and is found in the sphingolipids of the white matter of the human brain (Orengo, 2014). In diseases involving demyelination, such as adrenoleukodystrophy (ALD) and multiple sclerosis (MS), there is a considerable reduction in the levels of nervonic acid in sphingolipids. Nervonic acid has been studied as a raw material in the pharmaceutical industry for the production of drugs used in the symptomatic treatment of MS and ALD, of which rho oil is a good source, like oil. Camelina and new prototypes of brassicaceae (vegetable cabbage, rapeseed, watercress, horseradish, black mustard) that produce seed oil

(possibly rapeseed oil or camelina) with a high enriched content of nervonic acid for human and animal targets related to health. In micronutritional counseling one could propose a supplementation based on oil of *R. heudelotii* (Orengo, 2014).

## Conclusion

It could be concluded in view of the results of this investigation that *R. heudelotii*, *I. gabonensis*, *C. esculentus* and *C. colocynthis* seeds may be developed for oil production. The oilseeds of these tropical plants are predisposed to human consumption due to their low content in FFA and peroxide. Saponification values and physical properties of these oils make them suitable in cosmetic industries for skin care products as soaps. Oils extracted from *R. heudelotii* and *C. colocynthis* seeds are good source of EFA predominantly composed of linolenic acid. The fatty acids profile make them more nutritionally balanced than most of the conventional advisable oils. Linoleic and linolenic acid content confer to these oils a number of nutritional, cosmetic and dietetic properties.

In view of all these potentialities and qualities, *R. heudelotii*, *I. gabonensis*, *C. esculentus* and *C. colocynthis* seeds may be considered as new sources of non-conventional oils which could be use in pharmaceutical, cosmetic and food industries. This study could be improved by investigating the effects of the four oilseeds before using them as supplements in food, pharmaceutical and cosmetic industries.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## REFERENCES

- AFNOR ISO 5509 (2000). The french standards collection, fats, oleaginous seeds and by-products. 4th ed. AFNOR, Paris. p. 527.
- AFNOR ISO 734-1 (2006). The french standards collection, fats, oleaginous seeds and by-products.
- Alais C, Linden G (2009). Lipids. In: Masson, (ed). Food Biochemistry. Paris, pp. 55-73.
- Aluyor EO, Ozigagu CE, Oboh OI, Aluyor P (2009). Chromatographic analysis of vegetable oils: A review. Scientific Research and Essay 4(4):191-197.
- Anhwange BA, Ikyenge BA, Nyiatagher DT, Ageh JT (2010). Chemical analysis of *Citrullus lanatus* (Thumb), *Cucumeropsis mannii* (Naud) and *Telfairia occidentalis* (Hook F.) seeds oils. Journal of Applied Sciences Research 6:265-268.
- Anwar F, Chata SAS, Hussain AI (2007). Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage. Grasas y Aceites 58(4):390-395.
- Anwer T, Bhangar MI, Anwar F, Khan M, Shahaid R, Iqbal S (2006). A comparative characterization of different non-conventional oilseeds found in Pakistan. Journal of the Chemical Society of Pakistan 28(2):144-148.
- Besbes S, Blecker C, Deroanne C, Lognay G, Drira NE, Attia H (2004). Quality characteristics and oxidative stability of date seed oil during storage. Food Science and Technology International 10(5):333-338.
- Betti M, Perez TI, Zuidhof MJ, Renema RA (2009). Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipids classes. Poultry Science 88(8):1740-1754.
- Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G (2001). Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. Lipids 36(9):937-44.
- Codex Alimentarius (1993). Fats and Vegetable oils. FAO/WHO, Geneva, p. 160.
- Codex Alimentarius (1999). Codex Alimentarius Standards for Fats and Oils from Vegetable Sources. Section 2. Codex Alimentarius Standards for Named Vegetable oils. Codex Alimentarius-Stan 210.
- Combe N, Boue-Vaysse C (2004). Face aux besoins et a la realit e des consommations, quelles sont les specificit es des differentes sources d'acides gras omega 3 disponibles ? OCL-Oilseeds and Fats, Crops and Lipids 11(2):103-5.
- Das UN (2006). Essential fatty acids -a review. Current Pharmaceutical Biotechnology 7(6):467-482.
- DeMan JM (1992). Chemical and physical properties of fatty acids. In: Chow CK, (ed). Fatty acids in foods and their health implications. New York. pp. 18-46.
- Dhellit JR, Matouba E, Maloumbi MG, Nzikou JM, Ngoma DG, Linder M, Desobry S, Parmentier M (2006). Extraction, chemical composition and nutritional characterization of vegetable oils: case of *Amaranthus hybridus* (var 1 and 2) of Congo Brazzaville. African Journal of Biotechnology 5(11):1095-1101.
- Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. European Journal of Lipid Science and Technology 109(7):710-732
- Dyer WJ (2008). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 911 -917.
- Egbekun MK, Ehieze MU (1997). Proximate composition and functional properties of fullfat and defatted benissed (*Sesamum indicum* L.) flour. Plant Foods for Human Nutrition 51(1):35-41.
- Eka OU (1980). Proximate composition of bush mango tree and some properties of dikafat. Nigerian Journal of Nutritional Sciences 1:33-36.
- El-Adawy TA, Khalil AH (1994). Characteristics of roselle seeds as a new source of protein and lipid. Journal of Agricultural and Food Chemistry 42(9):1896-1900.
- FAO (2001). Nutrition in developing countries. 3rd ed. FAO, Geneva. p.515.
- Fokou E, Achu MB, Tchouanguep MF (2004). Preliminary Nutritional Evaluation of Five Species of Egusi Seeds in Cameroon. African Journal of Food, Agriculture, Nutrition and Development 4(1):11.
- Hedren E, Diaz V, Svanberg U (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. European Journal of Clinical Nutrition 56(5):425-30.
- Hunter K, Crosbie LC, Weir A, Miller GJ, Dutta-Roy AK (2000). A residential study comparing the effects of diets rich in stearic acid, oleic acid, and linoleic acid on fasting blood lipids, haemostatic variables and platelets in young healthy men. Journal of Nutritional Biochemistry 11(7-8):408-16.
- IUPAC (1979). Standard methods for the analysis of oils, fats and derivatives. 6th ed. Pergamon, Oxford. p. 190.
- Judde A (2004). Prevention of the fatty acids oxydation in a cosmetic product: mechanisms, consequences, determination methods, what antioxidants for what applications? OCL-Oilseeds and Fats, Crops and Lipids 11(6):414-418.
- Kamal-Eldin A (2006). Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. European Journal of Lipid Science and Technology 58(12):1051-1061.

- Kapseu C, Aboubakar Dandjouma AK, Kapseu C, Parmentier MJ (2007). Chemical Composition of Oil from *Coula edulis* (Bail.) nuts. *Journal of Food Engineering* 68:79-87.
- Kapseu C, Parmentier M (1997). Fatty acid composition of some vegetable oils from Cameroon. *Sciences des Aliments* 17(3):325-331.
- Kapseu C, Tchankou BC, Tchiegang C, Silou T, Parmentier M (2005). Some physicochemical properties of oils of non-conventional oleaginous from sub-saharan africa. Symposium GP3A, Ouagadougou, p. 4.
- Khosala P, Sundram K (1996). Effects of dietary fatty acids composition on plasma cholesterol. *Progress in Lipid Research* 35(2):93-132.
- Mahadevan N, Shivali A, Kamboj P (2009). *Hibiscus sabdariffa* Linn: an overview. *Natural Product Radiance* 8:77-83
- Maki KC, Rubin MR, Wong LG, Macmanus JF, Jensen CD, Lawless A (2011). Effects of vitamin D supplementation on 25-hydroxyvitamin D, high-density lipoprotein cholesterol, and other cardiovascular disease risk markers in subjects with elevated waist circumference. *International Journal of Food Sciences and Nutrition* 62(4):318-327
- Matos L, Nzikou JM, Matouba E, Pandzou-Yembe VN, Mapepoulou TG, Linder M, Desobry S (2009). Studies of *Irvingia gabonensis* seed kernels: oil technological applications. *Pakistan Journal of Nutrition* 8(2):151-157.
- Mbaye MS, Noba K, Sarr RS, Kane A, Sambou JM, Ba AT (2001). Systematic precision elements on adventitious species of the genus *Corchorus* L. (Tiliaceae) in Senegal. *African Journal of Science and Technology* 2:51-64.
- Meiattini F (1978). The 4-hydroxybenzoate/4-aminophenazone chromogenic system. *Clinical Chemistry* 24:2161-2165
- Mhanhmad S, Leewanich P, Punsuvon V, Chanprame S, Srinives P (2011). Seasonal effects on bunch components and fatty acid composition in Dura oil palm (*Elaeis guineensis*). *African Journal of Agricultural Research* 6(7):1835-1843.
- Nagakura T, Matsuda S, Shichijyo K, Sugimoto H, Hata K (2000). Dietary supplementation with fish oil rich in omega 3 polyunsaturated fatty acid in children with bronchial asthma. *European Respiratory Journal* 16(5):861-865.
- Naito HK (1988). Current status of blood cholesterol measurement in clinical laboratories in the United States: a report from the Laboratory Standardization Panel of the National Cholesterol Education Program. *Clinical Chemistry* 34(1):193-201.
- Naudet M, Soulier J, Farines M (1992). Principaux constituants chimiques des corps gras. In: Karleskind, (ed). *Manuel des Corps Gras*. Paris, pp. 65-115.
- Njoku OU, Muma AF, Ononogbu IC, Eleanya R (2001). Preliminary investigation on some nutritional and toxicological properties of *Ricinodendron heudelottii* seed oil. *Nigerian Journal of Biochemistry and Molecular Biology* 16:132-133.
- Nukhet A, Akpinar MA, Turkoglu S (2001). Total lipid content and fatty acid composition of the seeds of some *Vicia* L. species. *Food Chemistry* 74(4):444-453.
- Nzikou JM, Bouanga-Kalou G, Matos L, Ganongo-Po FB, MboungouMboussi PS, Moutoula FE, Panyoo-Akdowa E, Silou TH, Desobry S (2011). Characteristics and nutritional evaluation of seed oil from Roselle (*Hibiscus sabdariffa* L.) in Congo-Brazzaville. *Current Research Journal of Biological Sciences* 3(2):141-146
- Nzikou JM, Matos L, Bouanga-Kalou G, Ndangui CB, Pambou-Tobi NPG, Kimbonguila A, Silou T, Linder M, Desobry S (2009). Chemical composition on the seeds and oil of sesame (*Sesamum indicum* L.) grown in Congo-Brazzaville. *Advance Journal of Food Science and Technology* 1(1):6-11.
- Nzikou JM, Mvoula-Tsier M, Matos L, Matouba E, Ngakegni-Limbili AC, Linder M, Desobry S (2007). *Solanum nigrum* L. seeds as an alternative source of edible lipids and nutriment in Congo Brazzaville. *Journal of Applied Sciences* 7(8):1107-1115.
- Ogunka-Nnoka CU, Bravil M (2013). Toxic Effect of n-hexane oil Extract of two spices on Rat liver and Kidney functions. *Archives of Applied Science Research* 5(5):198-201.
- Orengo R (2014). Approche micro-nutritionnel des fibromyalgiques et des sportifs. PCP. <https://docplayer.fr/13556990-L-approche-micro-nutritionnelle-des-fibromyalgiques-et-des-sportifs.html>
- Rawsthorne S (2002). Carbone flux and fatty acid biosynthesis in plants. *Progress in lipid research* 41:182-196.
- Savag GP, Dutta PC, Mcneil DL (1999). Fatty acid and tocopherol content and oxidative stability of walnut oils. *Journal of the American Oil Chemists' Society* 76(9):1059-1060.
- Silou TH, Mampouya D, Loka Lonyange WD, Saadou M (1999). Composition globale et caractéristiques des huiles extraits de 5 espèces de Cucurbitacées du Niger. *La Rivista Italiana Delle Sostanze Grasse* LXXXVI:141-144 .
- Voelker T, Kinney AJ (2001). Variations in the biosynthesis of seed storage lipids. *Annual Review of Plant Physiology and plant Molecular Biology* 52:335-361.
- Wijendran V, Hayes KC (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annual Review of Nutrition* 24:597-615.