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Physicochemical and microbiological characterization of linolenic acid-rich oils from seeds of two tropical plants: *Corchorus olitorius* L. and *Hibiscus sabdariffa* L.

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This work was carried out to determine the potential applications of *Corchorus olitorius* and *Hibiscus sabdariffa* oil seeds by investigating their physicochemical and microbiological characteristics. Physicochemical parameters of the extracted oils were respectively as follow: refractive index (1.46 and 1.45), acid value (3.74 ± 1.62 and 2.80 mg KOH/g), peroxide value (6.67 ± 0.58 and 5.33 ± 0.60 meq O₂/kg), iodine value (132.54 ± 1.22 and 118.57 ± 1.22 g I₂/100 g), saponification value (183.63 ± 3.24 and 189.23 ± 1.62 mg KOH/g), unsaponifiable matter (0.88 ± 0.04 and 0.85 ± 0.05 %), vitamin A (0.45 ± 0.01 and 0.60 ± 0.01 mg/g), cholesterol (0.00 mg/g) for *C. olitorius* and *H. sabdariffa* respectively. Absorbances of the two oils decreased abruptly in the range of UV-B and UV-A wavelengths. *C. olitorius* and *H. sabdariffa* seeds showed relatively high content of linolenic acid (about 29 and 37% of total fatty acids) with extremely low n-6/n-3 ratio (0.26 and 0.46). Microbiological analysis revealed that the crude extracted oilseeds were pathogenic bacteria free and the lipolytic bacteria counted belonged to *Micrococcus* genus. All these interesting characteristics should arouse attention for the usage of these oilseeds in food, pharmaceutical and cosmetic industries.

Key words: *Corchorus olitorius*, *Hibiscus sabdariffa*, seed oils characterization, linolenic acid, lipolytic bacteria.

INTRODUCTION

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 (Ramadan et al., 2006). 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, 2006).

Besides the antioxidant effect of tocopherols, phenolic compounds and phospholipids (Lee et al., 2002), fatty acids composition determine the physical properties, stability, and nutritional value of lipids (Elfalleh et al., 2011). This nutritional value is linked to essential fatty acids (EFA) that are polyunsaturated fatty acids (PUFA). These compounds are essential for the human nutrition

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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 ω 3 (n-3) and ω 6 (n-6) ones (Alais and Linden, 1991). These families are similar as they both comprise a precursor, namely linoleic acid (LA) for the ω 6 and linolenic acid (ALA) for the ω 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). Apart 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 ω 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 ω 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 *Canarium schweinfurthii*, *Dacryodes edulis*, *Ricinodendron heudelotii*, *Coula edulis*, *Balanites egyptiaca*, *Vitellaria paradoxa*, *Telfairia occidentalis*, *Pentaclethra macrophylla*, *Parkia Africana*, *Cucumis melo*, *Tetracarpidium conophorum* and *Irvingia gabonensis* have been investigated (Badifu, 1993; Kapseu et al., 2005; Matos et al., 2009). 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 two tropical plants, namely *Corchorus olitorius* and *Hibiscus sabdariffa*.

C. olitorius and *H. sabdariffa* belong to the order Tiliaceae and Malvaceae, respectively. These species are annual herbaceous 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. Roughly studies showed that seeds of *C. olitorius* contain about 11 to 16% fat, 16 to 20% crude protein and 4.5 to 6.3% ash (Gadgil et al., 1989) while those of *H. sabdariffa* showed about 40.4% carbohydrate,

26.2% crude protein and 20.2% fat (El-Adawy and Khalil, 1994). Other studies have reported a traditional medicinal use of *C. olitorius* seeds (Sharaf and Negm, 1969; Mbaye et al., 2001) while those of *H. sabdariffa* were used as traditional condiments (Cissé et al., 2009). To date, we know little about the physicochemical and microbiological characteristics of extracted oils from seeds of the two species (Nzikou et al., 2011; Ibrahim and Fagbohun, 2011). 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

C. olitorius and *H. sabdariffa* seeds were collected from Abidjan district (Côte d'Ivoire) in June 2009. 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 Ali et al. (2008).

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) were from Merck (Germany). 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). Cholesterol enzymatic kit reagent was from Spinreact (Spain). Culture media (plate count agar, Sabouraud agar, Baird-Parker agar, violet red bile lactose (VRBL) agar, *Salmonella shigella* (SS) agar, tryptone-sulfite-neomycin (TSN) agar, Tween 80 agar, meat nutrient agar and tryptone-salt liquid) were provided from Bio-Mérieux (France).

Oilseeds 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 (Ejikeme et al., 2010) until further use for physicochemical and microbiological analysis.

Physicochemical analysis

Specific gravity and refractive index

Specific gravity and refractive index of oilseeds were determined at 25°C following the IUPAC (1979) method by using a pycnometer and a refractometer (Abbe, Optic Ivymen, Spain), respectively. Viscosity of oilseeds was determined at 25°C by using a viscometer tube (AFNOR, 1986).

UV-Vis spectra

UV-Vis spectra of oil samples were determined by measuring absorbance of hexanic oil solution (1%) by using a UV-Vis spectrophotometer (T80+, PG Instruments, England) in the range of 200 to 600 nm (Besbes et al., 2004).

Acid, peroxide, iodine and saponification values

Acid, peroxide, iodine and saponification values were determined following the AOAC (1997) 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.

Unsaponifiable matter

Unsaponifiable matter content of oil samples was determined following the IUPAC (1979) method. Vitamin A content was determined by using colorimetric method (Dungan et al., 1964). For this, 100 mg of unsaponifiable fraction was dissolved in 2 ml of chloroform. To 1 ml of aliquot, 4 ml of a trifluoroacetic-chloroform (1:3, v/v) solution was added. The absorbance was measured at 620 nm using a spectrophotometer (T80+, PG instruments, England). A standard curve of vitamin A was used as reference.

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.

Fatty acid composition

The fatty acids were converted to their methyl esters (FAMES) as described by the European Communities (1991). About 0.1 g of oil sample was mixed with 2 ml of n-heptane and 0.2 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 (Shimadzu, GC 14 A, Japan) equipped with a flame ionization detector (FID) and a capillary column TRD1 (60 m X 0.25 mm i.d. X 0.25 µm film thickness). The carrier gas was nitrogen and the flow rate was adjusted to 23 ml/min. Temperatures of detector and injector were 250°C. The initial column temperature was fixed to 100°C and programmed to increase by 5°C per min intervals until 220°C and, kept for 10 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 (%).

Microbiological analysis

Microbial enumeration and culture using lipids for the following parameters such as decimal dilution series of oilseeds in tryptone-salt, counting of microorganisms (total viable bacteria, yeasts and moulds, coliforms, *Staphylococcus*, anaerobic bacteria) and the search of *Salmonella* including pre-enrichment, enrichment and selective isolation were carried out by adopting protocols of AFNOR (1996).

Lipolytic bacteria count was done as described by Sierra (1957). 1 ml of decimal dilutions was inoculated onto Tween 80 agar medium and plates were incubated at 30°C for 72 h. The colonies were counted by using a colony counter (JP Selecta, Spain) and results were expressed as cfu/ml.

Lipolytic bacteria colonies on Tween 80 agar medium were sub-cultured on nutrient agar medium and plates were incubated at 30°C for 72 h. The colonies obtained were subjected to morphological characterization such as Gram staining and motility. The identification of lipolytic bacteria was confirmed by classic biochemical tests as described by Bergey and Holt (1994).

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 StatPlus 2008 (Analystsoft Inc) software. Statistical significance was measured at $p < 0.05$.

RESULTS

Oil yield

The oil content of *H. sabdariffa* seeds (24.53 ± 2.00) was more than twice of that of *C. olitorius* seeds which was 11.92 ± 1.09 (Table 1).

Physicochemical characteristics

There was no significant difference ($p < 0.05$) between most of the physicochemical parameters of the two seed oils except for viscosity (53.78 ± 0.13 and 52.70 ± 0.12 mPas), iodine values (132.54 ± 1.22 and 108.57 ± 1.22 g I₂/100 g) and vitamin A (0.45 ± 0.01 and 0.60 ± 0.01 mg/g), 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 1.6% and 6 meq O₂/kg, respectively. Oils extracted from *C. olitorius* and *H. sabdariffa* seeds were cholesterol free and their respective unsaponifiable matter contents were less than

Table 1. Extraction yield and physicochemical properties of *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds.

Parameter	Oilseed	
	<i>C. olitorius</i>	<i>H. sabdariffa</i>
Extraction yield	11.92 ± 1.09% ^a	24.53 ± 2.00% ^b
Specific gravity at 25°C	0.87 ± 0.01 ^a	0.85 ± 0.01 ^a
Refractive index at 25°C	1.46 ± 0.00 ^a	1.45 ± 0.00 ^a
Viscosity at 25°C (mPas)	53.78 ± 0.13 ^a	52.70 ± 0.12 ^b
pH at 25°C	5.65 ± 0.03 ^a	5.71 ± 0.02 ^a
Acid value (mg KOH/g)	3.74 ± 1.62 ^a	2.80 ± 0.00 ^a
Free fatty acids (% oleic acid)	1.87 ± 0.80 ^a	1.40 ± 0.00 ^a
Peroxide value (meq O ₂ /kg)	6.67 ± 0.58 ^a	5.33 ± 0.60 ^a
Iodine value (g I ₂ /100 g)	132.54 ± 1.22 ^a	118.57 ± 1.22 ^b
Saponification value (mg KOH/g)	183.63 ± 3.24 ^a	189.23 ± 1.62 ^a
Unsaponifiable matter (%)	0.88 ± 0.04 ^a	0.85 ± 0.05 ^a
Vitamin A (mg/g)	0.45 ± 0.01 ^a	0.60 ± 0.01 ^b
Cholesterol (mg/g)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

^{a,b}, Means in line with no common superscript differ significantly ($p < 0.05$).

1%. The saponification values of the two oilseeds were about 180 to 190 mg KOH/g (Table 1).

The UV-visible spectra of the studied oilseeds showed two fairly stacking graphs with maximum absorbances at 220 and 280 nm respectively. In addition, each oilseed sample showed rapid decrease from 0.64 to 0 in the range of 300 to 500 nm (Figure 1).

Fatty acid composition

Chromatographic profiles of fatty acids composition and their relative amounts in *C. olitorius* and *H. sabdariffa* oilseeds are given in Figure 2 and in Table 2, respectively. Fatty acid proportions of the studied oilseeds highlighted the presence of five main compounds namely palmitic, stearic, oleic, linoleic and linolenic acids (Figure 2). On average, these five fatty acids were about 88.82% and 87.37% of total fatty acids in *C. olitorius* and *H. sabdariffa* oilseeds, respectively (Table 2).

The two oilseeds were predominantly composed of saturated fatty acids (SFA) and PUFA which contents (about 42 to 47% of total fatty acids) showed significant difference ($p < 0.05$). The PUFA were made up of linoleic acid and a relatively high rate of linolenic acid (37.31 and 29.22% respectively for *C. olitorius* and *H. sabdariffa* oilseeds). These oilseeds were also composed of monounsaturated fatty acids (MUFA) that was essentially oleic acid (Figure 2) in the proportion of about 9 to 10% (Table 2). Ratios of linoleic acid to the linolenic one ($n-6/n-3$) calculated for *C. olitorius* and *H. sabdariffa*

oilseeds were significantly different ($p < 0.05$) with values of about 0.26 and 0.46, respectively (Table 2).

Microbiological characteristics

The counting of microorganisms isolated from *C. olitorius* and *H. sabdariffa* oilseeds is shown in Table 3. Crude extracted oilseeds of the two plants were fungus, anaerobic bacteria, coliforms, *Staphylococcus* and *Salmonella*-free. Nevertheless, oil samples were barely contaminated by total viable bacteria (5-11 cfu/ml) including lipolytic bacteria (1 cfu/ml) which belong to the genus *Micrococcus* (Table 4).

DISCUSSION

In regard to the oil content, *H. sabdariffa* seeds are lipid-rich than most of the conventional oilseeds such as cotton (13%), soybean (14%) and palm fruit (20%) (Nzikou et al., 2007) 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 reports (El-Adawy and Khalil, 1994; Nzikou et al., 2011). The oil content of *C. olitorius* seeds did not show much variation compared to previous report (Gadgil et al., 1989). However this oil content was lower than that (59.2%) reported by Ibrahim and Fagbohun (2011). These variations between oil yields in seeds could be attributed to their cultivation climate, ripening stage,

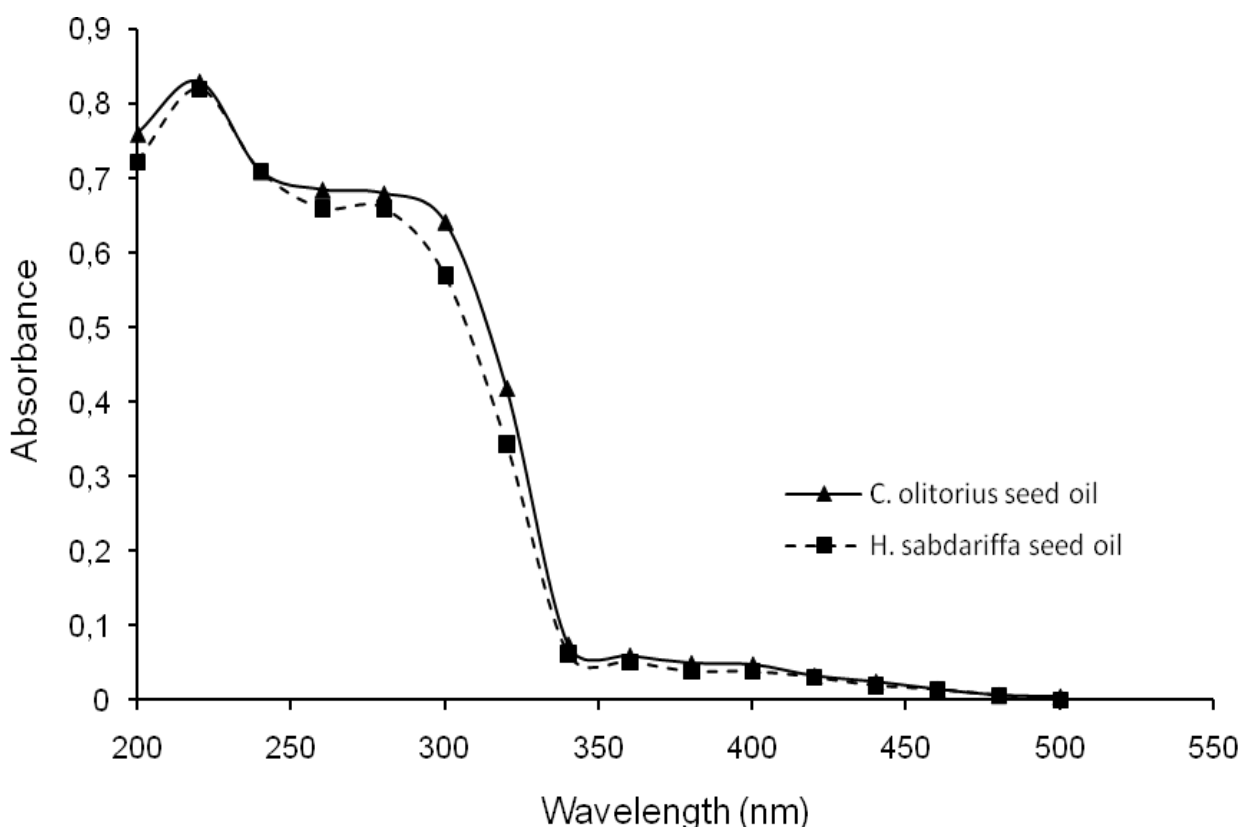


Figure 1. UV-Vis spectrum of *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds. Experiments were performed in triplicate by measuring absorbances of hexanic oilseeds (1%) in the wavelengths range varying from 200 to 600 nm. Data represents mean \pm SD.

harvesting time and the extraction method employed (Egbekun and Ehieze, 1997).

The specific gravity of *C. olitorius* and *H. sabdariffa* oilseeds are lower than those reported for most conventional oilseeds which are about 0.9 (Codex, 1993). In addition, the viscosity values of both of the oilseeds were in the range (50 to 100 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). The relatively low peroxide values of *C. olitorius* and *H. sabdariffa* 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 *C. olitorius* and *H. sabdariffa* 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). Another aspect for using these oils in cosmetics is the decrease in absorbance in the range of 290 to 400 nm. Indeed, this property may be advantageous for using *C. olitorius* and *H. sabdariffa* oilseeds in cosmetics formulation as UV protectors that provide protection against both UV-B and UV-A (Besbes et al., 2004).

The refractive indexes of the two oilseeds 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 I₂/100 g) and sunflower (110 to 143 g I₂/100 g) oils (Codex, 1993). However, these values are higher than those reported by Kapseu and Parmentier (1997) for other non-

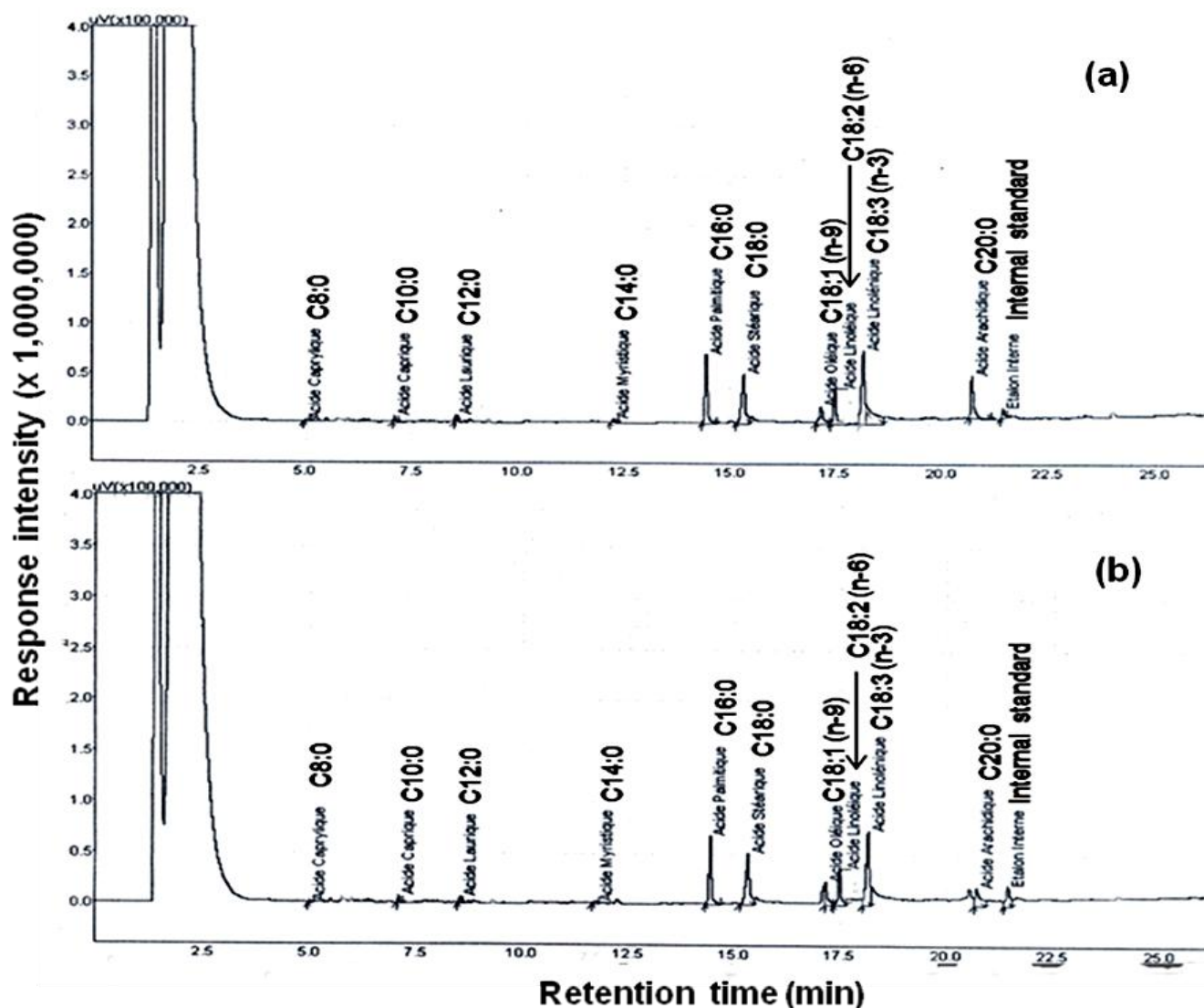


Figure 2. Gas chromatograms of fatty acids constituents of *Corchorus olitorius* (a) and *Hibiscus sabdariffa* (b) oilseeds. Experiments were performed in triplicate by gas chromatographic analysis (GC-FID) of fatty acids methyl esters derived from *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds. Data represents mean \pm SD.

conventional oilseeds such as *Coula edulis* (90-95 g I₂/100 g), *Dacryodes edulis* (60 to 80 g I₂/100 g) and *Canarium schweinfurthii* (71.1 to 94.9 g I₂/100 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 *C. olitorius* and *H. sabdariffa* oilseeds are higher than those reported for most of the non-conventional oilseeds as sheabutter (6.9%), avocado (15.5%), *Dacryodes edulis* (25.2%) and *Canarium*

schweinfurthii (28.8%) (Chalon, 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 linolenic acid content of *C. olitorius* and *H. sabdariffa* 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 linolenic oils and could be used in human diet for anti-inflammatory, anti-thrombotic, anti-hypertensive and anti-arrhythmic actions (Kamal-Eldin, 2006; Nzikou et al., 2007). In addition, linolenic acid content of *H. sabdariffa*

Table 2. Fatty acids composition of *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds.

Fatty acid (%)	Oilseed	
	<i>C. olitorius</i>	<i>H. sabdariffa</i>
Caprylic (C _{8:0})	0.15 ± 0.01 ^a	0.56 ± 0.01 ^b
Capric (C _{10:0})	0.10 ± 0.01 ^a	1.47 ± 0.01 ^b
Lauric (C _{12:0})	1.41 ± 0.00 ^a	1.38 ± 0.00 ^a
Myristic (C _{14:0})	0.90 ± 0.01 ^a	1.31 ± 0.01 ^a
Palmitic (C _{16:0})	17.61 ± 0.01 ^a	18.65 ± 0.05 ^a
Stearic (C _{18:0})	15.08 ± 0.01 ^a	15.92 ± 0.01 ^a
Arachidic (C _{20:0})	8.62 ± 0.01 ^a	7.91 ± 0.01 ^a
Oleic (C _{18:1})	9.02 ± 0.00 ^a	10.26 ± 0.01 ^a
Linoleic (C _{18:2 n-6})	9.80 ± 0.01 ^a	13.32 ± 0.01 ^b
Linolenic (C _{18:3 n-3})	37.31 ± 0.01 ^a	29.22 ± 0.02 ^b
SFA*	43.87 ± 0.06 ^a	47.20 ± 0.10 ^b
MUFA	9.02 ± 0.00 ^a	10.26 ± 0.01 ^a
PUFA	47.11 ± 0.02 ^a	42.54 ± 0.03 ^b
n-6/n-3	0.26 ^a	0.46 ^b

^{a,b}Means in the lines with no common superscript differ significantly ($p < 0.05$).

*SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.

Table 3. Microorganisms enumerated from *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds.

Microorganism (cfu/ml)	Oilseed	
	<i>C. olitorius</i>	<i>H. sabdariffa</i>
Total viable bacteria	9 ± 2 ^a	7 ± 2 ^a
Lipolytic bacteria	1 ± 0 ^a	1 ± 0 ^a
Yeasts and moulds	0 ^a	0 ^a
Anaerobic bacteria	0 ^a	0 ^a
Coliforms	0 ^a	0 ^a
<i>Staphylococcus</i>	0 ^a	0 ^a
<i>Salmonella</i>	0 ^a	0 ^a

^{a,b}Means in the lines with no common superscript differ significantly ($p < 0.05$).

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 LDL-cholesterol 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).

Ratios of linoleic acid to the linolenic one (n-6/n-3),

calculated for *C. olitorius* and *H. sabdariffa*, are extremely lower than the critical ratio (5.00) recommended by nutritionists to reflect the need for a nutritional equilibrium (Legrand et al., 2001). Therefore, *C. olitorius* and *H. sabdariffa* oilseeds are more nutritionally balanced than most of the conventional oils such as soybean, walnut, olive, corn, grape seed and sunflower oils with respective n-6/n-3 ratios of 8, 8.1, 34, 72.5, 233.3 and 670 (Codex alimentarius, 1993). Vitamin A contents of *C. olitorius* and *H. sabdariffa* 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

Table 4. Characteristics of lipolytic bacteria isolated from *Corchorus olitorius* and *Hibiscus sabdariffa* oilseeds.

Test	Characteristic
Motility	–
Gram reaction	+
Growth on nutrient agar	Yellow lemon colonies
Catalase	+
Oxidase	+
Coagulase	–
Mannitol	–
Glucose	–
Genus name	<i>Micrococcus</i>

+, Positive; –, negative.

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).

C. olitorius and *H. sabdariffa* oilseeds are exempt of pathogenic bacteria usually involved in food poisoning (Bourgeois et al., 1996). Compared with 500 cfu/ml that is the critical value of total viable bacteria advisable to vegetable oils consumption (Guiraud, 1998), the studied oilseeds were of high microbiological quality. The presence of *Micrococcus* in these oils is indicative of a human contamination because these bacteria are found as part of normal flora of human's skin (Jensen, 1997). Lipolytic activity of these bacteria may result in pH lowering by hydrolyzing triglycerides into fatty acids and increasing oils acidity (Hass, 2001).

Conclusions

It could be concluded in view of the results of this investigation that *C. olitorius* and *H. sabdariffa* 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, lather shaving and UV protector creams. Oils extracted from *C. olitorius* and *H. sabdariffa* seeds are good source of EFA predominantly composed of linolenic acid. The fatty acids profile and the n-6/n-3 ratio 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 addition, their microbiological qualities make them safe for human uses. In view of all these potentialities and qualities, *C. olitorius*

and *H. sabdariffa* 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 *in vitro* toxicological and antimicrobial effects of the two oilseeds before using them as supplements in food, pharmaceutical and cosmetic industries.

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REFERENCES

- Afane E, Lando G, Biyiti L, Ossima GA, Atchou G (1997). Boiling palm oil vapours, a broncho-annoying acid. *Med. Afr. Noire*, 44: 604-607.
- AFNOR (1986). The french standards collection, fats, oleaginous seeds and by-products. 4th ed. AFNOR, Paris. p. 527.
- AFNOR (1996). Microbiological analysis, food products quality control. 3rd ed. AFNOR, Paris. p. 521.
- Alais C, Linden G (1991). Lipids. In: Masson, (ed). *Food Biochem.* Paris. pp. 55-73.
- Ali MA, Sayeed MA, Reza MS, Yeasmin S, Khan AM (2008). Characteristics of seed oils and nutritional compositions of seeds from different varieties of *Momordica charantia* cultivated in Bangladesh. *Czech J. Food Sci.* 26: 275-283.
- Aluyor EO, Ozigagu CE, Oboh OI, Aluyor P (2009). Chromatographic analysis of vegetable oils: A review. *Sci. Res. Essay*, 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. *J. Appl. Sci. Res.* 6: 265-268.
- Anwar F, Chata SAS, Hussain AI (2007). Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage. *Grasas Acietes*, 58: 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. *J. Chem. Soc. Pak.* 28: 144-148.

- AOAC (1997). Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Washington DC. p. 684.
- Badifu G (1993). Food potentials of some non-conventional oilseeds grown in Nigeria. *Plant Food Hum. Nutr.* 43: 211-224.
- Bergey DH, Holt JG (1994). *Bergey's manual of determinative bacteriology*. 9th ed. Williams, Wilkins, USA. p. 787.
- Besbes S, Blecker C, Deroanne C, Lognay G, Drira NE, Attia H (2004). Quality characteristics and oxidative stability of date seed oil. *Food Sci. Technol. Int.* 10: 333-338.
- Bourgeois CM, Mesclé JF, Zucca J (1996). *Food microbiology*. 2nd ed. Technique and Documentation, Paris. p. 672.
- Chalon S (2001). Polyunsaturated fatty acids and functions. *OCL*, 8: 317-320.
- Cissé M, Dornier M, Sakho M, Ndiaye A, Reynes M, Sock O (2009). The Bissap (*Hibiscus sabdariffa* L.): composition and main uses. *Fruits*, 64: 179-193.
- Codex Alimentarius (1993). *Fats and Vegetable oils*. FAO/WHO, Geneva, p. 160.
- Das UN (2006). Essential fatty acids -a review. *Curr. Pharm. Biotechnol.* 7: 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.
- Dhellot 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. *Afr. J. Biotechnol.* 5: 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. *Eur. J. Lipid. Sci. Technol.* 109: 710-732.
- Dungan RE, Frigerio NA, Siebert JM (1964). Colorimetric determination of vitamin A and its derivatives with trifluoroacetic acid. *Anal. Chem.* 36: 114-117.
- Egbekun MK, Ehieze MU (1997). Proximate composition and functional properties of fullfat and defatted benisseed (*Sesamum indicum* L.) flour. *Plant Food Hum. Nutr.* 51: 35-41.
- Ejikeme PM, Obasi LN, Egbuonu AC (2010). Physicochemical and toxicological studies on *Azelia africana* seed and oil. *Afr. J. Biotechnol.* 9: 1959-1963.
- Eka OU (1980). Proximate composition of bush mango tree and some properties of dikafat. *Nig. J. Nutr. Sci.* 1: 33-36.
- El-Adawy TA, Khalil AH (1994). Characteristics of roselle seeds as a new source of protein and lipid. *J. Agric. Food Chem.* 42: 1896-1900.
- Elfalleh W, Ying M, Nasri N, Hua HS, Guasmi F, Ferchichi A (2011). Fatty acids from Tunisian and Chinese pomegranate (*Punica granatum* L.) seeds. *Int. J. Food Sci. Nutr.* 62: 200-206.
- European Communities (1991). Determination of free fatty acid methyl esters. In: OJ, (ed). *Characteristics of olive oil and the relevant methods of analysis*. Geneva, pp. 64-67.
- FAO (2001). *Nutrition in developing countries*. 3rd ed. FAO, Geneva. p. 515.
- Gadgil JD, Suseelan KN, Mitra R (1989). Chemical composition and electrophoretic pattern of seed storage protein of jute *Corchorus olerius*. *Seed Sci. Technol.* 17: 499-506.
- Guiraud JP (1998). *Food microbiology*. 2nd ed. Dunod, Paris. p. 652.
- Haas MJ (2001). Lipolytic microorganisms. In: American Public Health Association, (ed). *Compendium of methods for the microbiological examination of foods*. USA. pp. 175-181.
- Helme JP (1990). Lipids and Cosmetology. *Rev. F. Corps Gras* 11: 6-36.
- 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. *J. Nutr. Biochem.* 11: 408-416.
- Ibrahim TA, Fagbohun E (2011). Physicochemical properties and in vitro antibacterial activity of *Corchorus olerius* Linn. *Seed Oil. Life Sci. Leaflets*, 15: 499-505.
- IUPAC (1979). *Standard methods for the analysis of oils, fats and derivatives*. 6th ed. Pergamon, Oxford. p. 190.
- Jensen M (1997). *Microbiology for the health sciences*. 4th ed. Prentice Hall, USA. p. 700.
- Judde A (2004). Prevention of the fatty acids oxydation in a cosmetic product: mechanisms, consequences, determination methods, what antioxydants for what applications? *OCL*, 11: 414-418.
- Kamal-Eldin A (2006). Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *Eur. J. Lipid. Sci. Technol.* 58: 1051-1061.
- Kapseu C, Parmentier M (1997). Fatty acid composition of some vegetable oils from Cameroon *Sci. Aliment*, 17: 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. *Prog. Lipid Res.* 35: 93-132.
- Lee JC, Kim HR, Kim J, Jang YS (2002). Antioxidant property of an ethanol extract of the steam of *Opuntia ficus-indica* var Saboten. *J. Agric. Food Chem.* 50: 6490-6496.
- Legrand P, Bourre JM, Descomps B, Durand G, Renaud S (2001). Lipids. In: Martin A (ed). *The advisable nutritional benefits for French population*. Paris. pp. 63-82.
- Leung WTW, Busson F, Jardin C (1968). *Food composition table for use in Africa*. 1st ed. FAO, Roma. p. 306.
- Mahadevan N, Shivali A, Kamboj P (2009). *Hibiscus sabdariffa* Linn: an overview. *Nat. Prod. Rad.* 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. *Int. J. Food Sci. Nutr.* 62: 318-327.
- Matos L, Nzikou JM, Matouba E, Pandzou-Yembe VN, Mapepoulou TG, Linder M, Desobry S (2009). Studies of *Iringia gabonensis* seed kernels: oil technological applications. *Pak. J. Nutr.* 8: 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. *Afr. J. Sci. Technol.* 2: 51-64.
- Meiattini F (1978). The 4-hydroxybenzoate/4-aminophenazone chromogenic system. *Clin. Chem.* 24: 2161-2165.
- Mhahmad 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*). *Afr. J. Agric. Res.* 6: 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. *Eur. Resp. J.* 16: 861-865.
- Naito HK (1988). Current status of blood cholesterol measurement in clinical laboratories in the United States. *Clin. Chem.* 31: 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.
- Nukhet A, Akpinar MA, Turkoglu S (2001). Total lipid content and fatty acid composition of the seeds of some *Vicia* L. species. *Food Chem.* 74: 444-453.
- 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. *Adv. J. Food Sci. Technol.* 1: 6-11.
- Nzikou JM, Mvoula-Tsieri 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. *J. Appl. Sci.* 7: 1107-1115.
- Nzikou JM, Bouanga-Kalou G, Matos L, Ganongo-Po FB, Mboundou-Mboussi 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. *Curr. Res. J.*

- Biol. Sci. 3: 141-146.
- Ramadan MF, Sharanabasappa G, Seetharam YN, Seshagiri M, Moersel JT (2006). Characterisation of fatty acids and bioactive compounds of kachnar (*Bauhinia purpurea* L.) seed oil. Food Chem. 98: 359-365.
- Reiger M (1989). The apparent pH of the skin. Cosmet. Toilet, 104: 53-54.
- Rossell JB (1991). Vegetable oils and fats. In: Rossell JB (ed). Analysis of oilseeds, fats and fatty foods. London. pp. 261-325.
- Savag GP, Dutta PC, Mcneil DL (1999). Fatty acid and tocopherol content and oxidative stability of walnut oils. J. Am. Oil Chem. Soc. 76: 1059-1060.
- Sharaf A, Negm SAR (1969). Pharmacological study of *Corchorus olitorius* seed. Plant Food Hum. Nutr. 17: 305-312.
- Sierra G (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact cells and fatty substances. J. Microbiol. Serol. 23: 15-22.
- Wijendran V, Hayes KC (2004). Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Ann. Rev. Nutr. 24: 597-615.
- Wolf JP (1968). Fatty substances analysis manual. 1st ed. Azoulay, Paris. p. 552.