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Chemical survey of *Afraegle paniculata* fruit seeds from Côte d'Ivoire

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ABSTRACT

The chemical survey of the seeds of Afraegle paniculata fruit, a Rutaceae from Côte d'Ivoire has been achieved. The mineral analysis of ashes seeds by scanning electron microscope showed that they contain mineral elements Na, Mg, S, Cl, Ca, with a dominance of K and P. The quantitative analysis of oil extracted from the seeds, showed some contents of total chlorophylls (6.253 mg/100 g of oil), total carotenoids (4.658 mg/100 g of oil) and total phenols (83.27 ± 0.77 mg/100 g of oil). As for the one concerning the seeds of the aforesaid plant, it gave the contents of total sugars and reducing sugars respectively (205.183 µg/g of dry mass) and (20.44 µg/g of dry mass). The antioxidant power of the phenolic fraction (80.177mg/100 g of oil) has been valued also by the colorimetric method DPPH. GC/MS analysis of unsaponifiables from the oil highlighted 44 compounds left in 4 families of which hydrocarbons (33.76%), alcohols (7.48%), terpenes (12.90%) and sterols (11.55%).

Keywords: chemical study, seed, oil, *Afraegle paniculata*, Côte d'Ivoire

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INTRODUCTION

The fats bodies are organic matters of animal or plant origin. They are fluid, pasty or solid, unctuous, hydrophobic. The plants that produce them are varied and are called oleaginous. The lipid reserves observed at these are often noticed in the seeds or in the pulp that surrounds the core of the fruit [1]. *Afraegle paniculata* fruit is to be one of the unconventional oleaginous and very little known. Widespread on the West African coast from Senegal to Nigeria, *Afraegle paniculata* (Schumach. & Thonn.) Engl. is a plant of Rutaceae family. It is cultivated in the villages for its multiple uses (medicinal, nutritional, etc.) [2]. Of the recent studies led on the zest of Ivorian species fruit, revealed the presence of 40 compounds including hydrocarbon sesquiterpenes (64.49%), oxygenated sesquiterpenes (7.60%), oxygenated (5.78%) and hydrocarbon monoterpenes (7.82%) [3]. Besides the results of investigations made on the fat matter extracted from the seeds of the fruit of the aforesaid plant, relatively to the chemical composition and to some physical and physicochemical properties, have been reported [4].

The present survey makes an exposition of the results gotten of the chemical investigation of *Afraegle paniculata* fruit seeds from Côte d'Ivoire.

MATERIALS AND METHODS

Plant material

A. paniculata fruit seeds previously identified [4], were sprayed with an electric grinder after drying in an oven at 60 °C for one week.

Dosage of seeds mineral elements

The analysis was performed using a scanning electron microscope with variable pressure PHCD (SEM FEG Zeiss Supra 40 VP, variable voltage 0.1 kV to 30 keV). An X-ray detector (Oxford Instruments) connected to a micro analyzer platform EDS (Inca Cool Dry, without liquid nitrogen) was used. The sprayed seeds were incinerated in a furnace at 600 °C for 6 h. The ashes thus obtained were deposited on carbon pellets. For calibration, a probe of 30 mm to 120 mm diameter was selected. A magnification was made so as to observe a maximum of particles (magnification of 12 to 1 million times) with a resolution of 2 nm.

Determination of oil extinction coefficients

The oil was obtained from *A. paniculata* fruit seeds using a Soxhlet extractor [4]. The oil (0.1 g) was taken up in cyclohexane (10 mL). After homogenization, the specific extinction coefficients (K_{232} and K_{270}) were respectively calculated from the absorbance at 232 and 270 nm. As regards, the variation of the specific extinction (ΔK) was determined from the absorbance at 266, 270 and 274 nm [5]. Cyclohexane was used as reference. Reading is done in covered quartz tanks with a 1 cm optical path.

Determination of oil contents of chlorophylls and total carotenoids

The oil (7.5 g) was dissolved in cyclohexane (25 mL). The absorbance (A) of the solution was read at 670 and 470 nm using UV-visible spectrophotometer HP 8453 single beam, respectively for total chlorophylls and carotenoids [6]. The values of specific extinction coefficients used are $E_0 = 613$ for pheophytins and $E_0 = 2000$ for lutein, as they are the major components in the respective fractions of chlorophylls and total carotenoids [7, 8]. The contents of Chlorophylls and carotenoids were calculated using the following expressions:

Total chlorophylls= (mg/kg)= $(A_{670} \times 25 \times 10^4) / (613 \times 7.5)$

Total carotenoids= (mg/kg)= $(A_{470} \times 25 \times 10^4) / (2.10^3 \times 7.5)$

Dosage of seeds total sugars

Reducing and total sugars were respectively dosed using DNS colorimetric method [9] and phenol sulfuric assay [10]. Carbohydrate extract was obtained by mixing pulverized seeds (2 g) in ethanol (8 mL, 80%) and a pinch of Fontainebleau sand. The whole was centrifuged at 4000 tr/min for 10 min. The supernatant was collected and the volume was completed to 25 mL with the alcoholic solvent. The results of equivalent glucose were expressed in mg/g dm (dry matter).

Determination of oil total phenols

Total phenol contents were evaluated according Folin-Ciocalteu colorimetric method [11] with some modifications [12].The oil (2.5 g) was dissolved in hexane (5 mL) and 5 mL of MeOH/H_2O (60/40, v/v) were added. The mixture was vigorously stirred to extract the polyphenols. Phenolic fraction (1mL) was added to Folin-Ciocalteu reagent (0.5 mL, 0.5 N) then a solution of Na_2CO_3 (1 mL, 35%) and an amount of ultrapure water were added to it having a final volume (10 mL). The mixture was incubated for 1 hour in the dark at room temperature. The absorbance was read at 750 nm and the results were expressed in mg of caffeic acid per 100 g of oil.

Evaluation of oil antioxidant activity

The oil anti-radical power against DPPH, was evaluated according to the method described by Blois [13]. It was expressed by the following formula:

%I (Percent inhibition)= $[1-(A_{sample} / A_{Control})] \times 100$

A concentration curve of the extract as a function of %I was plotted to determine the IC_{50} [14, 15].

GC/MS analysis of oil unsaponifiables

The unsaponifiables were extracted according to the method reported [4]. Approximately 2 mg of unsaponifiables were mixed with 2 mL of pyridine/acetic anhydride (50/50, v/v), then analyzed using GC/MS spectrophotometer SHIMADZU, QP2010SE with a Zebron ZB-5ms column 20 m long, with an internal diameter of 0.18 mm and a film thickness of the stationary phase of 0.18 µm. Helium was used as carrier gas at a linear velocity of 0.9 mL/s. The oven temperature program was 50-280°C for 2 min and 280-300°C for 5 min, then maintained at 300°C for 18 min. The injector temperature was set at 250°C and the detector at 280°C. The injection was performed in splitless mode. The mass spectrometer parameters for electron impact mode are ion source temperature (230°C), electron energy (70 eV), scan speed (50 scans/s) and acquisition speed (10.000 a.m.u/s). The compounds identification was obtained by comparing retention times with those of reference compounds and spectral data obtained from NIST and Wiley libraries.

RESULTS AND DISCUSSION

Seeds mineral composition

The t**able 1** shows the contents of major mineral components in *A. paniculata* seeds, expressed as percentage of dry mass*.

Table 1: Mineral composition of *A. paniculata* seeds (% of dm)

Spectrum	C	Na	Mg	P	S	Cl	K	Ca	O	Total
Spectrum 1	18.29	**0.82**	**3.71**	**4.40**	**0.97**	**0.72**	**6.93**	**2.97**	61.19	100.00
Spectrum 2	15.49	**1.14**	**5.07**	**6.08**	**1.10**	**0.86**	**8.63**	**3.83**	57.79	100.00
Spectrum 3	14.65	**1.07**	**5.07**	**6.48**	**1.16**	**1.20**	**9.79**	**4.11**	56.48	100.00
Average value	16.14	**1.01**	**4.62**	**5.65**	**1.08**	**0.93**	**8.45**	**3.64**	58.48	100.00

** (% of dm)*

A. paniculata seeds contain several minerals. They are rich in K (8.45%) but also balanced in Mg (4.62%) and Ca (3.64%), what could assign to them hypotensive properties bound to diuretic virtues [16]. P can also be found with a high content (5.65%). The presence of Ca, P and Mg in the seeds procures them some nutritional qualities [17]. Indeed, Ca and P intervene in the dental solidification and ossification in the organism. Mg permits the activation of some enzymes and intervenes in the deterioration of the carbohydrates, the synthesis of the proteins, the transmission of the nervous impulse or the muscular contraction [18]. As for K, it plays an important role in the synthesis of amino acids and proteins [19]. Besides, to the look of the contents in Ca, Mg and Na *A. paniculata* oil would be rich in phospholipids nonhydratable than in hydratable phospholipids [20].

Oil specific extinction coefficient

The specific extinction coefficients of the oil K_{232} = 1.5956 ± 0.0005 and K_{270}= 0.3219 ± 0.0006 were determined in the UV. They provide information on the presence or absence of secondary oxidation products in the oil. Hydroperoxides of the first phase autoxidation absorb at 232 nm, whereas the secondary oxidation products such as ketones and unsaturated diketones absorb at 270 nm [21, 22].The absorbance in the UV can provide information on the quality of fat matter his state during its conservation. Also, it informs on the conditions (temperature, duration in particular) of the extraction method and the possible oxidation during the overexposure of the seeds to the open air at the time of the grinding [22].The absorbances in UV also show that K_{270}, ΔK= 0.0108 ± 0.0007 of oil from *A. paniculata* seeds, are slightly higher than values $K_{270} \leq 0.25$ $\Delta K \leq 0.01$ that correspond to IOC standard [23];what seems to indicate that the time put to grind the seeds, and the one of the oil extraction, must be reduced.

Contents of oil chlorophylls and total carotenoids

Chlorophylls and carotenoids are the minor components of the oils, that confer them organoleptic and nutritional qualities [24, 25]. Chlorophylls **a** and **b** and their immediate degradation products (pheophytins **a**, **b**) are photosensitizers [26]. It is suggested that β-carotene preserves oxygen activation by light [27]. The contents of carotenoids (4.658 ± 0.016 mg/kg oil) and chlorophylls (6.253 ± 0.071 mg/kg oil) of *A. paniculata* oil (**table 2**) are respectively superior to those of Tunisian olive oil (1.60 ± 0.03 mg/kg, 3.52 ± 0.04 mg/kg) [**28**]. These contents relatively raised in pigments, confer to *A. paniculata seeds* a protection against advanced oxidation [29, 30] and a source of vitamin A [31].

Table 2: Contents of pigments and total sugars of *A. paniculata* oil

Constituent	Content
Total chlorophylls (mg/kg oil)	6.253 ± 0.071
Total carotenoids (mg/kg oil)	4.658 ± 0.016
Total phenols (mg/100g oil)	83.27 ± 0.77
Total sugars (µg/g dm)	205.183 ± 0.617
Reducing sugars (µg/g dm)	20.44 ± 0.27

Oil Content of total phenols and antioxidant activity

It is known that the therapeutic effect of the oleaginous plants is determined not only by the quality of the unsaponifiables of their fat matter but also by the presence of polyphenols. It is reported that polyphenols are responsible for the manifestation of some biological properties between other antioxidant, antimicrobial and anticancer [**32**]. In this regard, it seemed sensible to extract polyphenols contained in *A. paniculata* seed oil and analyze them. Generally, phenolic acids are present in oleaginous as hydroxylated derivatives of benzoic and cinnamic acids. The content of total phenols determined in *A. paniculata* seeds oil is of 83.27mg of caffeic acid/100 g of oil (**table 2**). This value is higher than those of sesame oil (1.421 mg caffeic acid / 100 g of oil) [**8**] and olive (5.333 mg caffeic acid/100 g of oil), that is considered like an oil rich in phenolic compounds [**33**]. From this point

of view, oil extracted from seeds of *A. paniculata* could be a potential source in phenolic compounds. The polyphenols are often responsible of antioxidant activity of plants. That's why we have evaluated the antioxidant power of *A. paniculata* oil while determining its inhibitory concentration (IC_{50}) that is equal to 80.177±0.74 g/100 g. This value shows modestly the antiradical capacity of the oil tested opposite DPPH, which is not negligible compared to the one of vitamin C, whose IC_{50} is equal to 30.32±0.29 g/100 g).

Organic constituents of unsaponifiable fraction

More than 44 compounds have been identified in the unsaponifiable fraction (**table 3**). These are phytocompounds belonging to the hydrocarbons, alcohols, terpenes and phytosterols.

Table 3: Unsaponifiables of *A. paniculata* seeds oil

Peak	Retention time (min)	Identified phytocompound	Percentage (%)
1	5.898	Nonanal	0.22
2	6.244	5-methyldocosane	0.49
3	6.637	Piperidine	0.59
4	7.559	Glycerol	1.82
5	8.048	7-methyl-4-octanol	0.09
6	8.241	Caryophyllene	0.69
7	8.734	10,12-tricosadiynoic acid	1.84
8	9.093	Cis-1-chloro-9-octadecene	0.15
9	9.290	Caryophyllene oxide	1.27
10	9.453	Spiro[4,5]decane	0.10
11	9.869	3-ethyl-5-(2-ethylbuthyl)-Octadecane	0.40
12	10.156	Pentaerythritol	0.13
13	10.652	6,10,14-trimethyl-2-pentadecanone	0.33
14	11.491	**1-hexadecanol**	**4.79**
15	11.957	5-acetoxypentadecane	1.98
16	12.218	8-(1-hydroxyethyl)-4,9-dimethyl-2H-furo[2,3H] Chomen-2-one	0.07
17	12.508	**Phytol**	**7.87**
18	12.604	(2Z,6E)-3,7,11-Trimethyl-2,6-dodecadien-1-ol	0.22
19	12.652	3,7-dimethyl-6-nonen-1-ol	0.09
20	12.817	1-heptacosanol	0.11
21	12.956	Geranylgeraniol	1.55
22	13.378	3-[4-ethoxyphenyl]quinolin-4-ol	0.27
23	13.625	Hexanedioic acid,bis(2-ethylhexyl) ester	0.56
24	14.403	1-(1,2-dimethylpropyl)-1-methyl-2 –nonylcyclopropane	0.10
25	14.578	Tridecanol ,2-ethyl-2-methyl	0.07
26	14.913	1,2-benzenedicarboxylic acid,mono(2-ethylhexyl) ester	0.73
27	15.562	Tetratetracontane	0.05
28	16.347	Eicosane	0.06
29	16.767	Clionasterol	1.75
30	17.170	Squalene	2.79
31	18.741	2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene	0.02
32	19.634	Stigmasterol	0.12
33	19.894	Stigmast-5-en-3-ol	0.22
34	21.040	(+)-α-tocopherol	0.67
35	21.550	Isoindole-1,3(1H,3H)-dione,5-benzoyl-2-(4-methylphenyl	0.34
36	21.922	Cholesterol W	0.16
37	23.045	Desmosterol	2.72
38	23.329	Stigmasterol W	3.49
39	23.709	Campestanol	0.23
40	23.838	β-sitosterol	0.35
41	24.150	**Stigmastan-3,5-diene**	**30.33**
42	24.248	Cholestanol	0.60
43	24.810	(3β, 5α)-stigmast-7-en-3-ol (3β, 5α)-	1.97
44	24.810	5α-Stigmast-7,16,25-trien-3β-ol	0.16
	Others		26.38

We note that the unsaponifiable said oil composition is dominated by hydrocarbons (33.76%). The main hydrocarbon is stigmastan-3,5-diene (30.33%) (**fig. 1a**), followed by 5-acetoxypentadecane (1.98%) (**fig.1b**).

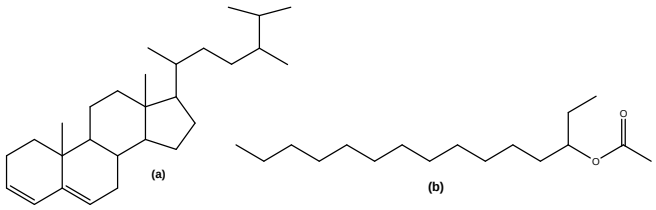

Figure 1: molecular structures of stigmastan-3,5-diene(a) and 5- acetoxypentadecane (b)

The high content of 3,5-stigmastan observed, could be justified by the presence of β-sitosterol [34]. Indeed, the temperature rise due to the drying environment of the chromatographic system could lead to dehydration of β-sitosterol in stigmastan-3,5-diene [35]. Moreover, this considerable content could confer to *A. paniculata* oil fibrinolytic and anti-inflammatory activities [36].

The alcohols whose rate is of 7.48% sign a relatively important proportion. Among them, 1-hexadecanol (1.82%) (**fig. 2c**) and glycerol (4.79%) (**fig. 2d**) are the most significant.

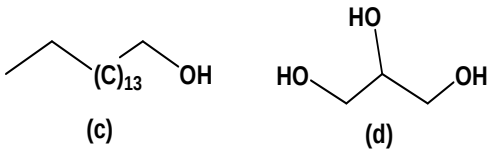

Figure 2: molecular structures of 1-hexadecanol(c) and glycerol (d)

After hydrocarbons, terpenes (12.90%) constitute important secondary metabolites of which phytol (7.87%), an oxygenated diterpene (**fig. 3**) is the most abundant.

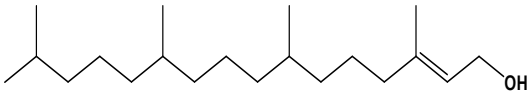

Figure 3: molecular structure of phytol

Its presence can procure to *A. paniculata* oil antioxidant and antifungal activities [**37**]. Phytosterols whose content is meaningful (11.55%), are among the main compounds of the unsaponifiable fraction of the oil. Stigmasterol W (3.49%) (**fig. 4f**) and desmosterol (2.72%) (**fig. 4g**) are the majority. Their presence gives to the oil its nutritional values [**20**]. Indeed, these natural sterols are precursors of provitamin D, and their existence may reduce the risk of certain cancers including those of the lung, breast, esophagus, stomach, colon and of ovary [**38**]. Besides, the presence of α-tocopherol (0.67%) (**fig. 4h**) protects *A. paniculata* oil of the oxidation.

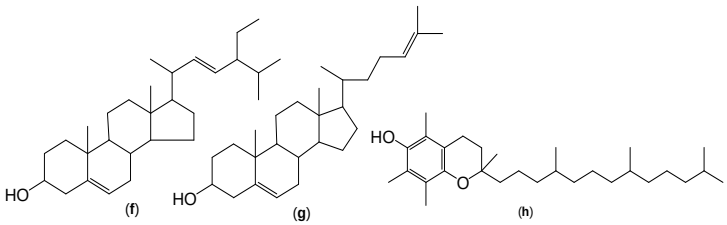

Figure 4: Molecular structures of stigmasterol W (f), desmosterol (g) and α-tocopherol (h)

It is a form of vitamin E that is preferentially absorbed and accumulated by human organism [39]. α-tocopherol used primarily in the treatment of hypercholesterolemia and is used also with satisfaction as a complement in the treatment of recalcitrant depression [40].Thus, the wealth in bio-important unsaponifiables of *A. paniculata* seeds oil could raise his recommendation as a dietary oil.

CONCLUSION

The present survey revealed the presence of major mineral elements Ca, Cl, Na, K, Mg, P, S in *Afraegle paniculata* seeds. The contents of chlorophylls, carotenoids, total phenols, total sugars and total reducing sugars of the seed oil have been determined. The phenolic fraction of the oil showed a moderate antioxidant power compared to vitamin C. The unsaponifiable fraction of the oil contains at least 44 compounds left in 4 families (hydrocarbons, alcohols, terpenes, sterols). The presence of stigmastan-3,5-diene (30.33%), phytol (7.8%) and phytosterols (11.55%) procures to *Afraegle paniculata* oil from Côte d'Ivoire nutritional and therapeutic properties.

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REFERENCES

[1] M. Bireche, B. Bakchiche, M. Maatallah, A. Gherib, *Int. J. Innovation Appl. Stud.*, **2014**, 9, 1944-1948.
[2] A. Chevalier, *Revue internationale de Botanique Appliquée et d'Agriculture Tropicale*, **1947**, 27(297), 264-270.
[3] N. S. Konan, B. A. Kouamé, A. M. Bossoh, J. A. Mamyrbékova-Békro, K. M. Konan, G. J. Nemlin, J-L. Pirat, Y-A. Békro, *European Journal of Scientific Research*, **2011**, 63(4), 482-488.
[4] S. Bamba, J. A. Mamyrbékova-Békro, D. Virieux, M. R. G. Kabran, J-L. Pirat, Y-A. Békro, *Der Chemica Sinica*, **2015**, 6(4), 47-50.
[5] H. Meftah, H. Latrache, F. Hamadi, H. Hanine, H. Zahir, M. El louali, *J. Mater. Environ. Sci*., **2014**, 5(2), 641-646.
[6] M. I. Minguez-Mosquera, L. Rejano, B. Gandul, A. H. Sanchez, J. Garrido, *J. Am. Oil Chem. Soc*., **1991**, 68, 332-336.
[7] M. Guerfel, M. B. Mansour, Y. Ouni, F. Guido, D. Boujnah, M. Zarrouk, *Grasas y aceites*, **2012**, 63, 290-295.
[8] C. Borchani, S. Besbes, C.H. Blecker, H. Attia, *J. Agr. Sci. Tech*., **2010**, 12, 585-596.
[9] R. N. Kouamé, C. Brahima, D. Emmmanuel, *Sciences & Nature*, **2010**, 7(2), 155-163.
[10] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, E. Smith, *Allal. Chem*., **1956**, 28, 350-356.
[11] V. L. Singleton, R. Ortofer, R. M. Lamuela-Raventos, *Methods in Enzymology*, **1999**, 299, 152-178.
[12] M. Tsimidou, G. Papadopoulos, D. Boskou, *J. Food Chemistry*, **1992**, 45, 141-144.
[13] M. S. Blois, *Nature*, **1958**, 181, 1199-1200.
[14] G. R. M. Kabran, N. C. Ambeu, J. A. Mamyrbékova-Békro, Y-A. Békro, *European Journal of Scientific Research*, **2012**, 68, 182-190.
[15] A. Merouane, A. Noui, H. Medjahed, K.M.A. Benhadj, A. Saadi, *Int. J. Biol. Chem. Sci*., **2014**, 8, 1865-1870.
[16] G. J. J. Flick, L. H. Aung, R. L. Ory, A. J. St-Angelo, *J. Food Sci*., **1977**, 42, 11-13.
[17] K. Doukani, S. Tabak, *Revue Nature & Technology*, **2015**, 12, 53-66.
[18] E. Dahouenon-Ahoussi, T. S. Djenontin, D. R. M. Codjia, F. P. Tchobo, A. G. Alitonou, J. Dangou, F. Avlessi, D. C. K. Sohounhloue, *Int. J. Biol. Chem. Sci.*, **2012**, 6(5), 2265.
[19] J. M. Nzikou, L. Matos, F. Mbemba, C. B. Ndangui, N. P. G. Pambou-Tobi, A. Kimbonguila, T. Silou, M. Linder, S. Desobry, *Res. J. Appl. Sci. Eng. Technol*., **2009**, 1(3), 154-159.
[20] S. T. Djenontin, J. Dangou, D. V. Wotto, K. C. D. Sohounhloue, P. Lozano, D. Pioch, *J. Soc. Ouest. Afr. Chim*., **2006**, 22, 59-67.
[21] R. Jeantet, T. Croguennec, P. Schuck, G. Brulé, Tec & Doc (Ed). Science des Aliments (Lavoisier, Paris, **2006**), 223.
[22] S. Ben Temime, W. Taamalli, B. Baccouri, L. Abaza, D. Daoud, M. Zarrouk, *J. Food Lipids*, **2006**, 13, 88-99.
[23] S. Boulfane, N. Maata, A. Anouar, S. Hilali, *J. Appl. Biosci*., **2015**, 87, 8029.
[24] B. Jacotot, *Olivae*, **2001**, 86, 27-29.
[25] G. Calabrese, *Olivae*, **2002**, 93, 19-20.
[26] E. Psomiadou, M. Tsimidou, *J. Agric Food Chem*., **2002**, 50(4), 722-727.
[27] M. Rahmani, L. Saad, *Rev. Fr. Corps Gras*, **1989**, 36(9/10), 355-360.
[28] I. Ben Tekaya, M. Hassouna, *OCL*, **2007**, 14(1), 60-67.
[29] A. K. A. Dandjouma, C. Tchiegang, M. Parmentier, *J. Biol. Chem. Sci*., **2008**, 2(3), 249-257.
[30] F. Mansouri, M. A. Ben, G. Lopez, M. L. Fauconnier, M. Sindic, H. Serghini-Caid, A. Elamrani, *Inside Food Symposium*, 9-12 Apr. **2013**, Leuven, Belgium, 12.
[31] A. Ali, D. Alhadji, C. Tchiegang, C. Saïdou, *Afr. J. Food Sci*., **2010**, 4(3), 115-119.
[32] E. B. M. Moreno, D. A. S. Lezcano, *Olivae*, **2015**,121, 21-28.
[33] S. Cheikh-Rouhou, B. Hentati, S. Besbes, C. Blecker, C. Deroanne, H. Attia, *Food Sci. Tech. Int*., **2006**, 15(5), 407-416.
[34] M. León-Camacho, S. M. Alvarez, C.E. Graciani, *Grasas y Aceites*, **2004**, 55, 227-232.
[35] A. Lanzón, T. Albi, A. Cert, J. Gracian, *Grasas y Aceites*, **1992**, 43, 271-276.
[36] M. Ravi, E. Palanisami, K. P. Ajay, *Int. J. Pharm Sci*., **2013**, 5(4), 655-659.
[37] A. Akpuaka, M. M. Ekwenchi, D. A. Dashak, A. Dildar, *N. Y. Sci. J.*, **2013**, 6(6), 119-124.
[38] B. R. Ghalem, H. Benhassaini, *Afrique Science*., **2007**, 03(3), 405-412.
[39] A. Rigotti, *Molecular Aspects of Medicine*, **2007**, 28(5/6), 423-436.
[40] https://fr.wikipedia.org/wiki/A-Tocophérol