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Analysis of a Rutaceae fat matter from Côte d'Ivoire

Souleymane Bamba[1,2], Janat Akhanovna Mamyrbekova-Bekro[1*], David Virieux[2], Guy Roger Mida Kabran[1], Jean-Luc Pirat[2] and Yves-Alain Bekro[1]

[1]Laboratoire de Chimie Bio Organique et de Substances Naturelles (LCBOSN), Unité de Formation et de Recherche des Sciences Fondamentales et Appliquées (UFR SFA), Université Nangui Abrogua, 02 BP 0801 Abidjan 02, Côte d'Ivoire.
[2]Laboratoire Architecture Moléculaire et Matériaux Nanostructurés, Ecole Nationale Supérieure de Chimie de Montpellier (ENSCM), 8 Rue de L'Ecole Normale F- 34296 Montpellier Cedex 5, France

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ABSTRACT

The fat matter from Afraegle paniculata has been extracted from its almonds by means of a Soxhlet apparatus with hexane (38.4% average grade). This is unconventional golden yellow colored oil, mainly consisting of 48.56% oleic acid, 19.52% stearic acid, palmitic acid 17.95% and 12.06% of linoleic acid. This oil presents itself like a source of essential fatty acids. Its content of unsaponifiable matter is 1.325%. Its Physical characteristics namely density (0.8844), the refractive index (1.468), were determined following the IUPAC standardized methods. The saponification value (200.55), acid value (2.08), ester value (197.75), iodine number (105.75), peroxide number (3.43) and the lower calorific value (39518.88 kJ/kg) evaluated according to the standardized methods, meet the physicochemical properties of this oil that confer its nutritional quality.

Keywords: *Afraegle paniculata*, almonds, fat matter, fatty acids, physicochemical characteristics, Côte d'Ivoire.

INTRODUCTION

In tropical countries of Africa, the interest of wild plants for rural populations' s food is widely recognized. Indeed, to cover their food needs, the populations have resorted to the agriculture of subsistence that they complete by edible wild species including oilseeds [**1, 2**]. Among these, we have *Afraegle paniculata*, a Rutaceae found on the West African coast from Senegal to Nigeria. *A. paniculata* is a shaft 8 to 15 m tall, with a trunk diameter between 25 and 40 cm. Its alternating leaves, leaflets 3 are 8 to 16 cm long. Its fruits are globular or ovoid, like a big orange (6-8 cm in diameter at the mature age) [**3**].

Of the previous studies led on the fruit of *A. paniculata* showed that the mucilage of the fruit of the Ghanaian species contains D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid [**4**]. In 1961, Quartey revealed the presence of ɣ-sitosterol and a coumarin in the fruit of the species [**5**]. The chemical composition of the essential oil extracted from the zest of the fruit of the species from Côte d'Ivoire showed 40 compounds including sesquiterpene hydrocarbons (64.49%), oxygenated sesquiterpenes (7.60%), monoterpene hydrocarbons (7.82%) and oxygenated (5.78%). The major compounds are δ-cadinene (11.71%), α-selinene (9.01%), α-cubebene (8.80%), o-menth-8-ene (6.06%) and β-caryophyllene (5.66%) [**6**]. However to our knowledge, the oil from the almonds of the same species from Côte d'Ivoire, was never the object of a survey. It seems to be a handicap for the valorization of this oleaginous that could present food potentialities.

Due to the consumption of *Afraegle paniculata* by rural populations of Côte d'Ivoire, it appears us by the way, to study oil extracted of its almonds for a good surveillance of its quality to the ends to guarantee a nutritional security.

MATERIALS AND METHODS

Almonds of *A. paniculata* were harvested in the garden of the Centre National de Floristique (CNF) of Felix Houphouët-Boigny University (Abidjan/Côte d'Ivoire) in June 2014. After identification by Professor Laurent AKE-ASSI, they were washed with water and dried in a steam room at 60°C for one week, then pulverized with a mechanical grinder.

Extraction of the fat matter

Almonds (10 g) of *A. paniculata* were intimately mixed with 3 g of anhydrous Na_2SO_4 in an agate mortar. The resulting mixture was introduced into a Soxhlet apparatus containing hexane (190 ml) heated to reflux in a water bath for 2 hours. The fat matter was dried, weighed, and analyzed after vacuum distillation of the solvent with a rotary evaporator.

Determination of some physical and physicochemical characteristics

The refractive index (Ir) was determined according to the ISO 6320 standard at 20°C with a thermostated Abbe refractometer (ATAGO Tliquid NAR-1). The determination of density was made with a pycnometer according to NF ISO 6883 standard. The saponification values (Sv) and acid value (Av) were determined according to the method described by Gnao *et al.*, [**7**] and AFNOR standards [**8**]. The iodine number (In) and peroxide number (Pn) were calculated respectively according to AOAC standards [**9**] and AOCS [**10**]. The ester value (Ev) was calculated based on the analytical data according to the formula Ia – Ie= Is. The calorific value (Cv) was calculated using the following formula: Cv = 47645-4.187In-38.31Sv (kJ/kg) [**11, 12**].

Determination of unsaponifiable matter content

Fat matter (5 g) was mixed with 50 ml of ethanolic KOH solution (2N). The resulting mixture was refluxed for 1 hour on a hot plate. After cooling, 100 ml of distilled water were added. The mixture was introduced into a separatory funnel. The organic phase was extracted with pentane (4 × 50 ml) followed by washing with distilled water until neutral pH is reached. The organic extract was then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure using a rotary evaporator. The residue obtained constituting the unsaponifiable fraction is dried and weighed.

The unsaponifiable matter content was calculated according to the expression:

$$Ins\ (\%) = (\frac{m_1}{m_2}) \times 100$$

Ins: unsaponifiable
m_1: mass of unsaponifiables, g
m_2: mass of the sample, g

Preparation of methyl esters of fatty acids

After extraction of the unsaponifiable components, soaps are decomposed by addition of 1 ml of HCl (5N). The fatty acids were extracted with ethyl acetate (3 × 25 ml). The solvent was evaporated to dryness under vacuum with a rotary evaporator to provide concentrated fatty acids. The methylation of the acids was carried out by addition of hydrochloric methanol (1 ml , 2N) that are heated to boiling on a hot plate [**13**]. After cooling the mixture, distilled water (20 ml) was added and methyl esters are extracted with ethyl acetate (25 ml). The solvent was evaporated under reduced pressure to give the fraction to be analyzed by GC/MS.

Qualitative analysis of the fat matter by GC/MS

The fatty acid analysis was conducted using a gas chromatograph coupled to a mass 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 from 50-280°C at 4°C/min and held at 250°C for 15 min. The injector temperature was set at 250°C and the detector at 280°C. The injection was performed in *splitl30* fashion. The parameters of the mass spectrometer for the electron impact ionization mode are the source temperature (230°C), electron energy (70 eV), the scan speed (50 scans/s) and acquisition speed (10.000 amu/s). The identification of the compounds was obtained by comparing the retention times with those of reference compounds and the spectral data obtained from NIST and Wiley libraries.

RESULTS AND DISCUSSION

Fat matter content

The fat matter content of *A. paniculata* is summarized in Table 1.

Table 1: Fat matter and unsaponifiable contents

Experiment	1	2	3	**Average value**
m_{obt}	3.85	3.75	3.91	
Fat matter content (%)	38.5	37.8	39.1	38.4±0.5
Unsaponifiable matter content (%)				1.325±0.075

m_{obt}: mass of the fat matter obtained (g).

The fat matter from *A. paniculata* almonds obtained with a yield of 38.4 ± 0.5% is golden yellow colored oil. This content is comparable to that of oilseed oils commonly used in factory oil (cotton 35% - 40% [**14**]; rapeseed 37.3% - 42%, palm almonds 50% - 33.3%, sunflower 36.7% - 47.7% [**15**]. Quantitatively, the proportion of oil used to classify this fruit among potential oilseeds to produce vegetable oil [**14**].

The unsaponifiable fat matter of *A. paniculata* is 1.325%. This rate is the average of the yields found in the literature [**16**].

Physical and physicochemical characteristics

The various physical and physicochemical parameters of *A. paniculata* oil are summarized in Table 2.

Table 2: Physical and physicochemical characteristics of the fat of *A. paniculata*

Characteristics studied	**Results obtained**
Physical state at room temperature	Liquid
Color	Yellow gold
Refractive index	1.468±0.005
Density	0.8844±0.0001
Net calorific value (kJ/kg)	39518.88±44.87
Molar mass (g/mol)	278.35±0.000
Iodine number (g of iodine/100 g oil)	105.75±2.11
Peroxide number (meq O_2/kg oil)	3.43±0.19
Saponification value	200.55±1.40
Acid value	2.08±0.91
Ester value	197.75±1.40

The saponification value generally indicates the content of fatty acids (esterified and free) of oil. And a high value of this physical characteristic permits to recommend the use of an oil in soap factory. The Sv of *A. paniculata* oil (Table 2) is close to the Sv palm oils (190-209), peanut (187-196) and cotton (189-198), but lower than those coconut (248-265) and palm (230-254) oils [**17**] fluently employees in soap factory. Therefore under its Sv, we believe that the fat matter extracted from *A. paniculata* almonds should not be recommended as an input in the production of soap [**18**]. Acid value of *A. paniculata* oil (Table 2) is less than the standard food (Av max= 4) [**17**]. This relatively low value of the Av obtained corresponds to a low content of free fatty acids in the oil [**14**]. The Iodine number allows to highlight the siccative properties of the oils. Thus, a non-siccative oil is called when In <100, semi-siccative when 100 < In <130 and 130 when siccative < In [**19]**. To the look of these values, we can classify the oil of *A. paniculata* from Côte d'Ivoire among the semi-siccatives oils. Besides this property, the high value of In proves that the fat matter of *A. paniculata* is rich in unsaturated fatty acids [**20**]. The peroxide number of *A. paniculata* oil is below the standard set by the Codex alimentarius [**17**], which classifies this oil among the oils indicating an acceptable level of oxidation [**21**] therefore can be used in gastronomy [**22**].

Afraegle paniculata fat matter possesses a calorific value superior to 35000 kJ/kg. This oil could be a source of energy (1g of lipids bring 9 kcal is about 37.6 kJ) on the nutritional plan. As could be used her like fuel and lubricant of the motors [**11**].

Qualitative analysis of the fat matter by GC/MS

Eight (8) fatty acids were identified by GC/MS in *A. paniculata* oil (Table 3), with a percentage of 39.12% saturated fatty acids (SFA) and 60.88% acid polyunsaturated fat (PUFA), a ratio (PUFA/SFA) of 1.56. The report PUFA/SFA and the iodine value show the potential nutritional qualities of this oil [**18**]. Majority fatty acids are oleic acid (48.56%), stearic acid (19.52%), palmitic acid (17.95%) and linoleic acid (12.06%). The oil has a high content of PUFA mainly oleic and linoleic acids representing 60.62% of the total fatty acids. Both PUFA involved in regulating cholesterol levels, decrease the risk of cardiovascular diseases [**23**].

Linoleic acid (component of vitamin E) is an essential fatty acid ω-6 type, which must be brought to the body through food. The presence of this acid in *A. paniculata* almonds (Table 3) makes it a potential source.

Table 3: Fatty acid composition of the fat matter extracted from *A. paniculata* almonds

Peak	Retention time(min)	Percentage (%)	Fatty acid
1	10.332	0.26	Palmitoleic acid
2	10.433	17.95	Palmitic acid
3	10.883	0.13	Margaric acid
4	11.197	12.06	Linoleic acid
5	11.236	48.56	Oleic acid
6	11.329	19.52	Stearic acid
7	12.138	1.40	Arachidic acid
8	12.937	0.12	Behenic acid
SFA	**39.12 (%)**		
PUFA	**60.88 (%)**		
PUFA /SFA	**1.56**		

CONCLUSION

The results of our survey show that *Afraegle paniculata* almonds are relatively high in fat matter (38.4%), which brings all the oils used in oil mill. The comparison of its physicochemical characteristics to those of the conventional food oils and its high PUFA content confer to this oil some nutritional, therapeutic and cosmetic potentialities. The analysis of its toxicity is in progress what will permit to appreciate its potentialities better.

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