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Research Article

Oleaginous Character and Profiles in Fatty Acids and in Triacylglycérols of the Seeds of *Allanblackia floribunda* Oliv. of Congo

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Abstract: The aim of this study was to investigate the oil extracted of seeds of different trees of *Allanblackia floribunda*. The analysis of the seeds of *Allanblackia floribunda* Oliv. permitted to value their oleaginous character and to establish their compositions in fatty acids and in triacylglycérols in order to promote the valorization of these seeds by diversifying lipid profiles of oils in the Congo basin. These seeds are very oleaginous with oil contents ranging between 60 and 65% after 5 h of extraction in the cyclohexane, permitting to classify *Allanblackia floribunda* Oliv. In the group of the oleaginous plants with high content in fat matter. The seeds of the seven studied trees show some contents in stearic acid superior to 60%, added to contents of more than 30% of oleic acid, the oil of the seeds of *Allanblackia floribunda* is characterized therefore by a predominantly saturated profile. The nutritional interest of this source of lipids resides in the neutrality of the stearic and oleic acids (98% cumulative percentage) opposite the blood lipids. The oils of *Allanblackia floribunda* Oliv. studied, whatever the tree highlights a stability of the profile in TAG of this species leading to fat with a very simple TAG profile which reduces to only one type of TAG constituting more than 88% of the total content in TAG for SSO.

Keywords: Allanblackia floribunda, Congo basin, fatty acid, oil content, triacylglycerol

INTRODUCTION

These last years, we are attending to a renewal of interest on the valorization of the local plant resources notably on the oleaginous plants of the countries of Congo basin. Indeed, the studies of the oleaginous plants in the Congo basin date of the colonial period and were achieved with rudimentary technical means. This study that uses finer techniques concerns the seeds of the fruits of Allanblackia floribunda Oliv of Congo and is interested in the assessment of the oleaginous character of these seeds and the study of their compositions in fatty acids and in triacylglycérols. The interest on the development of oilseeds in the Congo basin, has been dictated by on the one hand the existence of a protéo-lipidic malnutrition in some countries of the sub-region and on the other hand by the enormous exit of the currencies consecutive to the import of the fat matters, in an ecological zone with high oleaginous potentialities. Indeed, for a diet of 2500 Kcal capable to satisfy the daily energizing needs of the reference man, fat intake is 900 Kcal (Knia, 1983) and according to the food investigations achieved in Congo (Massamba, 1985), this contribution is about 20% for the populations of the urban centers and 16% for secondary centers. The reality shows that, the energizing contribution is of 1900 Kcal/day/individual

with a lipidic contribution of 11% instead of 20% (Massamba, 1985). These investigations prove that the Congolese diet is deficient in energy provided by fat. Furthermore, studies dating from the colonial period indicate real opportunities of sustainable development of the fat production sector. Thus, in the context of the development of the colonies, vast works of identification and localization of the oilseeds had been achieved, in particular by French and Belgian institutions of research for their colonies in Central Africa. From the years 1980, the research deepened themselves, with more modern means, notably within the network on the valorization of the safoutier and others non-conventional oilseeds, ASANET (African Safou Network), that regularly gathered together at each of its seminars, about thirty researchers from Central Africa around forty communications (Acts of seminars on the valorization of the safoutier and others non conventional oilseeds: Anonyme (1991, 1994, 1997, 2000, 2003, 2006)). Indeed, it notes that some non conventional oilseeds are used locally as medicines, cosmetics and foods, others on the contrary are marketed locally, regionally and internationally.

Allanblackia floribunda which is the subject of the present work, belongs to the family of the Clusiaceae. It is a species of the tropical Africa and its surface of distribution extends from Sierra Leone to Cabinda and

Corresponding Author: Bob Wilfrid Loumouamou, Equipe Pluridisciplinaire de Recherche en Alimentation et Nutrition: Pôle d'Excellence Régional en Alimentation et Nutrition B.P: 389, Faculté des Sciences, Brazzaville, Congo This work is licensed under a Creative Commons Attribution 4.0 International License (URL: http://creativecommons.org/licenses/by/4.0/). to Uganda. *Allanblackia floribunda* is widely distributed in the pluvial forest zone from Nigeria to Centrafrique and to the oriental area of the R. D. Congo and towards the south and the north of Angola (Orwa and Munjuga, 2007). It is a forests tree which can rich 30 m in height and 80 cm in diameter. The Fruits of *Allanblackia floribunda* are the berries in the shape of ellipsoid, capable to contain 40 to 100 seeds (Eyog *et al.*, 2005). This study investigates the oil extracted of seeds of different trees of *Allanblackia floribunda*.

MATERIALS AND METHODS

This study was carried out in the laboratory of Physical Chemistry of the multidisciplinary team of Food and Nutrition Research of Marien Ngouabi University and in the laboratory of Chemistry of Heterocycles and Carbohydrate, Chemistry of Essential Oils, Campus des Cezeaux, Blaise Pascal University of Clermont Ferrand, (France) for the period of January 2010 to October 2010.

Samples: Allanblackia floribunda fruits were collected from seven trees of Dzoumouna forest situated at about 20 km south of Brazzaville, capital of Congo. The seeds drowned in the pulp are separated, dried in an oven at 106°C during 24 h and finely ground into powder. The fruits of these seven trees represent the seven studied samples.

Fat extraction:

Soxhlet method: The dried and crushed seeds were introduced into a Soxhlet extractor. After 5 h of extraction with cyclohexane as solvent, the extract was dried with sodium sulfate. The solvent was evaporated in a rotary vacuum evaporator and the solvent traces were eliminated by drying oil in an oven at 103°C for 6 h. The mass of the fat matter has been measured and the content in lipids calculated by the following formula:

% lipids = $(M_1 - M_0) \times 100/M$

with,

M₁ : Mass of flask containing oil

 M_0 : Mass of empty flask

M : Mass of dried and crushed seeds used

Determination of the composition in fatty acids by gas chromatography: The gas chromatography is a separation method of the gaseous compounds or may be sprayed by heating without decomposition of the product. its principle is based on the balances of concentration of the present compounds between two non miscible phases, one of which, called stationary, nonvolatile, liquid, is jailed in a column and the other, called mobile (inert gas: H_2 , N_2 , He) moves to the contact of the first and transport the solute (Tranchant, 1964).The drive at different speeds of the compounds by the mobile phase leads to their separation. The separation of the compounds depends on the type of column used and therefore of the polarity of the stationary phase.

A Flame Ionization Detector (FID) marks the different compounds at the exit of the column. The constituents of the mixture which thus leave the column one after the other are identified and measured and the signals they produce at the detector are translated at a recorder by a succession of peaks (chromatogram), characterized by retention time (identification) and an area (dosage). The analysis of fat is therefore achieved in two stages: the separation of the products contained in the fat and the identification of these products, preceded by a liberation of the AG forming the TAG by méthanolysis.

After the trans esterification of fatty acids by sodium methylate (1 N) in methanol at ambient temperature during 2 min and under agitation, the methyl ester fat were analyzed on a Hewlett-Packard gas chromatograph model 5890, equipped with a FAME column (100 m×0.25 mm, 0.25 μ m), programming from 140°C (5 min) -240°C at 4°C/min, 15 min hold.

Hydrogen as carrier gas (0.7 mL/min); injection in split mode (1:40); injector and detector temperature, 280 and 300°C, respectively. FAMEs were identified by comparison of retention times with authentic standards and quantification was performed by internal normalization.

Determination of TAG profiles: Triacylglycerol (TAG) profile was obtained by High Performance Liquid Chromatography (Heron and *al.*, 2007). The chromatographic system consisted of a model LC-6A Pump (Shimadzu'Kyoto, Japan) Rheodyne loop a model 7125 injector valve with a 10 μ L loop (Rheodyne, Cotati, CA, the United States) and a model Sedex 75 Light-scarttering detector (Sedere, Alfortville, France). The nebulizing gas was air set 3, 5 bar, the evaporation temperature was set 37°C and the gain (PM) at 11. The column temperature was controlled using an igloo-cil column oven (Cluzeau, Sainte-Foyla-Grande, France) and set at 30°C evaporative detector with diffusion of light (Sedere Sedex 75).

TAG were separated at 20°C using a Kromacil C18 (5 μ m), 250×4.6 mm column (Part Number KR5C18-25QS mfg # 806610) and was eluted from the column with a mixture of acetonitrile/dichlorom ethane (70/30-40/60). Chromatograms were recorded using an Azur (v 3.0). Data acquisition software (Datalys, Saint Martin d'Heres, France).

Statistical analysis: Values represented are the means and standard deviations for three replicates. Statistics were processed using Excel Version 8.0 software.

RESULTS AND DISCUSSION

Oleaginous character of the seeds of *Allanblackia floribunda* Oliv. of Congo: Lipids are the fundamental

Allanblackia					Total			Total	
floribunda	Oil content (%)	C16:0	C18:0	Total *SFA	C18:1	**MUFA	C18:2	C18:3	***PUFA
Arbre 1	62.53±1.56	1.05	62.02	63.07	35.67	35.67	0.61	0.63	1.24
Arbre 2	64.17±0.18	1.19	62.17	63.36	35.33	35.33	0.64	0.64	1.28
Arbre 3	63.07±1.10	1.17	61.88	63.05	35.25	35.25	0.59	1.07	1.66
Arbre 4	62.55±1.94	0.88	60.97	61.85	36.72	36.72	0.65	0.68	1.33
Arbre 5	61.90±0.47	1.32	61.29	62.61	36.59	36.59	0.68		0.68
Arbre 6	62.48±0.91	1.05	62.02	63.07	35.65	35.65	0.60	0.61	1.21
Arbre 7	62.83±2.24	0.99	61.98	62.97	35.59	35.59	0.62	0.69	1.31

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*SFA: Saturated fatty acid; **MUFA: Mono unsaturated fatty acid; ***PUFA: Poly unsaturated fatty acid

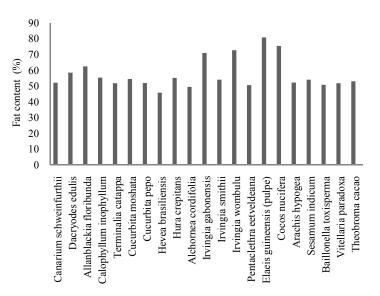


Fig. 1: Fat contents of some oleaginous plants of Congo basin

constituents of the totality of plants. With carbohydrates and protein, they are the primary metabolites of any plant. These are called: oil, fat and more inclusive "fat matters" or "grease".

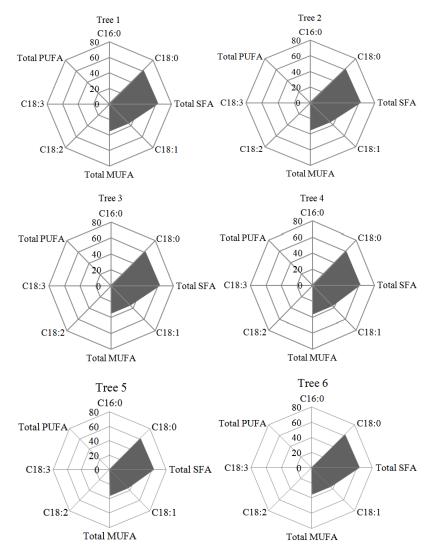
Oil plants are called oleaginous plants. All plants, at different contents, contain fat matters, as reserve constituents that are preferentially stored in the fruit (pulp or seed). The plant fat matters (oils or fats) play an important role in food and in economy. Their exploitation is therefore only interesting from a certain threshold of content in the plant. That's why, practically, are considered like oleaginous plants, those exceed this threshold. Conventionally, this threshold is fixed at 15-20% of content in fat matter. On the basis of this conventional threshold, we can distinguish the plants with low (%<20%), medium (20%<%<50%) and high (%>50%) content in fat matter (Loumouamou, 2011).

Table 1 shows the results of oil content of the seeds of seven trees of *Allanblackia floribunda* gotten after extraction to the soxhlet in the same conditions. Examination of this Table 1 shows that the seeds of *Allanblackia floribunda* Oliv. Are very oleaginous with oil contents ranging between 60 and 65% after 5 h of extraction in cyclohexane. *Allanblackia floribunda* can be classified therefore in the group of oleaginous plants with high content in fat matter. The lipid contents values obtained indicate a high degree of homogeneity of the plant material used seeing that the standard deviations of the three tests of extraction for each sample (tree) are extremely low (0.18 to 2.24) for content in lipids generally exceeding the 60%.

The oil extraction for a given sample is a perfectly reproducible operation, that is to say that the oil is homogeneously distributed throughout the powder obtained by grinding the seed.

In order to value the oleaginous character of *Allanblackia floribunda* seeds we have extracted in the same conditions the lipids of some major oleaginous encountered in the Congo basin by way of comparison.

The Fig. 1 shows the position of Allanblackia floribunda seeds among other oilseeds. It is clear by examining this figure that Allanblackia floribunda seeds with contents of just over 60% are well below those encountered in Elaeis guineensis (pulp), Cocos nucifera, Irvingia gabonensis and Irvingia wombulu that have some contents of more than 70%. However, they are higher or slightly higher than those encountered in Canarium schweinfurthii (almond), Dacryodes edulis, Calophyllum inophyllum, Terminalia catappa, Cucurbita moshata, Cucurbita pepo, Hevea brasiliensis, Hura crepitans, Alchornea cordifolia, Irvingia smithii, Pentaclethra eetveldeana, Arachis hypogea, Sesamum indicum, Baillonella toxisperma,



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Fig. 2: «Radar plots » representation of FA composition of Allanblackia floribunda seeds oil

Vitellaria paradoxa et Theobroma cacao which have some contents lower to 60%.

Allanblackia floribunda is therefore ranked among the major oilseeds of the Congo basin.

Fatty acid composition: The fatty acid composition of the oils is one of the first means of classification of oils or fats: saturated. unsaturated. mono or polyunsaturated, according to the quality of the dominant fatty acid (Delplanque, 2000). Table 1 gives the fatty acids composition of oils extracted from Allanblackia floribunda seeds. Examination of this table allows us to judge at first sight the quality of this oil on its value as a food oil because, the quality of foods fatty acids play an important role on the parameters related to atherosclerosis and more exactly to atherothrombosis, which is at the origin of the cardiovascular accidents (Delplangue, 2000). The effect of fatty acids on the cholesterolemia is known since the first studies done by Keys et al. (1965) and Hegsted et al. (1965) who found a positive correlation between

blood cholesterol rate and the incidence of cardiovascular diseases, then showing that saturated fatty acids were able to induce an increase of the cholesterolemia, whereas a balance of consumption between mono and polyunsaturated fatty acids in the form of vegetable oils of diverse origins reduces it (Delplanque, 2000; Keys et al., 1986; Baudet et al., 1980; Mattson and Grundy, 1985; Legrand, 2008). It is now well demonstrated that stearic (C18:0), palmitic (C16:0) and myristic (C14:0) or lauric (C12:0) acids have different metabolisms and we must not especially consider them in block (Legrand, 2008). These long saturated fatty acids are partly converted by D9desaturation in monounsaturated fatty acids, but with significantly different efficiency, increasing with chain length (Legrand et al., 2002). Stearic acid is the best substrate of D9-desaturase and its conversion into oleic acid is important (Legrand, 2008).

The examination of Table 1 bring to light for the seeds of seven trees of *Allanblackia floribunda* studied, contents in stearic acid (saturated fatty acid) superior to

60% added to contents of more than 30% of oleic acid (monounsaturated fatty acid). The oil of *Allanblackia floribunda* seeds is characterized by a predominantly saturated profile. The nutritional value of this source of fat is the neutrality of stearic and oleic acids (98% of cumulative percentage) opposite the blood lipids (Dubois *et al.*, 2008; Hunter *et al.*, 2000; Clarke *et al.*, 1997; Bonanome and Grundy, 1988).

Comparison of the composition in fatty acids of the oils of the seven trees: To compare the fatty acids composition of the oils from seeds of seven trees of *Allanblackia floribunda*, we used the representation in radar plots (Fig. 2), which permits an easier comparison than the histograms when we have samples with an important number of characteristic variables. This representation system allows to highlight the differences in the major compounds but not on those in small proportion (Silou *et al.*, 2004).

The Fig. 2 shows a perfect likeness of radar plots and highlighting the similarity of the fatty acids composition whatever the tree. This behavior is the same for the seven studied trees. we note for all studied trees that the oils of the seeds of *Allanblackia floribunda* are predominantly constituted by two major fatty acids, the stearic acid (C18: 0), the first constituent major and the oleic acid (C18: 1), the second constituent major; these two fatty acids have an accrued content of about 98% whatever the tree. The three acids, palmitic, alpha-linolenic and linoleic, are at the state of traces. This can be summarized in a very simple AG profile whatever the tree (Fig. 3):

% C18:0>% C18:1

Triacylglycerol (TAG) profiles of the oils studied: The knowledge of the fatty acids composition obtained by GC (Gas Chromatography) after transesterification of triacylglycerols provides extensive informations on the composition of oils and fats analyzed, however, various problems can arise:

• Fatty acids composition does not give a real reflection of a fat matter because we have no direct

composition in TAG. This can be obtained from statistical calculations, but these are not always the reflection of the real biological composition of the oil.

• The obtaining of fatty acid composition can be blemished of mistakes because of, firstly problems of injection causing the light compounds discrimination against those of higher mass and secondly the fact that the transesterification is not always quantitative (Silou *et al.*, 2004).

Various studies of direct analysis of triacylglycerols have been proposed in gas chromatography (Buchgraber et al., 2004; Ruiz-Gutiérrez and Barron, 1995). However, given the low volatility of these products, it requires working at very high temperatures, in conditions close to the limits of thermal stability of the stationary phase.

In order to overcome these problems of volatility, liquid chromatography analysis was considered and the reversed-phase chromatography, especially technique for separations based on both the difference in hydrocarbon volume of triacylglycérols and on their unsaturations number. The evaporative light-scattering detector is well suited to the analysis of high molecular weight compounds such as TAG. It allows a better separation of peaks that other detectors commonly used and in particular the refractometric detector. However, this detector is not giving an answer directly proportional to the injected quantity, quantitative analysis is not easy and on the other hand, mixed triacylglycerols are not available in standard, qualitative study proves to be difficult.

It can be made semi-quantitative by taking a reference triacylglycerol to which we will assign the same value of area and we can make a comparative analysis of triacylglycerols profiles of fat matter.

Moreover, under certain experimental precautions, the analysis in the evaporative light-scattering detector can lead to perfectly quantitative results (Héron *et al.*, 2010).

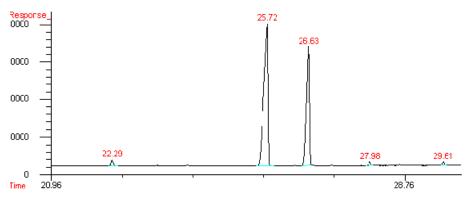


Fig. 3: Fatty acid profile of Allanblackia floribunda seed oil (by GC)

To study *Allanblackia floribunda* fat, we preferred to use the evaporative light-scattering detector instead of the other detectors for its quality of the resolution of triacylglycerol peaks.

The seeds of (7) trees of *Allanblackia floribunda* studied lead to fat with a very simple TAG profile which reduces to a single type of TAG constituting more than 88% of the total content of TAG for SSO (Table 2 and Fig. 4).

The oils of *Allanblackia floribunda* seeds studied, whatever the tree highlights a stability of fatty acid profile of this species (Table 1 and Fig. 5). We have finally the following profile:

% SSO>% SOO>% PSO>% OOO>% PSL (Fig. 4)

Table 2: TAG composition of *Allanblackia floribunda* seed oil

Allanblackia	000	PSL	SOO	PSO						
floribunda	(%)	(%)	(%)	(%)	SSO (%)					
Tree 1	0.74	0.31	8.34	1.73	88.83					
Tree 2	0.66	0.28	7.88	1.85	89.23					
Tree 3	nd	nd	nd	nd	nd					
Tree 4	0.87	0.44	9.11	0.98	88.52					
Tree 5	nd	nd	nd	nd	nd					
Tree 6	0.75	0.33	8.33	1.95	88.61					
Tree 7	nd	nd	nd	nd	nd					

nd: Not determined

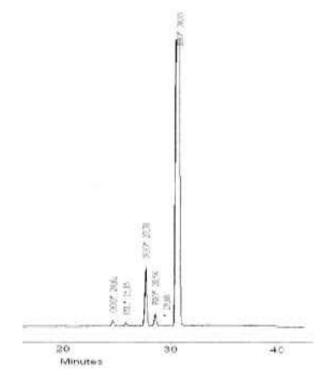
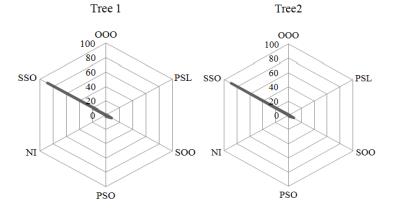


Fig. 4: Triacylglycerol profile of *Allanblackia floribunda* seed oil (by HPLC)





Tree 6

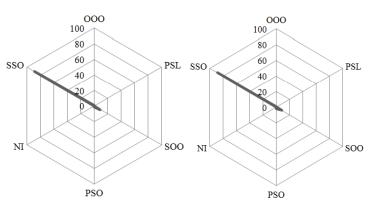


Fig. 5: «Radar plots » representation of TAG composition of Allanblackia floribunda seeds oil

Indeed we notes that SSO, the first TAG of *Allanblackia floribunda* seeds oil is widely major and is followed by SOO, which is the only minor TAG. PSO, OOO and PSL are present at the state of trace. The TAG profile of *Allanblackia floribunda* seeds oil can be summarized whatever the tree studied in the following very simple profile (Fig. 3):

% SSO>% SOO

The Fig. 5 built on the data of the Table 2 shows the similarity of the profiles of seed oils of the four trees analyzed. The content of each constituent, on the corresponding axis, defines the representative point of the constituent. These points joined by line segments define a geometric figure characteristic of the product, called "radar plots", which shows a single type of triacylglycerol (SSO) at 88% for lipids whatever the oil of tree studied.

CONCLUSION

The study showed that *Allanblackia floribunda* seed is a good source rich in oil and permitted to highlight the extreme simplicity of this oil. It consists of more than 98% of two fatty acids: S (Stearic acid) and O (Oleic acid) which lead to a single majority type of triacylglycerol (SSO) at 88% for the lipids of the seven trees studied. This TAG, once purified, can be used in standard chemical analysis including HPLC where the problem of mixed standard arises with acuteness.

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