PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 7 (6): 733-740, 2008
ISSN 1680-5194

Comparative Study of Fatty Acids and Triglycerids of *Luffa cylindrica* Versus Cucurbitaceae Seeds Consumed in Congo Brazzaville

M. Mvoula Tsieri[1], R. Kama Niamayoua[1], D. Mampouya[1],
Th. Silou[1], A. Trémolières[2], S. Héron[3] and A. Tchapla[3]
[1]Equipe Pluridisciplinaire de Recherche en Alimentation et Nutrition BP 389,
Brazzaville, Congo/BP 1286, Pointe-Noire, Congo
[2]Institut de Biotechnologie des Plantes, Université Paris Sud
[3]Groupe de Chimie Analytique de Paris Sud EA 3343,
IUT d'Orsay Plateau du Moulon, Orsay, France

Abstract: Fatty acids composition of various classes of lipids (neutral and polar lipids) was determined by gas chromatography (GC) and triacylglycerols (TAG) composition by high performance liquid chromatography (HPLC). The comparative study of fatty acids and triacylglycerols composition of the five cucurbitaceae species analyzed allow similarities within the species. The essence of the linoleic acid C 18:2 are concentrated in the TAG and the acids linoleic C 18:3 and palmitoleic C 16:1 can be used as factor discriminating the studied species.

Key words: Cucurbitaceae, lipids, fatty acids, triacylglycerols

Introduction

Cucurbitaceae are cultivated for human nutritional uses. The studies were carried out to analyze the composition from oil of five cucurbitaceous (Lepage, 1964) of Congo-Brazzaville must make it possible to carry out a selection the most interesting varieties from the point of view of the productivity and food quality.

A comparative study of seed oils of cucurbitaceous edible and no edible is necessary in order to release their food interest. We want to thus look further into the fundamental knowledge of cucurbitaceous from the nutritional point of view. The comparative study of the Fatty Acids (FA) and triglycerides (TAG) makes it possible to establish bringings together between the various species. From this study illustrating the extreme complexity of the distribution of AG in various classes of the lipids extracted seeds from five cucurbitaceous, it comes out clearly that the linoleic acid C 18:2 constitutes 43-74% of AG of the TAG. These oils have quantitative compositions in divergent TAG. The identification of the TAG was confirmed by results obtained on pilot oils of composition of theoretically known TAG. On the 35 compositions of theoretically possible TAG in this study, one observes in experiments 11 of comparable nature including 5 major and 6 minors.

Materials and Methods

Vegetable material: The study was carried out on five species the cucurbitaceous seeds: *Citrullus lanatus, Curcubita moschata, Cucurbita pepo, Lagenaria siceraria* and *Luffa cylindrica* (non-food species).

Citrullus lanatus (Picture 1): Citrullus lanatus is a herbaceous plant annual, monoïc, with angular stem with longitudinal furrows, crawling, covered with woolly hairs of white color.

The species had been described by Keraudren (1967), the sheets are petiolate (the petiole 6-12 cm long). Briefly the limb is oval. It is green clear, the two faces covered with hairs. The gimlets are bifides, pubescent at their bases, rather robust but short. Male and female flowers of yellow color are solitary. The petals are foliaceous, oval, blunt at the top and trinerves. The veins carry to the outside of the long and flexible hairs. Cheesecloths are inserted into the bottom of the floral cut.

The fruits are globulous, of yellowish green color in maturity. Pulp is soft and blanchâtre.

The seeds brown are flattened, small and are dispersed in pulp (mésocarpe). A fruit can contain 100-200 seeds.

The species is widespread in all the tropical, equatorial zones of the sphere and is probably originating in the semi-arid zones of the Southern Africa.

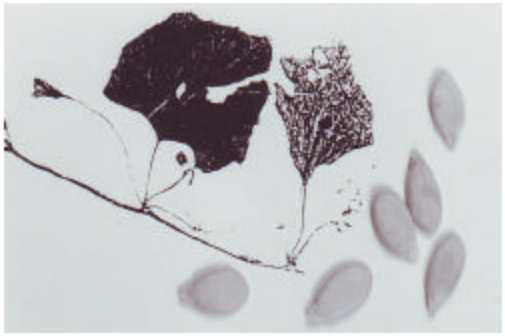
In Congo, this species is cultivated for its seeds for human consumption.

Cucurbita moschata (Picture 2): Cucurbita moschata is a monoïc herbaceous plant, crawling, with soft stem of circular section.

The sheets are petiolate (up to 20 cm); the limb is whole and wrinkled, of variable size being able to reach 20 cm length; they are lobed.

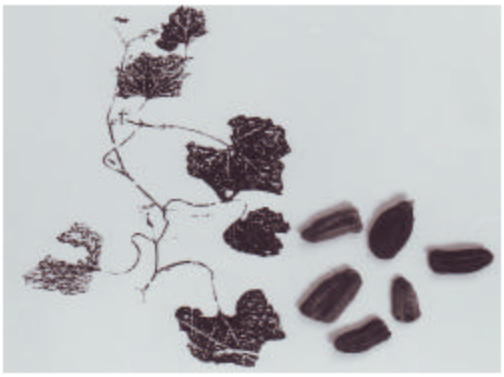
The gimlets multifides carry some hairs at the base of the rectilinear part.

Picture 1: Seeds and leaves of *Citrullus lanatus*



Picture 3: Seeds and leaves of *Cucurbita pepo*

Picture 2: Seeds and leaves of *Cucurbita moschata*



Picture 4: Seeds and leaves of *Lagenaria siceraria*

The flowers solitary, are synanthérées; the yellow corolla, with lobes welded at their base onto 1/3 their length.
Cheese cloths are grouped in the center of their perianth. The fruit-bearing stalk is thick. The fruits are ovoid or of varied form. Colouring varies from a fruit to another for the same species: fibrous pulp contains many seeds which are clearly margined on the edges. The sheets of this species, are used in our households like vegetable. This species is also cultivated for its seeds for human consumption.

Cucurbita pepo (Picture 3): Cucurbita pepo is an herbaceous, crawling, monoïc plant. The covered stem of hairs present of the short internodes.
The sheets are lengthily petiolate, with rather stiff hairs. The limb a little coriace, of variable size is long and broad, with acute lobes. The limbs are roughcast hairs on the two faces, on toothed board. The gimlets are multifides.
The male flowers with long pedicel are solitary. The sepals are small and the yellow corolla is bell-shaped. The corolla is long 5 cm and broad 3 cm approximately, acute lobe. The also solitary female flowers carry a variable ovary of form, containing many longitudinal ovules. The hard, angular fruit-bearing stalk is not extended to its insertion on the fruit.
The fruit of form and size variables contains a fibrous pulp containing of many largely oval seeds. The fruit is edible; it is cultivated by the peasants for its seeds which are much appreciated.
The species is largely cultivated on the surface of the sphere; only the polar areas are opposed to its culture (Keraudren, 1967). This species contains many varieties.

Lagenaria siceraria (Picture 4): Lagenaria siceraria is an annual, monoïc plant herbaceous climbing or crawling, with angular stem rather thick and covered with flexible and fine hairs.
Sheets lengthily petiolate (5-20 cm long thick petiole). The petioles cylindrical and are often dug below.
The whole limb is sometimes slightly trefoil, blunt or acute at the top, toothed on the edges. The two faces of the limb are covered with a pubescence fine, short, denser with the lower face.
The initially rectilinear gimlets, then bifides with branch subégale, are rolled up and pubescent in their lower part. The male and female flowers are solitary with white petals. The ovary is ovoid or cylindrical. The fruits

Picture 5: Seeds and leaves of *Luffa cylindrica*

of variable size, glabres with maturity are initially green then become blanchâtres or yellowish. The seeds of white color many and are flattened.
Lagenaria siceraria is largely widespread in all the hot or moderate zones of the world (Keraudren, 1967). Its fruit which can be presented in various forms is used by the local population like container.
In Congo, several species push naturally, some are cultivated.

***Luffa cylindrica* (Picture 5):** *Luffa cylindrica* is a herbaceous plant with stems lianescentes from 3 to 6 m length, marked fine longitudinal furrows.
The sheets have a petiole from 3-10 cm and a limb which is 3-5 cm palmatilobate. The plant clings to its support by gimlets.
The male flowers yellow, are gathered in racèmes carrying 10-20 flowers; the female flowers are solitary. The fruit is a bay hanging, cylindrical, from 15-20 cm length. The fruit has the aspect of a zucchini of which the interior is covered of a resistant fibrous system.
The young growths, the sheets, the buttons floral and the fruits can be consumed.
In the Antilles, the young pepos at coasts smooth are cooked in stew, ratatouille and curries of chicken or pig. The dried fruit is put to soak during several days, it is cleaned of its pulp and its seeds, then bleached. After drying, there remains a fibrous bread used like vegetable sponge, dermal exfoliated (massage glove), to therefore scour the kitchen utensils.
Originating from India, this plant is largely cultivated in the tropic areas.

Lipids extraction: It is a question of recovering all the neutral or polar lipids seeds. The seeds peeled before crushing are fixed in ebullient water. Boiling, maintained approximately 5 min, allows inactive the enzymes.
The extraction itself is done according to the method of Bligh and Dyer (1959). After crushing in methanol (10 mL), one adds the same quantities of chloroform and distilled water. In this manner, the lipids are released their interactions with the proteinic molecules and are found in the lower phase of the biphasic system obtained after centrifugation (10 mn, 4000 g). This phase is then recovered and the evaporated solvent. The total lipids thus extracted are preserved at 20°C in chloroform.

Separation of the lipids classes by Thin Layer Chromatography (TLC): The fractionation of the lipids in various classes is done by the chromatography of adsorption on thin layer of silica gel (plates including all taxes 60F-254, Merck). The method consists in depositing a aliquot total lipidic extract in chloroform to 2 cm of the lower edge of the plate under current of nitrogen. The witness used is the WT (tobacco). The plate is then placed in a hermetically closed tank containing solvent of polarity given.
The solvent Lepage (1964) whose composition is made of: chloroform/methanol/acetone/acetic acid/water (50/10/20/10/5, v/v/v/v/v) makes it possible to separate the neutral lipids from the polar lipids themselves split in various classes. Mangold (1964 solvent) is composed of: petroleum ether/ethylic ether/acetic acid (70/30/0,4, v/v/v) allows as for him the fractionation of the neutral lipids.

Revelation of lipids classes: The lipids separated on silica gel plate are revealed in a tank saturated with iodine vapour. The lipids appear in the form of yellow tasks. They are identified by comparison with the Rf of the witnesses. The spots detected on silica gel, which contain lipids, are scraped after evaporation of iodine in order to methylate their fatty acids.

Gas-chromatographic (GC) determination of total fatty acid:

- Trans methylation of the fatty acids.

The method of methylation used was previously described by Metcalfe *et al.* (1966). Briefly a aliquot of the total lipidic extract beforehand dried under nitrogen where the spots recovered on plate of silica are put to incubate with the bath Marie (60°C) during 20 min in 1 mL borontrifluoride-methanol 14% (v/v) as an acid catalyst. The reaction proceeds in a closed tube. After cooling of the tube of methylation, the methyl esters are extracted by 3 mL pentane and 0.5 mL water. Pentane is then evaporated at 50°C and the fatty acids methyl esters are preserved at the cold in methanol.

- Separation and analyzes of fatty acids methyl esters by GC.

The separation of fatty acids methyl esters was carried out on a gas chromatograph (Delsi series 30)
connected to an integrating tracer (Enica 21).

Chromatographic parameters were set up as fellows: fused carbowax capillary column (25×0.25mm id ×0.25µm film thickness); injector and detector temperatures 250°C. Helium was used with 0,5 bars at the column head and 3 mL min^{-1} of flow. The elution of various fatty acids esters was obtained done at constant temperature (temperature of the furnace of the column = 200°C).

Coupling Gas chromatography/mass spectrometry (CPG/SM): This coupling allows the non ambiguous identification of major methyl esters and the minor compounds.

The esters of the fatty acids constituting the seed oil were analyzed on a device made up of a chromatograph SHIMADZU GC-17A coupled to a mass spectrometer of the quadripolar type SHIMADZU QP 500.

The chromatograph is equipped with a column DB5 (J and W) (5% phenyl, 95% méthylsiloxane; 30×0.25×0.25 µm); helium is carrier gas (50 kPa); injection splitless (temperature injection 300°C); oven temperature programming from 40-130°C at 9°C min^{-1}, it then underwent a second heating up to 290°C at scan rate of 2°C min^{-1} and is kept at this temperature during 10 min.

High Performance Liquid Chromatography (HPLC) determination of TAG profile

Conditions of analysis: The conditions are those described by Heron and Tchapla (1994).

The chain breaks up into: a pump HP 1050 (Hewlett Packard, PALO-ALTO, CA, the United States), an injection valve Rheodyne model 7125 with a ball of 20 µL (Rheodyne, COTATI, CA, the United States), an evaporative detector with light diffusion Sedere Cedex 75 (Sedere, Alfortville, France). The temperature of the column was controlled using a furnace Croco-Cil (Cluzeau, Sainte-Foy-la-grande, France) with water circulation controlled by a thermostat-cryostat JULABO CPU F10 (Touzart and Matignon, the Ulis, France). The acquisition of the data was done using the software.

Azure V2.0 (Datalys, Saint-martin Of Heres, France). All the analyses were led to 20°C with column KromasilC18 (5µm) 250×4,6 mm (THERMO QUEST, the ULIS, France). The mobile phase is a binary mixture optimized CH_3CN/CH_2Cl_2 63/37 with a flow of 1 mL min^{-1}. The detector parameters were optimized and are: T = 37°C, P_{air} = 2 bars, Gain=11,

Time-constant = 1.

The acetonitrile (Acros, New Jersey, the United States) and dichloromethane (Carlo erba, rodano, Italy) are of quality rank HLPC. Oils were put in solution in a mixture $MeCN/CH_2Cl_2$ (50/50); concentration and volume injected were adapted in such way that a peak taken in reference (LLL) has the same surface always appreciably.

Trilinolein (LLL) standard used comes from Sigma Chemistry (St-Quentin Fallavier, France).

Oils comparison: The evaporative detector with diffusion of light does not give an answer directly proportional to the quantity injected (Dreux and Lafosse, 1995). The law of response of the detector used is form:

$$\text{Area} = a \times (\text{mass injected})^b \text{ with } 0.66<b<2.$$

In addition, the various TAG all did not answer in the same way. As they were not available out of standard, the quantitative analysis is thus not easy (one cannot know each coefficient of answer has and B of each TAG present, nor to make the assumption that they are equal).

For all the analyses, one injects a mass quantity constant (by modulation of the concentration and volume injected) of a TAG present in the oil and taken as reference. In this manner, whatever the chromatograms, the surface of the peak corresponding to this TAG was always quasi constant.

The comparison of various oils is thus done by calculating the ratio of the TAG area under area of the TAG reference. By comparing this ratio for various analyzed oils, for a given TAG, more this figure is raised, oil is rich in this TAG.

Data processing: The statistical processing was carried out with Microsoft Excel 8.0 software.

Results and Discussion

Comparative study of the fatty acids of the five cucurbitaceae seed oil: After having studied the nature and the composition of the classes of the lipids of four the cucurbitaceous species food ones of Congo-Brazzaville (Mvoula Tsieri *et al.*, 2005), we have in a comparative study to try to make bringings together with *Luffa cylindrica*, species not consumed in Congo.

As the food species, the major fatty acid of the residual lipids of *Luffa cylindrica* is $C_{18:2}$ (43%) followed $C_{16:0}$ (26%). C18:2 is the fatty acid most abundant in the di acylglycerol (DAG) with a content of 40%. On the level of free fatty acid, $C_{18:2}$ is the major fatty acid followed by $C_{16:0}$ and $C_{18:1}$. $C_{18:2}$ is the major fatty acid of the TAG and non esterified compounds.

$C_{18:2}$ and $C_{16:0}$ are abundant with prevalence of $C_{18:2}$ as well in neutral lipids as in PI, PC (polar lipids). On the other hand $C_{16:0}$ is the majority fatty acid in PE.

From this study, illustrating the extreme complexity of the distribution of FA in the various classes of the lipids extracted from cucurbitaceous seeds, it comes out clearly that the essence of $C_{18:2}$ is concentrated in the TAG and $C_{18:3}$ and $C_{16:1}$ acids can be used as factor discriminating the studied species.

Table 1: Fatty acids repartition in residual, neutral and polars lipids in five *cucurbitaceae*

Cucurbitaceae	Residual lipids	DAG	FFA	TAG	PI	PC	PE
CL	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$> $C_{18:1}$>$C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$> $C_{18:0}$>$C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$> $C_{18:1}$>$C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$ >$C_{18:0}$>$C_{18:3}$	$C_{18:0}$>$C_{18:2}$>$C_{18:1}$ >$C_{16:0}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$ >$C_{18:1}$>$C_{18:3}$	$C_{16:0}$>$C_{18:2}$>$C_{18:1}$> $C_{18:0}$>$C_{18:3}$>$C_{16:1}$
CM	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$> $C_{18:0}$>$C_{18:3}$	$C_{16:0}$>$C_{18:1}$>$C_{18:2}$> $C_{18:0}$>$C_{18:3}$	$C_{18:1}$>$C_{16:0}$>$C_{18:2}$> $C_{18:0}$>$C_{18:3}$	$C_{18:2}$>$C_{18:1}$>$C_{16:0}$ >$C_{18:0}$>$C_{18:3}$	$C_{16:0}$>$C_{18:0}$>$C_{18:3}$ >$C_{18:1}$>$C_{18:2}$	$C_{16:0}$>$C_{18:1}$>$C_{18:0}$ >$C_{18:2}$>$C_{18:3}$	$C_{16:0}$>$C_{18:1}$>$C_{18:0}$ >$C_{18:2}$
CP	$C_{16:0}$>$C_{18:2}$>$C_{18:0}$> $C_{18:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$> $C_{18:1}$>$C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$> $C_{18:1}$>$C_{18:3}$>$C_{16:1}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$ >$C_{18:0}$	$C_{18:0}$>$C_{16:0}$>$C_{18:2}$ >$C_{18:1}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$ >$C_{18:1}$	$C_{16:0}$>$C_{18:0}$>$C_{18:1}$ >$C_{16:1}$>$C_{18:3}$>$C_{18:2}$
LS	$C_{16:0}$>$C_{18:2}$>$C_{18:0}$> $C_{18:1}$>$C_{16:1}$>$C_{18:3}$	$C_{16:0}$>$C_{18:0}$>$C_{18:2}$> $C_{18:1}$>$C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{18:1}$>$C_{18:0}$> $C_{16:1}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:0}$ >$C_{18:3}$	$C_{16:0}$>$C_{18:0}$>$C_{18:1}$ >$C_{18:3}$>$C_{16:1}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$ >$C_{18:0}$>$C_{16:1}$	$C_{16:0}$>$C_{18:0}$>$C_{18:1}$ >$C_{16:1}$>$C_{18:3}$>$C_{18:2}$
LC	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$> $C_{18:0}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:3}$> $C_{18:1}$>$C_{18:0}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$> $C_{18:0}$>$C_{18:3}$	$C_{18:2}$>$C_{18:1}$>$C_{16:0}$> $C_{18:0}$>$C_{18:3}$	$C_{18:2}$>$C_{16:0}$>$C_{18:3}$ >$C_{18:1}$>$C_{18:0}$	$C_{18:2}$>$C_{16:0}$>$C_{18:1}$ >$C_{18:0}$>$C_{18:3}$	$C_{16:0}$>$C_{18:2}$>$C_{18:1}$ >$C_{18:0}$>$C_{18:3}$

$C_{16:0}$ is well represented in all the lipidic categories. The quantities of $C_{18:3}$ observed at *Luffa cylindrica* are definitely higher than those of *Cucurbita moschata*. The most significant differences seem to be the level of the fatty acids of the series C_{18}. If we consider the polar lipids (phospholipids), we noted that the three identified essential components are: PC (Phosphatidyl choline), PE (Phosphatidyl ethanolamine) and PI (Phosphatidyl inositol). PC and PE are most abundant in food species (*Citrullus lanatus*, *Cucurbita moschata*, *Cucurbita pepo and Lagenaria siceraria)*. On the other hand at *Luffa cylindrica* the PC and PI are most abundant.

The identified phospholipids (polar lipids) play of the very significant roles. They enter on to particular the constitution of the biological membranes and mainly the formation of the oléosomes where will be held the synthesis of the triacylglycérols (Gurr, 1980). They take part in the synthesis of TAG (Roughan and Slack, 1982).

The comparison of the data as the Table 1 shows it hereafter, on the one hand of *Citrullus lanatus, Cucurbita moschata, Cucurbita pepo Lagenaria siceraria* and on the other hand *Luffa cylindrica* makes it possible to make bringings together between the different cucurbitaceous ones.

On the level of the residual lipids, *Citrullus lanatus, Cucurbita moschata* and *Luffa cylindrica* are very close; *Cucurbita pepo* and *Lagenaria siceraria* are close. In the neutral lipids, on the level of the diglycérides and acid fats, *Citrullus lanatus, Cucurbita pepo* and *Luffa cylindrica are close;* on the other hand on the level of the TAG, *Citrullus lanatus, Cucurbita pepo* and *Lagenaria siceraria* are close (inversion of fatty acids per order of importance). Lastly, in the case of the polar lipids, *Cucurbita moschata* and *Lagenaria siceraria* are close (PI), *Citrullus lanatus, Cucurbita pepo, Lagenaria siceraria* and *Luffa cylindrica* are close (PC), *Citrullus lanatus, Cucurbita pepo* and *Luffa cylindrica* is close (PE).

Triacylglycérols profile of cucurbitaceae seeds oils: The triglycerides were identified thanks to pilot oils of composition perfectly known and studied under the same chromatographic conditions (Heron and Tchapla, 1994).

From the knowledge of different AG obtained by gas chromatography and data-processing program, one goes up with the theoretical composition in TAG. As we saw previously, the law of response of DEDL is form:

$$Area = am^b$$

LLL being a standard available, one established in experiments the equation of the calibration curve:

$$Area_{LLL} = 8{,}915\ m^{1{,}4}$$

So at first approximation, it is supposed that has and B are close, whatever the TAG, one can compare $(m_{XYZ}/m_{LLL})^{1,4}$ obtained thanks to the data-processing program and $Area_{XYZ}/Area_{LLL}$ values determined in experiments for the 5 studied species.

The Fig. 1-5 represent the chromatograms of seed oils of five studied species the cucurbitaceous ones.

On the 35 theoretically possible triglycerids compositions, one observe in experiments 11 of comparable nature in the four species the cucurbitaceous ones.

Five major TAG were identified: LLL, OLL, PLL, OOL, SLL and six minors: POL., PPL, OOO, GROUND, POO and PSL.

The contents of these TAG vary in a more or less significant way of a species to another; one leads according to case's to a maintenance or a modification of the general profile of the TAG of oils.

Thus, in *Citrullus lanatus* the quantitative composition of LLL, SLL, OLL and PLL represents more than 80% of total triglycerides, with prevalence of LLL (37%); in *Cucurbita moschata* the quantitative composition of OLL, LLL, PLL and POL. represents more than 60% of total triglycerides, with prevalence of OLL (21%); in *Cucurbita pepo*. The quantitative composition of LLL, OLL, PLL and SLL represents more than 70% of total triglycerides, with prevalence of LLL (25%) in *Lagenaria siceraria* the quantitative composition of LLL, PLL, OLL and SLL represents more than 80% of total triglycerides, with prevalence of LLL (42%) and in *Luffa cylindrica*, the quantitative composition of OLL, LLL, PLL and POL.

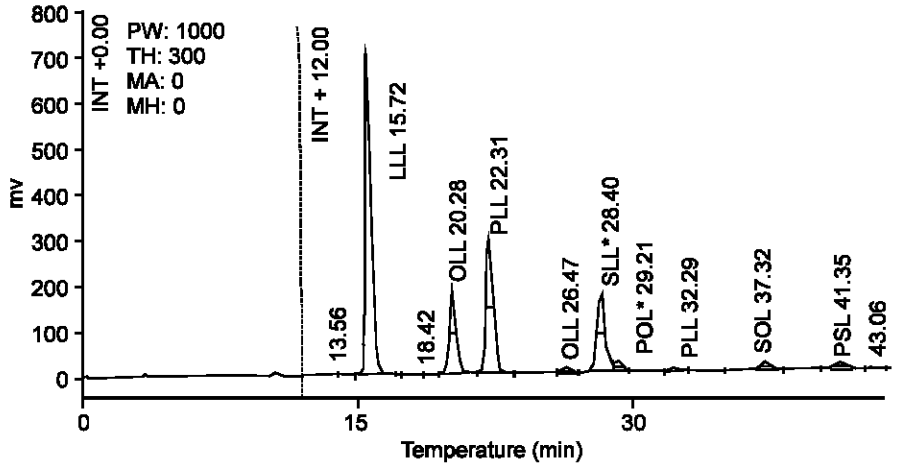


Fig. 1: TAG profile of Citrullus lanatus seed oil

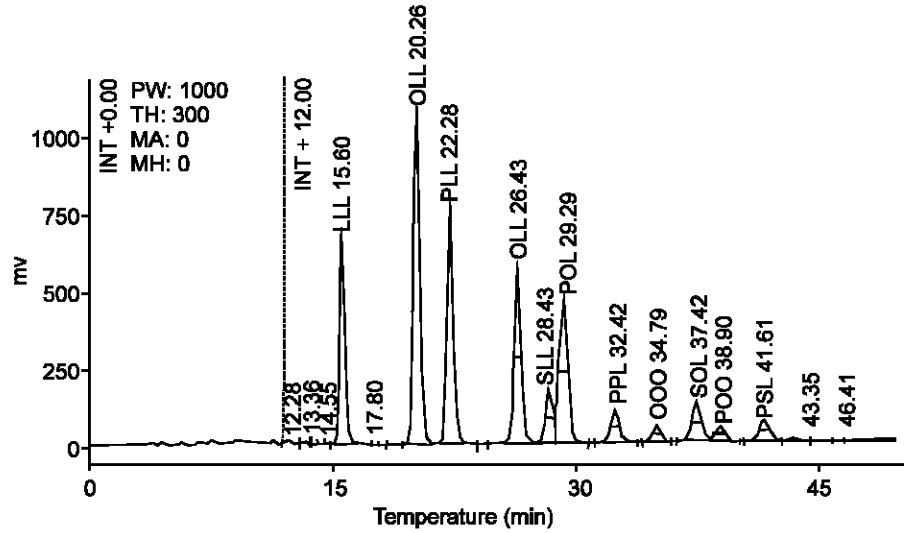


Fig. 2: TAG profile of *Cucurbita moschata* seed oil

represents more than 64% of total triglycerides with prevalence of OLL (21%). The method showed a good resolution of the peaks as shows it the various semi chromatograms while being quantitative. For an independent study, the same conclusion arises in Maloumbi thesis, 2006.

These oils have divergent quantitative compositions. In *Citrullus lanatus*, *Cucurbita pepo* and *Lagenaria siceraria*, the major triglycerides are: LLL, SLL, OLL and PLL; in *Cucurbita moschata* and *Luffa cylindrica* it is: OLL, LLL, PLL and POL. In all the cases, these triglycerides contain at least two unsaturated fatty acids. These results are in perfect agreement with those of the literature. We observe that concerning the cucurbitaceous ones usually consumed in Congo, three species out of four keep a similar profile in TAG; it is about *Citrullus lanatus*, *Cucurbita pepo* and *Lagenaria siceraria*. *Cucurbita moschata* presents a content of OLL which is five times higher than the average of this content for the three preceding species. It follows from there a modification of the hierarchy in the succession of the TAG, leading to a profile in TAG different from the precedent. These results confirm the resemblances observed in the compositions in fatty acids.

By considering *Cucurbita moschata* and *Luffa cylindrica*, one realizes that their only difference is on the quantities of OLL and OOL; one passes from *Luffa cylindrica* to *Cucurbita moschata* by simple increase in the quantity of OLL and OOL.

The oils extracted the species the cucurbitaceous ones resemble each other completely by their qualitative composition in TAG: 11 of the 35 awaited TAG were identified; they are the same ones for the five species. The individual content of the TAG varies from a species to another for *Citrullus lanatus*, *Cucurbita pepo* and *Lagenaria siceraria* there is conservation of

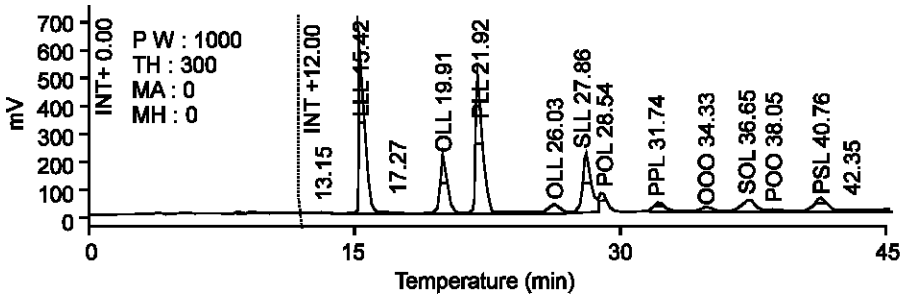


Fig. 3: TAG profile of *Cucurbita pepo* seed oil

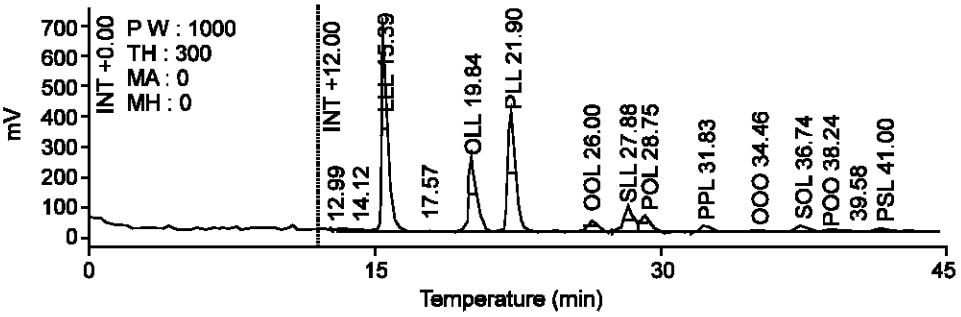


Fig. 4: TAG profile of *Lagenaria siceraria* seed oil

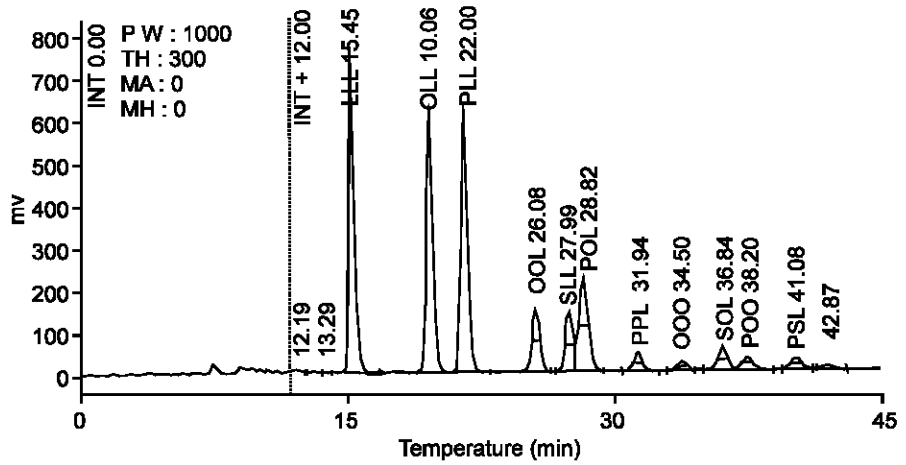


Fig. 5: TAG profile of *Luffa cylindrical* seed oil

the general profile in TAG of oil. *Cucurbita moschata* has a content of sufficiently high OLL thus involving a modification of this profile:-

Three of the five major TAG contain oleic and linoleic acids, the two remainders are linoleic combinations of two acids either with the palmitic acid, or with the stearic acid. Oleic and linoleic acids preferentially would thus be fixed in position 2 of glycerol and thus completely available for the organization. Such a possibility increases the nutritional interest of this botanical family.

Conclusion: The study of the lipidic classes (neutral and polar lipids) showed the extreme complexity of the distribution of the fatty acids. It arises clearly that the essence of the linoleic acid $C_{18:2}$ is concentrated in the TAG and the linolenic $C_{18:3}$ and palmitoleic $C_{16:1}$ acids can be used as factor discriminating for the species of cucurbitaceous studied. The cucurbitaceous ones analyzed do not present a great originality in the neutral lipids. The TAG constitutes the majority of the neutral lipids. On the level of the polar lipids, the three identified components are PC, PE and pi with PC and the PE like the most abundant lipids.

The cucurbitaceous ones show between them certain convergences. They are very rich in TAG. It is thought that the formation of the TAG coincides with the reduction in the rates of the DAG, AG and phospholipides. Our results show that it is possible to draw up points of

convergence between the cucurbitaceous ones. These results confirm once more that marrow oil is of the linoleic type whose majority fatty acid is $C_{18:2}$ essential fatty acid and essential to the man.

In addition to the studied parameters, unsaturated fatty acids/saturated fatty acids ratio shows that a classification can be established. *Cucurbita moschata* and *Luffa cylindrica* present a composition in fatty acids quantitatively similar. The respective proportions of acids palmitic, stearic, oleic and linoleic are very close. However the linoleic acid is more significant in *Luffa cylindrica*.

In an oil it quasi totality of the fatty acids do not exist in a free state, they enter the constitution of the TAG. It is thus on the level of the TAG that it would be necessary to seek the similarities and the differences.

References

Bligh, L.G. and A.J. Dyer, 1959. A rapid method for total lipid extraction and purification. Can.J. Biochem., 37: 911-917.

Gurr, M.I., 1980. The biosynthesis of triglycerols. In Biochem Plant. 4th Edn. Stumpf P.K. and Connee, pp: 205-248.

Heron, S. and A. Tchapla, 1994. Choix des phases stationnaires et mobiles pour la séparation en chromatographie en phase liquide de triglycérides de corps gras. Anal., 22: 114-126.

Keraudren, 1967. Flore du Cameroun. Cucurbitacées. Muséum National d'Histoire Naturelle. Paris.

Lepage, M., 1964. The separation and identification of plant phospholipids and glycolipids by two-dimensional thin-layer chromatography. J. Chrom., 13: 99-103.

Maloumbi, G., 2006. Etude de la biodiversité des graines de quelques cucurbitacées d'Afrique sub-saharienne. Mise au point d'une méthode de caractérisation de la fraction saponifiable. Thèse de doctorat d'état es-sciences. Université Marien Ngouabi Congo-Brazzaville.

Mangold, H.K., 1964. Thin-layer chromatography of lipids. J. Am. Oil Chem. Soc., 41: 762-773.

Metcalfe, L.D., A.A. Schmitz and J.R. Peka, 1966. Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. Anal. Chem., 38: 514-515.

Mvoula Tsieri, M., T. Silou and A. Tremolieres, 2005. Nayure et composition des classes des lipides de quatre espèces de cucurbitacées alimentaires du Congo-Brazzaville. La rivista italiana delle sostanze grasse. Vol LXXXII- Maggio/Giugno, pp: 147-151.

Roughan, P.G. and C.R. Slack, 1982. Cellular organization of glycerols metabolism. Ann. Rev. Plant Physiol., 33: 97-132.

Abbreviation: CL: *Citrullus lanatus*. CM: *Cucurbita moschata*, CP: *Cucurbita pepo*, DAG: Diacyl glycerol, FA: Fatty acid, FFA: Free Fatty acid, LC: *Luffa cylindrica*, LLL: Trilinolein, LS: *Lagenaria siceraria*, OLL: Oleodilinolein, OOL: Dioleolinolein, OOO: Triolein, Pc: Phosphatidyl choline, PE: Phosphatidyl ethanolamine, PI : Phosphatidyl inositol, PLL: Palmitodilinolein, POL: Palmitooleolinolein, POO: Palmitodiolein, PPL: Dipalmitolinolein, PSL: Palmitostearolinolein, SLL: Stearodilinolein, TAG: Triacyl glycerol