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Determination of Triacylglycerols, Fatty Acids and Total Lipid Content in Oil of 2 *Macrotermitinaes* Varieties from Congo

[1]Victor N'Goka and [2]Diane Julien-David

[1]Departement of Chimie, Faculte des Sciences, Universite Marien Ngouabi, Republique du Congo
[2]Laboratoire of Chimie Analytique des Molecules Bioactives, Faculte de Pharmacie, Universite Louis Pasteur, 74 route du Rhin, 67400 Illkirch, France

Corresponding Author: Victor N'Goka, Laboratoire de Pharmacochimie et Pharmacotechnie des Plantes Médicinales Chired Congo, B.P. 13.922, Brazzaville Tel: +242 06 663 76 06

ABSTRACT

The triacylglycerols and fatty acids composition of the oils of the *Bellicositermes natalensis* Haviland obtained from two different regions of Congo were qualitatively and quantitatively determined by gas chromatography/SM. This insects contained oil in the range of 48-53% dry matter, comparable with oleaginous plants like palm tree (45-50%), peanut (45-54%), more than soya (18-25%) and sunflower (22-50%). Levels of lipid content probably could be considered sufficient for economic industrial exploitation for every usage like human's consumption and medical usages. The pre-dominant triacylglycerol was palmitoyl-Oleoyl-Palmitoylglycerol (POP), 15.6-19.2%; followed by Palmitoyl-Oleoyl-Stearoylglycerol (POS), 14.6-18.1%, the minor were stearoyl-oleoyl-stearoylglycerol (SOS), 1.7-1.9%. The pre-dominant fatty acid was oleic (C18:1) and its content was about 57-60%, followed by palmitic acid (28-30%), stearic acid (9-11%), palmitoleic acid (3.5-4.0%), myristic acid (0.4%) and undetermined acid (0.2%). High qualitative similarity with butter fatty acids composition was observed and more, the content of this oil in monounsaturated fatty acids (60.5-62.5%), is near of the favourite olive oil which is 67.2%.

Key words: Termite, *Termitidae*, *Macrotermitinae*, *Bellicositermes natalensis*, insects, total lipids; triacylglycerol, fatty acids

INTRODUCTION

In general the entomophagy; the eating of insects is practiced throughout the world, except in Europe and in North-America. The edible fresh termites are consumed raw or cook, ground or entire. Dried termites are consumed entire or ground. Mignon (2002) descript that it is well known that some insects contain three or four time amount of proteins then chicken and pig. This author cited several results about nutritional values of insects. There in, he described amount of fat of insects from central Africans. Fats ware found to be comprised between 8.1 to 35% of dry matter with amount of linolenic acid more than 33% of the fatty acids.

The lipid composition of fruit and vegetable has lately received particular attention (Phillips *et al*., 2005; Celebi and Utlu, 2006; Sorho *et al*., 2006; Miladi *et al*., 2007; Nour *et al*., 2009; Okpuzor *et al*., 2009; Kim *et al*., 2010; Okoye *et al*., 2011). The saturated/unsaturated fatty acid ratio may be important in diet so adulterations are common and discrimination methods of oils are developed (Carelli and Cert, 1993; Ashurt and Dennis, 1996; Marikkar *et al*., 2005a, b; Cunha and Oliveira, 2006). However, this ratio is not sufficient to understand the vegetable or animal origins of the corresponding oil. We can see that, the saturated/unsaturated ratio is found to be, over 7 for prickly pear seed oils species, *Opuntia ficus indica* and *Opuntia stricta* (Ennouri *et al*., 2005) but is variable from 2.70 to 20 for some varieties of pomegranate seed oil (Fadavi *et al*., 2006). Studies of *Irvingia gabonensis* seed oils for two origins reported the saturated/unsaturated rates of 33 and 50 (Matos *et al*., 2009). One study of some legumes from Nigeria shows this ratio between 0.70 to 3.54 (Ajayi *et al*., 2006) for vegetable oil and in the other hand the same ratio is going from 0.5 to 2.1 for animals fat (Marikkar *et al*., 2005b; Jabeen and Chaudrhy, 2011). Krichene *et al*. (2009), showed that the rate of saturated fatty acids/unsaturated fatty acids also varies according to geographic site from 0.17 to 0.25 for chemlali virgin olive oils. Essential fatty acids are asking with emphasis because of the health potential of polyunsaturated fatty acids. It is considered that these fatty acids play a natural preventive role in cardiovascular disease and in alleviation of some other health problem (Kuller, 1997; Simopoulos, 1999; Hooper *et al*., 2001; Dodin, 2005; Adebote *et al*., 2006; Faremi and Ekanem, 2011). Polyunsaturated fatty acids promote the reduction of both total and HDL cholesterol and better; monounsaturated tends to lower LDL cholesterol. (Perz-Jimenez *et al*., 2002; Rawashdeh, 2003; Ausman *et al*., 2005; Ajayi and Ajayi, 2009).

In database (www.NutriStrategy.com) "fats, cooking oils and fatty acids" it have shown that 62% of fatty acid of butter fat were saturated and 33% unsaturated. Ninteen percent of fatty acid of vegetable shortening was saturated and 65% unsaturated. And margarine contains 31% of saturated fatty acids and 65% of unsaturated. Palm oil contains 49% of saturated fatty acids and 46% of unsaturated. Cmelik (1971) described composition of the lipids from the guts of termite queens which contained large amount of oleic acid. But, little attention has been paid to the study of chemical composition and particularly the fatty acid composition of termites' fat. The present study reports the total lipid content as well as the nature of triacylglycerols and fatty acids of *Bellicositermes natalenis* Haviland from 2 types of drying corresponding with two important regions of Congo.

MATERIALS AND METHODS

Samples: The termites are the same species of *Bellicositermes natalenis* Haviland obtained from two origins Sangha and Plateaux-Cuvette, respectively named termites S and termites PC. Sample was buying at the market in Brazzaville Congo on 2005 September. These two origins (Sangha and Plateaux-Cuvette), represent large amount of the total production of termites in Congo. Commercially dries termites were harvested during September to December from different regions. Termites were transported to the laboratory where they were conserved at -18°C.

Chemical materials: All solvent/chemicals used were of analytical grade and obtained from Sigma (MO, USA) and Merck (Germany).

Extraction of Bellicositermes natalenis fatty oil: Three individual 10 g samples of dry termites of each origin were refluxed with 300 mL of heptan in weighed flasks using a soxhlet apparatus according to the AACC (1987) method. The fat matter of the termites has been extracted with the help of a soxhlet equipped with extraction cartridges in cellulose. The termites ground, with the help of a homogenizer to knives, have been submitted to an extraction under ebullition of heptan during 9 h. The oils were recovered by distilling the solvent in a rotary evaporator at 45°C, then dried to constant weight in a vacuum oven at 90°C for 1 h and weighed.

Triacylglycerols analysis: A fat matter 0.5 mg mL^{-1} solution in the heptan has been prepared for the two types of termites, 1 µL of these solutions have been analysed with the help of chromatograph in sparkling phase Varian of type 3400 CX equipped of a "on-column" injector of SPI type and a detector to ionization of flame. The separation of the triacylglycerols has been done on a HT-5 column (SGE, Ringwood, Australia, stationary phase, 5% phenyl (equiv) polycarbonate-siloxane, thickness : 0.1 µm), 12 m long and 0.22 mm of internal diameter, with an external coating made of aluminum to resist the high temperatures. The initial temperature of the column was of 130°C maintained during 2 min. The column has been programmed then to reach the temperature of 400°C with a speed of 20°C min^{-1}, remained then to this temperature during 20 min. The initial temperature of the injector was of 130°C. It has been programmed to reach the temperature of 350°C with a speed of 100°C min^{-1}, remained then to this temperature until the end of the analysis. The temperature of the detector was, as for it, maintained to 400°C during all the analysis. The vector gas was helium (purity 99.9995%) with a debit of 1 mL min^{-1}.

Identification has been done by comparison of the retention times of a mixture of triacylglycerol knew (POP, POS, SOS with P: palmitic acid; O: oleic acid; S: stearic acid). The triacylglycerol (Cm:n, m = No. of carbon and n = No. of insaturation) separated according to their number of carbon.

Some free fatty acids have also been detected in the extracted fat matter. They have been identified by comparison of the retention times of a mixture of known fatty acid.

Fatty acid analysis: Fatty Acid Methyl Esters (FAMEs) were prepared, following the procedure described by international norm, ISO (1978). The principle is of saponification the triacylglycerols, then to change the free fatty acids into ester, in presence of boron trifluorure. The samples have been analyzed then with the help of a chromatograph in phase sparkling Varian of type 3400 CX equipped of a "on-column" injector of SPI type coupled to a spectrometer of mass (in fashion electronic impact, EI, energy of ionization 70 eV and on scale of mass of m/z 40-600. The separation of the derivative fatty acids has been done on a column capillary Factor Oven VF-5 ms (Varian, stationary phase: 5% phenyl, 95% diméthylpolysiloxane, thickness of 0.1 µm), 60 m long and 0.25 mm of internal diameter. The initial temperature of the column was of 70°C (maintained during 2 min). The column has been programmed then to reach the temperature of 230°C with a speed of 15°C min^{-1}. This temperature has been kept during 20 min. The initial temperature of the injector was of 70°C. It has been programmed to reach a temperature of 230°C with a speed of 100°C min^{-1}. The volume of injection was of 1 µL. The vector gas was helium (purity of 99.9995%) with a debit of 1 mL min^{-1}.

The peaks were identified based on their retention times and by their mass spectres. All samples were made in triplets.

Statistical analysis: Result, means and Standard Deviation (SD) of three simultaneous assays were carried out in all the methods.

RESULTS AND DISCUSSION

The amount of total lipid on dry matter ranged between 48 and 53% (w/w) in both origins but in termites S lipid content was 48%, whereas in termites PC, it was 53% (Table 1). The observation on Sangha and Plateaux-Cuvette origins was in agreement with the system of their transformation. In fact Sangha termite's are obtained after drying in fair whereas Plateaux-

Table 1: Lipid, free fatty acid and triacylglycerol composition of oil of two termites

Component	Termites S (%)	Termites PC (%)
Lipids	48	53
C14:0	7.3	11.1
C16:0	21.6	23.8
C18:1	25.8	34.6
POP	15.6	19.2
POS	14.6	18.1
SOS	1.9	1.7
TAG uknown	2.1	2.4

POP: Palmitoyl-oleoyl-palmitoylglycerol, POS: Palmitoyl-oleoyl-stearoyl-glycerol, SOS: Stearoyl-oleoyl-stearoylglycerol

Table 2: Fatty acid composition of oil from termites of two origins and butter

Component	Termites S (%)	Termites PC (%)	Butter (%)
C12:0			3.7
C14:0	0.5	0.43	12.6
C16:0	28.0	26.95	39.0
C16:1	3.5	1.9	1.8
C18:0	11.0	10.1	14.4
C18:1	57.0	60.7	28.4
SFA	39.5	37.5	69.7
USFA = MUSFA	60.5	62.6	30.2
SFA/USFA	0.65	0.60	2.31

SFA: Saturated fatty acids, USFA: Unsaturated fatty acids, MUSFA: Monounsaturated fatty acids

Cuvette termite's are drying by sun. Fair drying system occurs with more lost of oil. Our Central African insect contain more lipid then descript by Malaisse (2000) which will contained 8.1-35.0%. Finally, these lipid contents of termites (48-53%), could probably be considered sufficient for economic industrial exploitation compared with those of conventional vegetable like palm oil (45-50%), soya (18-25%), sunflower (22-50%) and peanut (45-54%).

The analysis of triacylglycerol of termite's fat allowed us to found that the major were POP (15.6-19.2%) and POS (14.6-18.1%). The minor were SOS (1.7-1.9%) and the undetermined (2.1-2.4%). Some free fatty acids observed were oleic acid (25.8-34.6%), palmitic acid (21.6-23.8%) and myristic acid (7.3-11.1%).

Concerning fatty acids, six were present in termites' oils but five was identified. Individual percentages of each fatty acid are given in Table 2. Oleic acid was determined to be the predominant fatty acid in termites. Its amounts ranged around 57%. Palmitic acid was determined to be the second most abundant in these samples. Percent of palmitic acid was between 28.5 and 30.0%. Stearic and palmitoleic acids were observed in termites. Their amounts ranged between 9.0-11.0 and 3.5-4.0%, respectively. To a lesser extent, the saturated myristic (0.4%) and undetermined acid (0.2%) were also found in these samples. Based on qualitative scale, it is curious to see the similarity of fat of *Bellicositermes natalensis* with butter fat. In fact we found as previously described that butter fatty acid components are oleic acid, palmitic acid, stearic acid, palmitoleic acid, myristic acid and lauric acid. The difference concern the saturated lauric acid which is in small amounts in butter but absent in termites. Based on quantitative scale, we observed that in butter, palmitic acid represent 39.5% followed by oleic acid 28.4%, stearic acid 14.4% and myristic acid 12.6%. The little amount is observed for lauric acid (3.7%) and palmitoleic acid (1.9%). The oleic acid was the main fatty acid in termites'fat about 57%, before palmitic acid which is in about half amount (29%). However, the main fatty acid in butter fat is palmitic acid 39% followed by oleic acid 28%. Then, the more quantitative different between butter and termites'fat is that butter contain 10% more in palmitic acid (39% against 29%) and about half amount of oleic acid (28% against 57%). It has been observed that the unsaturated fatty acids were predominant in termites than in butter fat with respectively 62.6 and 30.2%. The saturated/unsaturated acid ratio of termites' oil is low about 0.6 (Table 2). This ratio is lower than of butter 2.31. Several results are available from where saturated unsaturated ratio may be found (Marikkar *et al.*, 2005b; Ennouri *et al.*, 2005; Zimba *et al.*, 2005; Cunha and Oliveira, 2006; Kokten *et al.*, 2011). From animal fat to vegetable oil, these ratios do not give significative information about origins of fat or oil. The amount in monounsaturated fatty acid in termites is found to be between 60.5 and 62.6%. This ratio is near 67.2% which is described for the favorite olive oil by Rawashdeh (2003). Based on monounsaturated fatty acid, we described year new *Bellicositermes natalensis* fat that takes the second place after olive oil from conventional oils. And then this oil can have nutritional or medicinal utility.

CONCLUSION

Based on this study, *Bellicositermes natalensis* may be considered as the oleaginous source as some vegetables. Termites of Sangha and Plateaux-Cuvette so far had similar (but not identical). Interorigins differences in free fatty acids composition were found and they can be used to establish drying differences. A strong qualitative similarity in fatty acids was shown between butter and termites. Their fatty acids compositions contained high amount of oleic acid. Their monounsaturated fatty acids amount ranged this animal oil in the second place, just after the prestigious vegetable olive oil. Further studies are in coming to show nutritional or medicinal utility of this oil.

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