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Analysis of fatty acids levels of freeze-dried termite queen *Macrotermes gilvus* Hagen using gas chromatography-mass spectrometry

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Abstract

Objective: The objective of the study was to evaluate the fatty acids composition of freeze dried termite queen *Macrotermes gilvus* Hagen by gas chromatography (GC) combined with mass spectrometry (MS) and the most important physical-chemicals parameters.

Methods: Freeze dried termite queen oil that was used for analysis has been obtained by soxhlet extraction method. It is known that individual fatty acids can be identified by GC because of their different retention times, the samples of freeze dried termite queen oil were esterified to bring them into a vaporous phase, transforming the fatty acid from freeze dried termite queen oil into fatty acids methyl esters.

Results: The results showed that the major component of freeze dried termite queen oil was oleic acid (omega-9), linoleic acid (omega-6) and EPA (omega-3) contents 32812.70 mg/100 g (32.81 %), 345.15 mg/100 g (0.34 %) and 72.20 mg/100 g (0.07%) respectively.

Conclusion: It can be concluded that freeze dried termite queen oil is an excellent source of essential fatty acids omega-9 (oleic acid).

Keywords: Fatty Acids, Freeze Dried, Dried Termite Queen Oil, *Macrotermes gilvus*, Omega-9

Introduction

Edible insects have served as traditionally and nutritionally important food for Africans, Asians, Australians and Latin Americans for many years [1]. Insects are high in protein, energy (calories) and various vitamins and minerals [1, 2]. Edible insects are important dietary components in many developing countries. Insects commonly consumed include locusts, termites, grasshoppers, weevils and various caterpillars [3]. Many studies have shown that edible insects contain appreciable amounts of proteins of good quality and high digestibility [4, 5]. They have also been found to be rich sources of fat, vitamins and minerals, especially iron and zinc [2, 6, 7]. Phytophagous insects such *as Lepidopterous larvae* have been reported to contain appreciable amounts of the polyunsaturated fatty acids [4].

This beneficial effect on health was associated to the hypocholesterolemic effect of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contained in certain edible insects [8]. Unfortunately, these insects are not very often consumed for their beneficial effects. In Africa, insects are one of the immediate sources of fatty acids and proteins used to satisfy the elementary need. As a result, edible insects must be looked upon as alternative sources for the food problems faced in Africa. The objective of this paper was to give highlights on the nutritional aspects of insects consumed in sub-Saharan Africa, based on some examples of insects consumed in Cameroon and to show their potential as a valuable source of lipids and essential fatty acids.

Macrotermes gilvus Hagen (Order: Isoptera, Family: Termitidae, Subfamily: Macrotermitinae) is a common mound-building termite in the whole of South-East Asia, from Malaya and Indo-China to Indonesia (Sumatera, Java, Borneo, etc.) and the Philippines. The queen termite *Macrotermes gilvus* Hagen has been used traditionally in Indonesia as skin disease, hypertension, diabetic, vertigo, Alzheimer, others. *Macrotermes gilvus* Hagen have been investigated as burn healing effect topically applied in doses of 0.5 g/ 10 g, immunomodulatory activity at 10 mg/kg BW, antihiperlipidemic activity at 300 mg/kg BB and acute toxicity and sub acute toxicity (LD_{50}) at 1000 mg/kg BW [9]. The queen of termites was the nutrient-rich insects that can supply adequate quantities of various amino acids for pharmaceutical nutrient [10].

Such composition data would be very useful for pharmaceutical studies, in updating composition tables and pharmacologic effect. The purpose of this study was to determine the fatty acid profile of termite queen *Macrotermes gilvus* Hagen.

Materials and methods

Materials

The queen termite *Macrotermes gilvus* Hagen, the fatty acid methyl esters (FAMEs) (Sigma Chemicals, USA), n-hexane

(Merck), potassium hydroxide (Merck), hydrochloric acid (Merck), methanol (Merck).

Equipment

High performance gas chromatography system and Mass spectrometry (Hawlet, USA).

Procedure

Sample collection and processing

The queen termite *Macrotermes gilvus* Hagen were collected in the early morning hrs of September 2015, from palm plantation, Taluak Kuantan, Riau, Indonesia. They were opened from nest, freeze-dried to fine powder. The processed termite sample was packaged in labeled dry glass jar and stored at 4 ºC until analysis [11].

Fatty acids analysis

The oil, powder and fresh form in a portion, 5 g of the ground sample was extracted for 8 h with 20 mL of n-hexane. Then 0.8 g of the extracted oil was saponified for 1 h with 15 mL of KOH in methanol at 90 ºC, treated with 2 ml of 1.4 N HCl for another 1 h and allowed to cool to room temperature. To the cooled solution, 15 mL of n-heptane and 200 mL of brine were added, mixed thoroughly and 5 µL of the fatty acid methyl esters (FAMEs) solution was analyzed with the aid of a high performance gas chromatography system (Hawlet, USA) for the fatty acids content. The FAMEs extract was co-analyzed with authentic FAME standards (Sigma Chemicals, USA) of known structures.

Results and discussion

Table 1 showed the fatty acid profile of lipid content of freeze dried termite queen oil *M. gilvus* Hagen. Oleic acid (C18:1) was found to be the fatty acid with the highest percentage composition (32812.70 mg/100 g) in the sample, while linolenic acid was the least at 345.15 mg/100 g (0.34 %) and for palmitic acid content 13.13 %.

Table 1: Analysis of freeze dried termite queen oil by GC-MS

No	Parameter	Unit	Result	Limit of Detection	Method
1.	Oleic Acid	mg / 100 g	32812.70	-	18-6-1/MU/SMM-SIG, GC
2.	Linolenic acid	mg / 100 g	345.15	-	18-6-1/MU/SMM-SIG, GC
3.	EPA	mg / 100 g	72.20	-	18-6-1/MU/SMM-SIG, GC
4.	Palmitic acid	%	13.13	-	18-6-1/MU/SMM-SIG, GC
5.	Stearic acid	%	7.42	-	18-6-1/MU/SMM-SIG, GC

Table 2: Analysis of freeze dried termite queen powder by GC-MS

No	Parameter	Unit	Result	Limit of Detection	Method
1.	Oleic Acid	mg / 100 g	13709.70	-	18-6-1/MU/SMM-SIG, GC
2.	Linolenic acid	mg / 100 g	49.10	-	18-6-1/MU/SMM-SIG, GC
3.	EPA	mg / 100 g	8.15	-	18-6-1/MU/SMM-SIG, GC
4.	Palmitic acid	%	4.90	-	18-6-1/MU/SMM-SIG, GC
5.	Stearic acid	%	2.83	-	18-6-1/MU/SMM-SIG, GC

Table 3: Analysis of termite queen fresh by GC-MS

No	Parameter	Unit	Result	Limit of Detection	Method
1.	Oleic Acid	mg / 100 g	4357.20	-	18-6-1/MU/SMM-SIG, GC
2.	Linolenic acid	mg / 100 g	113.85	-	18-6-1/MU/SMM-SIG, GC
3.	EPA	mg / 100 g	33.65	-	18-6-1/MU/SMM-SIG, GC
4.	Palmitic acid	%	2.10	-	18-6-1/MU/SMM-SIG, GC
5.	Stearic acid	%	1.00	-	18-6-1/MU/SMM-SIG, GC

Table 2 showed the fatty acid profile of lipid content of freeze dried termite queen *M. gilvus* Hagen. Oleic acid (C18:1) was found to be the fatty acid with the highest percentage composition (13709.90 mg/100 g) in the sample but its lower than freeze dried termite queen oil, while linolenic acid was the least at 49.10 mg/100 g and for palmitic acid content 4.90 %. Table 3 showed the fatty acid profile of lipid content of termite queen fresh *M. gilvus* Hagen but the content was lower compared than oil and freeze dried form (Figure 1).

The lipid content of the termite is appreciably high in comparison with the reported mean lipid content of 20% for most adult insects on a dry weight basis [4]. Our observation is in agreement with reported oil content of 28.37 ± 0.00% for *M. nigeriensis*, 22.5% for *M. notalensis* and 28.2% [12] and 36.12 ± 0.28% for *M. bellicosus* [13]. The lipid content we obtained, along with those mentioned above, are however lower than a lipid value of 46.1% (moisture free basis) earlier reported for *M. bellicosus* [14].

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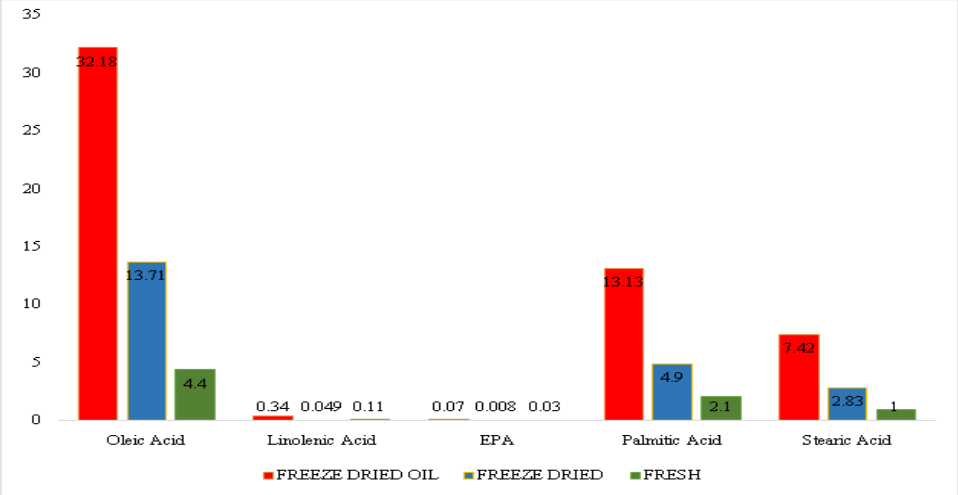

Figure 1: Chromatogram of amino acids of queen termite

Dietary fats increase the palatability of food by absorbing and retaining flavor. Hence, the high lipid content observed in this termite could have contributed to its highly acceptable flavor when fried or roasted, and may contribute to the reduced need of oil in the preparation of its delicacy. *M. gilvusis* rich in oleic acid, palmitic acid and linoleic acid, an essential fatty acid, but poor in stearic acid andeicopentanoic acid. These observations are in agreement with the reports on *M. bellicosus* [15] and other insect species [4]. The total saturated fatty acid content of the termite is 39.35%, while that of the unsaturated fatty acids is 60.64%, with monosaturated fatty acids (MUFA) comprising 53.07% and polyunsaturated fatty acids (PUFA) making up 7.57% only.

Some research present the beneficial aspects of some insects consumed in the world such as sub-Saharan Africa, Cameroon, southern Nigeria [5]. The main components of termite oil Macrotermes sp., in Cameroon are palmitic acid (30.47%), oleic acid (47.52 %) and linoleic acid (8.79%) [6]. *Macrotermes bellicosus* was extracted 31.46% ± 0.57% and major fatty acids were palmitic acids 42.45% ± 0.20 and linoleic acids 24.24% ± 1.08, roasted *Macrotermes bellicous* contained 34.4 gram fat/100 g portion [7].

Physicochemical properties of the oils of four castes of subterranean termite, *Macrotermes subhyalinus* in queen termite was 157.18 free fatty acid. Winged termites *Macrotermes falciger* contain of crude fat 43.0% ± 0.1. *Trinervitermes germinatus* contain of crude fat 47.50 ± 2.50% [8]. Fatty acids composition of soldier termites *Coptotermes gestroi* content 42.27% and the major unsaturated fatty acid was linoleic acid 8.60% accounted for total fatty acids [16].

Saturated fatty acids are not good for human consumption because they have been implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging [17]. Low saturated fatty acid content makes *M. gilvusan* important food component for those who have high blood cholesterol content and may be at risk of cardiovascular disease. Omega-9 is a MUFA effects with lower LDL and raise HDL. A diet providing 1-2% of its caloric energy as fats has been reported to be sufficient for human consumption [17].

Diet with excessive amounts of omega-6 PUFA and a very high omega-6/omega-3 ratio, has been reported to promote the pathogenesis of many diseases, including cardiovascular disease, cancer and inflammatory and autoimmune diseases [18]. However, consumption of a lower ratio of omega-6/omega-3 diet resulted in suppressing these effects. Diet with a ratio of omega-6/omega-3 ranging from about 2/1 to 5/1 benefited human health, such as reduction of mortal rate in patients with cardiovascular disease, reduction of colorectal cancer proliferation, repression of inflammation in patients with rheumatoid arthritis, and beneficial effect on patients with asthma [6, 18].

Conclusion

The results showed that the major component of freeze dried termite queen oil were oleic acid (omega-9); linolenic acid (omega-6) and EPA (omega-3) and its contents were 32812.70 mg/100 g (32.81%), 345.15 mg/100 g (0.34%) and 72.20 mg/100g (0.07%) respectively. It can be concluded that freeze dried termite queen oil is an excellent source of essential fatty acids omega-9 (oleic acid).

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